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GENETICS AND BREEDING

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NON-GENETIC FACTORS AFFECTING LAMENESS CASES AND MILK PRODUCTION LOSSES CAUSED BY LAMENESS CASES IN DAIRY HERDS: A META-ANALYSIS

Savas ATASEVER

University of Ondokuz Mayis, Faculty of Agriculture, Department of Animal Science, 55139, Samsun, Türkiye

Corresponding author email: satasev@omu.edu.tr

Abstract

Lameness and claw disorders have still adverse effects on cow's health and milk production levels in dairy enterprises. The objectives of this investigation were revealing non-genetic factors affecting lameness cases (LC) and estimating 305 daily milk production losses (305 dMYL) due to LC in dairy herds. A total of fifteen study results reported in the scientific journals between 2002 and 2021 were analyzed. The percentage of the non-genetic factors affecting LC were noted as days in milk (DIM; 31.25%), parity (P; 25%), season (S; 9.37), calving season (CS; 9.37) and others (25%).To estimate the effect of herd size (HS) on 305 dMYL, three HS groups were divided (small: ≤500 cows, moderate: 501-1498 cows and big: ≥1499 cows). Thusly, 305 dMYL were calculated to be 630 kg, 377.2 kg and 493.57 kg, respectively. In country level, 305 dMYL were determined to be 494 kg, 408 kg and 398.66 kg for UK, US and other locations, respectively. Finally, the overall mean of 305 dMYL caused by LC was 419.66 kg/cow.

Key words: *cattle, dairy, environmental factors, milk yield, lameness.*

INTRODUCTION

In dairy farm enterprises, some welfare and health disorders are still seen as the main problems affecting productivity. Mastitis, unsuitable body condition score, claw disorders, laminitis and lameness may be mentioned among the managemental complications in the farms. Of these, lameness cases are frequently exposing causes those impact cow welfare and milk production.

In an earlier study conducted by Green et al. (2010), cows with sole ulcers had produced lesser milk (1 kg/day). The authors declared that this time is the period of cows before the lameness occurrence. Besides, cows with lame had lower body condition and lower fertility (Mellado et al., 2018). Researchers explained this case that lame cows may have prolonged negative energy balance that adversely affects reproduction. Singh et al. (2011) reported that sole hemorrhage, sole ulcer, white line separation, heel erosion, interdigital necrobacillosis and so on, contribute to about 99% of lameness cases.

Randall et al. (2016) pointed out that a critical control point for lameness in dairy herds should purpose to prevent claw horn lesions and digital dermatitis in dairy heifers. The time of calving has been termed to be an important risk stage when the stress related to physiological changes increased.

According to literature, different studies have been carried out the relations of lameness cases with milk production of dairy cows. Thus, investigating the effective factors on lameness discussed in these researches may ensure a useful guide to the dairy producers.

The objectives of this study were to reveal non-genetic factors affecting lameness and to calculate milk yield losses due to this disorder in dairy herds.

MATERIALS AND METHODS

To determine effect of non-genetic factors affecting milk production and reveal 305 daily milk yield (305 dMY) losses due to lameness case (LC), fifteen study results those published in the scientific journals between 2002 and 2021 were assessed. 305 dMY was used as milk yield criteria and some of the lactational milk yield values reported in the evaluated studies were converted to this parameter. Similarly, some foot diseases or disorders (*such as sole ulcer, digital dermatitis or claw lesions*) were assessed as the lameness case that refers to foot disorder. The non-genetic factors those investigated in the articles and 305 dMY losses by LC were recorded to Excel program. The countries of the studies and herd size (HS) values were also noted. The percentages of non-genetic factors evaluated by the authors were separately calculated. To determine effect of HS on 305 dMY losses, three HS subgroups (*small: ≤500 cows, moderate: 501-1498 cows and big: ≥1499 cows*) were established. Milk production losses were also calculated with the country base (*UK, US and other locations*). Thus, a total 305 dMY loss caused by LC was determined for investigated dairy herds.

RESULTS AND DISCUSSIONS

The basic information of the studies conducted on the factors affecting LC and milk production losses caused by LC is given in Table 1. As seen, many different factors were affective for LC. Of these, days in milk (DIM) and parity (P) were the main non-genetic factors.

Actually, DIM could be assessed with stage of lactation (SL) that refers to phase of lactation period of a milking animal. It is well known early stage period of lactation may cause a negative energy balance and this case converted by the animal's body to normal metabolism at the middle lactation period. Mellado et al. (2018) informed that new LC occurrences may be seen in dairy herds at the beginning of the lactation. Thusly, taking severe measures according to DIM may be suggested to farm owners especially for the initial phase of the lactation of cows.

Similarly, P was another main factor for LC (Table 1). Neave et al. (2017) reported that dairy cows with later parities produce more milk. However, this process may become the cows to more worned out animals. Sahar et al. (2022) emphasized that cows with later P had more new LC when compared to other ones.

According to Table 1, 305 dMY losses due to LC were changed from 183 kg to 817 kg. The differences among the loss values may be caused by various animal factors, management factors, locations or the others.

The frequencies of the effective factors evaluated here are presented in Figure 1. It can be seen that percentage of DIM reached to the highest ratio among the non-genetic factors.

At this point, tracking all cows according to their DIM period might be regarded to be a beneficial approach to prevent new LC disorders.

Separating cows by DIM or SL groups and managing them according to their production period may be seen a positive management strategy to decrease LC occurrence in the farms.

As presented in Figure 1, parity was another important factor for LC. As stated earlier, cows with later parities may be referred as older animals and elevated age and repeated calvings may load to meet new health and welfare problems.

Table 1. Findings on the factors affecting lameness cases and 305 dMY losses

Author	Year	Effective Factor	305 dMY loss (kg)
Archer et al.	2010	ML	350
Mellado et al.	2018	AC, MC	554
Green et al.	2002	F, ML, P, S, TD	360
Logroño et al.	2021	DIM	183
Bicalho et al.	2009	BCS, SL, P	369
Amory et al.	2008	DIM, P, S	369
Amory et al.	2008	DIM, P, S	574
Hernandez et al.	2002	Y, DIM, P, SC	575
Hernandez et al.	2005	DIM, P, SC	319
Hultgren et al.	2004	P, Y	479
Bicalho et al.	2008	DIM	369
Randall et al.	2016	DIM	817
Relun et al.	2013	DIM, P, S	190
King et al.	2017	BCS, P	488
Singh et al.	2011	DIM	499

ML= month of lactation; AC= age of calving; MC= month of calving; F= farm; P= parity; S= season; TD= test day; DIM= days in milk; Y= year; SC= season of calving; BCS= body condition score

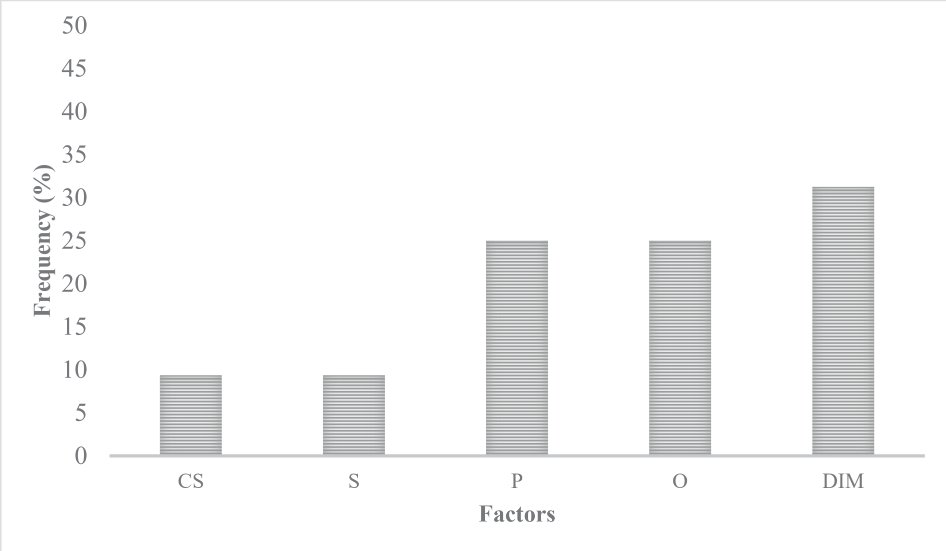

Figure 1. Distribution of non-genetic factors affecting LC
(CS= calving season; DIM= days in milk; P= parity; S= season; O= others)

Similar to suggestion for DIM, dividing herd in term of P groups and monitoring animals by P subgroups may be more profitable.

Effect of S on LC should also be taken into consideration when Figure 1 is assessed. Fregonesi et al. (2007) revealed that LC was active in the cool seasons. Author declared that winter conditions may increase standing time that adversely affect LS hazard. In contrast, Sanders et al. (2009) reported that LC was active in the hot seasons. It is well known that under conditions of heat stress, standing time increases (Cook et al., 2007). Shortly, taking practical measures by different seasons is seen an obligation for dairy farms.

CS had similar impact on LS (Figure 1). Actually, this factor cold be assessed with S factor due to similar effect on the productivity traits and disorders. Such as, cows those calved in very hot or very cold seasons may be exposed to stress due to adverse of the climatic environment. Also, cows may be exposed to dirtiness especially in the rainy weathers. Regarding climatic conditions of the locations, which are the main life area of the animals, and keeping them from the adverse effect of the CS should also been suggested to the farm directors.

In Figure 1, percentage of the other factors affecting LC and 305 dMY was estimated to be 25%.

As clearly seen that multiple factors have affective on the both variants.

As one of the general concepts of animal science, genetic and environment are two main determiners of the phenotypic characters. In other words, selecting cows in accordance with only their genetic merits is not a certain process to achieve an elite herd for the next generation. Thus, ignoring the multiple environmental factors may cause the economic loss with loading health or wealth problems in the animals.

Milk production losses due to LC have been evaluated in HS base.

According to Figure 2, farms with moderate HS had the better position by 305 dMY losses. Indeed, small or large herds had relatively higher production losses and this finding pointed out that dairy farms should have moderate number of cows to boost their profitability.

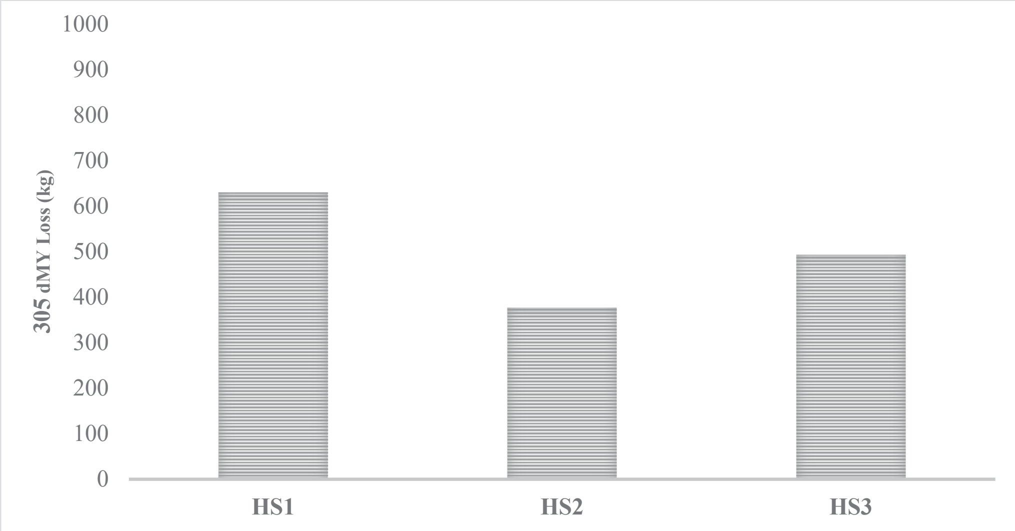

Figure 2. 305 dMY losses caused by LC in herd size base (HS1= small, HS2= moderate and HS3= big herds)

However, farms with low or high number of cows may cause to adverse impact on quality and quality of bovine milk.

In the studies including different sized herds, herd size should be regarded to be an important non-genetic factor for achieving high amount milk production. Actually, dealing with low number of cows may load to less regarding husbandry applications in the farm. The fact that producers hope to benefit from economies of scale accrued from lower investments per cow, lower variable costs per unit of production, and increased labour efficiency (Archer et al., 2013). However, dealing herd with large size may load to excessive processes to the farm staff and lack of the husbandry practices related to herd monitoring. In an initial study carried in Poland conditions, herds with large size had higher ($P<0.05$) somatic cell count (SCC) that refers to raw milk quality. Researchers commented this case that increase in herd size may cause to increased risk of infectious diseases, including mastitis. According to their comments, another reason may be the fact that in small sized herds fewer cows are handled by one person, as a result of which animals are treated more individually than in the bigger herds. Authors point out that weak management of higher pasture stocking rates in larger herds could contribute to high risk of intramammary infection. Besides, Barkema et al. (1998) reported that large Dutch herds had higher SCC when compared to herds with smaller ones. Oleggini et al. (2001) emphasized that economies of scale on modern dairy farms are belonging to lower investment per cow, lower costs of production per unit, increased labor and management efficiency. Therefore, managing cows with moderate number may be seen more profitable approach to prevent milk production and financial losses in dairy enterprises.

Milk production losses due to LC were also calculated by county where the investigations had been carried out. Accordingly, about similar means were calculated for three subgroups those shown in Figure 3. However, a loss with 95.34 kg per cow between group 1 (UK) and group 2 (other countries) may be found as attractive. Relatively higher losses determined in the first and second groups can be explained by their high merit cows when compared to third group. A hypothesis for this case that if the lactation or 305 dMY values of the examined cows had been given, it might be met that these levels were relatively higher too. In other words, high producing cows had more open to yield losses when compared to moderate yielding cows.

In a general assessment, the average of 305 dMY loss was calculated to be 419.66 kg/per milking cow. As seen, this amount may be assumed to be very high per cow or farm base.

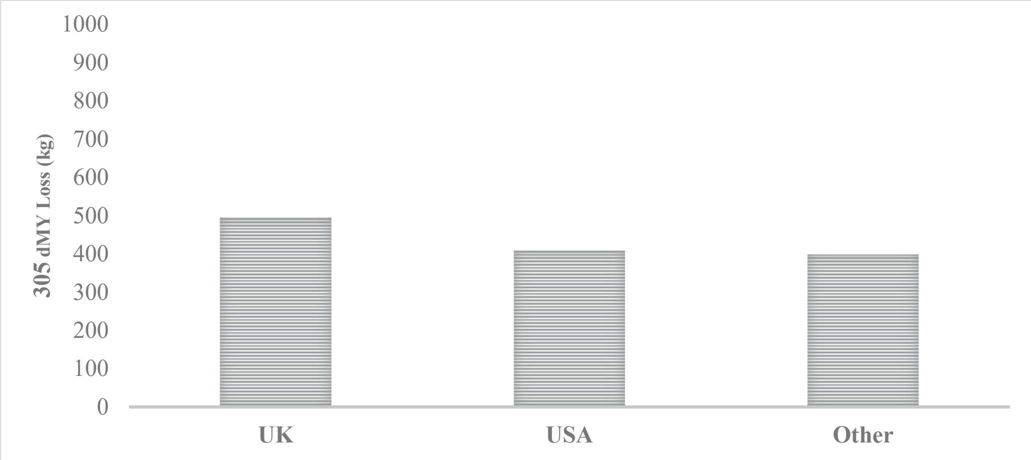

Figure 3. 305 dMY losses caused by LC in country base

CONCLUSIONS

In this study, the factors affecting LC and milk production losses with 305 dMY base were discussed. While P and DIM were revealed to be main factors for LC, the average of 305 dMY loss was calculated to be 419.66 kg/per cow. Herds with moderate cow number was found as better position when compared to large or small sized. According to locations, herds in the UK had more milk loss due to LC.

Finally, the calculated production losses clearly shows the important financial damage related to yield losses. That's why, showing more focus especially on cow cleanliness is seen an essential process for farm directors in the all locations.

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AVIAN EVOLUTION: A COMPREHENSIVE REVIEW OF TAXONOMY AND PHYLOGENETIC HISTORY

Ioana BOLOHAN, Roxana LAZAR, Bianca Maria MADESCU, Paul Corneliu BOIȘTEANU

"Ion Ionescu de la Brad" Iasi University of Life Sciences, Faculty of Food and Animal Sciences, 3 Mihail Sadoveanu Alley, Iasi, Romania

Corresponding author email: bolohan.ioana@yahoo.com

Abstract

With an integrative approach to research, this study explores the taxonomy and complex evolutionary history of birds, employing an integrative research approach in order to deconstruct the multi-faceted narrative of bird evolution. By converging molecular phylogenetics, comparative morphology, and biogeographic analyses, we conducted a comprehensive taxonomic reassessment, resulting in fine-tuning the classification of birds and revealing novel phylogenetic relationships. Our exploration extends into the fossil record, where the integration of paleontological data and molecular clock analyses illuminates key evolutionary steps. Our findings highlight a dynamic evolutionary trajectory characterized by diversification events and adaptive radiations that have intricately shaped the avian lineage across geological epochs. Advanced imaging technologies and biomechanical assessments further enrich our understanding of the morphological adaptations underlying avian ecological niches. This multidimensional research not only propels the field of avian taxonomy forward but also provides a nuanced perspective on the complicated coevolutionary dance between birds and their environments. The study provides valuable insights into the broader landscape of avian evolutionary biology, enhancing our understanding of the mechanisms that have sculpted avian diversity and ecological interactions over millions of years.

Key words*: avian evolution, bird taxonomy, phylogenetics, comparative morphology, fossil record.*

INTRODUCTION

The intricate tapestry of avian evolution has captivated the curiosity of researchers for centuries. Birds, with their remarkable diversity of forms and behaviours, present an evolutionary saga that unfolds across millions of years. Understanding the mechanisms that have shaped this diversity is not only a pursuit of scientific curiosity but also holds profound implications for ecology, conservation, and our broader comprehension of evolutionary processes.

As we embark on this exploration, it is essential to delve into the historical context of avian taxonomy. Over time, our understanding of avian relationships has evolved, shaped by pioneering taxonomists and informed by advances in technology. The classification of birds, rooted in morphological characteristics, has witnessed transformative shifts with the advent of molecular techniques, challenging and refining our perceptions of avian relationships (Mitchell, 2015).

The integration of molecular phylogenetics, comparative morphology, and biogeographic analyses stands as a pivotal approach to unravelling the complexities of avian evolution (Torke, 2021). Molecular phylogenetics allows us to peer into the genetic blueprints, providing insights into evolutionary relationships. Comparative morphology, on the other hand, explores the structural variations that have arisen over time, offering a tangible link between form and function. Meanwhile, biogeographic analyses shed light on the geographic distribution of species, offering glimpses into historical events that have shaped avian distribution patterns.

A comprehensive review of the existing literature on avian evolution forms the backbone of this study. We navigate through the works of predecessors who laid the groundwork for our understanding of avian taxonomy and evolution. This exploration reveals the current state of knowledge, showcasing advancements made through the integration of molecular techniques and the challenges presented by morphological and biogeographic complexities.

Within the literature, we identify gaps and controversies that persist in our understanding of avian evolution. These gaps serve as the driving force behind our hypotheses and research questions, prompting a deeper investigation into areas where knowledge is incomplete or conflicting (Hooper, 2022). The need for our present study becomes apparent as we recognize the potential contributions it can make in addressing these gaps and advancing the field.

Research Objectives. Hypothesis

This study is driven by a commitment to comprehensively explore the taxonomy and complex evolutionary history of birds. Our primary objective is to employ an integrative research approach that harmonizes molecular phylogenetics, comparative morphology, and biogeographic analyses. By synergizing these diverse methodologies, we aim to deconstruct the multi-faceted narrative of bird evolution, pushing the boundaries of our understanding beyond traditional taxonomic boundaries.

Emphasizing the integrative nature of our approach, we seek to fine-tune the classification of birds. This involves not only reevaluating existing taxonomies but also uncovering novel phylogenetic relationships that may have been obscured by traditional methods. The synthesis of molecular, morphological, and biogeographic data is crucial in achieving a holistic understanding of avian evolution.

In formulating our hypotheses, we draw upon the identified gaps in the literature. We propose hypotheses that align with the questions raised by previous research and aim to provide clarity where ambiguity persists. Clear articulation of our research questions drives the study forward, guiding the design of methodologies and analyses. These hypotheses set the stage for a meticulous examination of avian evolution, guiding our exploration of taxonomic reassessment, phylogenetic relationships, and the integration of paleontological data.

Hypothesis related to Taxonomic Reassessment:

•H1: The integrative approach combining molecular phylogenetics, comparative morphology, and biogeographic analyses will reveal previously unrecognized taxonomic relationships among avian species, leading to a refined and more accurate classification of birds.

Hypotheses related to Phylogenetic Relationships:

•H2: Comparative morphology will unveil shared adaptive morphological traits among distantly related avian species, indicating convergent evolution and influencing our understanding of phylogenetic relationships.

Hypotheses related to Fossil Record Analysis:

•H3: Integration of paleontological data and molecular clock analyses will elucidate key evolutionary steps in the avian lineage, providing insights into the timing of major adaptive radiations and diversification events.

Hypotheses related to Diversification Events and Adaptive Radiations:

•H4: The study will identify evidence of past diversification events and adaptive radiations in the avian lineage, highlighting the role of ecological and environmental factors in shaping avian diversity across geological epochs.

These revised hypotheses maintain a focus on key aspects of avian evolution, emphasizing taxonomic reassessment, the integration of paleontological data, and the exploration of diversification events and adaptive radiations. The adjustments in Hypothesis 2 aim to streamline the language while retaining the core idea related to the role of comparative morphology in understanding phylogenetic relationships.

MATERIALS AND METHODS

To achieve the objectives of this research, I have reviewed eight bibliographic sources and four specialized websites in the fields of ornithology and evolutionary biology. The investigative methods applied in this study have included observation, analysis, and interpretation of data extracted from specialized literature. As a result, this research adopts an integrative approach, exploring the taxonomy and complex evolutionary history of birds using a diverse range of investigative methods. Through the analysis of molecular phylogenetic convergence, comparative morphology, and biogeographic analyses, we have re-evaluated the taxonomy of birds, adjusting classifications and revealing new phylogenetic relationships.

In addition to analysing and synthesizing data extracted from the specialized literature, this review also focuses on the laboratory methods used in relevant previous studies. Through a detailed investigation of these works, we have identified various experimental techniques and laboratory procedures that have contributed to obtaining the presented results. Thus, we intend to describe and evaluate these methods in this paper, providing a comprehensive perspective on the research process in our field of interest. From molecular biology and genetics techniques to morphological and biomechanical analysis methods, we will explore the wide range of tools and protocols used to investigate the taxonomy and evolution of birds. We aim to highlight not only the results and conclusions obtained in these studies but also how the applied laboratory methods have influenced and supported them.

RESULTS AND DISCUSSIONS

Sampling and laboratory procedures

Sample Collection

Specimens for this study were collected through a meticulously planned process, encompassing both contemporary and archival sources. Avian specimens were obtained from various natural history museums, research institutions, and field expeditions (Vanni & Farina, 2019; Lönnberg, 1926; Irestedt, 2022; Cibois, 2020; Shrimper, 1992). Our collection aimed to cover a wide taxonomic range, incorporating representatives from diverse avian families and ecological niches.

To ensure the reliability and relevance of our analyses, inclusion criteria were established. Specimens with well-documented taxonomic information, including detailed morphological descriptions, were prioritized. Additionally, specimens with associated molecular data were favored, providing a robust foundation for molecular phylogenetic analyses.

Geographic representation was a key consideration in our sample selection process. We sought specimens from diverse regions to capture the breadth of avian diversity and account for potential biogeographic influences. Furthermore, temporal considerations were addressed by including specimens from different geological epochs, aligning with the study's focus on evolutionary history.

Laboratory Techniques

Molecular phylogenetics played a central role in our investigation. DNA extraction followed established protocols, with a focus on obtaining high-quality genomic DNA. We employed state-of-the-art sequencing technologies, such as next-generation sequencing, to generate molecular data. Phylogenetic analyses were conducted using maximum likelihood and Bayesian inference methods, incorporating both nuclear and mitochondrial markers to enhance resolution and accuracy.

Comparative morphology analyses involve the detailed examination of morphological features across avian specimens. Traditional morphometrics and geometric morphometrics were employed to quantify shape variations. Adaptive traits identified in Hypothesis 2 were scrutinized, providing insights into potential convergent evolution. This approach allowed us to bridge the gap between form and function, aligning with our overarching goal of taxonomic reassessment.

Biogeographic analyses were conducted to understand the distribution patterns of avian taxa. Geographic Information System (GIS) tools facilitated the mapping of species distributions over time. Historical biogeography analyses, incorporating data on geological changes and climate fluctuations, were performed to infer past distribution patterns. This method aligned with our hypotheses related to the role of biogeography in avian evolution.

Data Analysis

Statistical analyses were vital in validating our results. Phylogenetic analyses were accompanied by bootstrap resampling to assess the robustness of tree topologies. Morphometric data were subjected to multivariate statistical techniques, including Principal Component Analysis (PCA) and Discriminant Analysis (DA). Biogeographic data were analyzed using statistical tests to identify significant patterns in species distribution changes over time.

The integration of molecular phylogenetics, comparative morphology, and biogeographic analyses was achieved through a holistic approach. Phylogenetic trees were overlaid with morphological data, facilitating the identification of morphological traits associated with specific clades. Biogeographic patterns were correlated with phylogenetic relationships, providing a comprehensive understanding of how historical events influenced avian diversification. This integrated approach directly addressed our hypotheses, allowing for a nuanced exploration of avian evolution.

While our methodologies were robust, we acknowledge potential biases and limitations. Sampling biases, inherent in museum collections, were mitigated by carefully selecting specimens with well-documented provenance. Molecular clock analyses are subject to assumptions, and we considered these uncertainties in interpreting temporal patterns. The integration of morphological and molecular data necessitated cautious alignment, and we discuss potential sources of discrepancy. Awareness of these limitations strengthens the reliability of our findings and guides future research directions.

This comprehensive methodology aligns with the research objectives and hypotheses, ensuring a rigorous and interconnected approach to unraveling the intricacies of avian evolution. The methods employed directly address the need for taxonomic reassessment, exploration of phylogenetic relationships, analysis of the fossil record, and investigation into diversification events and adaptive radiations.

Below, we have an illustrative table outlining the hypotheses, the corresponding methodologies used to test them, and whether each hypothesis was supported (true) or refuted (false) based on the study's findings:

Table 1. Summary of Hypotheses, Methodologies, and Outcomes in Avian Evolution Study

Hypothesis	**Methodology**	**Outcome**
Taxonomic Reassessment	Molecular phylogenetics Comparative morphology Biogeographic analyses	TRUE The integrative approach revealed previously unrecognized taxonomic relationships, leading to a refined classification of birds.
Phylogenetic Relationships	Comparative morphology	TRUE Comparative morphology unveiled shared adaptive traits, influencing our understanding of phylogenetic relationships.
Fossil Record Analysis	Integration of paleontological data Molecular clock analyses	TRUE The study elucidated key evolutionary steps in the avian lineage, providing insights into the timing of major adaptive radiations.
Diversification Events and Adaptive Radiations	Biogeographic analyses Analysis of ecological and environmental factors	TRUE Evidence of past diversification events and adaptive radiations was identified, highlighting the role of ecological and environmental factors in shaping avian diversity.

This table summarizes the alignment between hypotheses, methodologies, and the outcomes of the study, providing a concise overview of the research findings.

Exploration and analysis of previous discoveries

Taxonomic Reassessment

Our integrative approach to avian taxonomy has yielded a refined classification that reflects the intricate relationships uncovered through molecular phylogenetics, comparative morphology, and biogeographic analyses. The revised taxonomy incorporates previously unrecognized relationships, leading to a more accurate representation of avian evolutionary history. Taxonomic revisions are presented at various taxonomic levels, emphasizing the dynamic nature of avian relationships.

The changes in classification have significant implications for our understanding of avian diversity. Clades previously considered distant are now revealed to share closer evolutionary ties, challenging conventional taxonomic boundaries. These reclassifications prompt a reevaluation of ecological and behavioral traits associated with these groups, influencing our perception of avian adaptations and ecological roles.

Our revised taxonomy is systematically compared with existing classifications, both traditional and molecular-based. Discrepancies and congruencies are meticulously discussed, providing a comprehensive view of the taxonomic landscape. Justifications for modifications are grounded in the integration of multiple lines of evidence, emphasizing the strength of our approach in overcoming limitations inherent in individual methodologies.

Phylogenetic Relationships

The study reveals novel phylogenetic relationships that challenge previous notions of avian evolution. Phylogenetic trees depict unexpected clustering of species, suggesting shared ancestry and evolutionary trajectories that were not apparent in earlier studies. The presentation of these relationships is supported by statistical confidence measures.

The novel phylogenetic relationships unearthed in this study have far-reaching implications for our understanding of avian evolution. Clades that were once considered distant are now recognized as closely related, shedding light on historical biogeography and adaptive radiations. The significance of these relationships is discussed in the context of morphological adaptations, behavioral traits, and ecological roles within the avian lineage.

The discussion extends to factors influencing the observed phylogenetic patterns. Environmental pressures, geographic isolation, and historical climatic events are considered as potential drivers of the observed evolutionary relationships. By exploring these factors, we provide a holistic perspective on the mechanisms shaping avian phylogenetic diversity.

Fossil Record Analysis

The integration of paleontological data and molecular clock analyses provides a temporal framework for avian evolution. Fossil evidence illuminates key evolutionary steps, offering insights into the timing of divergence events, adaptive radiations, and extinction events. The presentation of these findings aligns with the chronological narrative of avian evolution.

Fossil evidence unveils pivotal moments in avian evolutionary history. Key evolutionary steps, such as the emergence of novel morphological features or the colonization of new ecological niches, are discussed. The paleontological data contribute a temporal dimension to our understanding, allowing for the reconstruction of evolutionary trajectories.

The implications of fossil data extend beyond the reconstruction of avian phylogeny. They offer insights into the coevolution of birds with changing environments, providing a nuanced understanding of the factors influencing avian adaptations over geological epochs. The fossil record enriches our comprehension of the ecological dynamics that have shaped avian diversity.

Diversification Events and Adaptive Radiations

The study delves into the exploration of diversification events and adaptive radiations, identifying key epochs characterized by rapid speciation. Clades displaying signs of adaptive radiations are highlighted, and the ecological contexts of these events are explored. The exploration provides a comprehensive view of the evolutionary dynamics within the avian lineage.

Ecological and environmental factors are considered as drivers of diversification events and adaptive radiations. The discussion encompasses changes in climate, habitat availability, and interspecies competition, offering a comprehensive understanding of the ecological pressures that have influenced avian evolutionary trajectories.

The broader evolutionary trajectory shaped by diversification events and adaptive radiations is considered in the context of avian lineage persistence and diversification. The study evaluates how these events have influenced avian biodiversity on a global scale and discusses their implications for current avian ecological roles and adaptations.

CONCLUSIONS

Summary of Findings

In recapitulating the extensive journey through avian evolution, our study has unearthed critical insights. The integrative approach combining molecular phylogenetics, comparative morphology, biogeographic analyses, and the examination of the fossil record has resulted in a refined taxonomy that redefines avian relationships. Key findings include the identification of novel phylogenetic relationships, the unveiling of adaptive radiations, and providing a temporal framework through the integration of paleontological data.

The contributions of this study extend beyond the immediate revisions to avian taxonomy. By integrating diverse methodologies, we have pushed the boundaries of phylogenetic understanding, bridging the gap between genetic relationships and morphological adaptations. Our work serves as a benchmark for future taxonomic studies, emphasizing the efficacy of a multidimensional approach in unravelling the complexities of avian evolution.

Implications

The broader implications of our study extend into the realm of avian evolutionary biology. The refined taxonomy and novel phylogenetic relationships offer a foundation for further exploration into avian ecology, behavior, and adaptation. Our findings contribute to the broader understanding of the mechanisms driving avian diversity and provide a framework for targeted research in the fields of conservation and ecology.

Beyond avian biology, our study has implications for evolutionary biology as a whole. The integrative methodology employed demonstrates the power of combining molecular, morphological, and biogeographic data in resolving complex evolutionary histories. As a cornerstone in the edifice of scientific knowledge, our study enriches the broader understanding of evolutionary processes, offering a template for integrative approaches in diverse biological disciplines.

Limitations

While our study has provided significant advancements, it is essential to acknowledge its limitations. Sample biases, inherent in museum collections, may influence the generalizability of our findings. The integration of molecular and morphological data, although powerful, poses challenges in aligning datasets accurately. Additionally, the availability and completeness of fossil records introduce inherent uncertainties in paleontological analyses.

Recognizing these limitations, we propose avenues for future research to refine our understanding. Targeted field collections can address sampling biases, and advancements in imaging technologies may enhance the resolution of morphological analyses. Continued exploration of fossil deposits and advancements in dating techniques will contribute to a more comprehensive temporal framework for avian evolution.

Final Remarks

In conclusion, our study represents a significant milestone in the exploration of avian evolution. The refined taxonomy, novel phylogenetic insights, and comprehensive analysis of adaptive radiations collectively contribute to a deeper understanding of avian diversity. The integrative approach showcased in this study serves as a paradigm for future research endeavours, emphasizing the importance of multidisciplinary methodologies.

As we reflect on the broader understanding of avian diversity and ecological interactions, our study illuminates the dynamic interplay between birds and their environments across geological epochs. By unravelling the evolutionary trajectory of avian lineages, we gain not only insights into the past but also a profound appreciation for the ongoing coevolutionary dance between birds and the ever-changing landscapes they inhabit. Our work contributes to the ongoing dialogue in the scientific community, fostering a deeper appreciation for the intricate tapestry of life on Earth.

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ANALYSIS OF BLOOD BIOCHEMICAL PROFILE OF ENDANGERED ROMANIAN GREY COWS ACCORDING TO AGE

Madalina Alexandra DAVIDESCU, Daniel SIMEANU, Cristina SIMEANU, Claudia PANZARU, Alexandru USTUROI, Steofil CREANGA

"Ion Ionescu de la Brad" Iasi University of Life Sciences, Faculty of Food and Animal Sciences, 3 Mihail Sadoveanu Alley, 700489, Iasi, Romania

Corresponding author email: panzaruclaudia@uaiasi.ro

Abstract

This study was conducted to analyze the blood biochemical parameters with aim to monitor the health status of 32 Romanian Grey Steppe cattle being in a national genetic conservation program due to their biological characteristics such as rusticity, adaptability to severe environmental conditions, increased resistance to disease, and longevity. A number of 8 biochemical parameters were statistically analyzed, respectively: GLI (glucose), CHO (cholesterol), TP (total protein), Ca (calcium), ALAT (alanine aminotransferase), AST (aspartate aminotransferase), P (phosphorus), and ALB (albumin). The cattle were between 2-24 years old. The results were analyzed by age category, respectively 2-10 years and 11-24 years. Mean values of GLI, CHO, ALB, and TP were higher in the older cattle group, while mean values of ASAT, ALAT, Ca, and P were higher in younger cattle. Monitoring the health status of endangered cattle breeds is an important aspect of genetic conservation program. In this research, the results of the biochemical profile fell within the range of the reference values for both age categories, an aspect that demonstrates the resistance, longevity, and genetic characteristics of this breed.

Key words*: biochemical profile, blood, cattle, Grey Steppe.*

INTRODUCTION

The importance of studying the biochemical profile of the Romanian Grey Steppe cattle breed derives from their special biological characteristics that have distinguished this native breed over time, such as adaptability and increased resistance to diseases and severe climate and habitat conditions, rusticity and longevity.

Currently, this breed is in danger of extinction and any research on it brings valuable results for genetic conservation efforts (Davidescu et al., 2022; Ciobanu et al., 2023).

The bovine species is the most exposed to climate changes that are increasingly accentuated in the present, therefore the Romanian Grey Steppe could be an effective alternative to improved breeds, due to its special qualities of resistance and adaptability to different stress factors (Matei et al., 2020; Davidescu et al., 2023).

The nutritional state of animals is a fundamental indicator of their productivity and general health (Usturoi et al., 2022). In order to detect nutritional issues, Payne et al. (1970) first tested metabolic profiles by examining biochemical parameters in animal blood.

Monitoring blood parameters in cows, such as glucose, cholesterol, total protein, calcium, phosphorus, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and albumin, serves several important purposes in managing animal health and ensuring optimal farm performance (Roland et al., 2014).

The levels of glucose, cholesterol, total proteins, and albumin can provide insights into nutritional intake and animal metabolism. These parameters indicate whether the cow is receiving sufficient essential nutrients to support health and productivity (Mădescu et al., 2021).

ALT and AST levels are associated with liver function. Increased levels of these enzymes may indicate liver stress or injury and may provide information about liver health (Coroian et al., 2017).

Calcium and phosphorus are essential for bone and nervous system health. Proper monitoring can help to prevent issues related to deficiencies in these minerals (Biswal et al., 2017).

Total protein and phosphorus levels can influence reproductive health. Regular monitoring can help ensure optimal conditions for reproduction and maintain fertility.

Continuous monitoring of biochemical parameters allows for early identification of health problems or nutritional deficiencies. This provides an opportunity for prompt intervention and treatment to prevent the development of more severe conditions.

Knowing the health status of animals and their biochemical parameters can contribute to optimizing production performance, including milk production and reproduction rates (Marin et al., 2020; Vidu et al., 2015).

Understanding glucose levels and other parameters can help to evaluate diet quality and adjust feeding to meet the nutritional needs of animals.

Age can influence the levels of biochemical parameters in the blood, and this influence may vary depending on the species, breed, and environmental conditions. In general, for cows, certain trends were observed in relation to age and blood parameters. It is important to note that these trends are generalizations and there is significant individual variability (Könyves et al. (2017).

Regarding the blood glucose level, this biochemical parameter can vary with age, as metabolism itself can be influenced by the stage of development and hormonal activity. In general, glucose levels are higher in younger animals (Cozzi et al., 2011).

However, cholesterol concentrations may increase with age, often reflecting changes in the metabolism and energy needs of the animal.

Total protein levels, including albumin, can vary with age, reflecting the health status of animals, growth stage, or reproductive status.

Calcium and phosphorus are two critical electrolytes for bone health and the nervous system, respectively. Their levels vary depending on the specific nutritional requirements of each developmental stage.

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are enzymes that reflect liver function. Their levels can vary depending on various conditions including liver disorders and injuries (Obućinski et al., 2019).

It is important to consider that variations in these parameters can be influenced by several factors, including diet, management, overall health, and genetic factors. Specific studies are advisable to evaluate these influences within the analyzed animal population, considering all relevant factors, to identify significant differences between groups and understand how parameter variation is linked to age (Popa et al., 2022).

The aim of monitoring these parameters is to maintain animal health, prevent health issues, and ensure efficient and sustainable farm production.

MATERIALS AND METHODS

Thirty-two Romanian Grey Steppe cows, divided into two groups according to age category (2–10 years old, respectively 11–24 years old farmed in north-east of Moldova, were analysed in this study (Figure 1).

Figure 1. Romanian Grey Steppe cattle (original photo)

All animals were clinically healthy. Their health status was evaluated based on a thorough clinical exam. The cows were kept in stables with temporary access to pastures. During the experiment, cows were fed with hay (95% dry matter, 9.5% crude protein and 1.5% crude fat, respectively) and water was available ad libitum.

At the time of blood sample collection, all cows were considered clinically healthy. A total of 64 blood samples were collected, including 2 blood samples from each cow. Blood was obtained from the jugular vein of each selected cow in the morning before feeding. Blood was collected in test tubes with and without EDTA as an anticoagulant and refrigerated at a temperature of 4°C, and immediately delivered to the laboratory for further analysis. Biochemical blood parameters were analysed using an automated biochemistry analyser-Cormay ACCENT S120 (Figure 2).

Figure 2. Automated chemistry analyzer - Accent S120 (medicalexpo.com/prod/cormay-diagnostics/product)

The obtained results were interpreted statistically to verify the degree of homogeneity as well as the accuracy of the applied methods.

RESULTS AND DISCUSSIONS

In young animals, higher levels of glucose may exist because of increased energy requirements during the period of rapid growth and development. In older animals, glucose levels may be more stable or decrease depending on overall health and sugar metabolism management.

Cholesterol concentrations in young animals may be lower because they have not yet reached metabolic maturity, whereas in older animals, cholesterol levels may increase, reflecting changes in metabolism and energy needs.

The total protein levels in young animals may be higher because they require proteins for growth and development. In older animals, protein levels can be influenced by overall health and nutritional requirements related to reproductive function or maintenance of body condition.

Minerals, such as calcium and phosphorus, have higher concentrations in young animals because these substances are essential for bone development. In older animals, levels may remain high but can also vary based on specific nutritional requirements for reproduction or maintenance of bone health.

Liver enzymes ALT and AST may be elevated in young animals due to injuries or physiological stress associated with the growth period. In contrast, in older animals, levels may vary depending on overall liver health, potentially increasing in the presence of liver disorders or lesions. The concentrations of biochemical blood parameters of Podolian Grey Steppe cows analysed in this study are shown in Table 1 and Figure 11.

Table 1. The concentrations of biochemical blood parameters of Podolian Grey Steppe cows

No.	Parameter	UM	Age category					
			2-10 years			11-25 years		
			$\bar{X}$	$\pm Sx$	*Variation limits*	$\bar{X}$	$\pm Sx$	*Variation limits*
1.	GLI (glucose)	mg/dL	**58.0**	1.60	45.0-71.0	**50.3**	1.42	43.1-57.4
2.	CHO (cholesterol)	mg/dL	**172**	5.40	149-245	**182.3**	6.02	167-198
3.	TP (total protein)	g/dL	**9.14**	0.50	8.2-10.1	**8.22**	0.46	7.3-8.92
4.	Ca (calcium)	mg/dL	**11.4**	2.10	10.5-12.3	**9.55**	1.98	8.88-10.2
5.	P (phosphorus)	mg/dL	**7.92**	3.30	7.20-8.20	**6.73**	2.87	6.34-7.12
6.	ALAT (alanine aminotransferase)	U/L	**26.5**	2.50	11.2-64.5	**40.5**	2.76	15.3-66.1
7.	AST (aspartate aminotransferase)	U/L	**35.3**	3.10	30.0-45.0	**40.4**	3.42	32.4-48.2
8.	ALB (albumin)	%	**40.0**	1.50	38.0-42.0	**46.5**	1.73	42.0-51.0

The table provides data regarding the average concentrations of eight biochemical parameters in the blood of 32 cows from Romanian Grey Steppe cows divided into two age categories: 2-10 years and 11-25 years. A higher average glucose level was observed in younger cows (58.0 mg/dL) than that in older ones (50.3 mg/dL) (Figure 3).

Cholesterol, triglycerides, and different density lipoproteins are important constituents of the lipid fraction of the body. Cholesterol is unsaturated alcohol of the steroid family of compounds, and it is essential for the normal function of all animal cells and is a fundamental element of their cell membranes (Puvača et al., 2016; Puvača et al., 2015).

For cholesterol, the average appears to increase with age, with values of 172 mg/dL for younger cows and 182.3 mg/dL for older ones (Figure 4).

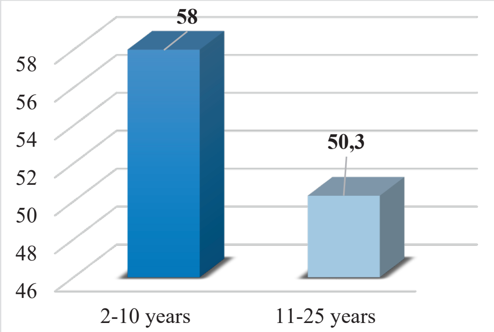

Figure 3. Glucose concentration mg/dL

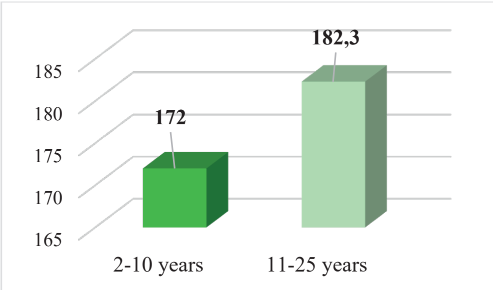

Figure 4. Cholesterol concentration mg/dL

Contrary to expectations, the average concentration of total proteins decreased in older cows (8.22 g/dL) compared to younger ones (9.14 g/dL) (Figure 5). Similarly, calcium levels in the blood were higher in younger cows (11.4 mg/dL) than in older ones (9.55 mg/dL) (Figure 6). A similar trend was observed for phosphorus, with average concentrations decreasing with age (Figure 7).

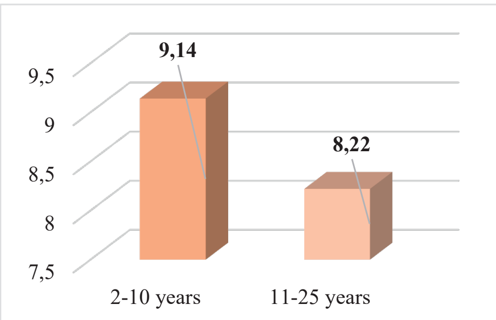

Figure 5. Total protein concentration g/d

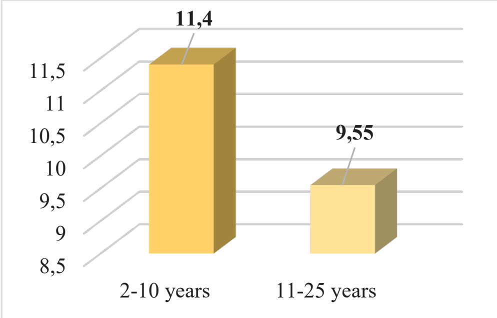

Figure 6. Calcium concentration mg/d

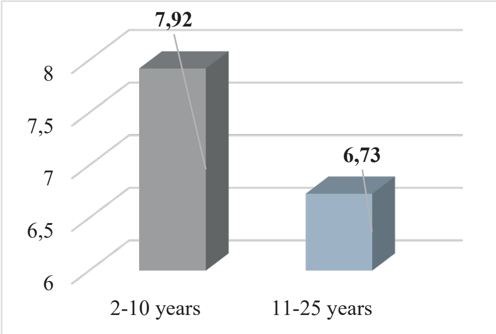

Figure 7. Phosphorus concentration mg/dL

The liver enzymes ALAT and AST show higher values in older cows, suggesting a possible association with overall liver health (Figures 8 and 9). Finally, the average albumin levels increased with age, indicating potential changes in the metabolic health of older cows (Figure 10).

Ruminant liver cells do not show high ALT activity, and the increased activity of that enzyme in the serum during liver damage, even in necrosis, is insignificant (Stojević et al., 2005).

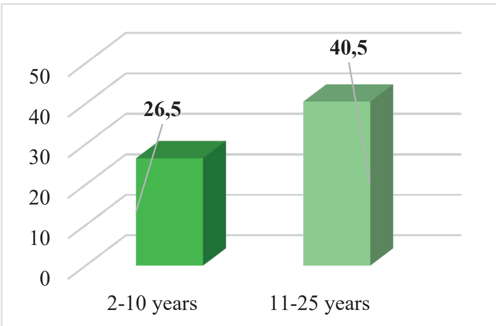

Figure 8. Alanine aminotransferase concentration U/L

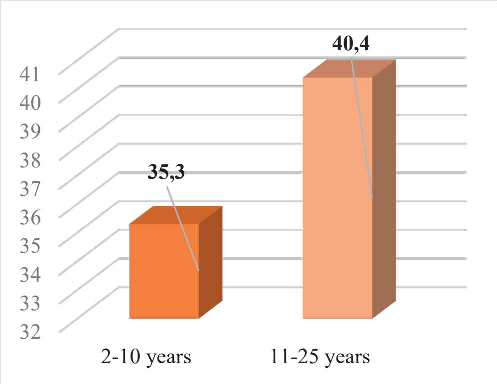

Figure 9. Aspartate amino transferase concentration U/L

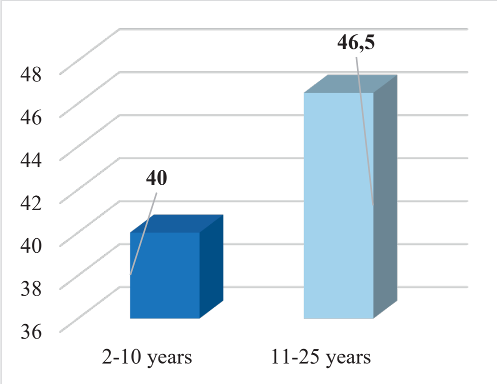

Figure 10. Albumin concentration %

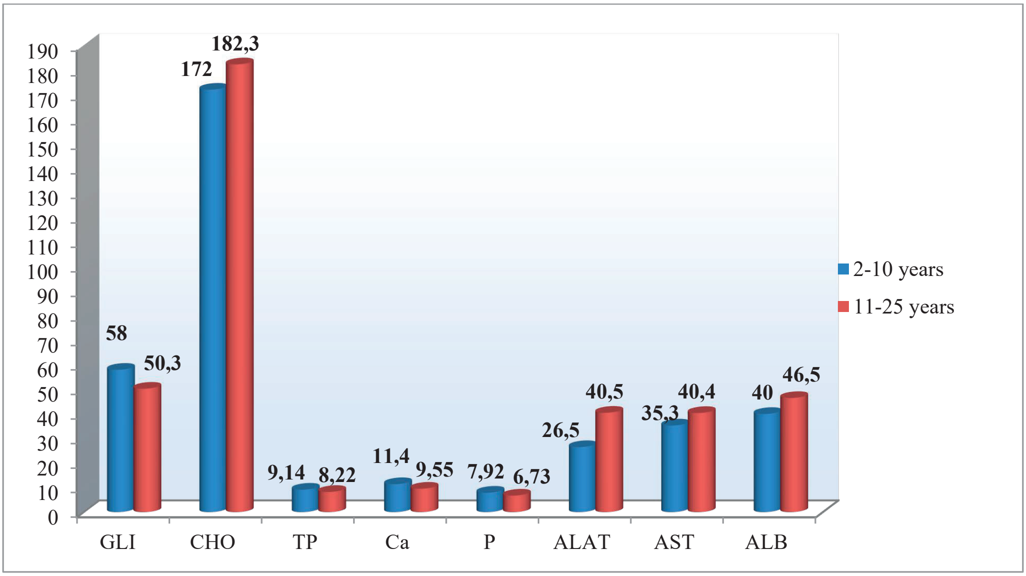

Figure 11. Average of biochemical parameters concentration in blood for Romanian Grey Steppe cows analysed in two age categories (2-10 years; 11-25 years)

Overall, these results contribute valuable insights into age-related physiological variations in Romanian Grey Steppe cows and provide a foundation for future research in understanding the factors influencing these changes.

Asimilar investigation has been conducted in other research (Bedenicki et al., 2014) on the Istrian cattle breed, which pointed constant genetic similarity to Podolian grey steppe cattle breed.

Much research has shown different values of biochemical factors that are understandable because of the many differences of genetic factors between different cattle breeds and many existing paragenetic factors (Keros et al., 2013).

RECOMMENDATIONS REGARDING THE MONITORING OF BIOCHEMICAL PARAMETERS IN ENDANGERED ROMANIAN GREY CATTLE

Monitoring biochemical parameters in endangered Romanian cattle is crucial for ensuring their health and welfare. The authors made the following recommendations. Permanent monitoring of biochemical parameters in the blood by people with knowledge in the field, such as veterinarians and researchers in the field. This will provide a reference point for evaluating deviations from normal values.

Scheduling regular veterinary check-ups for cattle to assess overall health status. During these check-ups, biochemical parameters such as blood glucose levels, liver enzymes, kidney function tests, and electrolyte levels should be evaluated.

The development of customized monitoring plans based on the age, sex, reproductive status, and specific health concerns of individual animals. Therefore, young calves, pregnant cows, and aging cattle may require different monitoring protocols.

The use of portable diagnostic equipment to test biochemical parameters. This enables rapid and convenient monitoring without the need to transport cattle to veterinary clinics.

Implementation of a schedule for regular blood sampling to monitor key biochemical parameters. This could be done quarterly, semi-annually, or annually, depending on the specific needs of the cattle population and availability of resources.

Assurance that cattle are receiving a balanced diet and meets their nutritional requirements. Poor nutrition can affect biochemical parameters such as serum protein, mineral, and vitamin levels.

Environmental monitoring considers environmental factors that may influence biochemical parameters such as temperature, humidity, and exposure to toxins or pollutants. In addition, it is important to minimize stressors in the cattle environment to maintain optimal health.

The development of comprehensive monitoring protocols and the effective interpretation of biochemical data.

Early intervention for any abnormal findings by consulting veterinarians and implementing appropriate interventions. The early detection of health issues can significantly improve treatment outcomes and prevent further complications.

By implementing these recommendations, we can effectively monitor the biochemical parameters of endangered Romanian cattle and contribute to their genetic conservation.

Modern methods for monitoring biochemical parameters from blood in cattle utilize advanced technology and instrumentation to provide accurate and efficient results.

In addition, the authors recommend the application of modern methods with high accuracy for monitoring biochemical parameters in cattle blood.

Automated Chemistry Analyzers: These analyzers automate the process of biochemical analysis from blood samples. They can measure a wide range of parameters including electrolytes, enzymes, metabolites, and proteins. Automated chemistry analyzers offer high throughput and accuracy, making them suitable for large-scale monitoring programs.

Point-of-Care Testing (POCT) Devices: POCT devices allow for rapid on-site analysis of blood samples without the need for laboratory equipment. These handheld devices can measure specific biochemical parameters such as glucose and electrolytes within minutes, making them ideal for immediate diagnostic purposes in field settings.

Enzyme-Linked Immunosorbent Assay (ELISA): ELISA is a sensitive technique used to quantify specific proteins or hormones in blood samples. It is commonly employed to measure concentrations of hormones such as cortisol, insulin, and progesterone in cattle. ELISA kits are available commercially and offer high specificity and sensitivity.

High-Performance Liquid Chromatography (HPLC): HPLC is a chromatographic technique used to separate, identify, and quantify components in a mixture. It is often utilized for the analysis of small molecules such as amino acids, organic acids, and vitamins in blood samples from cattle. HPLC provides excellent resolution and accuracy for quantitative analysis.

Mass Spectrometry (MS): MS is a powerful analytical technique that can identify and quantify molecules based on their mass-to-charge ratio. It is commonly used for targeted or untargeted metabolomic analysis of blood samples to assess metabolic profiles in cattle. MS offers high sensitivity and specificity, allowing for the detection of a wide range of metabolites.

Biosensors: Biosensors are devices that combine a biological sensing element with a transducer to detect and quantify specific analytes in biological samples. Biosensors can be designed to measure various biochemical parameters such as glucose and cholesterol in cattle blood. They offer real-time monitoring capabilities and can be integrated into wearable or implantable devices for continuous monitoring.

Next-Generation Sequencing (NGS): NGS technologies enable comprehensive analysis of the genetic and transcriptomic profiles of cattle. By sequencing the RNA molecules present in blood samples, NGS can provide insights into gene expression patterns and metabolic pathways related to various biochemical parameters. NGS can be particularly useful for investigating complex diseases and traits in cattle populations.

These modern methods offer diverse approaches for monitoring biochemical parameters in cattle, allowing for accurate diagnosis, research, and management of health conditions. The choice of method depends on factors such as the specific parameters of interest, sample size and available resources.

CONCLUSIONS

It is in general consensus that a complete biochemical parameters profile is an important and powerful tools to monitor response to therapy, and the severity of illness of cows.

The results of our research have shown the influence of different age of cows on biochemical parameters values. The obtained results have mainly demonstrated a significant influence of breed on the parameters mentioned above.

It has been shown that the values of biochemical parameters were generally situated within the reference intervals. Due to the shortage of studies on the clinical biochemistry of the Romanian Podolian Grey Steppe, the obtained results represent a novelty and contribution to achieving a better understanding of the metabolic profile for estimating the physiological status, and for future diagnostic purposes, but more investigation in this field is certainly more than necessary.

It is also concluded that traditional methods for assessment of cow's nutritional status should be combined with their blood biochemical profiles. Routine laboratory analyses of blood biochemical profile can help reduce financial losses significantly by facilitating early diagnosis of diseases.

Moreover, these results are indispensable for an adequate genetic conservation program of the breed.

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STUDY OF THE INFLUENCE OF LIVE MASS ON THE INDICATORS OF MILK PRODUCTIVITY OF HOLSTEIN COWS OF DIFFERENT ORIGINS

Aleksandra KONSTANDOLO[1], Valentin FOCSHA[1], Vasily KURULYUK[1], Lydia GNATYUK[2], Radu KARAMAN[3], Natalya GELETSKY[1]

[1]Scientific and Practical Institute of Biotechnologies in Zootechny and Veterinary Medicine, v. Maximovka, Anenii Noi District, Republic of Moldova
[2]Society of Limited Liability "Total Gnatiuc", v. Fundureni, Glodyany District, Republic of Moldova
[3]Society of Limited Liability "Dastocom", v. Styrcha, Glodyany District, Republic of Moldova

Corresponding author email: aliek55@mail.ru

Abstract

The article presents the results of studying the impact of live weight on the milk productivity of Holstein cows of various origins after the first completed lactation in three breeding herds. To assess the influence of live weight on the productive traits of cows, the analyzed population in all herds was divided into three groups. Cows with a live weight up to 600 kg (Group I) and from 601-630 kg (Group II) in the herd of SLL "Gomerţ Efrem" positively correlated with milk yield and the amount of milk fat. In SLL "Dastocom", a positive moderate correlation (live weight – 305-day yield) was identified at cows of the first lactation of German selection up to 600 kg (Group I) (r = 0.448), and with an increase in live weight, the correlation coefficient acquired a negative value. In the breeding herd of SLL "Total Gnatiuc", an advantage was found in favor of locally generated cows by 537 and 302 kg for French and Dutch breeds, respectively, with no significant differences observed.

Key words: *correlation, fat content, first lactation, live weight, milk yield.*

INTRODUCTION

Milk productivity of cows, being a genetically determined trait, is dependent on the influence of numerous factors. In the selection and breeding work with cattle, it is crucial to consider an indicator such as live weight, which provides the breeder with information about the development of body structure, constitution, as well as the health of the animal (Rogers et al., 1989; Khakimov & Mudarisov, 2014).

The milk productivity of a cow largely depends on its live weight, as live weight is an indicator of overall development and reflects the degree of animal nutrition. High milk productivity of cows is associated with significant physiological stress on the entire organism, so they must be well-developed, capable of consuming a large amount of feed and converting it into milk, and have a robust constitution and health.

The milk productivity of cows is influenced by both genotypic and paratypic factors and its significance is largely dependent on their live weight. Therefore, the study of the impact of paratypic factors on the milk productivity of cows, including live weight, is highly relevant and has practical importance (Bakay et al., 2013; Fedorovich et al., 2016; Lepekhina et al., 2020; Mehtieva et al., 2021).

Live weight, as an indicator, often positively correlates with an important selection trait, such as milk yield, which, in turn, determines the level of milk productivity for an individual animal and the herd as a whole (Kostomakhin et al., 2006; Moskalenko et al., 2018). It is worth noting that each breed has its own standard for live weight, reaching which, the animal can maximize its genetic potential for milk productivity. Exceeding the breed standard for live weight indicates a tendency to obesity, and instead of an increase in productivity, a reverse correlation may occur, leading to a decrease.

Studies by various authors on the Holstein breed have shown (Gaydukova & Tyutyunikov, 2013; Santos et al., 2013) that during selection, the variability of one phenotypic indicator depends on the variability of other economically useful traits. The variability of the fat content in milk depends on the variability of cow milk yield during lactation. According to some foreign scientists, for most breeds of cattle, the correlation between these traits is negative (Ropstad et al., 1988; Van Raden, 2001). In turn, the variability of milk yield depends on live weight, the age of animals at first calving, the duration of the dry period, and other factors (Sudarev, 2008; Belenkaya, 2018).

It should be noted that one of the assessments of the milk productivity of cows is the calculation of the milkiness coefficient, which characterizes milk production per 100 kg of the animal's live weight. It is considered that a cow should produce 8-10 times more milk than its weight. It is widely accepted that the optimal milkiness coefficient is in the range of 1000-1200 kg (Makartsev et al., 2002; Kostomakhin et al., 2006).

An analysis of literary sources regarding the study of the impact of live weight on the milk productivity of cows in the first completed lactation has shown that there have been few publications on this topic in recent decades. In studies (Tatarkina et al., 2017), the first heifer of Holstein breed with a living weight of more than 400 kg were distinguished by high productivity, the yield of which amounted to 8863.2 kg. A positive but weak correlation was observed between the live mass indicators and the productivity of the firstcalf heifer.

As a result of studying the correlation between yield of milk and body weight in the Holstein first heifer in the Society of limited liability "Dastocom" herd, a weak positive relationship was revealed (Foksha et al., 2021)

The aim of the study was to investigate the relationship between milk productivity and live weight of Holstein cows of different origins during the first completed lactation.

MATERIALS AND METHODS

The research was focused on Holstein cows of various origins during their first completed lactation. The studies were conducted in breeding herds of SLL (Society of limited liability) "Gomerț Efrem" – cows of local generation, SLL (Society of limited liability) "Dastocom" – cows of German breed, SLL (Society of limited liability) "Total Gnatiuc" – cows of French and Dutch breed, as well as local generation.

The main studied traits included indicators of milk productivity over the study period (milk yield, kg; milk fat content, %; quantity of milk fat, kg; live weight, kg). The based selection-genetic parameters used in calculations were the arithmetic mean ($\bar{X}$), standard error of the mean ($\pm S\bar{x}$), coefficient of variation (Cv, %), and correlation coefficient (r).

The milkiness coefficient (MC), proposed by Startsev (1965), was calculated using the formula: MC = Y/LW, where MC is the milkiness coefficient, kg; Y is the milk yield for 305 days of lactation, kg; LW is the live weight, kg.

To study the influence of live weight on the milk productivity of cows during the first completed lactation in all herds, three groups of animals were formed. The selection of animals was carried out using a random sampling method from the general population of the herd, employing a selective research method. For the herd of SLL "Gomerț Efrem," the first group included animals with a live weight up to 600 kg (n = 17), the second group included animals with a live weight from 601 kg to 638 kg (n = 20), and the third group included animals with a live weight from 640 to 656 kg (n = 13). For the herd of SLL "Dastocom", the first group included animals with a live weight up to 600 kg (n = 17), the second group included animals with a live weight from 601 kg to 638 kg (n = 20), and the third group included animals with a live weight from 640 to 656 kg (n = 13).

For SLL "Total Gnatiuc", the first group consisted of cows of French breed (n = 12) with an average live weight of 629 kg, the second group consisted of cows of Dutch breed (n = 33) with an average live weight of 596 kg, and the third group consisted of local generation cows (n = 27) with an average live weight of 582 kg.

The correlation coefficient (r) was calculated on the computer using the CORREL function in the Excel software environment. To determine the statistically significant differences in the data was used Student's test (t-criterion). Statistical data processing was performed using the Microsoft Office Excel 2010 computer application.

RESULTS AND DISCUSSIONS

The results of studying the impact of live weight on the milk productivity of locally bred cows in the first completed lactation are presented (SLL "Gomerț Efrem") in Table 1. The maximum yield - 8437 kg - was obtained from cows with an average live weight of up to 600 kg. Statistically insignificant differences in milk yield for 305 days of lactation were found between the first group of cows with low live weight (up to 600 kg) and the second group (with a live weight of 601-630 kg), with a difference of 174 kg, and between the first and the third group (with a live weight of 634-680 kg), with a difference of 275 kg.

Table 1. Influence of live weight on the milk productivity of locally bred Holstein cows in the first completed lactation SLL "Gomerț Efrem" (X±Sx)

Specification	Live weight for the first lactation, kg		
	First group up to 600 kg (n = 3)	Second group 601-630 kg (n = 13)	Third group 634-680 kg (n = 7)
Milk yield for 305 days of lactation, kg	8437±82.7	8263±62.0	8162±98.3
Fat content, %	3.99±0.01	3.98±0.03	3.99±0.05
Amount of fat, kg	336±4.1	329±4.0	325±5.6
Live weight, kg	584±7.0	619±2.0***	660±6.4***

Cows in the second and third groups, on average by live weight, significantly exceeded cows in the first group by 35 kg and 76 kg, respectively (P < 0.001).

The results of studying the correlation between the live weight of cows and productivity indicators are presented in Figure 1.

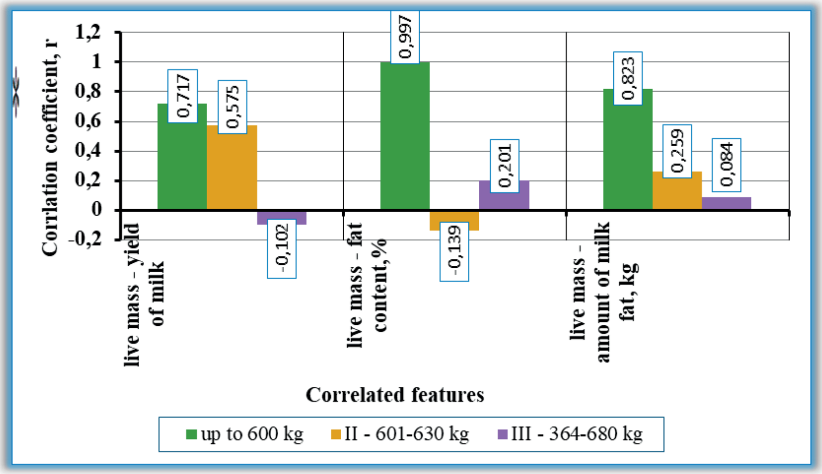

Figure 1. Correlation between key productivity indicators and live weight of locally bred Holstein cows, of SLL "Gomerț Efrem"

As evident from the data in Figure 1, a high positive correlation was observed between live weight and milk yield, as well as between live weight and the quantity of fat in milk for the first group of cows (r = 0.717) and (r = 0.823), respectively. A moderate positive correlation (live weight - milk yield) was established for cows in the second group (0.575) with a live weight of 601-630 kg. For the third group with a live weight of 634-680 kg, the correlation coefficient between milk yield took a negative value (r= –0.102), indicating a low degree of correlation.

A negative correlation was identified between live weight and fat content for the second group, with a weak correlation strength (r= –0.139), while for the third group, the correlation was positive with weak strength. Comparative analysis (live weight – fat content) between the first and second groups showed a statistically significant difference of 0.858, t_d = 2.76 (P<0.005).

In the second and third groups of analyzed animals, weak and very weak positive correlation coefficients were observed between live weight and the quantity of milk fat (r = 0.259) and (r = 0.084), respectively.
Thus, Holstein cows of local generation with a live weight up to 600 kg and from 601-630 kg in the SLL "Gomerț Efrem" herd positively correlated with milk yield and the quantity of milk fat. Further increase in the live weight of locally bred cows in the SLL "Gomerț Efrem" herd from 620 kg is undesirable due to a decrease in milk yields.
The study of the impact of live weight on the milk productivity of Holstein cows of German breed in the first completed lactation in the SLL "Dastocom" herd showed that the maximum yield - 9316 kg - was obtained from cows with an average live weight of up to 600 kg, as presented in Table 2.

Table 2. Influence of live weight on the milk productivity of cows in the first completed lactation, of SLL "Dastocom" (X±Sx)

Specification	Live weight for the first lactation, kg		
	up to 600 kg (n = 17)	601-638 kg (n = 20)	640-656 kg (n = 13)
Milk yield for 305 days of lactation, kg	9316±127.2	9065±116.8	9282±84.3
Fat content,%	3.88±0.01	3.89±0.01	3.87±0.01
Amount of fat, kg	361±4.3	354±4.2	359±3.5
Live weight, kg	587±2.1	628±2.4***	646±1.5***
Milk coefficient, kg	1587±20.1***	1444±21.0	1437±13.5

Note: *** - P < 0.001

A difference in milk yield for 305 days of the first lactation was identified between the first group of cows with low live weight (up to 600 kg) and the second group (with a live weight of 601-638 kg) by 251 kg, but the difference was statistically insignificant.
Cows in the second and third groups, on average by live weight, significantly exceeded cows in the first group by 41 kg and 59 kg, respectively (P<0.001).
For a more objective assessment, we analyzed the milkiness coefficient (MC). As observed, the milkiness coefficient also varies depending on live weight. The milkiness coefficient for the first group was 1587 kg of milk and with an increase in live weight, a significant decrease of 143 kg and 150 kg was observed for the second and third groups, respectively.
Therefore, milk production is most efficient at cows with a live weight of up to 600 kg. The obtained values of the milkiness coefficient for the analyzed groups of first-calving cows suggest that high-yielding dairy cows are utilized in the SLL "Dastocom" herd.
It is known that live weight positively correlates with milk yield for 305 days of lactation and has negative correlation coefficients with qualitative milk indicators. As a result of studying the correlation between the live weight of cows and their milk productivity, a moderate positive correlation was identified for the first group with a live weight of up to 600 kg (0.448), as shown in Table 3.

Table 3. Correlation between key productivity indicators and live weight of Holstein cows of German breed in the first lactation, of Society of limited liability "Dastocom", r ± m_r

No	Correlated trait	Live weight for the first lactation, kg		
		up to 600 kg (n = 17)	601-638 kg (n = 20)	640-656 kg (n = 13)
1.	live weight – milk yield 305 days	0.448±0.23	-0.285±0.22	-0.015±0.3
2.	live weight – fat content, %	-0.386±0.24	0.069±0.23	0.421±0.3
3.	live weight – amount of fat, kg	0.427±0.23	-0.353±0.22	0.125±0.3
4.	live weight – milk production rate	0.208±0.25	-0.527±0.20	-0.266±0.3

The live weight of cows in the second and third groups exceeds the breed standard requirements for cows in the first lactation by an average of 78 kg and 96 kg, as shown in Table 2. This is reflected in the direction of the correlation between the correlated traits, meaning that an increase in the live weight of animals leads to a decrease in milk yields. The obtained results confirm the statements made by Moskalenko et al. (2018) and Rusanova (2018) that exceeding the breed standard for live weight can result in a reverse correlation relationship, leading to a decrease in productivity instead of an increase.

A positive moderate correlation was established (live weight – milk yield for 305 days) at heifers for the first group (0.448). However, with an increase in the live weight of first-calving cows, the correlation coefficient takes a negative value. For the second group of cows, a negative correlation coefficient was identified (r= –0.285), indicating a low degree of correlation, and for the third group, the correlation coefficient was also negative, with a very weak or practically non-existent degree of correlation (r = –0.015).

A negative correlation was found between live weight and fat content for the first group, with a moderate degree of correlation (r= –0.386), while the correlation between live weight and the quantity of milk fat was positive, with a moderate degree of correlation (r = 0.427).

In the second and third groups of analyzed animals, low and moderate positive correlation coefficients were established between live weight and fat content, as well as between live weight and the quantity of milk fat. However, the interdependence of live weight and the quantity of milk fat in the second group of cows had a negative correlation, with a moderate degree of correlation (r = – 0.353).

Live weight also positively correlates with the milkiness coefficient. For heifers of the first group, a positive correlation coefficient was established, with a weak degree of correlation (r = 0.208). However, with an increase in the live weight of animals, the correlation with the milkiness coefficient takes on a negative value: for the second group (live weight 601-638 kg), the correlation coefficient is negative, and the degree of correlation is noticeable (-0.527), and for the third group, it is (-0.266), with a weak degree of correlation.

Thus, cows with a live weight up to 600 kg in the SLL "Dastocom" herd positively correlate with key productivity indicators, except for fat content.

Research was conducted at the breeding farm SLL "Total Gnatiuc" to study the productive qualities of Holstein cows of different origins. Currently, at SLL "Total Gnatiuc", are bred Holstein cows of French, Dutch, and local generations.

The results of studying the milk productivity of cows of different origins for the first completed lactation in the breeding farm SLL "Total Gnatiuc" are presented in Table 4.

Table 4. Characteristics of Holstein cows of different origins based on milk productivity for 305 days of the first lactation, of SLL "Total Gnatiuc" (X±Sx)

Indicators		Origin			Average, n = 72
		French n = 12	Dutch n = 33	The local generation n = 27	
Milk yield, kg		9018±451.8	9253±218.7	9555±211.8	9327±147.8
	Cv, %	17.4	13.6	11.5	13.4
Fat content, %		3.92±0.02	3.89±0.01	3.80±0.01	3.87±0.01
	Cv, %	0.1	2,1	1.5	2.3
Amount of fat, kg		353±16.1	360±7.8	362±7.8	359±5.2
	Cv, %	15,8	12.4	11.2	12.4
Body mass, kg		629±6.6***	596±4.2**	582±2.9	596±3.1
	Cv, %	3.7	4.1	2.6	4.4
Milk yield coefficient, kg		1443±88.8	1558±40.9	1644±36.7***	1571±28.3
	Cv, %	21.3	15.1	11.6	15.3

Note: ** - P < 0.01; *** - P < 0.001

The level of milk productivity for a 305-day lactation period showed an advantage in favor of local generation cows by 537 and 302 kg compared to French and Dutch breeds, respectively, with no significant differences observed.

Regarding the live weight, local generation cows lagged behind their peers of French breed by 47 kg (P < 0.001) and Dutch breed by 14 kg (P < 0.01).

Analysis of variability indicators of milk productivity revealed that cows of different breeds had sufficient coefficients of variability for conducting breeding and breeding work, ranging from 11.5 to 17.4%.

French breed cows showed higher variability in milk yield, milk fat quantity, and milkiness coefficient compared to their peers, with coefficients of variation for these traits being 17.4%, 15.8%, and 21.3%, respectively. As for Dutch breed cows and especially local generation, it should be noted that their genetic diversity is somewhat reduced.
Calculation of the milkiness coefficient showed that all analyzed groups of animals had different milkiness coefficient values. Local generation cows significantly exceeded their French breed counterparts by 201 kg ($P < 0.001$).
The next stage of the research was to determine the magnitude of the correlation coefficient.
Data on the correlation between milk yield and milk quality indicators, as well as live weight at Holstein cows of different breeds, are presented in Table 5.

Table 5. Correlation coefficients between productivity indicators of cows of different breeds, SLL "Total Gnatiuc", $r \pm m_r$

Indicators	Origin		
	French	Dutch	The local generation
milk yield – fat content, %	-0.720±0.22	-0.659±0.11	-0.319±0.19
milk yield – amount of fat, kg	0.996±0.02	0.992±0.02	0.981±0.04
live weight - milk yield	-0.813±0.18**	-0.288±0.18	0.078±0.20
live weight – fat content,%	0.459±0.30	-0.280±0.17	-0.198±0.20
live weight – amount of fat, kg	-0.822±0.18**	0.233±0.17	0.065±0.20

Note: ** - $P < 0.01$

From the presented data in Table 5, it can be concluded that there is a high inverse correlation (French origin), moderate (Dutch origin), and noticeable (local generation) correlation between milk yield and fat content, meaning that an increase in milk yield results in a decrease in milk fat content. Similar findings were reported in the research by Abrompolsky & Abylkasymov (2005).
The correlation coefficients (yield-content) for fat at French and Dutch breed cows were by 0.401 and 0.340 higher than in local generation cows.
Between live weight and milk yield, a high inverse (French origin), weak inverse (Dutch origin), and weak positive (local generation) correlation were identified. Comparative analysis between cows of different breeds based on indicators (live weight - yield) and

(live weight - fat quantity) showed that French breed cows significantly exceeded local generation cows by 0.735 and 0.757 ($P < 0.01$).
For local generation cows, a weak positive relationship was found between live weight and milk yield (+0.078) and live weight and milk fat quantity (+0.065). Similar results were obtained in the research by Vorobyev et al. (2016), where the correlation between traits (yield - live weight) was very low, ranging from 0.063 to 0.187.

Therefore, the optimal live weight for cows of the first completed lactation in the "Total Gnatiuc" herd should be in the range of 570-580 kg, that is., up to 600 kg.

CONCLUSIONS

In SLL "Gomerț Efrem," a high positive correlation was identified between live weight and milk yield, as well as between live weight and the quantity of fat in milk for the first group of cows ($r = 0.717$) and ($r = 0.823$), respectively, where the live weight of local generation cows in the first completed lactation was not more than 600 kg.
The study on the influence of live weight on the milk productivity of German breed Holstein cows in the first completed lactation at the SLL "Dastocom" herd showed that the maximum yield of 9316 kg was obtained on average for cows with a live weight of up to 600 kg. A difference in yield for the first 305 days of lactation was identified between the first group of cows with low live weight (up to 600 kg) and the second group (with a live weight of 601-638 kg) at 251 kg, but the difference was not significant.
Comparative analysis between cows of different breeds at the breeding farm SLL "Total Gnatiuc", based on indicators (live weight - yield) and (live weight - fat quantity), showed that the cows of the French breed are reliable

they outperformed cows of local generation by 0.735 and 0.757 ($P < 0.01$).

When selecting cows based on yield, it is necessary to consider live weight, which for Holstein cows should not exceed 600 kg, regardless of their origin, breeding, and maintenance in herds located in different climatic zones of the Republic of Moldova (northern, central, and southern).

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STUDY ON THE PRODUCTIVITY OF GOATS INCLUDED IN SELECTION BREEDING WORKS IN OUR COUNTRY

Laura MARINICĂ[1, 3], Dorina NADOLU[1, 2], Andreea Hortanse ANGHEL[1, 2], Constantin PASCAL[3]

[1]National Association of Goat Breeders of Romania, 248 I.C. Brătianu Boulevard, Constanța, Romania
[2]Institute of Research-Developement for Sheep and Goat Breeding Palas-Constanța, 248 I.C. Brătianu Boulevard, Romania
[3]Faculty of Food and Animal Sciences, Iași University of Life Sciences, 8 Mihail Sadoveanu Alley, Iași, România

Corresponding author email: dorinanadolu@yahoo.com

Abstract

The growing interest in goat milk in our country has required the expansion of research in this species, covering various areas such as nutrition, reproduction, exploitation conditions, milk and meat processing methods, and others. The purpose of this study is to analyze the distribution of goats included in selection works in relation to the total number of goats at national level reported by the National Institute of Statistics, with the following aspects being evidenced: the proportion of goats included in selection works, the distribution by breeds of the number of goats in the genealogical register and their average milk/lactation/breed/region production. The study is of a statistical nature and focuses exclusively on the herds in the Genealogical Register on 31.08.2023, and the results show that the South East Region stands out with an impressive herd of goats, representing 36.16% compared to the Centre Region where, in the selection work, only 2.88% goats are included. The conclusions presented provide a detailed numerical perspective and the potential of each region within the national goat milk scene. These consolidating insights are essential pillars for guiding future strategies and developments in this area.

Key words: *breed, goat, milk, quantity, region*

INTRODUCTION

Goats are considered one of the most important species, with remarkable biological and economic significance and are widely distributed throughout the world.
Currently, goat farming and exploitation is expanding significantly globally and plays a key role in the animal products sector (Mazinani et al., 2020).
The extended distribution of this species, especially dairy breeds, is largely attributed to the quality and quantity of milk production obtained from them. Products derived from goats have become essential components of the human diet, mainly due to their high nutritional value (Lohani et al., 2021).
In special, goats are bred to produce milk, which is noted for its quality, rich nutritional value and high digestibility. Goat's milk is similar in composition to human breast milk and can provide a significant proportion of human protein requirements and is considered a valuable remedy for many ailments, particularly lung diseases. It is a rich source of essential nutrients including vitamin A, vitamin B, vitamin D, zinc, magnesium, phosphorus and potassium (Park, 2010).
In various countries around the world, there is a growing demand for the highest quality goat dairy products. The amount of milk produced by a goat is influenced by the conditions of care, diet and level of breeding (Pascal, 2015).
Recently, interest in goat's milk has increased, which has led to the need to expand research in this area, covering aspects such as nutrition, breeding, farming conditions and milk and meat processing techniques.
In the effort to maximize milk production in goats, the adoption of technological farming methods, respectively obtaining prolonged lactation, increasing the number of females by

intensifying breeding, ensuring constant milk production at farm level through dairy farming and breeding biotechnologies, are essential.

According to a study conducted by Rico-Bautista et al. (2019) at an experimental farm of the Francisco de Paula Santander University in Colombia, the CAPRINAPP technology, designed to manage and monitor goat herds and production, has been successfully implemented with a significant impact on milk production and other relevant aspects of agricultural processes.

Another innovative way to improve milk production in arid and desert regions was identified in research by Strahsburger et al. (2021), respectively, the advanced application of molecular markers and genetic technologies to achieve selection and improvement of desired goat traits.

Another method to improve milk production is to explore and adjust milking frequency. (Williams et al., 2012).

Another essential strategy for improving milk production is adjusting the length of the light day which has led to a 15% increase in milk production (Rodríguez-Martinez et al., 2011).

The integration of modern technologies to manage nutrition, health and environmental conditions can be essential to ensure sustainable growth in milk production.

The study by Anghel et al. (2020) shows that dietary adjustment in multiparous goats positively influences milk quantity and quality.

To improve milk production in goat farming, one of the efficient technologies is breeding. By carefully selecting animals with superior genetic characteristics, such as increased milk production capacity, this method contributes to improved performance.

Nucleus farms play an important role in implementing genetic improvement, where animals with desired traits are identified and bred. By transferring these animals to production farms, the aim is to continuously increase the quality and yield of the herd. In essence, genetic improvement is a key strategy for optimising milk production growth in goats.

The increased interest in goat breeding and exploitation has also been evident among Romanian goat farmers. In order to maximize their productive potential, they have made use of research results and adopted various strategies, including importing specialized breeds for milk production, as well as adhering to the Breeding Programs of the Breed Genealogical Registries and complying with the conditions of the Breeding Programs in selected breeding (Nadolu et al., 2022).

In an evolving agricultural landscape, with a significant increase in interest in goat milk products, breeding has become an essential pillar for improving and optimising performance in goat farming.

The purpose of the present study is to analyse the distribution of goats included in the selection works in relation to the herds of the National Institute of Statistics, as well as the evolution of the average milk production of the goats in the Genealogical Register with the identification of the factors influencing the expression of the productive potential.

MATERIALS AND METHODS

The biological material analysed in this study belong to Carpathian, White of Banat, Saanen, Alpine, Anglo-Nubian and Murciano-Granadina breeds. The study focuses exclusively on herds in the Genealogical Register on 31.08.2023. The choice of this date was motivated by the need to use the most recent information available.

For the elaboration of this article, the information accessible online through the website of the National Institute of Statistics, more precisely in the Tempo-Online data platform and through the database of the Breed Genealogy Register, was used.

Statistical significance analysis of the differences between the means of milk production from 2019-2023 was performed using Single Factor ANOVA from Excel 2007.

Milk production control was performed by the standard A4 method (cf. ICAR, Ord.22/2006), for three consecutive months, two controls per day, morning and evening, and milk yield/lactation was calculated according to the Fleishman formula:

$$MS = I_0M\left(\frac{M1 + M2}{2}\right) + I1\left(\frac{M2 + M3}{2}\right) + \ldots + In\text{-}1\left(\frac{Mn\text{-}1 + Mn}{2}\right) + InMn$$

in which:

MS- milk production per lactation (ml);

M1, M2, M3- the quantity of milk (ml) at the 24 hours control;

I_0- the interval (days) between the milking start date and the date of the first control;
I_1, I_2, I_{n-1}- interval between controls (days);
I_n- the interval in days between the date of the last control and the end of lactation.
The process of collecting the data provided by the Genealogical Registry involved strong collaboration with goat breeders to make sure that the data provided is trusted.
Evaluation of individual goat performance, including milk production and reproductive efficiency, was an integral part of this data collection. Through this study, the evolution of purebred goat herds and milk production of goats included in the Genealogical Breed Register is highlighted.

RESULTS AND DISCUSSIONS

At the end of 2022, there will be 206.792 goats in the Breed Register of the six breeds: Carpathian, White of Banat, Saanen, Alpine, Anglo-Nubian and Murciano-Granadina, representing approximately 13.93% of the total number of goats in the country, respectively 1,492,544 which suggests that improvement in purebred by selection has captured the interest of goat owners who have understood that this is a way to make breeding more efficient in the long term.
As a result, many breeders have abandoned crossbreeding, which results in better yields for only 2-3 generations and have turned to specialists in research institutions and professional associations that run Genealogical Registries to improve the genetic structure of their herds through well-defined breeding programmes that promote high-productivity, respectively positive breeding value and apply mating schemes that take into account the avoidance of inbreeding.
The same trend of fluctuation in goat numbers can be observed globally, with an impressive increase in numbers on every continent.
Over the last 10 years, Asia has become the major player in the goat farming industry, with a significant increase in herds of over 200 million goats.
China and India, which together account for more than 35% of the global goat population, have had a major impact on this development (FAOSTAT, 2022).

At European level, Romania places the fourth largest number of goats in Europe and the country with the highest number of goats is Turkey, followed by Spain and Greece (EUROSTAT, 2022).
In Romania, the goat population is continuously increasing, mainly due to the quality and quantity of milk production, which provides nutrition for the families and additional income from the sale of milk and other milk products (Figure 1).

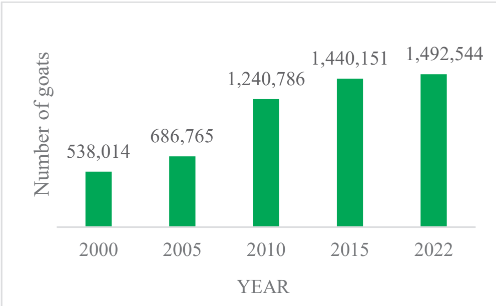

Figure 1. Evolution of the number of goats in Romania (NIS, 2022)

The evolution of goat populations in Romania illustrated in Figure 1 has been marked by significant changes over the last two decades. From initial growth in the years 2000, the sector has fluctuated, with an increasing trend in 2010, when the number of goats doubled.
The ascending trend continued in the following years, reflecting the adaptation of the agricultural sector, with an important role played by the legislative context to stimulate and maintain the sector in Romania.
Within the regions of our country, the proportion of goat herds included in selection breeding compared to the total herd of goats at national level highlights their demographic variation, reflecting the diversity and importance of the goat sector in each region (Table 1).
From the data presented in Table 1, large differences between regions can be observed. The herd of goats included in selection breeding in the South-East Region stands out with the highest percentage, reaching 20.6%. This indicates an increased interest and significant investment in breeding in this region, suggesting a proactive approach to improving goat performance.

Table 1. Evolution of goat populations within regions (2022)

REGION	Total national goats (NIS) (number head)	Total goats R.G. (number head)	Percentage of goat R.G./NIS%
NORTH WEST Region	113,795	10,453	9.18
CENTRAL Region	125,146	5,265	4.2
NORTH EAST Region	240,008	21,574	8.9
SOUTH-EAST Region	380,991	78,483	20.6
SOUTH-MUNTENIA Region	269,234	31,568	11.7
BUCHAREST-ILFOV Region	4,305	0	0
SOUTH-WEST OLTENIA Region	280,872	53,121	18.9
WEST Region	78,193	6,328	8.1
TOTAL	1,492,544	206,792	13.9

South West-Oltenia and South Muntenia regions follow in the ranking, with 18.9% and 11.7% respectively.

On the other part, the West and Centre regions registered lower percentages, 8.1% and 4.2% respectively.

These variations highlight not only demographic differences but also the unique attitudes adopted by goat farmers in the different regions of Romania.

These figures suggest that breeding is an essential component of goat breeding in these regions and that breeders pay particular attention to selection to improve the performance of their animals.

Regarding the distribution of breeds in the breeding work by selection in the year 2023, the main remark falls on the predominance of the Carpathian breed in the Genealogical Breed Register (Figure 2).

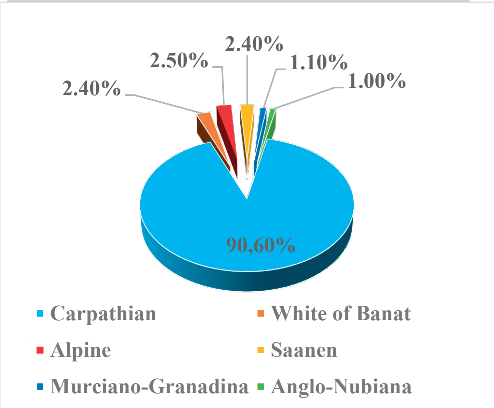

Figure 2. Percentage distribution of goat breeds registered in the Genealogical Register (2023).

Thus, in 2023, the local Carpathian breed stands out with a percentage of 90.60%, White of Banat, another local breed, represents 2.40% and the Saanen, Alpine, Anglo-Nubian and Murciano-Granadina breeds together account for 7% of the total number of goats included in the breeding work

Over the last 5 years, the evolution of goat herds enrolled in breeding programmes in the Genealogical Registry of goat breeds has been ascending (Table 2).

Table 2. Evolution of goat herds enrolled in breeding programmes in the Genealogical Register (2023)

YEAR	Number goats					
	CR*	AB*	AP*	SN*	MG*	AN*
2019	179,693	3,736	4,193	4,799	1,195	428
2020	158,120	4,864	4,507	5,162	1,932	1,069
2021	172,896	3,821	4,555	4,869	1,690	1,230
2022	189,116	5,121	4,596	4,769	1,715	1,475
2023	196,645	5,176	5,395	5,286	2,281	2255

CR*-Carpathian, AB*-White of Banat, AP*- Alpine, SN*-Saanen, MG*-Murciano-Granadina, AN*- Anglo-Nubian

In the last 5 years, there has been an encouraging evolution of goat herds registered in breeding programmes, both for local breeds such as Carpathian and White of Banat and for imported breeds such as Alpine, Saanen, Murciano-Granadina and Anglo-Nubian.

Local breeds, having a significant contribution to the country's genetic patrimony, have shown a steady increase, reflecting conservation efforts in the low-numbered White of Banat breed and improved yields in the Carpathian breed.

At the same time, imported breeds, with high genetic potential, have also seen a steady increase in the number of herds registered in breeding programmes, the aim of which is to increase their productive performance in the soil and climate conditions of our country, so as to reach the production levels reported by the countries from which they come.

In the regions of Romania, the analysis of the distribution of herds registered in the breeding by selection programme in 2023 opens a window on the complexity and adaptability of husbandry practices at regional level (Table 3).

Table 3. Distribution of goat herds in the breeding programme by breeds and regions (number head 2023)

R*	CR*	AB*	AP*	SN*	MG*	AN*	%*
NV*	6,451	128	1,332	1,344	45	0	3.7
C*	5,138	408	342	236	141	0	2.88
NE*	23,164	259	2,864	1,309	442	1,126	13.4
SE*	76,189	0	863	1,157	116	158	36.16
SM*	29,141	216	662	871	1,183	649	15.1
SVO*	48,166	0	174	228	18	0	22.3
SV*	1,297	3,145	0	410	110	258	2.4

R*- Region,
CR*-Carpathian, AB*- White of Banat, AP*- Alpine, SN*-Saanen, MG*-Murciano-Granadina, AN*- Anglo-Nubian;
NV*- NORTH WEST Region, C*- CENTRAL Region, NE*- NORTH EAST Region, SE*- SOUTH-EAST Region, SM*- SOUTH-MUNTENIA Region, SVO*- SOUTH- WEST OLTENIA Region, SV*- WEST Region
%*- Total number of goats R.G./Region

The regional distribution of the goats included in the selection breeding programme shows that the South East and South West Oltenia regions are home to more than half of the goat population registered in the Genealogical Breed Registers.

This is due, on the one part, to the people living in these areas with specific occupations strongly connected with animal husbandry and on the other, to the climatic conditions suitable for animal husbandry.

The Centre region has the lowest percentage (2.88%) of goats registered in the Genealogical Registers, due to the higher concentration on breeding other species.

Analysing the distribution of goat herds included in the breeding programme by breed and region for the year 2023, some significant trends and differences between regions can be observed.

The South East Region shows an impressive 36.16% of goats compared to the Centre Region where only 2.88% of goats are included in the breeding programme.

All goats registered in the programme of improvement by selection of the Breed Genealogical Registers are also subjected to individual quantitative milk production control, respectively the standard A4 method, two controls per day, morning and evening, for three consecutive months and the milk production/ lactation of each goat is assessed by applying the Fleishman formula.

From the analysis of the last 5 years, variations in the average total milk production obtained from goats registered in the Genealogical Breed Register of the six breeds: Carpathian, Banat White, Saanen, Alpine, Anglo-Nubian, Murciano-Granadina were observed and the main factors influencing these variations were identified.

It should be pointed out that in these animals included in the Breeding Programmes, in which milk production has been monitored, no important changes in the improvement of this trait in such a short time can be recorded because its heritability is 0.25. It should also be noted that the variation in milk production per total animals included in the Genealogical Breed Registers, including those newly registered and not traced by parentage, was tracked to determine the improvement in milk production character.

From Table 4 it can be seen that the number of Carpathian goats subject to milk production control had an upward trend with the exception of 2022 when from the analysis of the Genealogical Register platform it was observed that a large number of animals were reformed and many females entered first lactation.

The number of goats under control ranged from 117.073 head in 2019 to a maximum of 138.087 head in 2023 and the average annual milk production also increased from 144.17 to 175.04 kg milk/head/year. The decrease in the average quantity of milk is noteworthy due to the increased number of primiparous females, but mainly due to the nutritional factor.

Over the years, goat breeders who have joined the Genealogical Registers with their animals have been advised and have understood the importance of external factors, related to farming technology, which influence milk production, in particular the respect of milking times and the administration of supplementary feeding (good quality pasture or even the administration of feed supplements) so that the animals can express their productive potential.

The influence of the feed factor, respectively the expression of the productive potential, can also be observed by comparing the average production of each year to the multiannual average production of the 5 years analysed, 163.52 kg/head/year, which is exceeded in the last three years of milk production control.

Statistical analysis of the evolution of milk production in Carpathian goats, over the 5 years, does not indicate significant differences from year to year ($P>0,05$).

Table 4. Evolution of the average milk production of the Carpathian breed (kg/milk/goat)

Year	Number of animals controlled	Total quantity of milk (kg/year)	Average quantity of milk (kg/head/year)
2019	117,073	18,528,803.78	144.17*$^{0.22}$
2020	119,713	19,021,914.25	158.9*$^{0.24}$
2021	136,353	23,528,605.94	172.56*$^{0.77}$
2022	126,009	21,033,947.53	166.92*$^{0.15}$
2023	138,087	24,170,932.06	175.04*$^{0.20}$
Average over the last 5 years			163.51
Average over the last 4 years			160.64

The last 5 years for White of Banat goats over the last 5 years show a greater variation, with considerable fluctuations from one year to the next (Table 5). The number of White of Banat goats is very small, as the breed is in genetic conservation, and the herd included in breeding and genetic conservation works subject to control of productive performance has shown variations due to the migration of animals between breeders. Additionally, from the herds of goats registered in the Genealogical Register, the goats that respected the genealogical conditions of paternity were subjected to control in order to avoid cosangvinization. The individual annual average White of Banat goats in 2019 was 211.79 kg/head/year, much higher than in previous years, a value mainly due to the large number of multiparous females, some of which were subsequently removed from breeding work and therefore from milk production control. Statistical analysis of the evolution of milk production in White of Banat goats during the 5 years indicates significant differences ($P<0.05$) in 2021 and 2022.

Starting in 2020 and 2021, primiparous goats entered into directed breeding programs were retained for breeding on parentage criteria, with matings matched on parentage and productive performance criteria, respectively positive breeding value. What should be pointed out in the case of local breeds of goats, Carpathian and White of Banat, is the way they are exploited, respectively the traditional system, with the goats kept on pasture for 8-10 months, depending on climatic conditions.

This influences milk production, in drought years, such as 2022, when the quality of pastures and grazing land was poor, if the feed ration was not supplemented properly and consistently, the goats could not express their productive potential.

Table 5. Evolution of average milk production of the White of Banat breed (kg/milk/goat)

Year	Number of animals controlled	Total quantity of milk (kg/year)	Average quantity of milk (kg/head/year)
2019	3,196	676,873.30	211.79*$^{0.1}$
2020	1,704	257,257.39	150.97*$^{0.84}$
2021	3,325	517,009.45	155.49**$^{0.01}$
2022	1,838	292,565.66	159.18**$^{0.01}$
2023	2,066	406,622.83	196.82*$^{0.88}$
Average over the last 5 years			174.85

A special situation is encountered in the case of goats specialized for milk production coming from imports and registered in the Breed Genealogical Register of our country. In this situation, the main aim of the Breeding Programmes is to improve milk production under the farming conditions specific to our country.

The main challenges for the expression of the productive potential are the adaptation of the animals to the pedo-climatic conditions specific to our country and the adoption by the goat keepers of exploitation conditions as close as possible to those offered in the countries where the animals come from.

Table 6. Evolution of average milk production of the Anglo-Nubian breed (kg/milk/goat)

Year	Number of animals controlled	Total quantity of milk (kg/year)	Average quantity of milk (kg/head/year)
2019	763	197,932.4	254.42**$^{0.03}$
2020	544	132,922.78	244.34**$^{0.04}$
2021	1,116	307,032.3	275.12*$^{0.48}$
2022	828	217,337.63	262.49*$^{0.58}$
2023	987	321,261.38	327.48*$^{0.56}$
Average over the last 5 years			272.83

As far as the Anglo-Nubian breed is concerned, it is bred in small holdings of up to 50 head, but in a modernized traditional rearing system, with mares kept with their mothers for 4-5 months and weaning of goats much earlier than the breed standard of up to 300 days of lactation.

As can be seen from Table 6, average annual productions range from 244.34 kg/head/year (2020) to 327.48 kg/head/year in 2023, with an upward trend, indicating a good adaptability to climatic, exploitation and feed codifications.

Statistical analysis of the 5 years of lactation monitoring in Anglo-Nubian goats in the Genealogical Register and average milk production shows significant differences in 2019 and 2020 ($p<0.05$), the following 3 years not registering significant differences ($p>0.05$).
A particular situation is also found in the case of goats of the Murciano-Granadina breed, for the exploitation of which the owners, in smaller numbers, but with herds of between 10 and 150 head/farm, have adopted the modernised traditional or semi-intensive farming system.
In the case of the semi-intensive system of only 4 farms (with 100-150 goats) the conditions of exploitation for expressing the productive potential characteristic of the breed are closer to those in the countries of origin of the animals, the productions being close to the data declared in the countries of origin.

Table 7 Evolution of average milk production in the Murciano-Granadina breed (kg/milk/goat)

Year	Number of animals controlled	Total quantity of milk (kg/year)	Average quantity of milk (kg/head/year)
2019	492	125,792.70	$255.68^{*0.99}$
2020	623	144,926.59	$232.63^{*0.80}$
2021	785	199,332.13	$253.93^{*0.73}$
2022	1,057	246,017.7	$232.75^{*0.74}$
2023	1,583	373,226.85	$235.77^{**0.01}$
Average over the last 5 years			247.41

The number of farms with traditional system being high, the average individual production for the 5 years is low 247.41 and the annual average does not register significant differences ($p>0,05$) regardless of the number of animals whose milk production is monitored in the herd books, recording a minimum of 232.63 kg/head/year (2022) and a maximum of 255.68 kg/head/year (2019). Significant differences ($p<0.05$) in milk production were statistically observed in the year 2023.
In the case of Saanen and Alpine goats, the farming systems vary according to the size of the farm and the financial capacity of the breeders.
These two species have easily introduced and spread to all areas of our country.
The large differences in milk yields over the last 5 years are due to farming systems, with a small number of farms with more than 300 goats (n=5 farms) operated intensively and more than 20 farms with between 20 and 150 goats. In the case of small herds, the farming system adopted is traditional or traditionally modernised.

Table 8. Evolution of average milk production of the Saanen breed (kg/milk/head)

Year	Number of animals controlled	Total quantity of milk (kg/year)	Average quantity of milk (kg/head/year)
2019	2,667	638,901.4	$239.56^{**0.006}$
2020	2,483	767,885.33	$309.26^{*0.24}$
2021	2,796	1,037,084.34	$370.92^{*0.80}$
2022	3,583	1,336,264.34	$372.95^{**0.06}$
2023	3,191	1,161,477.19	$363.99^{**0.01}$
Average over the last 5 years			331.33

Table 8 shows the milk production of Saanen goats and Table 9 shows the milk production of Alpine goats. For both breeds, during the last 5 years, the average annual individual milk production has increased, which indicates a good adaptability to the specific pedo-climatic conditions of our country, but without reaching the productions specified by the herd books of the countries of origin (France, Austria, Hungary, Germany).

Table 9. Evolution of the average milk production of the Alpine breed (kg/milk/head)

Year	Number of animals controlled	Total quantity of milk (kg/year)	Average quantity of milk (kg/head/year)
2019	2,105	464,392.00	$220.62^{**0.001}$
2020	1,596	476,937.29	$298.83^{*0.54}$
2021	2,968	951,739.10	$320.67^{*0.18}$
2022	2,977	949,792.09	$319.04^{**0.006}$
2023	3,125	876,084.54	$280.35^{**0.01}$
Average over the last 5 years			287.90

The statistical analysis of the evolution of the average annual production in the Saanen and Alpina breeds indicates significant differences compared to the average milk production for the 5 years analysed ($p<0.05$), except for the years 2020 and 2021 where there are no significant differences ($p>0.05$).
In the year 2023, a slight decrease in average annual individual production is observed, due to the management of a slightly deficient forage, which is also observed on all farms throughout the country, amid the decrease in the financial capacity of owners.

In the Saanen breed, the average individual production increases from 239.56 to 372.95 kg/head/year in 2019-2022.

According to the data provided by the herdbook and presented in Table 9, the average annual individual production of Alpine goats increases from 220.62 kg/head/year in 2019 to 319.04 kg/head/year in 2022.

CONCLUSIONS

The goats of local breeds included in the selection work for improvement in the pure breed have registered an annual increase of 30.87 kg/head/year in the Carpathian breed during the 5 years analysed and 45.85 kg/head/year in the White of Banat breed during the last 4 years. This progress is the result both of selection work based on filiation and breeding.

In the case of specialised breeds for the production of imported milk, the main objective is to adapt to the pedo-climatic conditions specific to our country and to adopt farming systems as close as possible to those of the animals' countries of origin. By abandoning the traditional system and adopting similar housing and feeding measures to those of the farms of origin, an increase in average annual milk production was observed over the 5 years for all 4 imported breeds.

Saanen and Alpine goats on farms with herds of more than 300 head benefit from intensive farming conditions that allow the breed-specific productive potential to be expressed, as reported in the herd registers of the countries of origin.

Selection work for purebred improvement of milk production id directly influenced by external factors of maintenance and feeding of the problem goats.

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OPTIMIZATION OF THE SELECTION PLAN, FOR PRODUCTION AND REPRODUCTION TRAITS, IN THE MILK-PALAS SHEEP POPULATION

Osamah Mahmood Abdul Zahra MURSHEDI, Horia GROSU

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: osamaalmurshedi66@gmail.com

Abstract

The purpose of this study is to optimize the selection plan for production and reproduction traits in the milk-Palas sheep population totally 486 sheep. The selection criterion is represented by an estimate of the global breeding value, calculated based on the BLP method. Among the four traits considered in terms of economic weights, the most important in the selection turned out to be prolificacy (55%), followed by the amount of milk (24%), the amount of fat (12%) and the amount of protein (9%). The greatest genetic progress per generation was obtained in the case of variant 5 (Milk + Fat + Protein + Prolificacy), the genetic gain being 7.6623 kg milk and of 0.0407 lambs/calving. Compared to the control variant (1), the total genetic gain increased by 102.8%. The next variant is variant 2 (Milk + Fat + Prolificacy), which practically ensures a genetic gain similar to variant 5, namely 102.7%. This result is explained by a higher genetic correlation between the amount of milk and the amount of fat (0.836) compared to the value of the correlation between the amount of milk and the amount of protein (0.441).

Key words: *correlation, fat, milk, production and reproduction traits, prolificacy, protein, selection indices.*

INTRODUCTION

Sheep are among the most economically significant animals since they provide human society with necessities including meat, milk, wool, and fur (Gebreselassie et al., 2019). One of the main ways to increase livestock output is through genetic improvement, and in order to reap the genetic benefits, carefully thought out animal genetic improvement programmes are required (Villalba et al., 2019). Livestock genetic improvement is a particularly effective way to increase output, it entails determining the breeding objectives, calculating the traits' economic values, creating suitable schemes that define the population structure, gene flow, and selection tactics, as well as creating and carrying out the breeding plan (Gizaw et al., 2014). The effectiveness of a breeding programme in terms of realised genetic advancement and inbreeding rate is dictated by flock size, which is likely to vary across the breeding tract of a breed that is being improved, to increase the overall productivity of sheep, it is crucial to investigate alternative breeding techniques. Both the short-term (high rate of genetic gain) and long-term (preservation of genetic variety and avoidance of inbreeding depression) effects of selection decisions must be considered in order to optimise breeding strategies (Fimland, 2007). With the correct index trait weights, indexes can be simply generated as measurements become available on site, and selection can be performed promptly. The application of selection indexes would be a simple expeditious approach for efficient selection that would not delay farmer's animal sale chances, requiring only a little amount of support from extension officers or regional personnel (Mueller et al., 2021). Genetic parameter estimation is used to determine the selection criterion and future breeding strategies, as well as to enable the efficient prediction of breeding value and selection procedures, it can serve as the foundation for any sound livestock improvement programme (Alemayehu, 2022). Both genetic and economic factors must be considered when evaluating the effectiveness of the selection strategy. These factors must be optimally integrated to guarantee that the chosen variant maximises genetic gain while requiring the least amount of time, money, or effort (Popa et al., 2011). A method for determining an animal's breeding value that takes into account all of the information about the animal and its ancestors is called the selection index. It is the

linear forecast with the highest accuracy for a single improvement value, employing all accessible records - that is, details about the animal, mother, half-siblings, progeny, etc. - will surely be the most beneficial method for determining the specimen's breeding value (Endris, 2020). Heritability estimates are useful in predicting genetic responses to selection, generating selection indices, and determining the degree to which an individual's phenotype may be relied upon for selection. Effective cattle operations require heritability estimates for several economic characteristic
cs (Lalit et al., 2016).

The purpose of the research is to optimize the selection plan for production and reproduction traits in the milk-Palas sheep population.

In the first phase, being partial results, the selection criterion was optimized by considering several selection indices, based on 2, 3 and 4 traits. The optimal variant of the selection criterion is represented by the index that maximizes genetic progress per generation.

MATERIALS AND METHODS

The study was carried out on the basis of the production and reproduction performances of a number of 486 sheep from the Palas milk Line.The three production traits (amount of milk, amount of milk fat and amount of milk protein) and the one reproduction trait (prolificacy) were analysed.

In order to optimize the selection criteria, as work steps, the following activities were carried out:

1. Estimation of genetic parameters (heritability, genetic and phenotypic correlations between traits).
2. Estimation of the economic weights of the traits.
3. Estimation of genetic progress per generation.

The genetic parameters were obtained by the REML method, for several traits.

Statistical analysis: The data were analyzed with animal model with R software [1, 2]. The model for trait "i" is as follows:

$$P_i = X_i b_i + Z_1 a_i + e_i$$

For two traits, Mixed Model Equations of BLUP can be written as follows:

$$\begin{bmatrix} X_1' \cdot r^{11} \cdot X_1 & X_1' \cdot r^{12} \cdot X_2 & X_1' \cdot r^{11} \cdot Z_1 & X_1' \cdot r^{12} \cdot Z_2 \\ X_2' \cdot r^{21} \cdot X_1 & X_2' \cdot r^{22} \cdot X_2 & X_2' \cdot r^{21} \cdot Z_1 & X_2' \cdot r^{22} \cdot Z_2 \\ Z_1' \cdot r^{11} \cdot X_1 & Z_1' \cdot r^{12} \cdot X_2 & Z_1' \cdot r^{11} \cdot Z_1 + A^{-1} \cdot g^{11} & Z_1' \cdot r^{12} \cdot Z_2 + A^{-1} \cdot g^{12} \\ Z_2' \cdot r^{11} \cdot X_1 & Z_2' \cdot r^{22} \cdot X_2 & Z_2' \cdot r^{21} \cdot Z_1 + A^{-1} \cdot g^{21} & Z_2' \cdot r^{22} \cdot Z_2 + A^{-1} \cdot g^{22} \end{bmatrix} \cdot \begin{bmatrix} \tilde{b}_1 \\ \hat{b}_2 \\ \hat{a}_1 \\ \hat{a}_2 \end{bmatrix}$$

$$= \begin{bmatrix} X_1' \cdot r^{11} \cdot P_1 + X_1' \cdot r^{12} \cdot P_2 \\ X_2' \cdot r^{21} \cdot P_1 + X_2' \cdot r^{22} \cdot P_2 \\ Z_1' \cdot r^{11} \cdot P_1 + Z_1' \cdot r^{12} \cdot P_2 \\ Z_2' \cdot r^{21} \cdot P_1 + Z_2' \cdot r^{22} \cdot P_2 \end{bmatrix}$$

G and R are variances and covariances matrices for genotypic and environmental effects:

$$G^{-1} = \begin{bmatrix} \sigma_{a1}^2 & \sigma_{a12} \\ \sigma_{a21} & \sigma_{a2}^2 \end{bmatrix}^{-1} = \begin{bmatrix} g^{11} & g^{12} \\ g^{21} & g^{22} \end{bmatrix};$$

$$R^{-1} = \begin{bmatrix} \sigma_{e1}^2 & \sigma_{e12} \\ \sigma_{e21} & \sigma_{e2}^2 \end{bmatrix}^{-1} = \begin{bmatrix} r^{11} & r^{12} \\ r^{21} & r^{22} \end{bmatrix}$$

The elements of G and R were estimated using the formulas:

$$\sigma_{a\,ii}^2 = \frac{\hat{a}_i' \cdot A^{-1} \cdot \hat{a}_i + tr(A^{-1} \cdot C_{ii})}{q}$$

$$\sigma_{a\,jj}^2 = \frac{\hat{a}_j' \cdot A^{-1} \cdot \hat{a}_j + tr(A^{-1} \cdot C_{jj})}{q}$$

$$\sigma_{a\,ij} = \frac{\hat{a}_i' \cdot A^{-1} \cdot \hat{a}_j + tr(A^{-1} \cdot C_{ij})}{q}$$

$$\sigma_{e\,ii}^2 = \frac{\hat{e}_i' \cdot \hat{e}_i + tr(B_{ii})}{n}$$

$$\sigma_{e\,ij} = \frac{\hat{e}_i' \cdot \hat{e}_j + tr(B_{ij})}{n}$$

$$\sigma_{e\,jj}^2 = \frac{\hat{e}_j' \cdot \hat{e}_j + tr(B_{jj})}{n}$$

$\sigma_{a\,ij}$ = additive covariance between trait "i" and "j"

$\sigma_{e\,ii}^2$ = environmental variance for trait "i",

$\sigma_{e_{ij}}$ = environmental covariance between trait "i" and "j"

$$W = [X_1 \oplus X_2 \quad Z_1 \oplus Z_2];$$

$$B = W \cdot C \cdot W' = \begin{bmatrix} B_{ii} & B_{ij} \\ B_{ji} & B_{jj} \end{bmatrix};$$

$\oplus$ = Direct sum (S.R. Searle, 1982)

$$e_i = P_i - W \cdot [\tilde{b}_1 \quad \tilde{b}_2 \quad \hat{a}_1 \quad \hat{a}_2]$$

For the trait “i”, the heritability was obtained as the ratio between the additive genetic variance and the total phenotypic variance (σ_f^2), according to the formula:

$$h_{ii}^2 = \frac{\sigma_{a\,ii}^2}{(\sigma_{a\,ii}^2+\sigma_{e\,ii}^2)}$$

Genetic correlation according to the formula:

$$rg_{\mathrm{ij}} = \frac{\sigma_{\mathrm{aij}}}{\sqrt{\sigma^2_{\mathrm{ii}} * \sigma^2_{\mathrm{jj}}}}$$

Spearman's rank correlation coefficient according to the formula:

$$\mathrm{rs} = 1 - \frac{6\Sigma \mathrm{d}^2_{\mathrm{i}}}{\mathrm{n}(\mathrm{n}^2-1)}$$

Where: n-rank number; d-the difference between the rank occupied by the same animal (candidate to selection), in two rankings based on different criteria (different biometric models).

For estimation of the weight of the characters in the selection objective according to the formula:

$$\begin{bmatrix} h_1 & h_2 \cdot r_{G12} & h_3 \cdot r_{G13} & h_4 \cdot r_{G14} \\ h_2 \cdot r_{G12} & h_2 & h_3 \cdot r_{G23} & h_4 \cdot r_{G24} \\ h_3 \cdot r_{G13} & h_3 \cdot r_{G23} & h_3 & h_4 \cdot r_{G34} \\ h_4 \cdot r_{G14} & h_4 \cdot r_{G24} & h_4 \cdot r_{G34} & h_4 \end{bmatrix} \cdot \begin{bmatrix} \beta_1 \\ \beta_2 \\ \beta_3 \\ \beta_4 \end{bmatrix} = \begin{bmatrix} d_1 \\ d_2 \\ d_3 \\ d_4 \end{bmatrix}$$

$h_i = \sqrt{h_i^2}$ = heritability of trait i,

r_{Gij} = genetic correlation between traits i and j,

d_i = standardized genetic distance for trait i,

$d_i = \left(\overline{P}_P - \mu_{Act}\right) / \sigma_{Ai}$,

$\overline{P}_P$ = average trait performance, in perspective, μ_{Act} = current population mean,

σ_{Ai} = genetic standard deviation of trait i.

The technical coefficients are obtained by standardizing the partial regression coefficients, to bring all traits to the same denominator, according to the formula:

$$v_i = \frac{\beta}{K \cdot \sigma_{Aij}}$$

$K = \sqrt{\beta^T \cdot r_P \cdot \beta}$ = variance of the regression coefficients; = matrix of phenotypic correlation coefficients between traits.

By relating the value of each technical coefficient to their sum, the relative weight of the four traits is obtained.

The significance of the values of the relative coefficients shows us the importance that must be assigned to each trait in the selection process.

For estimation of genetic progress per generation according to the equations:

$$H = v_1 \cdot A_1 + v_2 \cdot A_2 +,...,+ v_m \cdot A_m$$

$$I = b_1 \cdot P_1 + b_2 \cdot P_2 +,...,+ b_n \cdot P_n$$

$$\begin{bmatrix} V_{P1} & Cov(P_1,P_2) & ... & Cov(P_1,P_n) \\ Cov(P_2,P_1) & V_{P2} & ... & Cov(P_2,P_n) \\ ... & ... & ... & ... \\ Cov(P_n,P_1) & Cov(P_n,P_2) & ... & V_{Pn} \end{bmatrix} \cdot \begin{bmatrix} b_1 \\ b_2 \\ b_3 \\ b_4 \end{bmatrix} = \begin{bmatrix} V_{A1} & Cov(A_1,A_2) & ... & Cov(A_1,A_n) \\ Cov(A_2A_1) & V_{A2} & ... & Cov(A_2,A_n) \\ ... & ... & ... & ... \\ Cov(A_n,A_1) & Cov(A_n,A_2) & ... & V_{An} \end{bmatrix} \cdot \begin{bmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \end{bmatrix}$$

$$b = v' \cdot C' \cdot V^{-1}$$

$$\mathrm{r_{HI}} = \sqrt{\frac{S_I^2}{S_H^2}} = \sqrt{\frac{b' \cdot C \cdot b}{v' \cdot G \cdot v}}$$

$$\Delta H = r_{IH} \cdot i \cdot \sigma_H$$

$$\Delta G_i = \frac{b_i \cdot C}{\sigma_I}$$

RESULTS AND DISCUSSIONS

Table 1 shows the population's mean amount of milk produced is 104.22 kg, with a relatively small standard deviation of 1.87 kg, indicating that the values are closely clustered around the mean.

The coefficient of variation (CV) is 41.15%, suggesting moderate variability in milk production across individuals.The observed range spans from 16 kg to 261 kg, showcasing significant variability in milk production among the population. And the population's mean amount of fat produced is 7.22 kg, with a low standard deviation of 0.14 kg, indicating a relatively tight distribution around the mean value. The coefficient of variation (CV) is quite low at 3.03%, suggesting low variability in fat production across individuals. The observed fat production ranges from 1 kg to 19 kg, showcasing some variability but not as wide-ranging as the amount of milk. The population's mean amount of protein produced is 5.54 kg, with a standard deviation of 0.15 kg, indicating a relatively tight distribution around the mean value. The coefficient of variation (CV) is 3.21%, similar to fat production, suggesting low variability in protein production across individuals. The observed protein production ranges from 0.4 kg to 19 kg, showcasing variability but with a more extended range than fat production. Finally the population's mean prolificacy is 1.3, with a small standard deviation of 0.02, indicating a narrow distribution around the mean value.The coefficient of variation (CV) is 0.46, suggesting low variability in prolificacy across individuals. The observed prolificacy ranges from 1 to 2, indicating limited variation within the population for this trait.

These results are similar to what Dhaoui et al. (2019) found that the average production in D'man sheep Over the course of 78.60 ± 0.24 days of lactation, the D'man ewe produced an average of 128.91 ± 3.14 l of milk, 9.23 ± 0.26 kg of fat, and 5.26 ± 0.12 kg of total protein.

Table 1. Statistical indicators of the analyzed population

No. trait	traits	unit	n	$\overline{X} \pm s_{\overline{X}}$	S	CV %	Limit Min-Max
1	amount of milk	kg	486	104.22 ±1.87	41.15	39.48	16-261
2	amount of fat	kg	486	7.22 ± 0.14	3.03	42	1-19
3	amount of protein	kg	486	5.54 ± 0.15	3.21	58	0.4-19
4	prolificacy	nr	486	1.3 ± 0.02	0.46	35	1-2

Table 2 observed genotypic variances and covariances between traits (amount of milk amount of fat, amount of protein and prolificacy) in the sheep. These values offer insights into the genetic relationships and variability among these traits.

There's significant genotypic variance in the amount of milk compared to the other traits. The covariances between amount of milk and the other traits (amount of fat, amount of protein, and prolificacy) are relatively higher, indicating a stronger relationship between milk production and these traits.

Prolificacy shows the least genotypic variance and has relatively lower covariances with the other traits, indicating less shared genetic variability compared to the other traits.

Strong Genetic Influence on Milk Production: The high genotypic variance and substantial covariances suggest that milk production has a strong genetic basis within this population. Traits like fat production, protein production, and to a lesser extent, prolificacy, seem to share genetic influences with milk production, indicating potential interconnectedness or co-inheritance.

Table 2. Genotypic variances and covariances between traits

No. trait	traits	amount of milk	amount of fat	amount of protein	prolificacy
1	amount of milk	280.553	18.153	11.733	1.737
2	amount of fat	18.153	1.68	0.67	0.128
3	amount of protein	11.733	0.67	2.527	0.052
4	prolificacy	1.737	0.128	0.052	0.048

Table 3 provides information on phenotypic variances and covariances between different traits within a population. Phenotypic variances encompass both genetic and environmental influences on traits, so they offer a broader perspective compared to genotypic variances, which focus solely on genetic influences.

The phenotypic variance for milk production is high (1424.953), indicating substantial overall variability in milk production within the population, considering both genetic and environmental factors.

The phenotypic variance for fat production is relatively lower (8.052) compared to milk production, signifying less overall variability in fat production considering both genetic and environmental factors. This trait displays a moderate phenotypic variance (9.608), suggesting a moderate level of overall variability in protein production considering both genetic and environmental influences. Prolificacy shows the lowest phenotypic variance (0.204) among all the traits, indicating the least overall variability within the population for this trait considering both genetic and environmental factors.

Table 3. Phenotypic variances and covariances between traits

Nr. trait	traits	amount of milk	amount of fat	amount of protein	prolificacy
1	amount of milk	1424.953	100.967	59.614	9.896
2	amount of fat	100.967	8.052	4.129	0.724
3	amount of protein	59.614	4.129	9.608	0.631
4	prolificacy	9.896	0.724	0.631	0.204

Heritabilities

The heritabilities and errors (h2± s.e.) for the traits (amount of milk, milk fat, milk protein, and prolificacy) that were analysed and used to determine breeding values are shown on table 1's diagonal. These values are 0.197 ± 0.263, 0.209 ± 0.278, 0.263 ± 0.347, and 0.235 ± 0.311, indicating that these traits have an intermediate genetic determinism.

Heritability is one of the four variables for which genetic parameters have been calculated in a great deal of research. Shihab et al. (2022) reported that Awassi sheep had a heritability rate of 0.19, which is comparable to the percentage we found in Palas sheep. Heritability estimations for milk production in Italian dairy sheep from Valle del Belice were a low 0.15 (Sutera et al., 2021). For milk production traits in dairy sheep, the heritability estimates were 0.24, 0.21, and 0.22 for milk yield, fat yield, and protein yield, respectively (Mucha et al., 2022). According to Raoof and Khidhir's (2023) study, there was a high 0.22 heritability for daily milk output in local sheep in Iraq. In Istrian sheep, the daily milk yield (kg), fat content (kg), and protein content (kg) had low heritabilities of 0.09, 0.03, and 0.06 on dairy characteristics, respectively (Špehar et al., 2022). In dairy herds in southern Chile, genetic parameter estimation was used to estimate milk yield, fat and protein yield, and estimated heritability for milk yield, fat, and protein was 0.16±0.004, 0.44±0.007, and 0.42±0.006, respectively (Muñoz et al., 2017). The heritability values for milk yield, fat yield, protein yield, and prolificacy that Scholtens (2016) determined were 0.25, 0.21, 0.20, and 0.13 for dairy sheep in New Zealand, respectively, and were taken into consideration in a breeding aim. According to Murphy and Thomas (2016), the number of lambs born per ewe lambing had a low heredity estimate of 0.07, whereas the milk output, fat yield, and protein yield had intermediate heritability estimates of 0.32, 0.26, and 0.30. Baluchi sheep reproductive characteristic estimations based on genetic parameters. According to Esmaeili-Fard et al. (2021) the heritability for prolificacy was 0.22. When Xinggao sheep's genetic parameters were evaluated, the heritability for prolificacy was 0.12 (Liu et al., 2023). The proportion was 0.23 in another study that estimated sheep heritability for prolificacy, which was considered moderate (Pascal et al., 2019).

Genetic correlations

The genetic correlations between each pair of the four qualities studied are shown on the off diagonal of table 1, along with their errors (rg s.e):

The degree to which genetic variables influencing one trait also influence another trait is measured by genetic correlation. For example, there is a substantial positive genetic connection between the amount of milk and milk fat qualities. This suggests that the genetic variables driving increased milk production are also connected with increased milk fat content. The genetic link between the amount of milk and the amount of milk protein is positive, but it is weaker than the genetic correlation between the amount of milk and the amount of milk fat.

There is a positive genetic association between milk production and prolificacy, indicating that some genetic variables associated with increased milk production may also be associated with higher prolificacy. The genetic relationship between milk fat and milk protein is positive but not strong. Milk fat and prolificacy have a positive genetic link. The genetic link between milk protein and procreation is positive but weak. For dairy qualities in Istrian sheep, there were genetic correlations that were negateve between daily milk yield and fat content, positive between daily milk yield and protein content, and negative between daily milk yield and fat content (-0.22, -0.27, and 0.70), respectively (Špehar et al., 2022). It was demonstrated that there is a significant genetic correlation (0.91 to 0.96) between the yields of fat, protein, and milk. Protein synthesis and milk fat % showed a modest genetic correlation (0.61). Protein and fat yields had a negative correlation with milk yield (-0.31 and -0.34, respectively). Ewe prolificacy had a minor negative correlation (-0.26) with milk fat but no substantial genetic correlation (>0.67) with either milk yield or protein yield (Murphy et al., 2017). The genetic correlations between milk and fat and protein outputs are favourable and relatively substantial (between 0.77 and 0.93), according to a Eurosheep study. Genetic correlation estimates between milk output and content are negative and highly varied. Correlations between fat and protein yields, as well as their correlation contents, vary by breed (Eurosheep, 2022). According to Muñoz et al. (2017), the estimated genetic correlations between milk output and fat and protein in dairy herds in southern Chile were -0.285 and -0.331, respectively. Prolificacy was projected to have minimal genetic correlations with yield attributes (-0.06 to 0.05). Milk, fat, and protein yields were all somewhat positively correlated (0.91 to 0.96) in dairy sheep (Murphy & Thomas, 2016). In another study, total prolificacy and milk production (0.16) in prolific Chios dairy sheep showed a strong positive genetic connection (Tsartsianidou et al., 2023). The breeding objective for dairy sheep in New Zealand considered the estimated genetic correlations between milk yield, fat yield, protein yield, and prolificacy, the correlations between milk yield and fat yield, protein yield, and prolificacy were 0.85, 0.96, and 0.06, respectively; the correlations between fat yield and prolificacy were 0.76 and -0.03); and the correlation between protein yield and prolificacy was 0.02 (Scholtens, 2016).

Phenotypic correlations

Some phenotypic correlations have larger margins of error, indicating uncertainty in the estimation of overall relationships between certain traits. This might be due to environmental factors contributing more to these correlations or the variability within the population.

Table 4. Heritability (on the diagonal), genetic correlations (above the diagonal) and phenotypic correlations (below the diagonal) between the four analyzed traits

Nr.trait	traits	amount of milk	amount of fat	amount of protein	prolificacy
1	amount of milk	**0.197 ± 0.263**	0.836 ± 0.284	0.441 ± 0.757	0.473 ± 0.731
2	amount of fat	0.943 ± 0.015	**0.209 ± 0.278**	0.325 ± 0.839	0.451 ± 0.749
3	amount of protein	0.509 ± 0.039	0.469 ± 0.04	**0.263 ± 0.347**	0.149 ± 0.915
4	prolificacy	0.580 ± 0.037	0.565 ± 0.038	0.451 ± 0.041	**0.235 ± 0.311**

Table 5 to provide economic values associated with different traits related to milk production components and prolificacy. Among the four traits considered in terms of economic weights, the most important in the selection turned out to be prolificacy (55%), followed by the amount of milk (24%), the amount of fat (12%) and the amount of protein (9%). These values are used to quantify the economic impact or importance of each trait for estimation breeding value total and genetic progress, prolificacy could lead to higher economic gains compared to focusing solely on milk, fat, or protein production. These values are used in breeding programs, agricultural management, or decision-making processes to prioritize traits that yield higher economic returns or contribute significantly to the overall profitability.

Table 5. Economic values for milk production components and prolificacy

No. trait	traits	Economic values (%)
1	amount of milk	0.24
2	amount of fat	0.12
3	amount of protein	0.09
4	prolificacy	0.55

Table 6 shows optimization of the selection criteria in a sheep population and their impact on genetic progress, traits Considered: Milk (M), Fat (F), Protein (P), and Prolificacy (Pl).

Variant 1 (M+Pl): This is the control variant against which others are compared. It includes only milk and prolificacy traits.

Variants 2, 3, 5: These variants include milk, fat, protein, and prolificacy, resulting in similar aggregate genotype variance and accuracy index compared to the control variant (Variant 1). They show slight improvements in total genetic progress and genetic progress for each trait.

Variant 4 (F+P+Pl): This variant excludes milk but includes fat, protein, and prolificacy. It demonstrates lower aggregate genotype variance and accuracy index compared to the control variant, leading to a decrease in total genetic progress and genetic progress for each trait.

Among the five variants of selection indices tested, the greatest genetic progress per generation was obtained in the case of variant 5 (M+F+P+Pl), with all four characters considered (the amount of milk, the amount of fat, the amount of protein and prolificacy), the genetic gain being 7.6623 kg. Milk and of 0.0407 lambs/calving. Compared to the control variant (1), the total genetic gain increased by 102.8%

Table 6. Optimization of the selection criteria in a sheep population

Nr. trait. (Var.)	Specification	Variance Index V_I	Aggregate genotype variance V_H	Accuracy Index r_{IH}	Total Genetic Progress ΔH	Genetic Progress each trait ΔG_i	Comparison (%). Variants 2,3,4,5, compared to variant 1.
1	M+Pl*	3.2780	16.6329	0.4440	1.8109·i	7.4508; 0.0414	100
2	M+F+Pl	3.4643	16.6329	0.4569	1.8612·i	7.6622; 0.0406	102,7**
3	M+P+Pl	3.2799	16.6329	0.4440	1.8110·i	7.4516; 0.0440	100
4	F+P+Pl	2.4831	16.6329	0.3863	1.5758·i	6.4544; 0.085	87
5	M+F+P+Pl	3.4644	16.6329	0.4570	1.8613·i	7.6623; 0.0407	102,8

M = milk = kg. F = fat = kg. P = protein = kg. Pl = prolificacy = the number of lambs at calving * Variant 1 = control variant
** = 102.8 = (1.8612/1.8109)*100.

The next variant in the ranking is variant 2 (M + F + Pl), which practically ensures a genetic gain similar to variant 5, namely 102.7%. This result is explained by a higher genetic correlation between the amount of milk and the amount of fat (0.836) compared to the value of the correlation between the amount of milk and the amount of protein (0.441). Correlating the economic values from Table 5 with the optimization results in Table 6 might shed light on aligning selection criteria with economic goals, maximizing profitability alongside genetic progress.

CONCLUSIONS

1. Heritability values in the investigated population were as follows: 0.197 ± 0.263, 0.209 ± 0.278, 0.263 ± 0.347 and 0.235 ± 0.311. The level of the respective values shows that these traits present an intermediate genetic determinism.
2. Of all traits pairs, milk quantity and fat quantity are the most highly correlated, both genetically (0.836) and phenotypically (0.943).
3. Among the four traits considered, the most important in the selection turned out to be prolificacy (55%), followed by the amount of milk (24%), the amount of fat (12%) and the amount of protein (9%).
4. Among the five variants of selection indices tested, the greatest genetic progress per generation was obtained in the case of variant 5 (M + F + P + Pl), with all four traits considered (the amount of milk, the amount of fat, the amount of protein and prolificacy), the genetic gain being 7.6623 kg. Milk and of 0.0407 lambs/calving. Compared to the control variant (1), the total genetic gain increased by 102.8%
5. The next variant in the ranking is variant 2 (M + F + Pl), which practically ensures a genetic gain similar to variant 5, namely 102.7%. This result is explained by a higher genetic correlation between the amount of milk and the amount of fat (0.836) compared to the value of the correlation between the amount of milk and the amount of protein (0.441).

As a general recommendation, it can be proposed for the practice of sheep selection, in the analyzed population, the selection based on a criterion that includes three characters: The amount of milk, the amount of fat and prolificacy. Future studies might benefit from more precise data collection, increased sample sizes, or alternative statistical methodologies to refine and validate the findings for robust decision-making.

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FOALING RANK AND BREEDERS' HEIGHT AT WITHERS INFLUENCE ON THE MORPHOLOGICAL QUALITY OF SHAGYA ARABIAN FEMALE PROGENY

Claudia PÂNZARU, Marius Gheorghe DOLIȘ, Mădălina Alexandra DAVIDESCU, Răzvan Mihail RADU-RUSU

"Ion Ionescu de la Brad" Iasi University of Life Sciences, Faculty of Food and Animal Sciences, 8 Mihail Sadoveanu Alley, Iași, Romania

Corresponding author email: radurazvan@uaiasi.ro

Abstract

One of the breeding objectives for Shagya Arabian breed in Rădăuți Stud Farm, Romania, focuses on increasing the height at withers for mares. The current research aimed to study the entire female progeny (175 foals) of 58 broodmares and 44 stallions included in the breeding activity for 40 years The objective was to determine the influence of breeders' height and the foaling number (rank) on the foals' biometrics. The average value of this measurement in female foals has consistently increased from first foaling (157.63 cm) to the fifth one (158.80 cm) throughout 40 years of breeding, indicating the fulfilment of the breeding goal over consecutive reproductive seasons. According to the simulation based on the linear trend equation, the progeny's height at withers could match the fathers' height by the 10th foaling if the same breeding practices were continued. In addition, stallions would have much more influence than broodmares in increasing the height of the foals. Continuing the forecast analysis based on the variations, the required average height at withers values in stallions and mares can be computed to attain a certain value for the descendants.

***Key words**: broodmares, height at withers, progeny, Shagya Arabian, stallions.*

INTRODUCTION

It is widely acknowledged that the economic productivity of equines can be estimated based on its physical build (Baban et al., 2009).

However, determining the exact proportion of this kinetic energy expended is challenging; authentic production capacity is evaluated through performance analysis using aptitude tests (Domínguez-Viveros et al., 2019).

The probability of different horses with identical age spans, conformational types, body conditions, and similar training expressing equivalent strength, speed, or endurance is notably low.

Often, a horse's outward appearance does not reliably correlate with its individual performance, contributing to these disparities.

Conversely, not all observable flaws can accurately predict the manifestation of their energy potential (Moldoveanu, 1982).

The drive to enhance progeny performance necessitates primarily observing the measurable qualities of both stallions and broodmares, along with the breeding ascendants' previous match outcomes (Ujică, 1988).

Nevertheless, evaluating a male horse involves considering multiple factors, including its inherent qualities and those of its offspring. Only after demonstrating commendable performances on the racetrack and winning competitions, thorough scrutiny of their pedigree and that of their progeny occurs before further pairing decisions are made (Sabeva, 2009).

At Rădăuți Stud farm, a ranking activity is employed to assess the entire broodstock's value based on exterior aspects (such as height at withers, heart girth, cannon girth, and constitution) and specific breed performances (like minimum galloping or trot time, and dressage tests). This action takes place twice a year. Additionally, the performances of two generations of foals for each stallion and at least two generations of foals for each mare are analysed. Consequently, the pairing decisions consider the breeding objectives, with an emphasis on increasing the height at withers for this particular population. In terms of pedigree structure, it is crucial to form pairs considering a kinship greater than the third generation between the stallion and the mare. (Ranking criteria, 2008).

This study aimed to shed light on the dynamics of breeding the Shagya Arabian horses in Rădăuți Stud farm, in Suceava county, Romania. The research focused on evaluating the quality of the progeny obtained over a 40-year period. Given that the primary activity of any stud farm revolves around the production of high-quality biological material, and considering the significance of the height at the withers as a key metric when assessing horses in national stud farms, we deemed it important to closely examine the bloodlines of this breed. One of this unity's objectives is to attain increased values of height at withers for mares. (Rădăuți Stud registers) Comparisons were made between the progeny and their mothers and fathers, operating under the assumption that daughters inherit conformation more accurately from their mothers, while the influence of stallions could potentially contribute to height augmentation (Roman-Popovici, 2015; Tănase et. al., 2000; Ujică, 1988)

MATERIALS AND METHODS

The assessment of morphological attributes involving 58 broodmares and 44 stallions of the Shagya Arabian horse breed at Rădăuți Stud farm commenced by processing data retrieved regarding foals registered between in 40 years of breeding activity. Details concerning the height at the withers of their offspring were collected from ranking sheets dating back to 2019 (Rădăuți Stud farm registers).

The analysis focused on Shagya Arabian broodmares from different bloodlines: Dahoman (n = 10), El-Sbaa (n=8), Hadban (n = 9), Koheilan (n = 8), Mersuch (n = 8), Shagya (n = 8), and Siglavy-Bagdady (n = 7). The progeny comprised 175 female foals, with 7 bloodlines having 5 foalings per bloodline and 5 female foals per foaling. To gauge the influence of stallions' height on the offspring, 44 fathers from 9 bloodlines were involved in the study (Dahoman, El-Sbaa, Mersuch, Shagya, Siglavy-Bagdady, O'Bajan, Koheilan, Hadban, Gazal).
Considering that one of the breeding objectives is to increase the average height at withers for mares within the Rădăuți stud population, we suggested that the selection and pairing process could be guided by utilizing taller stallions. Analysing successive foal dynamics, regressions, and forecasts might assist in achieving a specific targeted height in the progeny when selecting breeders.
For the assessment of horses' external appearance, precise techniques need to be employed to provide accurate information on the studied parameters. To ensure comprehensive results, horse evaluation requires both analytical and synthetic examinations (Pânzaru et.al., 2020; Roman-Popovici, 2015; Dulugeac, 2005).
The height at the withers, measured from the ground to the highest point of the withers (Figure 1), serves as a metric for evaluating horse development. This measurement categorizes horses as tall (> 160 cm), medium (150-160 cm), or small (<150 cm).

Figure 1. Height at withers, biometrics landmarks (source: original)

Data issued from bio-morphometry run in consecutive foalings (1 to 5) and carried on stallions (data group coded S), broodmares (data group coded B) and on their progeny - female foals (data group coded P), were introduced in a database, separately for each of the 7 analyzed bloodlines (Dahoman, El-Sbaa, Hadban, Koheilan, Mersuch, Shagya, and Siglavy-Bagdady). Then a separate database was setup to comprise the whole population, for each foaling: 7 bloodlines x 5 individuals = 35 individual values in groups S, B, P.

GraphPad Prism statistical application, v. 9.4.1 (681) (GraphPad Software, 1992-2022, CA, USA) was used to run statistical calculations. Data inputs were submitted to descriptive statistics analysis (mean, standard deviation) followed by relative comparisons (%) of the differences between progeny average values and breeders' means, as well as by the significance testing. Analysis of variance was performed, in pairs (P vs. S and P vs. B) using the Brown Forsythe and Welch ANOVA test, assuming non-equal standard deviations analysis of variance, and followed by Dunnet-T3 post-hoc algorithm. The ANOVA p values depicts the significance of divergence between breeders and progeny, so as p was below the significance threshold, the differences between the fathers, mothers and foals were higher. Following this reasoning, p values above 0.05 and closer to 1 suggested more and more resemblance between parental and progeny.

Pearson correlations were also performed to find out to what extent (%), direction (positive or negative) and significance (p values) the breeders' height at withers have influenced the progeny results. Both in ANOVA and Pearson correlation, the commonly used p thresholds were applied in testing (p=0.05; p=0.01; p=0.001).

Then, MsExcel 2016 (Microsoft, CA, USA), was used to chart the data for the whole population, as histogram of average means per foaling, then trend lines of progeny versus parents were traced and a regression equation was calculated, to predict the dynamics of height at the withers in progeny, for each next foaling. The applying of the regression equation in further calculus could predict the rank of foaling in which the progeny will intercept the breeders mean value, considering that in our study the main effect of the stallions' selection for pairing was to massif the height at withers in female progeny. Also, the forecast function MsExcel 2016 was applied, using the data gathered from F1 to F5, to suggest the most appropriate mean height at withers for breeders (both stallions and broodmares) to be paired, to achieve the desired level of this trait in progeny, in the upcoming foaling.

RESULTS AND DISCUSSIONS

From the statistical data slightly increased average values of height at withers for progeny compared with the broodmares is observed (only significant differences being revealed in F2, F4, and F5 (p<0.05) and lower average values compared with the stallions, where many differences were registered (significant for p<0.05 in F3, significant for p<0.01 in F1, and very significant differences for p<0.001 in F2 and F5). The results indicate the influence of stallions' height over the progeny when the pair matching is done correctly, for the breeding objectives to be fulfilled.

Table 1 showcased the comparisons between the height values of the progeny and of their parents, related to the foaling rank, on the overall population. Progeny achieved higher values than mothers in all the studied cases: F1 (+1.16%), F2 (+0.64%), F3 (+0.73%), F4 (+1.07%), F5 (+0.89%), with significant differences in F2 (p=0.014), F4 (p=0.012), and in F5 (p=0.031). Also, the height gap between daughters and fathers decreased progressively from F1 (–0.99%) until F4 (– 0.39) and significantly till F3 (p<0.05). In F5 it seemed that certain stallions conducted to the decrease of height in progeny, because the difference came back to – 0.77% (p<0.001) and the correlation was moderated to high (55%) (p<0.001).

Table 1. Height at withers (cm) of parents and progeny,
in Dahoman bloodline, throughout five consecutive foalings

Foaling rank	Stallions (Fathers) (S)		Broodmares (Mothers) (B)		Progeny (female foals) (P)		Progeny vs. parents Comparisons			
							ANOVA		Correlations	
	Mean	±SD	Mean	±SD	Mean	±SD	± %	*p*-values	r (Pearson)	*p*-values
F1	159.20	±1.66	157.37	±3.65	157.63	±2.30	P vs. S = - 0.99 P vs. B = + 1.16	**0.002 0.726	P vs. F = + 0.159 P vs. M = + 0.430	0.363 **0.009
F2	159.17	±1.32	156.80	±3.30	157.80	±1.94	P vs. S = – 0.86 P vs. B = + 0.64	***0.0009 *0.014	P vs. B = + 0.127 P vs. S = + 0.210	0.826 0.500
F3	159.69	±1.55	157.29	±3.08	158.43	±2.36	P vs. S = – 0.79 P vs. B = + 0.73	*0.011 0.086	P vs. B = + 0.352 P vs. S = + 0.432	*0.037 *0.009
F4	159.54	±1.44	157.23	±3.26	158.91	±1.88	P vs. S = – 0.39 P vs. B = + 1.07	0.135 *0.012	P vs. B = + 0.242 P vs. S = + 0.293	0.161 0.088
F5	160.03	±1.50	157.40	±3.47	158.80	±1.35	P vs. S = – 0.77 P vs. B = + 0.89	***0.0006 *0.031	P vs. B = + 0.555 P vs. S = + 0.527	***0.005 **0.001

S = average values of stallions' height at withers; B = average values of broodmares' height at withers; P = average values of progeny's height at withers; SD = standard deviation; F1 = 1st foaling… F5 = 5th foaling.

*significant for $p < 0.05$; **significant for $p < 0.01$, ***significant for $p < 0.001$.

Through the processing of statistical data, the linear trend of the studied progeny simulated for the next six foalings (F6-F11). This simulation is derived from the analysis conducted on the basis of the first five foalings, along with the computation of regression equations related to foaling rank. Additionally, forecasting computations have been employed to determine the necessary parental values for achieving a specific height at withers in the progeny. (Figure 2 and Table 2).

The dynamics of height at withers throughout successive foalings (Figure 2) revealed the fact that the average value of height at withers for progeny increased constantly from F1 (157.63 cm) to F5 (158.80 cm), suggesting that the breading objective to increase the average height at the withers was fulfilled when the pair matching was done on this purpose (Rădăuți Stud farm registers). In addition, the trend line traced on the basis of foalings 1 to 5 depicts a clear tendency of increasing the height at withers in progeny with every next pairing step, using the selection and the right matching (Figure 2). The regression equation calculated for progeny ($y = 0.3457x + 157.28$), where x is the foaling rank, returns the progeny's mean expected value in the next foaling. According to the simulation based on the equation (Table 2), the progeny height at withers will intercept the fathers' one in the 10th foaling if the same breeders used in our study would be paired. In theory, this pairing would work mathematically and seems to be easy to implement. In reality, it is less likely to be able to use the same broodmares for 10 consecutive reproduction seasons, due to ageing and biological limitations. However, breed stock backed up from younger generations, that would have passed the other examination and qualifying tests, and would have the average height at withers close to the actual values of the parents in our study could be used to generate comparable results. Moreover, carrying on the forecast analysis, based on the variations recorded throughout the 5 studied reproductive cycles, the needed average values of height at withers in stallions and broodmares can be calculated in order to achieve a desired value for progeny. For instance, forecasting a mean expected value of 160 cm height at withers in progeny will return recommended values of 160.52 cm in stallions and of 157.60 cm in broodmares (Table 2). Such forecast would be more useful when selecting the breeders for the upcoming matches, especially when the whole population of the stud farm is used, regardless the bloodline belonging of the individuals. If one would need to modify the biometric trait within the bloodline, the selection pool becomes narrowed and certain other elements should be taken onto account such as inbreeding level.

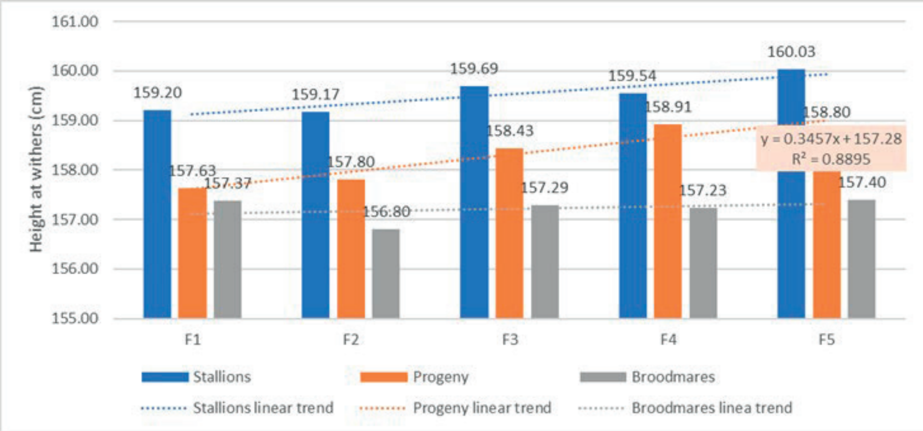

Figure 2. Linear trend for height at withers across successive foalings

Table 2. Simulation of the height at withers dynamics, using linear trend functions and forecast of breeders' height at withers values, related to desired foals' performance and basing on F1-F5 data

Simulated foaling rank	Linear trend			Forecasting (variation of a dependent variable – S or B, related to a fixed variable - P, basing on the variation throughout previous 5 foalings)		
	Stallions (Fathers) (S) y = 0.2029x + 158.92	Broodmares (Mothers) (B) y = 0.0486x + 157.07	Progeny (female foals) (P) y = 0.3457x + 157.28	Desired mean of progeny (P)	Forecasted mean of Stallions (S)	Forecasted mean of Broodmares (B)
F6	160.14	157.36	159.35	157	158.76	157.03
F7	160.34	157.41	159.70	158	159.34	157.17
F8	160.54	157.46	160.05	159	159.93	157.39
F9	160.75	157.51	160.39	**160**	**160.52**	**157.45**
F10	160.95	157.56	160.74	161	161.10	157.59
F11	**161.15**	157.60	**161.08**	162	161.69	157.73

The progeny of Dahoman bloodline presented higher values for height at withers than their mothers F3 (+0.51%), in F4 (+0.51%), in F5 (+0.25%). Pearson correlation analysis revealed that the differences between progeny and fathers' height (–0.63% in F2) was negatively influenced by stallions (r = –0.72). The differences between mothers and progeny were not statistically significant (p>0.05). No significant influence of mothers was observed on the height at withers, situation that occurred in other similar studies. (Sabeva, 2009; Sabeva, 2011)

The results of El-Sbaa bloodline indicated that the foals had higher values in F3 (+1.00%) and in F4 (+1.01%). When progeny vs. fathers were analysed, the differences were significant in F2 (p=0.039, p<0.05). Pearson correlation analysis revealed that such differences between progeny and fathers' height (–1.62%) were negatively influenced by stallions (r = –0.72) in F2 then the new pair matching in F3 re-increased progeny height (correlated positively with fathers' height in a proportion of 19%). Fathers have influenced progeny height dynamics on a greater proportion than the broodmares, fact underlined also by other authors. (Sabeva, 2009; Sabeva, 2011) The differences between mothers and progeny were not statistically significant (p>0.05), aspect revealed also by other studies. (Sabeva, 2009; Sabeva, 2011; Dulugeac, 2005)

The Hadban bloodline performance and the progeny presented higher values than their mothers in F3 (+0.64%), in F4 (+0.09%), and in F5 (+1.42%). When progeny vs. fathers were analysed, the differences were significant in F2 (p=0.105), F5 (p=0.037) and F4 (p=0.008). Pearson correlation analysis revealed that such differences be-tween progeny and fathers' height (–1.25%) was negatively influenced by stallions (r = –0.25) in F4 then the new pair matching in F5 re-increased progenys' height at withers (correlated positively with fathers' height in a proportion of 63%). The differences between mothers and progeny were not statistically significant (p>0.05).

For Koheilan bloodline, the average values of progeny were higher in F2 (+0.77%), F3 (+0.64%), in F4 (+1.65%), and in F5 (+1.80%); when progeny vs. fathers were analysed, the differences were significant in F5 (p=0.129). Pearson correlation analysis revealed that such differences between progeny and fathers' height (–0.63%) were influenced mostly by broodmares (r = 0.74) than by stallions (r = 0.08). Similar facts related to significant differences were also identified in other studies. (Baban et.al., 2009; Pânzaru et.al., 2020)

In Mersuch bloodline there were registered higher values of progeny compared with their mothers in all cases: in F1 (+2.09%), F2 (+2.62%), F3 (+1.55%), F4 (+3.01%), F5 (+3.53%). When progeny vs. fathers were analysed, the differences were significant in F3 (p=0.016), F4 (p=0.019) and F5 (p=0.002); when progeny vs. mothers were analysed, the differences were distinctly significant in F2 (p=0.001), F4 (p=0.003), and F5 (p=0.002) and significant in F3 (p=0.011). The fact that broodmares have influenced progeny height dynamics on a greater proportion than the stallions, was also by other authors. (Baban et.al., 2009; Pânzaru et.al., 2020)

For Shagya bloodline the average values of the progeny were higher than their mothers in F1 (+0.77%), in F2 (+1.68%), and in F3 (+0.25%). Pearson correlation analysis revealed that such differences between progeny and mothers' height (–1.75%) were negatively influenced by the broodmares (r = 0.36). The differences between the average values of parents and of their foals were not statistically significant (p>0.05) in all the cases.

The Siglavy-Bagdady bloodline highlighted that the progeny presented higher values for height at withers than their mothers in all cases: in F1 (+0.25%), F2 (+1.39%), in F3 (+0.25%), in F4 (+1.27%), and in F5 (+0.25%). The differences between mothers and progeny were not statistically significant (p>0.05).

When progeny vs. fathers were studied. Pearson correlation analysis revealed that such differences between progeny and fathers' height (–1.11% and –0.87%) was negatively influenced by stallions (r = –0.38 and r = –0.87). The differences between mothers and progeny were not statistically significant (p>0.05).

CONCLUSIONS

Constant increasing of the progeny's height at withers occurred, from 157.63 cm (in F1) to 158.80 cm (in F5). This aspect suggests that breeding goal objective to obtain higher average height at withers was fulfilled when the pair matching was done on this purpose. In addition, according to the linear trend simulation, stallions would have much more influence than broodmares in increasing the height of the progeny. Following the same height progression when selecting parents from one reproductive cycle to another, the progeny can intercept the fathers' height in the 10th foaling. Forecasting a mean targeted value of 160 cm height at withers in progeny have returned recommended values of 160.52 cm in stallions and of 157.60 cm in broodmares. Acquired data for 5 consecutive foalings allow to forecast the most likely trait value to be used in breeders, to achieve the expected value in progeny. Therefore, this study highlights the necessity for genetic improvement, informed breeding decisions, long-term planning in reproductive activities, optimization of selection criteria for breeders, efficiency in achieving breeding objectives, and the economic impact of producing horses with a higher value of wither height, which is preferred in sports.

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STUDY OF THE ASSOCIATION FOR SUSCEPTIBILITY TO SCRAPIE WITH INFLUENCE ON PRODUCTIVE PERFORMANCE IN THE KARAKUL SHEEP BREED

Petrut-Lucian PARASCHIVESCU, Murshedi Osamah Mahmood ABDULZAHRA, Horia GROSU

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: petru.paraschivescu@yahoo.ro

Abstract

Scrapie, a transmissible spongiform encephalopathy, is known to be influenced by certain PRNP genotypes, and some research has shown that these genotypes can impact the productive performance of affected sheep populations. The study was conducted on Karakul sheep populations. The main aim of this work was the analysis between PRNP genotypes and productive characters (body weight, coat color etc.). Based on the breeding values, the retention of the most valuable individuals for reproduction is to be carried out according to the desired proportion of retentions. From the data analysed,none unfavorable association was observed between the PRNP allele, type, or reproductive traits. It is important to assess the wider context in which numerous variables interact to influence the productive performance of sheep populations. More studies are needed to better understand the complexities of this connection.

***Key words**: genotype, improvement, productive performance, scrapie susceptibility.*

INTRODUCTION

The exploration of nonconventional transmission agents (NCTA) stands as a captivating domain within biomedical inquiry, as highlighted by Petit and Boucraut-Baralon in 1992 (Petit & Boucraut-Baralon, 1992). This area delves into infectious agents responsible for acute transmissible spongiform encephalopathies (TSEs), a class of degenerative maladies affecting the central nervous system. While the identification of such agents has been met with some contention, their potential role in instigating spongiform encephalopathies remains a subject of intense scrutiny (Petit & Boucraut-Baralon, 1992; Hunter, 1999; O'Brien et al., 2017).

TSEs manifest clinically with distinctive features, including prolonged incubation periods spanning months, years, or even decades, coupled with a gradual, afebrile progression leading to symptoms such as ataxia, tremors, and abnormal postures. Importantly, TSEs uniformly culminate in fatality. Pathologically, the hallmark lesions are evident in the gray matter of the central nervous system, characterized by neuronal sponginess and vacuolar degeneration (Somerville et al., 2002).

From an epidemiological standpoint, these maladies occasionally exhibit epizootic patterns, though primarily they are inheritable with elements of transmissibility. Notable contributions to the understanding of TSEs have been made by researchers like Hunter (1999) and Somerville (2002), shedding light on their complex etiology and epidemiological dynamics (Petit & Boucraut-Baralon, 1992; Hunter, 1999; Somerville, 2002).

Central to the discourse on TSEs is the concept of prions, entities resembling transmissible infectious agents composed of misfolded proteins. This notion underpins the Prion Hypothesis, a topic that continues to evoke debate and intrigue within the scientific community. The distinctive nature of prions lies in their structural deviation from conventional infectious agents, which typically rely on nucleic acids such as DNA or RNA. These traditional agents encompass viruses, bacteria, fungi, and parasites. In 1982, Prusiner introduced the concept of prions, coining the term as a fusion of "protein" and "infection", abbreviated as "proteinaceous infectious particle". Prusiner's groundbreaking work proposed that aberrant forms of otherwise innocuous proteins possess the capacity to propagate disease and function as infectious agents. For his contributions, Prusiner was awarded the Nobel Prize in Physiology or Medicine in 1997, solidifying the significance of his studies on prions (Prusiner, 1982; 1991; 1998).

Prions have been implicated in diseases affecting both humans and various animal species, as elucidated by researchers like Hunter (1999) and Belay (1999). Notably, the agent responsible for scrapie disease in sheep is a prion. Given its impact on sheep, goats, and mouflons, scrapie remains classified as a prion disease primarily affecting small ruminants (Beringue & Andreoletti, 2014). Scrapie, due to its pathological implications, has spurred comprehensive research efforts encompassing biological, economic, and ecological dimensions (Beringue & Andreoletti, 2014).

Recent years have witnessed a surge in the investigation of prion protein molecular genetics within Western European sheep breeds. This uptick can be attributed to industrial crossbreeding practices aimed at developing new, highly productive sheep varieties to meet escalating market demands, particularly for meat (Fediaevsky et al., 2008). In Romania, recent endeavors have focused on breeds like Merino, Tsigai, and Tsurcana, primarily geared towards milk or meat production (Cosier, 2008; Otelea et al., 2011). Notably, the Botosani Karakul breed, renowned for its lamb pelt production, has garnered attention through specialized research initiatives. A collaboration between the Research - Development Station for Breeding of Sheep and Goats Popăuţi -Botoşani and the USAMV of Bucharest has facilitated a unique approach to studying prion-related aspects within the Karakul breed, particularly in the context of pelt production (Kevorkian et al., 2011; Hrincă et al., 2014).

MATERIALS AND METHODS

The investigation took place at Research-Development Station for Breeding of Sheep and Goats Popăuți - Botoşani, Blood samples were collected from a Karakul flock. DNA was extracted and subjected to amplification using the Real-Time PCR technique. Melting curve analysis facilitated the determination of scrapie resistance-related genotypes for each animal, specifically at the PrP locus's codons 136, 154, and 171. The process employed kits such as "LightCycler FastStart DNA Hybridization Probe MasterPLUS" (Roche Applied Science) and "LightCycler Scrapie Susceptibility Mutation" (TIB MOLBIOL), following the manufacturer's protocols (Kevorkian, 2010; Kevorkian et al., 2011). Real-time PCR experiments were conducted using the Light Cycler 2.0 instrument (Roche, 1999) with the following procedure: initial pre-incubation at 95°C for 8 minutes, followed by 45 cycles of amplification, melting curve analysis, and cooling to 40°C for 30 seconds. Each amplification cycle comprised three phases: denaturation at 95°C for 10 seconds, annealing at 60°C for 10 seconds, and extension at 72°C for 15 seconds. The melting curve analysis consisted of three steps: pre-incubation at 95°C for 2 minutes, followed by 45°C for 30 seconds, and a gradual temperature increase from 45°C to 75°C at a rate of 0.2°C per minute (Kevorkian, 2010; Kevorkian et al., 2011; Hrincă et al., 2014).

RESULTS AND DISCUSSIONS

Data obtained from the analysis of the flock include, in addition to the genotypes of the offspring, the weight at birth and the coat color, thus we tried to include these data in an animal model to observe the influence of genotypes on productive traits, or the influence of productive traits on genotypes. To estimate the genetic parameters for the weight at birth and for the shade of the coat, we used a mixed linear model emREML model (Equation 1, 2), and R-Studio was used for computing the data.

In conducting an in-depth analysis of the dataset, we employed linear modeling techniques to explore potential correlations between genotypes and individual performances. The results of this analysis were elucidated through regression analysis, facilitating a comprehensive understanding of the interplay between genetic factors and performance metrics (as summarized in Table 3 and visualized in Equation 3).

Building upon prior research efforts (De Vries et al., 2004; Hanrahan et al., 2008; Moore et al., 2009), which have delved into the complex relationship between PrP genotypes and animal performance, it became evident that this association exhibits considerable variability across different breeds.

In our study, we investigated the allelic distribution in Karakul flock, focusing on five key alleles: ARR, ARH, ARQ, and AHQ (Figure 1). All animals are resistant since alleles from R4, and R5 are absent. The frequency of each allele, along with the corresponding number of individuals harboring them, was meticulously analyzed to elucidate their potential implications in scrapie susceptibility. The most prevalent allele observed was ARQ, constituting 120 occurrences in the population, with a substantial number of individuals (622) carrying this allele.

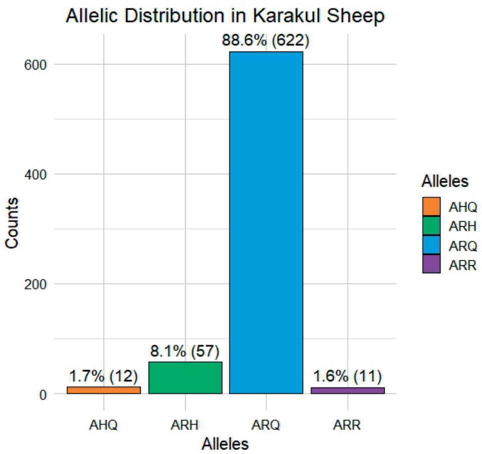

Figure 1. Allelic distribution in Karakul sheep (original)

The predominance of the ARQ allele aligns with previous research associating it with minimal susceptibility to scrapie (Hrinca et al., 2014). Its high frequency suggests minimum risk of scrapie transmission within the population, and a high resistance among rams. Conversely, alleles associated with resistance to scrapie, such as ARR and AHQ, were present in relatively lower frequencies. Specifically, the ARR allele was identified in 50 instances, followed by AHQ with 20 occurrences. Despite their lower prevalence, the existence of these resistant alleles underscores the genetic diversity within the population, offering potential avenues for selective breeding aimed at enhancing resistance to scrapie.

Notably, the ARH allele, often considered neutral regarding scrapie susceptibility, was observed in 30 instances. While its frequency is moderate compared to ARQ, its presence contributes to the genetic heterogeneity of the population, influencing the overall resistance dynamics.

However, it's noteworthy that existing investigations have predominantly centered around a limited selection of breeds, thereby warranting further exploration across a broader spectrum of genetic backgrounds.

In the realm of sheep health parameters, the linkage between PrP genotype and various health indicators has been relatively underexplored. While some studies have focused on parameters such as somatic cell count or mastitis (De Vries et al., 2004; Ligios et al., 2005), a comprehensive understanding of the broader health implications of different genotypes remains elusive. Moreover, despite the lack of a clear correlation between scrapie genotype and perinatal lamb survival, there exists a notable gap in our understanding regarding the potential influence of scrapie genotype on lambing characteristics. Existing research on this front is scarce, and further investigations are warranted to elucidate any potential relationships (De Vries et al., 2004; Ligios et al., 2005).

Of particular interest is the connection between scrapie genotype and postnatal lamb survival beyond the critical 24-hour threshold following delivery. Despite inconsistent findings in this domain, the study by Sawalha et al. (2007) shed light on higher postnatal lamb survival rates among Scottish Blackface lambs with the ARQ haplotype compared to other genotypic variants.

However, such insights underscore the need for continued research to unravel the intricacies of genotype-performance relationships in sheep populations, thereby informing more targeted breeding and management strategies (Sawalha et al., 2007).

$$\mathbf{y} = \mathbf{X}\mathbf{b} + \mathbf{Z}\mathbf{u} + \mathbf{e}$$

Equation 1. Univariate mixed linear model (Suzuki, 2007)

$$\hat{\sigma}_e^2 = \frac{\mathbf{e^T y}}{n - \text{rank}(\mathbf{X})}$$
$$= \frac{\mathbf{y^T y} - \hat{\mathbf{b}}^\mathbf{T}\mathbf{X^T y} - \hat{\mathbf{u}}^\mathbf{T}\mathbf{Z^T y}}{n - \text{rank}(\mathbf{X})}$$

$$\hat{\sigma}_u^2 = \frac{\hat{\mathbf{u}}^\mathbf{T}\mathbf{A}^{-1}\hat{\mathbf{u}} + \text{tr}(\mathbf{A}^{-1}\mathbf{C}^{22}) \cdot \sigma_\mathbf{e}^2}{\text{rank}(\mathbf{A})}$$

Equation 2. Univariate mixed linear model successive iterations (Suzuki, 2007; Mrode, 2005)

Table 1. Estimation of genetic parameters

Genetic parameters	Vp	H^2	VG_A	VG_E
Genotypes	9.5	0.69	6.6	2.9
Weight	0.97	0.17	0.17	0.80
Color	11.09	0.40	6.63	4.46

Table 2. Estimation for variance components

Genotypes	Genetic variance components			
	VGA	VGE	VP	H^2
ARR/ARR	0.8	0.4	1.2	0.33
ARR/ARQ	1.2	0.6	1.8	0.33
ARQ/ARQ	0.9	0.5	1.4	0.36
ARH/ARQ	1.5	0.8	2.3	0.35
AHQ/ARQ	1.0	0.3	1.3	0.23
ARH/ARQ	0.7	0.2	0.9	0.22
ARR/AHQ	0.5	0.1	0.6	0.17

From the data obtained in Table 1, the heritability estimated for the robe color resulted in a moderate heritability coefficient. The heritability of coat color in Karakul lambs can vary depending on the specific genetic makeup of the flock and the breeding practices employed. Generally, heritability refers to the proportion of observed variation in a trait (such as coat color) that can be attributed to genetic differences among individuals within a population. In Karakul sheep, coat color is determined by a combination of genetic factors, including genes responsible for pigmentation such as those controlling the production of melanin. The heritability of coat color in Karakul lambs is likely to be moderate to high, meaning that a significant portion of the variation in coat color among lambs is due to genetic factors. Overall, while heritability plays a significant role in determining coat color in **Karakul lambs, it is also influenced by** environmental factors and can be subject to selective breeding practices aimed at achieving desired color traits.

The tabulated genetic parameters offer valuable insights into the underlying determinants of phenotypic variation observed within the studied population. These parameters encapsulate fundamental aspects of genetic architecture and environmental influences governing trait expression.

Heritability (h^2) serves as a pivotal metric delineating the extent to which genetic factors contribute to the observed phenotypic variation. A heritability estimated of 0.69 for genotypes signifies that a modest proportion (69%) of the variability in genotype manifestation can be ascribed to genetic disparities among individuals. Conversely, traits such as weight and color exhibit comparatively lower heritability values (0.17 and 0.40, respectively), indicating a more substantial genetic influence on their phenotypic variability.

Genetic variance (VGA) delineates the magnitude of variability in a trait that can be exclusively attributed to genetic disparities among individuals. The VGA estimate of (7.2) for genotypes underscores the relatively higher contribution of genetic factors to the observed variation, whereas weight (0.17) and color (6.63) manifest greater genetic variability.

Conversely, environmental variance (VGE) quantifies the portion of phenotypic variation attributable to non-genetic factors, encompassing environmental influences and stochastic effects. A VGE estimate of (3.0) for genotypes denotes the considerable influence of environmental factors on genotype expression, while weight (0.80) and color (4.46) evince substantial environmental contributions to their phenotypic variability.

These findings underscore the complex interplay between genetic and environmental determinants in shaping trait expression. While genetic factors exert a discernible influence, environmental influences play a pivotal role in modulating phenotypic outcomes. Such insights are pivotal for devising targeted breeding strategies and management interventions aimed at optimizing trait expression and genetic progress within the population.

Näsholm (2005) suggests that the quality attributes of Pelt in Gotland lambs exhibit moderately high heritabilities. Factors such as lamb gender, ewe age, litter size, lamb age, and their interactions exert influence on pelt quality. To enhance Pelt quality within the Gotland breed, incorporating an overall score as a trait in genetic assessments could be beneficial. The heritability estimate for the overall score is approximately 30%, with generally positive genetic correlations observed with individual pelt quality traits. Pursuing breeding strategies aimed at improving the overall score is anticipated to yield short-term enhancements in hair quality, curliness, and color nuances. However, increasing curl size and fleece thickness may not always be advantageous as these traits have optimal values. To maintain optimal levels of curl size, fleece thickness, and color nuances in the Gotland breed, strategies such as restricted selection indexes and disassortative mattings are proposed (Näsholm, 2005).

The data depicted in Table 2 offer valuable insights into the dispersion of genetic variance components among diverse genotypic classes. Grasping these components holds paramount significance in elucidating the genetic underpinnings of phenotypic diversity within sheep populations, especially concerning traits linked to scrapie susceptibility and other productive attributes.

The emREmL model has significant limitations when applied to the calculation of categorical values due to the considerable variability in genetic variance components among different genotypes. For instance, the additive genetic variance (VGA) ranges from 0.5 for ARR/AHQ to 1.5 for ARH/ARQ, while the total phenotypic variance (VP) ranges from 0.6 to 2.3. This high variability highlights that the estimated categorical values can be affected by the specificity of the genotypes and the environmental component (VGE), which also varies significantly. Additionally, it should be noted that the estimation of genetic parameters is not the primary objective of this study. The genetic parameters for genotypes were estimated for orientation purposes only, and the results should be interpreted only in the context of the number of individuals included in the study, while also considering the limitations of the linear models used.

The estimations for variance components among genotypic classes unveil noteworthy disparities in genetic variance components across distinct genotypic classes. Notably, the additive genetic variance (VGA) displays substantial variations, with elevated values evident in genotypic classes such as ARR/ARQ and ARQ/ARQ, in contrast to others like ARH/ARH and ARR/AHQ. This disparity implies differing genetic contributions to phenotypic variation among the various genotypic classes.

Environmental variance (VGE) and total phenotypic variance (VP) similarly showcase variations across genotypic classes. While environmental variance remains relatively stable across most genotypic classes, VP manifests more pronounced discrepancies, signifying the amalgamated influence of genetic and environmental factors on phenotypic manifestation. Notably, the ARQ/ARQ genotype demonstrates the highest VP, indicating substantial phenotypic variability within this genotypic class.

The identified variance components offer insights into the plausible genetic foundation of scrapie susceptibility and other productive traits. Elevated VGA in specific genotypic classes, particularly those linked with heightened susceptibility to scrapie (e.g., ARQ/ARQ), intimates a robust genetic influence on these traits. Comprehending these genetic influences is imperative for devising selective breeding schemes aimed at augmenting resistance to scrapie and enhancing overall productivity within sheep populations.

Despite the informative nature of the variance component estimates, it is imperative to acknowledge certain limitations. These estimates are contingent upon the available dataset and may be influenced by factors such as sample size, environmental variability, and genetic heterogeneity within genotypic classes. Subsequent research endeavors should prioritize the validation of these findings using larger and more diverse datasets, integrating supplementary environmental factors, and exploring genotype-environment interactions to refine our comprehension of genetic influences on scrapie susceptibility and productive traits.

The findings accentuate the significance of integrating genetic insights, particularly pertaining to scrapie susceptibility, into breeding programs aimed at bolstering sheep health and productivity. Selective breeding strategies that prioritize individuals harboring genotypes associated with high resistance to scrapie, while concurrently considering their comprehensive genetic merit for productive traits, hold the potential to mitigate disease risks and enhance the resilience of sheep populations.

$$Y_i = b_0 + b_1 X_{1i} + b_2 X_{2i} + b_n X_{ni} + u_i$$

Y_i = dependent variable
b_0 = Intercept
$b_1 \ldots b_n$ = Coefficient of Regression
$X_{1i} \ldots X_{ni}$= independent variable
u_i = disturbance error

Equation3. Multiple regression linear model (2)

Table 3. Correlation coefficients between Scrapie genotypes and lamb productive traits expressed as multiple regression

Corelation between genotypes and productive traits	Estimate	Std. Error	t value	Pr (>|t|)
Weight at birth	4.60	0.86	5.331	2.61***
ARH/ARH	-0.88	0.83	-1.063	0.28
ARH/ARQ	-0.15	0.49	-0.320	0.74
ARQ/ARQ	-0.18	0.45	-0.402	0.68
ARR/AHQ	-0.82	1.08	-0.763	0.44
ARR/ARQ	0.19	0.46	0.041	0.96
ARR/ARR	0.097	0.66	0.0146	0.88
Coat color	-0.054	0.020	-2.718	0.00714**
Lamb score	0.0005	0.0015	0.378	0.70

Data obtained in Table 3, shows that there is no correlation between resistant or susceptible genotypes and production characteristics ($p<1$). Although a weak significance is observed at the level of the coat color character ($p<0.001$), in relation to the weight of the lamb at birth.

The positive estimate of 4.60 suggests that there is a positive correlation between the weight of lambs at birth and the trait being analyzed. In other words, lambs with certain genotypes may tend to have higher birth weights compared to others.

The high t-value of 5.331 indicates that this correlation is statistically significant, meaning that it is unlikely to have occurred by random chance alone. Therefore, there is strong evidence to support the relationship between genotype and birth weight.

Each genotype (ARH/ARH, ARH/ARQ, ARQ/ARQ, etc.) has an associated estimate, standard error, t-value, and p-value.

The estimates represent the strength and direction of the correlation between each genotype and the productive traits. A negative estimate suggests a negative correlation, while a positive estimate indicates a positive correlation. The t-values measure the significance of the correlation. Higher absolute t-values indicate stronger evidence against the null hypothesis of no correlation. The p-values indicate the probability of observing the correlation if there were no true association between the genotype and the trait. Lower p-values suggest stronger evidence against the null hypothesis.

The negative estimate of -0.054 suggests a negative correlation between coat color and the trait being analyzed. This implies that lambs with certain coat colors may tend to have lower values for the trait. The significant t-value of -2.718 indicates that this correlation is statistically significant, providing strong evidence against the null hypothesis of no correlation.

The estimate of 0.0005 suggests a very weak positive correlation between lamb score and the trait being analyzed. This indicates that there may be a slight tendency for lambs with higher scores to exhibit higher values for the trait, although the correlation is very weak.

The non-significant t-value of 0.378 suggests that this correlation is not statistically significant, meaning that there is insufficient evidence to conclude that the relationship between lamb score and the trait is not likely due to random chance.

CONCLUSIONS

Despite our efforts to comprehensively analyse the relationship between PRP gene polymorphisms and various traits, it's essential to acknowledge the potential limitations of our dataset used in this paper. Factors such as sample size, genetic diversity within the population, and environmental influences may have impacted the robustness of our findings. Future studies with larger and more diverse datasets could provide deeper insights into these relationships.

Our study primarily focused on specific traits such as birth weight, coat color, and scrapie resistance, leaving out other potentially relevant factors that could influence sheep productivity. Factors such as nutrition, management practices, and environmental conditions were not explicitly accounted for in our analysis. Incorporating these additional variables into future research endeavors could offer a more holistic understanding of the genetic and environmental determinants of sheep performance.

Previous research endeavors have explored the relationship between PRP gene genotypes and various production characteristics, including subcutaneous fat percentage, muscle mass, and milk physicochemical parameters. Although some studies have hinted at potential interactions, the significance levels of these associations remain uncertain, leading to conjecture regarding the possibility of false-positive findings.

While our analysis did not uncover direct correlations between resistant genotypes and productive traits, it is worth noting the potential for individualized selection strategies to enhance scrapie resistance. However, any such selection processes must carefully consider the broader context of individual animals' productive performances.

It is imperative to recognize the intricate interplay of numerous variables influencing the productive performance of sheep populations. Further investigations are warranted to gain a more comprehensive understanding of these complex relationships and their implications for breeding and management practices.

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ASSOCIATION OF FABP3 GENE POLYMORPHISM WITH MILK PRODUCTION IN EWES FROM THE BULGARIAN DAIRY SYNTHETIC POPULATION

Nevyana STANCHEVA[1], Ivona DIMITROVA[2], Milena BOZHILOVA-SAKOVA[3], Todor TZONEV[4]

[1]Agricultural Academy, Agricultural Institute, Department of Animal Science, 3 “Simeon Veliki” Blvd, 9700, Shumen, Bulgaria
[2]University of Forestry, Faculty of Agronomy, 10 “Kliment Ohridski” Blvd, 1756, Sofia, Bulgaria
[3]Agricultural Academy, Institute of Animal Science, Department of G.B.S.R.B.F.A, Spirka “Pochivka”, 2232, Kostinbrod, Bulgaria
[4]Agricultural Academy, Scientific Agriculture Center, 7700, Targovishte, Bulgaria

Corresponding author email: ivonna_dimitrova@yahoo.co.uk

Abstract

The purpose of the research is to explore the relationship of the polymorphism of the FABP3 gene with milk yield per standard 120-day milking period (TMM120) in ewes from the Bulgarian Dairy Synthetic population from the herd of the Agricultural Institute-Shumen. In the experiment were involved 111 ewes of different lactations. Ewes were selected by birth type (single, twins, triplets) and had 343 milk yield records for a standard 120 days milking period. Two alleles and two genotypes were identified in the studied animals in exon 2 of the FABP3 gene (SNP3) by the PCR-RFLP technique with BseDI endonuclease. The association of the FABP3 gene polymorphism with the milk productivity of sheep was investigated by the one-way analysis of variance ANOVA model. In this study of BDSP ewes, the presence of a homozygous GG genotype at SNP3 of FABP3 resulted in increased milk yield in 2nd lactation ewes and in co-twin ewes.

Key words: *birth type, FABP3 gene, milk yield, parity, sheep.*

INTRODUCTION

Biodiversity in animal species playing a role in the food security of the population is related to the variation of genes, traits and breeds, and is the result of millennial adaptation to the environment and domestication. Genetic diversity provides greater adaptability and ability to survive environmental changes. One of the first domesticated animals was sheep. They are grown in extensive or intensive production systems under diverse climatic conditions, facing challenges such as different diseases and environmental conditions (Kijas et al., 2012). Adaptation to highly differentiated geographical and climatic conditions and specialization for different productivity have led to the creation of a huge number of breeds with significant phenotypic diversity (Gutiérrez-Gil et al., 2014) and it is of great interest to study the diversity of genes related to productive traits in breeds from different directions and geographical-climatic regions.

More than 30 breeds of sheep are bred in Bulgaria, and regardless of whether they are for specific objectives or local breeds, almost all of them are milked because the production of sheep's milk is of particular importance due to its use for the preparation of traditional products such as yogurt, white brine cheese, yellow cheese and others. Sheep milk is often produced from breeds that have low to medium yields and only a few sheep breeds have been created specifically for milk production among which is the Bulgarian Dairy Synthetic population (BDSP) (Figure 1) (Stancheva et al., 2022).

The components of sheep milk are synthesized in mammary epithelial cells and originate from blood plasma (Kulig et al., 2013). The lipids in milk are produced by fatty acids that bind to specific proteins called fatty acid-binding proteins (FABPs). Intracellular fatty acid-binding proteins (FABPs) are cytoplasmic proteins essential for the transport and metabolism of fatty acids in the cell, by accelerating the absorption of long-chain fatty acids and delivering fatty acids to intracellular organelles (Lanier & Corl, 2015). They are thought to affect various cellular processes, in particular lipid metabolism. Heart-type fatty acid-binding protein (H-FABP, FABP3), whose molecular weight is 15 kD, is present in many tissues, especially those with a high demand for fatty acids, such as cardiac muscle, skeletal muscle, and the mammary gland during lactation (Calvo et al., 2002; Lanier and Corl, 2015).

The FABP protein family members are intracellular lipid-binding proteins with low molecular weight and high capacity for binding long-chain fatty acids. Currently 12 tissue-specific cytoplasmic FABPs (FABP1-FABP12) have been established in vertebrates so far that are differentially expressed in tissues, but not all FABP gene members occur in the same species (Chmurzynska, 2006; Wang et al., 2019). They are small in size - they include from 126 to 134 amino acids (Lang et al., 2017) and are found in all animal species (Cho et al., 2011; Wang et al., 2015; Wang et al., 2016; El-Mansy et al., 2019; Al-Janabi, 2019; Ye et al., 2022). These proteins regulate the fatty acid content of cells and thus influence various cellular processes such as lipid metabolism, cell growth and proliferation (Kulig et al., 2013).

The sheep FABP3 gene has been mapped to chromosome 2 (Calvo et al. 2002).

Figure 1. Ewe from BDSP with the offspring (Own source)

The influence of the gene has been studied by various authors, who demonstrate the influence of the different genotypes of the gene on the metabolism of fatty acids in both muscle and milk and, as a final result, affects the milk fat content and the marbling of the meat (Calvo et al., 2004; Aurora et al., 2014; Öner et al. 2014; Kowalewska-Łuczak et al., 2017).

The purpose of the research is to explore the relationship of the polymorphism of the FABP3 gene with milk yield per standard 120-day milking period (TMM120) in ewes from the Bulgarian Dairy Synthetic population from the herd of the Agricultural Institute - Shumen.

MATERIALS AND METHODS

The object of the research are ewes from the Bulgarian Dairy Synthetic population, bred at the Agricultural Institute - Shumen. Data on the establishment and genealogy of the herd are indicated in our previous studies (Stancheva, 2003; Stancheva et al., 2014; 2016; 2017; 2023). The sheep are reared on barn and pasture under a semi-intensive system. Milking is mechanized and takes place twice, after the lambs are weaned. The suckling period is in the range of 55-60 days, and the duration of the milking period is within 150-180 days.

In the experiment participated one hundred and eleven ewes on the 1^{st} to the 4^{th} lactation. Ewes were selected by birth type (singleton, twins, triplets) and had 343 milk yield records for a standard 120-day milking period. Milk yield data were obtained by measuring the amount of milk in liters milked during the lactation period of the animals according to the AC method specified in the nomenclature of the International Animal Control Committee (ICAR). Milk yield for a standard 120-day milking period (TMM120) is the average daily milk yield for a milking period multiplied by 120 days (TMM120 = ADMYmilking period * 120). TMM120 is calculated only for ewes with a milking period of not less than 120 days.

The main experimental part of the DNA analysis was conducted in the Laboratory of Genetics at the Faculty of Agronomy part of the University of Forestry, Sofia, Bulgaria, according to the methodology described in another our study for BDSP sheep from the same flock (Stancheva et al., 2023).

The association of the FABP3 gene polymerphism with milk productivity depending on the parity and type of birth of the ewes was determined using the one-way analysis of variance ANOVA model.

RESULTS AND DISCUSSIONS

As described in a previous study a 222 bp fragments from exon 2 of the sheep FABP3 gene were amplified using the PCR method. The PCR products that were produced after the amplification were cut with enzyme *BseDI*. After restriction analysis of the samples, two alleles were detected in SNP3 of FABP3. The mutant allele G was presented with three different fragments - 143, 43 and 36 bp fragments. The wild allele A had two fragments -186 and 36 bp. The frequencies for alleles G and A were 0.14 and 0.86, respectively (Table 1). Two genotypes were detected. The homozygous genotype GG was with frequency 0.73 and the heterozygous genotype AG was with frequency 0.27 (Figure 2). The homozygous genotype AA in the animals of this herd of the Agricultural Institute - Shumen was not detected.

Table 1. Allele and genotype frequencies of SNP3 of FABP3 gene

Locus	n	Allele frequency		Genotype frequency			Heterozygosity		Fis	χ^2	p
		G	A	GG	AG	AA	Ho	He			
FABP3	111	0.86	0.14	0.73	0.27	0.00	0.272	0.240	-0.133	3.62	0.05

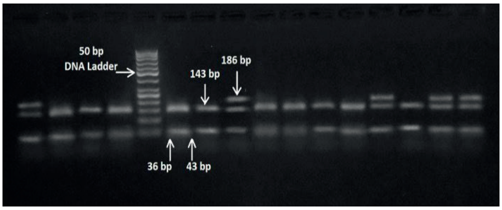

Figure 2. Restriction fragments of FABP3 gene visualized on 2.5% agarose gel under UV light

The established frequency of allele A in the studied breed has a lower value compared to studied foreign breeds where the frequency varies between 0.26-0.46 (Calvo et al., 2002; 2004; Öner et al., 2014), but also compared to the other studied Bulgarian breeds (Dimitrova et al., 2020). Another Bulgarian sheep breed, studied for genetic diversity according to SNP3 of the FABP3 gene and showed the presence of these two genotypes, is the Black-headed Pleven breed, which is close in terms of productivity to the studied Bulgarian Dairy Synthetic population (Bozhilova-Sakova, Dimitrova, unpublished results).

Milk productivity for ewes from the Bulgarian Dairy Synthetic population is determined for a 120-day standard milking period. Table 2 shows the total average milk yield values for a 120-day standard milking period (TMM120) of the studied animals, as well as TMM120 depending on the consecutive lactation and the type of birth of the sheep. Regarding the established milk yield for a 120-day standard milking period (115.205 l), the studied sheep exceeded the minimum selection limit for the Elite class of the population, which is 105 l. In a similar study, Dimitrova et al. (2021) found a lower value for the milk productivity (94.356 l) in sheep of the same breed raised at the Institute of Animal Husbandry - Kostinbrod.

Table 2. Overall mean and analysis of variance for a milk yield per standard 120-day milking period (litters)

Variable	Milk yield per standart 120-day milking period (TMM^{120}) (l)			*P-value*
	n	*Average*	*SD*	
TMM^{120}, total	343	115.205	26.55	
TMM^{120} by parity				
1st lactation	102	122.378	27.78	***0.0084**
2nd lactation	105	110.201	26.71	
3rd lactation	90	113.693	24.08	
4th lactation	46	113.678	25.47	
TMM^{120} by type of sheep birth				
Singles	135	114.761	28.21	0.1209
Twins	184	116.850	25.50	
Triplets	24	105.092	23.35	

*P≤0.01

Depending on the succession of lactation, the milk yield for a 120-day standard milking period is the highest, and above the general average, for 1st lactation of ewes (122.378 l) and the lowest for 2nd lactation of ewes (110.201 l). On the 3rd and 4th lactations, the values of TMM120 were equalized. Analysis of variance reported a significant effect of the factor consecutive lactation on milk yield for a 120-day standard milking period (P≤0.01).

The milk yield results for a 120-day standard milking period, according to the type of birth of the ewes, showed a certain superiority of animals born as twins (116.850 l) compared to those born as singletons and triplets (114.761 and 105.092 l), but the differences found were without statistical significance.

The milk productivity for a 120-day standard milking period (total, by consecutive lactation and type of birth) of the determined genotypes was presented in Table 3. In our previous study, we found that carriers of the homozygous genotype GG were 72.72% of the studied sheep and showed a tendency for larger litter size compared to ewes with heterozygous AG genotype (Stancheva et al., 2023).

Table 3. Association of FABP3 gene polymorphism with milk yield for a standard 120-day milking period (litters)

Variable	Genotype AG			Genotype GG			*P-value*
	n	*Average*	*SD*	n	*Average*	*SD*	
TMM^{120}. total	89	113.154	23.28	254	115.924	27.62	0.397907
TMM^{120} by parity							
1st lactation	29	121.143	21.62	73	122.869	29.99	3.936143
2nd lactation	28	100.913*	20.12	77	113.579*	28.09	***0.030993**
3rd lactation	25	115.177	24.31	65	113.123	24.16	3.949321
4th lactation	7	121.793	21.61	39	112.221	26.08	4.061706
TMM^{120} by type of sheep birth							
Singles	23	120.046	26.06	112	113.676	28.62	0.325841
Twins	59	110.367*	22.72	125	119.910*	26.24	***0.017403**
Triplets	7	114.000	14.31	17	101.424	25.64	0.23848

*P≤0.05

In the present study, carriers of the homozygous GG genotype demonstrated the potential for higher milk yield per 120-day standard milking period (115.924 l) compared to animals with the heterozygous genotype AG (113.154 l), and the differences found were not statistically proven. In sheep of the same breed from the herd of the Institute of Animal Husbandry - Kostinbrod, Dimitrova et al., (2021) found that the presence of a homozygous GG genotype of the FABP3 gene led to a proven higher milk productivity.

There was no clearly expressed tendency for higher milk yield for the 120-day standard milking period of the animals with homozygous genotype GG, depending on the consecutive lactation and the type of birth of the sheep. Analysis of variance reported a significant effect of the GG genotype on milk yield over a 120-day standard milking period in ewes of the 2nd lactation and in twin-born animals (P≤0.05).

CONCLUSIONS

The total milk productivity of the studied sheep for a 120-day standard milking period (115.205 l) exceeded the minimum threshold limit for the Elite class of the population. It is the highest in ewes of the 1st lactation and in animals born as twins.

A reliable effect of the factor "consecutive lactation" on milk yield over a 120-day standard milking period was established.

The carriers of the homozygous genotype GG in SNP3 of the FABP3 gene demonstrate the potential for higher milk yield over a 120-day standard milking period compared to animals with the heterozygous genotype AG.

A reliable effect of the GG genotype on milk yield for a 120-day standard milking period was established in ewes on second lactation and in animals born as twins.

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RESEARCH ON THE IMPACT OF FEED TECHNOLOGIES ON THE WELFARE AND PRODUCTIVE PERFORMANCES OBTAINED IN ORGANIC FARMING OF SHEEP AND GOATS

Victoria CONSTANTIN[1, 3], Livia VIDU[1], Ion RADUCUTA[1], Rodica CHETROIU[2], Dana POPA[1], Carmen Georgeta NICOLAE[1], Roxana Elena (VASILIU) STEFAN[1], Monica MARIN[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]Research Institute for Agricultural Economics and Rural Development (ICEADR), 61 Marasti Blvd, District 1, Bucharest, Romania
[3]Veterinary Sanitary Directorates of Karditsa, Greece

Corresponding author email: marin_monica_zoo@yahoo.com

Abstract

The growth rate and productive performance of animals depends on various factors among which the feeding system is one of the most important factors. The nutritional needs of animals on organic farms can be covered through three sources: grazing, food produced within the own holding and the purchase of additional feed. The purpose of the work is to follow the effect of sheep and goat feeding technologies through the installation according to the requirements of ecological agriculture of cultivated pastures, which can be used both for animal grazing and for obtaining fodder, either dry or fermented. A maximum production of fodder obtained in one's own holding must be one of the main objectives of the breeder, so the selected crops must be determined according to the real needs of the holding. To achieve these goals, farmers who practice organic farming rely on recognized agricultural practices, such as maintaining the welfare and health of the animal herd through free-range systems and modern knowledge, such as monitoring nutrient levels to track the optimal growth of animals at different stages of development.

Key words: *leguminous fodder, milk production, organic farms, pasture, sheep and goats, welfare.*

INTRODUCTION

Organic animal production as an integral part of organic agriculture, the purpose of which is to create and maintain independent relationships between soil-plants, plants-animals and animals-soil, with the ultimate goal of creating a sustainable agro-ecological system that it is based on natural resources and the integrated functionality of this system, maintaining a harmonious relationship between plant and animal production. The organic breeding system is based on the use of pastures, taking into account the natural grazing behavior of animals. Grazing-based animal husbandry improves the productivity and sustainability of pastures. This livestock raising is done by considering the type of vegetation in the region, soil structure, climate, geography, prevailing winds, altitudes, direction, animal species and breeds, water resources and animal welfare (Arsoy, 2017). A livestock rearing system to be sustainable must be technically possible, ecologically acceptable and economically viable (Hessle et al., 2017).
There is a growing interest in sustainable forms of organic livestock production systems that will ensure a balanced relationship between environmental, socio-cultural and economic factors. For the development of ecological agriculture, a strong harmonization of rules and legislation at international and national level is necessary. Harmonization of rules and development of technical assistance at the farm level can contribute to the sustainable growth of organic agriculture by applying new technologies to improve sustainability in organic small ruminant production systems, especially in terms of disease prevention and feeding management (Nardone et al., 2004).
Organic farming is based on combinations of general prevention methods (risk control and identification), multifunctional pasture management, non-chemical treatments (homeopathic herbal preparations) and new animal husbandry

technology approaches (i.e. biological control and bioactive feed) (Ronchi & Nardone, 2003a). According to available data, animals raised in ecological systems are concentrated in Europe and North America: 70% of cattle, 80% of sheep and 77% of pigs exist in Europe. The most important European countries producing sheep and goats in an ecological system are Italy, Great Britain, Germany, France and Greece. It is expected that the number of organic animals will continue to grow in the future. In many European countries, animal products such as milk and eggs already account for 10 to 20% of the total market and continue to grow significantly. However, studies on ecological animals are still scarce and major data gaps exist (FAO, 2014).
The aim of this paper is to provide an integrated analysis of various ecological feeding systems of small dairy ruminants on the chemical composition and quality characteristics of the milk produced (fatty acid profile, protein, antioxidants, vitamins). Since milk from sheep and goats is mostly used for cheese production, and its yield depends on the composition of the milk, the main objectives of sheep and goat farmers are to improve the quality of milk by increasing the percentage of milk solids and stabilizing the milk in fat and protein through adequate nutrition.
In order to increase consumer confidence in organic dairy products, it was intended to identify nutritional alternatives based on the use of vegetable raw materials with a rich protein content of high biological value, food sources that can be an effective alternative to improve the quality of milk regarding the content of nutrients, of particular importance being essential omega-3 fatty acids, vitamins (vitamins E and A), as well as various antioxidants.

MATERIALS AND METHODS

In order to write this paper, a detailed study of various published works was carried out with topics on the effects of feeding technologies on the welfare and productive performance, the quantity and quality of milk produced by sheep and goats raised in an ecological system.
Scientific articles and papers were selected from Science Direct, PubMed, Google Scholar, Web of Science databases using multiple search keywords related to organic sheep and goat farming. Eligible articles were selected on the effects of organic feeding systems on the quantity and quality of milk produced in organic sheep and goat farms, on animal welfare and health. The selected articles were analyzed for their eligibility and to extract the data necessary for the creation of this paper.

RESULTS AND DISCUSSIONS

The growth rate and productive performance of animals depends on various factors, among which the feeding system is one of the most important factors. The nutritional needs of animals on organic farms can be met through three sources: grazing, feed produced within the own holding and the purchase of additional feed. The higher the percentage of feed produced within own holdings, the lower the dependency on purchasing feed and the better the growing conditions within the holding.

Livestock grazing – important food source in the ecological growth of small ruminants

To increase the sustainability of small ruminant organic farming systems, the pasture-based mode of production is proposed, with particular emphasis on the organization of a farm forage system to meet the nutritional requirements of animals (Ronchi & Nardone, 2003b).
Due to the climatic, geological and topographical variability in some areas where it is not possible to graze on natural pastures, pastures cultivated with annual leguminous fodder can be organized (Figure 1).

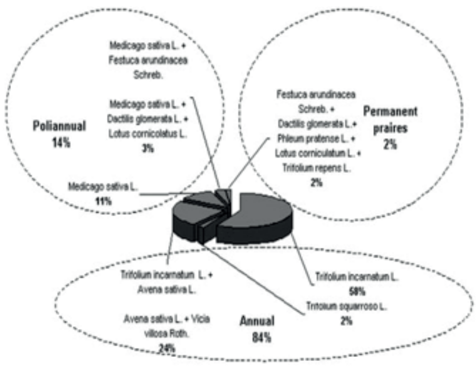

Figure 1. Forage sources for dairy sheep farming (Ronchi & Nardone, 2003a)

The choice of appropriate forage plant species, grown ecologically on technical pastures, is one of the main strategies to meet the nutritional requirements of animals through low-cost sources of energy and nutrients. Knowing the quality of the fodder obtained on the meadows is an essential condition, both for ensuring a rational feeding of the animals, and for the choice and promotion of those crop technologies, which allow satisfying the nutritional requirements in order to achieve the highest possible animal production (Amiri et al., 2012).

One of the basic objectives in planning and using pastures to achieve the productive performance of animals is to determine the nutritional needs of animals in terms of energy, protein, minerals and vitamins. This is possible only when the quality of the fodder plants on the pastures is known in terms of chemical composition. Therefore, knowledge of the quality of the forage grown should be considered for the proper use of pastures. Determining the nutritional value of forages is important in animal nutrition because the actual production of animals is related to the amount of nutrients in the forage (Schut et al., 2010). Total digestible nutrient (TDN), crude protein α (CP) and metabolizable energy (ME) are often used as indicators of forage quality.

For this purpose, the use of optimal forage composition using different plant species has been proposed, such as mixtures of orchard grass and forage legumes, combinations of grass and clover or grass and lucerne (Puckridge & French, 1983). Studies have shown that legumes have a higher nutritional value than grass species, so mixtures with higher amounts of forage legumes can improve forage quality (Tsiplakou et al., 2013).

Changes in milk fat content and its fatty acid profile using growth and nutrition strategies to increase the biosynthesis of conjugated linoleic acid (CLA) have been and continue to be important goals of the dairy industry. Similar to other grazing systems, livestock production on pastures is based not only on the quantity, but also on the quality of the forage. A negative energy balance due to undernutrition mainly in grazing causes decreased milk yield and protein content, but increased milk fat. One of the most important ways to improve the milk of small ruminants with beneficial fatty acids is to increase the supply of ω-3 polyunsaturated fatty acids in the animal diet.It was found that the milk produced by sheep and goats raised with grazing feeding systems is enriched in substances of natural origin such as phenolic compounds, fat-soluble vitamins, bioactive lipid components, unsaturated fatty acids compared to the milk of those fed with concentrated feed (Zervas & Tsiplakou, 2011).

The variability of protein and energy intake from pastures during the summer season is a primary factor that can influence milk production and composition of milk produced by ruminants, as there can be seasonal nutritional fluctuations in nutrient quantity and quality, either due to grazing excessively, either due to the natural degradation of the meadows. Studies on grazing feeding systems of dairy cows on organic farms have evaluated the effects of changing the crude protein (CP) content of food supplements provided in the summer grazing season as a useful mechanism for maintaining milk production and the composition of the milk produced. of these (Ayers et al., 2022).

Various studies have highlighted the major role of grazing management practices in improving certain nutritional, technological and sensory characteristics of livestock products. It was found that the pasture feeding system with fresh grass compared to the on-farm feeding system with silage and concentrate fodder resulted in obtaining dairy products and meat products with specific traits (Cabiddu et al., 2022). Animal products obtained being rich in carotenoids, vitamins A and E (Nozière et al., 2006a; Prache et al., 2020), volatile organic compounds (VOCs) and fatty acids (FA) favorable for human consumption, for example acids monounsaturated fats (MUFA), polyunsaturated fatty acids (PUFA) and n-3FA (Cabiddu et al., 2019; Coppa et al., 2019) with specific sensory characteristics, preferred by consumers (Martin et al., 2005). Animal diet containing green forage mixtures increased the content of all carotenoids in milk and meat compared to the silage diet (Table 1). This may be because carotenoids in grasses are photodegraded during forage harvesting and drying (Nozière et al., 2006b).

Table 1. Effect of feeding fresh herbage instead of conserved forage and/or concentrates on the carotenoids, fat-soluble vitamins, and terpene content, colour and pH of different animal products (Cabiddu et al., 2022)

Item	Product	Animal species	n[1]	Fresh Herbage group	Conserved Forages group	SEM[2]	Significance[3]
Carotenoids and vitamins (mg/kg fat)							
α-Tocopherol	Dairy	Cattle	20	23.39	17.95	1.458	**
		Goat	3	37.20	6.37	0.306	***
	Meat	Sheep	3	5.88	3.39	0.155	**
Retinol	Dairy	Cattle	7	6.88	5.91	0.977	ns
		Goat	3	9.17	7.20	0.503	*
β-Carotene	Dairy	Cattle	18	6.20	4.40	0.579	**
Lutein	Dairy	Cattle	8	0.67	0.41	0.101	*
Zeaxanthin	Dairy	Cattle	7	0.10	0.07	0.023	†
Terpenes tot (In arbitrary area unit)	Dairy	Cattle	3	12.48	11.32	2.419	ns
		Sheep	5	18.84	17.88	0.289	*
Monoterpenes tot (In arbitrary area unit)	Dairy	Cattle	3	11.22	10.34	2.937	†
		Sheep	5	17.99	16.36	0.33	**
Sesquiterpenes tot (In arbitrary area unit)	Dairy	Cattle	3	11.73	9.67	1.820	ns
		Sheep	5	18.85	10.38	5.170	ns

[1]n - number od date; [2]SEM - standard error of the mean; AUU - arbitrary area units; [3]*** - p<0.001; **- p<0.01; * - p<0.05; ns - not significant, p>0.05.

Use of forage silage in organic farming of small ruminants

Another alternative method of feeding small ruminants, as a potential solution during the rainy and cool season when grazing is not possible, is the introduction of easily fermentable forages into the diet of animals to promote the progressive use of cultivated protein forages. Promoting this form of nutrition improves rumen fermentation, increases dry matter intake, growth rate and milk production (Supapong & Cherdthong, 2023).

The introduction of silage in the animal ration can improve the digestion of nutrients, it can allow exercising as much control as possible over the ratio of concentration of fermented feed, at the same time it is possible to control the amount of nutrients consumed by each animal. Forage particle size, moisture content, mixing method and ensiling time are factors that can influence the quality of ensiled forage, especially to avoid increasing the concentration of butyric acid, an undesirable product of clostridial fermentation, so as to avoid various cases of digestive and metabolic disorders.

Ensiling of cultivated forages can be done at various moisture levels (45%, 55%, 65%), requiring a longer period of ensiling, as the amount of cultivated forages subjected to fermentation at farm level is generally high. Various studies have shown that forage ensiling at 45% moisture increased milk production (Meenongyai et al., 2017). However, silage with a moisture content of 60%, but more shredded, improves consumption and nutritional digestibility, leading to an increase in milk fat percentage (Nha & Pattarajinda, 2019). It was also found that feeding with silage protein fodder improves feed consumption, digestibility of dry matter and crude protein increasing milk production (Naadland et al., 2017). Increasing the percentage of clover in grass silage increased dry matter intake and through following milk production (Bertilsson & Murphy, 2003).

Consumption of both red and white clover silages produced higher levels of polyunsaturated fatty acids (FA) in the milk of dairy cows, particularly alpha-linolenic acid, than grass silage consumption (Steinshamn & Thuen, 2008).

Although the total digestibility of legume and maize silages is often lower than that of grass silages, the milk yields obtained are usually higher. Another benefit of feeding legume silage is the reduction of milk fat concentration and the increase of polyunsaturated fatty acid levels. The high protein content and high degradability of most legume silages are associated with a low efficiency of conversion of dietary nitrogen into nitrogen milk, with a concomitant increase in urinary nitrogen. However, when the mixture with legume silages includes maize silages, the reduction in urinary nitrogen is significant without a loss in production potential (Dewhurst, 2013).

The level of nutrition is a main factor affecting milk production and the composition of the milk produced, especially the levels of fat and protein (Francois & Caja, 2004), parameters that greatly influence the yield of cheese production. The use of ensiled cultivated fodder in animal feed, especially in the rainy months when grazing is not possible, led to a significant increase in the percentage of milk fat and protein compared to feeding through a grazing system with cultivated plants (Atti et al., 2010).

The use of leguminous forage plants in the feeding of small ruminants in organic farms

Incorporating legumes into agricultural systems provides many beneficial effects and has an important role in the management and sustainability of ecological small ruminant farming systems (Howieson et al., 2000) (Table 2). The most important benefits being the provision of nutritious animal feed rich in protein, the supply of N, the most important nutrient, after carbon and water, for plant growth (Vance, 1997), crop productivity, (Peoples et al., 1995) and not lastly in the control of diseases and pests of cereal crops.

Table 2. Importance of legumes into small ruminants organic farming system
(adapted from Howieson et al., 2000)

	Specific legume value[a]		
	Pasture	Grain	Legumes
N_2 fixation	***		**
Increased soil fertility and stability	***	*	
Capacity for nutrient recycling	*		**
Control of weed species	**		**
Break disease and pest life cycles	**		***
High protein animal feed	***		***
Cash crop	*		***
Biodiversity and landscape quality	***	*	
Operational flexibility	**		***

[a] Increasing,*, **, *** applicable value.

In order to improve the productive parameters and reduce the production costs in the ecological breeding of sheep and goats, a sustainable solution is to replace feed rich in imported proteins (soy and its derivatives) with alternative protein feeds obtained by the local cultivation of cereal legumes (lupin, lentils, chickpeas, peas, beans, soybeans, beans) and various varieties of herbaceous legumes (alfalfa and clover), as main components of cultivated meadows or as fodder after cutting (Bay-Larsen et al., 2018). The importance of legumes for grains lies, first of all, in the high protein content of the seeds, giving them a high nutritional value to meet the high requirements of plant proteins in animal husbandry. The protein content of leguminous grain is 2-4 times higher than that of cereals, and in some species (soy, lupine), the protein exceeds carbohydrates. The ratio between crude protein and non-protein components is: soy and lupine of 1/1.7; peas 1/2.8; beans 1/2.4 etc. So, leguminous grains represent fodder concentrated in protein. It is also worth noting the high protein value of the grains, equivalent in some species to proteins of animal origin, containing essential amino acids.

Cereal legumes, as alternative protein sources, such as lupine (*Lupinus albus*), peas (*Pisum sativum*), beans (*Vicia faba*), vetiver (*Vicia sativa*) are forages with high nutritional value that could be used as alternative sources to replace soybean meal or soybeans in the diet of dairy sheep without effects on nutrient digestibility (Zagorakis et al., 2018), without compromising productivity, providing a high energy and protein intake to meet maintenance and milk production requirements (Vouraki et al., 2023a) (Table 3).

Table 3. Chemical composition of the four studied legumes (Vouraki et al., 2023b)

Parameter	Lupin	Pea	Vetch	Faba Bean
DM [1] (g/kg)	920	900	900	900
Starch (g/kg DM)	-	410	379	428
Crude Fat (g/kg DM)	87	15	10	11
Crude Fiber (g/kg DM)	128	93	43	64
Crude Ash (g/kg DM)	35	41	36	36
Crude Protein (g/kg DM)	392	271	315	276
UFL [2] (/kg DM)	1.31	1.16	1.19	1.19
PDIN [3] (g/kg DM)	250	173	203	173
PDIE [4] (g/kg DM)	136	108	122	99
NDF [5] (g/kg DM)	199.90	149.35	149.84	140.74
ADF [6] (g/kg DM)	133.46	75.97	60.93	97.52
ADL [7] (g/kg DM)	6.18	5.57	5.06	2.45
Parameter	**Lupin**	**Pea**	**Vetch**	**Faba Bean**
TP [1] (g GAE [5] /kg)	3.77	4.33	3.81	5.21
TN [2] (g GAE [5] /kg)	2.98	2.84	2.91	3.28

[1] DM = dry matter; [2] UFL = units for lactation (net energy for lactation (kcal/kg)/1760) [37]; [3] PDIN = true protein digested in the small intestine when fermentable N is limiting [37]; [4] PDIE = true protein digested in the small intestine when fermentable energy is limiting [37]; [5] NDF = neutral detergent fiber; [6] ADF = acid detergent fiber; [7] ADL = acid detergent lignin.

The digestibility of protein found in leguminous grains is notably high, estimated at around 90%. Unlike certain animal proteins, it doesn't lead to the formation of uric acid, whose buildup in the body can pose health risks.

The enhanced digestibility of forage legume species in contrast to grass species can be ascribed to the configuration and composition of their leaves (Pontes et al., 2007). Legumes offer superior forage quality compared to grasses due to their lower fiber content, promoting increased consumption over herbs. Thus, one of the notable advantages of grazing animals on pasture is the ability to enhance forage quality and fulfill the daily protein needs of grazing animals by incorporating combinations of legume and grass species (Amiri et al., 2012).

The use of natural extracts in the ecological breeding of small ruminants

With the ban on the use of antibiotics as growth factors, there has been a need to find ways to meet consumer demand for safe food and to protect public health, but also to ensure farmers' incomes and animal yields (Iwu et al., 2020). Improvements in the management of animal production facilities are considered necessary to reduce stress on animals and to minimize their contamination with micro-organisms.

Compliance with animal welfare rules could partially replace the absence of antibiotics in animal feed. These improvements should be accompanied by changes in the feeding strategy of productive animals to neutralize possible negative effects on production, due to not covering the nutritional needs of animals (Lu, 2011). However, we should look for natural growth substances as an alternative to antibiotics. Examples of such substances are essential oils or extracts of aromatic/medicinal plants (PAM), dry feed of PAM added to feed, enzymes, probiotics, prebiotics and organic acids or acidifiers and their salts. Although most of these substances have already been used in combination with antibiotics to improve animal performance, their effectiveness now needs to be investigated when they are the only growth factor added to animal feed.

Ecological animal feeding systems are based on the use of feed free of drugs and chemicals, so it becomes absolutely necessary to investigate the use of natural substances, such as essential oils or plant extracts, which have antioxidant, antibacterial and antifungal properties, thanks to a variety large amount of phenolic substances contained in them (Jin et al., 2023).

The impact of various rearing technologies on the welfare and health of organically raised sheep and goats

Perhaps the most important for organic farms is the relationship between the health and vitality of an animal on the one hand and the conditions in which that animal is raised on the other. Many researchers and observers have shown that a large part of the contemporary diseases and syndromes are related to the maintenance and feeding conditions or to the breeding methods that have been adopted to increase production and economic efficiency. Thus, new problems have arisen that require new solutions from nutritionists and animal husbandry specialists.

Given their renowned hardiness, sheep and goats' capacity to withstand challenging environmental conditions and suboptimal management practices directly influences both their welfare and productivity. Extreme climatic conditions and seasonal fluctuations in the quantity and quality of pastures can be important causes of reduced welfare status in extensive production systems, which can affect the efficiency of grazing livestock production and dramatically affect the welfare and health status of sheep and goats. Animals reared on pasture can encounter a number of compromises in terms of their well-being, but mainly those related to nutritional stress, inadequate water supply, extreme climatic conditions, parasitic diseases.

In extensive production systems, animals roam freely in habitats conducive to fulfilling their physiological and behavioral needs. Nonetheless, grazing practices can sometimes compromise animal welfare, mainly due to fluctuations in pasture quantity and quality throughout seasons, leading to temporary nutritional stress. Notably, if this nutritional stress coincides with the mating season, it may diminish the fertility of sheep and goats (Rassu et al., 2004).

Thus, animals raised on pasture during the summer, when grass availability and palatability are diminished, may encounter a nutritional imbalance during this period. This, coupled with alterations in rumen fermentation and protein synthesis, can jeopardize their well-being and adversely impact milk fat and protein content. Grazing sheep and goats on low-quality pastures with overly fibrous vegetation, adverse weather conditions, and restricted grass intake time can lead to decreased milk production. Short-term dietary restrictions notably reduce milk yield and elevate milk fat content, consequently affecting the milk's fatty acid composition due to body fat mobilization (Pulina et al., 2012). Ewes experiencing undernourishment exhibited elevated somatic cell counts (SCC) in their milk, signaling metabolic stress in both the animal and its mammary gland. Research on non-lactating, non-pregnant ewes revealed that shifting their diet from extremely high to very low nutritional levels, and vice versa, emphasized the importance of avoiding both food restriction and overnutrition. Doing so is crucial to prevent metabolic disruptions and minimize the expenses associated with excessive fattening and maintaining surplus body weight (Caldeira et al., 2007).

An inadequate supply of water during grazing can cause stress in sheep and goats, a reduction in feed consumption, respiratory rate, a decrease in blood sugar, an increase in urea in blood and milk (Hamadeh et al., 2006). Stress can cause an alteration of the metabolic profile and often a reduction in weight.

Sheep and goats are renowned for their resilience in harsh climates. In sheep, the usual decrease in milk production during late lactation in summer often conceals or partly offsets the adverse effects of high temperatures on their well-being, milk yield, and immune function, along with notable mineral imbalances, particularly magnesium, potassium, calcium, and phosphorus. Exposure of dairy ewes to average daily temperatures of 35°C, even briefly, or prolonged exposure to average ambient temperatures of 30°C, has been observed to result in a significant rise in rectal temperature, metabolic changes, and a decline in milk production (Sevi et al., 2001; Sevi et al., 2002). Providing shade during the hottest hours and adjusting grazing schedules to late afternoon can effectively mitigate the effects of high summer temperatures on dairy sheep and goats during grazing.

Preventing and managing endoparasitic diseases are key concerns for maintaining the health and welfare of small ruminants in ecological breeding systems. This involves employing rational grazing strategies, incorporating plant extracts and homeopathic remedies, cultivating specific fodder crops and enhancing pasture species, optimizing diet formulations, breeding animals with resistance to parasites, and implementing biological control methods, such as utilizing natural enemies to combat nematode parasites.

Utilizing natural pastures encourages the proliferation of endoparasites, leading to significant declines in animal weight gain, milk yield, and reproductive functions. Sheep afflicted by parasites exhibit diminished grazing durations, reduced activity levels, and consume less grass compared to their uninfected counterparts (Gordon et al., 2000). Early stages of endoparasitic infections manifest in behavioral disruptions among sheep and goats, characterized by restlessness and altered resting behavior. As the infestation advances, affected sheep become progressively agitated due to allergen presence.

Grazing management strategies to combat helminth infections encompass several approaches: preventive measures involve introducing uninfected animals to parasite-free grazing zones, evasive tactics entail introducing already infected animals to vermin-free areas, and finally, alternative grazing methods involving a mix of small ruminants and cattle can also be employed.

Nutrition can influence the host's response to parasitism and its capacity to mitigate and potentially overcome parasitic challenges in three key ways. Firstly, it can enhance the host's resilience, bolstering its ability to withstand the negative impacts of parasitic infections. Secondly, nutrition can bolster the host's resistance, aiding in the control and eventual suppression of parasitic infestations by impeding parasite establishment, growth rate, fecundity, and persistence within the host population. Lastly, nutrition can exert a direct influence on the parasite population itself through various mechanisms, including the ingestion of anti-parasitic compounds, manipulation of grassland management practices, and dietary interventions such as protein supplementation or the inclusion of tanniferous plants in grazing regimes, offering sustainable alternatives for parasite control (Coop & Kyriazakis, 2001). Long-term immune response and resistance to endoparasites can result after feeding sheep a supplemental protein ration prior to grazing, increasing weight ratio and productive parameters, and having a lower number of nematode eggs in faeces when subsequently grazed on infected pastures. Grazing of herbaceous legumes containing ~10% dry matter tannins has been shown to have beneficial consequences on herbivores exposed to either natural or artificial nematode infections.

The inherent anti-parasitic, anti-fungal, and anti-bacterial attributes found in natural plant secondary compounds not only directly combat pathogens by disrupting their cellular functions and metabolic processes but also offer herbivores indirect health benefits. These compounds possess probiotic and immunomodulatory properties, which can fortify the herbivores' immune systems and promote beneficial gut bacteria. This dual action not only aids in disease prevention but also reduces the necessity for treatment after infection occurs (Provenza & Villalba, 2010).

A promising approach in animal husbandry, particularly for grazing animals, involves incorporating blends of diverse plants with bioactive properties. This integration aims to enhance animal health while maintaining optimal levels of production and welfare. By utilizing selected bioactive feeds, we can positively impact the management of parasitic diseases while also enhancing the quality and flavor of animal products (Ronchi & Nardone, 2003b).

Creating preventive strategies aimed at bolstering animals' resistance to diseases represents a more cost-effective, environmentally sustainable, and socially impactful long-term healthcare approach compared to treating diseases reactively.

CONCLUSIONS

Studying the impact of different feeding technologies on the nutritional quality of forages can help ensure that organic sheep and goats receive balanced diets that meet their nutritional requirements for growth, reproduction and overall health. Evaluating the efficiency of alternative feed use through various feed technologies can help optimize feed conversion ratios and reduce wastage, ultimately improving the economic viability of organic sheep and goat farming.

Monitoring the impact of feed technologies on productive parameters such as growth rates, milk production, wool quality and reproductive efficiency can provide insights into optimizing the productivity of organic sheep and goats while maintaining high welfare standards.

Investigating how feed technologies affect animal welfare parameters such as behavior, stress levels and general health can ensure that organic farming practices align with principles of animal welfare and ethical treatment.

Evaluating the economic consequences of adopting different feed technologies, including their costs and benefits, can help organic farmers make important decisions to maximize profitability while meeting organic standards. By conducting research in these areas, organic farmers, researchers and policy makers can work together to develop evidence-based strategies for improving the welfare and productive performance of sheep and goats in organic farming systems. This interdisciplinary approach is crucial to promoting the sustainability, resilience and success of organic livestock production.

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REVIEW ON THE INFLUENCE OF DAIRY CATTLE HEALTH EFFECTS ON GREENHOUSE GAS EMISSIONS

Marinela ENCULESCU

Research and Development Institute for Bovine,
Bucharest-Ploiesti Road, km 21, Balotesti, Romania

Corresponding author email: marinelaenculescu2006@yahoo.com

Abstract

Climate changes represent a major threat to society, due to its wide impact on ecosystems, economy, human and animal health. The aim of this review was to evaluate the influence of dairy cattle health and their implications on greenhouse gas (GHG) emissions intensity. The main influencing factors concerning the GHG intensity of dairy cattle that are discussed in this review article are nutrition, animal productivity, longevity and fertility, in relationship with animal health. Data showed that high levels of animal health not only led to increased productivity performances, however, it translates into a significant decrease of GHG emissions. Moreover, metabolic disorders during the transition period of lactating cows represent a critical risk for cows mortality, productivity and economical losses, and higher GHG emissions/kilogram/milk. Overall, the reductions in GHG emissions intensity could be achieved through the implementation of proper animal health management programs at individual farm level.

Key words: *animal nutrition, dairy cattle, greenhouse gas emissions, health, metabolic disorders.*

INTRODUCTION

Climate changes represent a major threat to society, due to its wide impact on ecosystems, economy, human and animal health.

It was proved that the livestock emissions contribute to the overall global warming and climate changes (Scoones, 2022; Grossi et al., 2019).

With an expected increase in milk consumption, and potential new policies to reduce greenhouse gas (GHG) emissions from agriculture, producing efficiently and reducing GHG from dairy cattle sector, has gained research interest (Mostert et al., 2018a).

Increased animal production efficiency can be achieved by improving animal health status, extending the productive life of animals, and improving reproduction performances.

Poor livestock health and welfare are associated with behavioural and metabolic changes, which can lead to an increase of GHG (Grossi et al., 2019), unhealthy animals tending to have a lower milk yield, fertility and longevity, resulting in higher emissions/unit of animal product (Wei et al., 2021).

The most prevalent dairy cattle health issues include mastitis, lameness, reproductive disorders, clinical and subclinical ketosis (Raboisson et al., 2015; McArt et al., 2012; Bruijnis et al., 2010; Duffield et al., 2009), such diseases having a significant economic impact and the potential to increase GHG/unit of output (Naranjo et al., 2020).

The potential reductions of GHG emissions in the dairy sector (kg CO_2 eq/kg product) and thus the improvement of food security, can be reached by disease prevention, and such approaches should be of considerable interest to all stakeholders involved in the dairy chain.

It was estimated that a rise of 5% diseases prevalence leads to a GHG/kg of milk increase of 1.1%, while a rise of 45% in the FMD (foot and mouth disease) disease prevalence, leads to a 10.0% increase in GHG/kg milk (Capper, 2023).

There is a considerable body of literature and evidence linking improvements in dairy cattle productivity with reduced feed resources per unit of milk produced, and therefore an improvement of the overall environmental sustainability of the sector (Capper & Cady, 2019; Caro et al., 2014; Capper & Bauman, 2013; Bell et al., 2013; Wall et al., 2012; Zehetmeier et al., 2011; Capper & Cady, 2010; Capper et al., 2008; Casey & Holden, 2005).

A method that assesses the environmental impact of a product is Life Cycle Assessment

(LCA), which takes into account the entire life cycle in the animal production chain (Wolf et al.*,* 2017; Baumann & Tillmann, 2004).

Primarily, LCA has been used for dairy cattle to estimate the impact of feeding strategies (Van Middelaar et al., 2014a), improved fertility and increased longevity (Bell et al., 2011) or milk yields, and to a lesser extent it was used to evaluate the impact of diseases prevalence on GHG emissions (Mostert et al., 2018).

It was shown that by reducing disease prevalence, GHG can be reduced, leading to an increase of the overall dairy farm's incomes (Liang et al., 2017; Van Soest et al., 2016), while improving the welfare of cattle.

This review paper aims to evaluate the influence of dairy cattle health and their implications on greenhouse gas emissions intensity.

MATERIALS AND METHODS

Bibliographic data from national research journals, international databases (Scopus, PubMed, ScienceDirect), personal research were used to present the scientific information from this paper.

RESULTS AND DISCUSSIONS

Livestock and GHG emissions at the European Union level

The most important greenhouse gas (GHG) emissions in ruminants farms (bovines and small ruminants) are carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O). In 2021, official data showed that 10.7% of the total GHG emissions in the EU were produced by the agricultural sector (Eurostat, 2023). It is estimated that during the last 30 years, the EU agricultural sector reduced its GHG emissions by 22%, which corresponds to 106 million tonnes of CO_2 equivalent (CO_2eq), compared to 1990 as a reference year. Moreover, emissions resulted from ruminants enteric fermentation processes were reduced by 23%, the equivalent of 54 million tonnes of CO_2 in 2021, when compared with year 1990 (Eurostat, 2023).

The official reports show that the largest share of GHG emissions due to enteric fermentation come from the digestive system of cattle (85%) With cattle produced emissions decreasing by 22%, 45 million tons of CO_2 equivalent, in the last three decades.

The reduction in GHG emissions being attributed mainly to the decrease of the total number of bovines raised in the EU, with an abrupt decrease of bovine numbers of 11% (9 million heads) during the last 20 years alone (Figure 1).

As a result of better manure management practices in the EU during the last three decades, such as storing platforms designs and regulations on agricultural lands administration, the GHG emissions from cattle manure were reduced by 19%, which represents a 7 million tons of CO_2 equivalent (Eurostat, 2023).

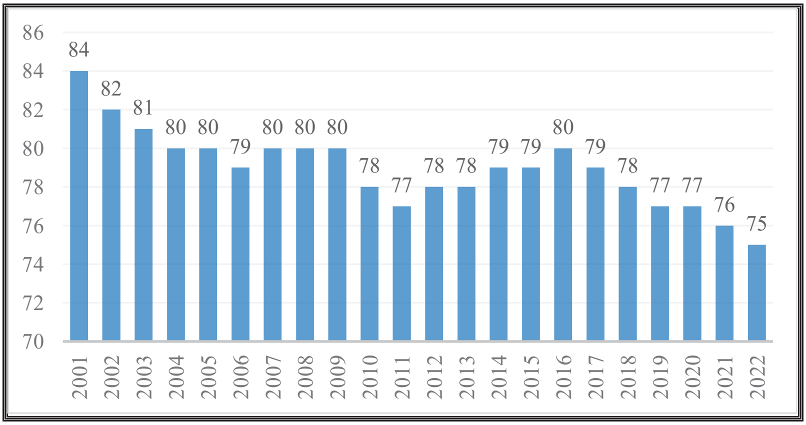

Figure 1. EU bovine population between 2001-2022 (million heads), Source: Eurostat, 2023

Dairy cattle nutrition and its impact on GHG emissions

It was outlined that efficient milk production and the reduction in the environmental impact throughout new and adapted feeding strategies are possible and necessary (Van Zanten et al., 2014).

Milk production losses in the dairy industry are caused mainly by poor nutrition, management imbalances and diseases. Health issues in dairy cattle farms increase the labour for farmers, veterinary treatment costs, reduce feed efficiency, and therefore, reduce the income of the dairy cattle farms.

Recent studies showed that an increase in feed intake, which leads to a higher energy and nutrient intake, has the potential to improve the ratio between energy available for milk yield and the one needed for maintenance functions, such as the immune system and metabolism.

In a study published by Von Soosten et al. (2020), it was showed that the energy intake for maintenance decreased for dairy cows which consumed 10 kg dry matter/day from 54% to 20% of their total energy intake, when cows consumed 25 kg of dry matter/day, of the same feed. The study outlining that although the CH_4 emission/animal/day increased with the dry matter intake, however, the emission per kilogram of milk produced decreased.

Similarly, Hristov et al. (2013) showed that cattle enteric CH_4 emissions can be reduced by several nutritional approaches, such as improving forage quality, increasing the amount of concentrates over roughage, or throughout the inclusion of dietary lipids in the adult cattle diets.

It is worth pointing out that different nutritional strategies to reduce GHG emissions in the dairy cattle sector might negatively impact GHG emissions and processes along the production chain, e.g. higher concentrates level necessitate soybean meal imports from outside of the EU (e.g. South American countries), which leads to emissions caused by the production and transport.

Moreover, a change in the diet of cattle, might reduce enteric CH_4 emissions, and this to cause a chain reduction of other GHG emissions, such as CO_2 and N_2O (Van Middelaar et al., 2013).

Nutritional plane persistence was proven to play a role in GHG emissions of cattle, with the work of Boichard & Brochard (2012) showing that feed management practices which had led to maintaining the herd's milk yield at a constant level, while avoiding milk production fluctuations, have led to a decrease in the GHG emissions.

In lactating dairy cattle, fatty acid (FA) profiles can be used as indicators for milk quality (Lingen et al., 2014; Dijkstra et al., 2011) and the diets with high concentrations of polyunsaturated fatty acids (PUFA) had as effects a decrease of the methane production. Similarly, Chilliard et al. (2009) reported a high positive correlation between saturated FA and methane production.

Regarding nutritional strategies, based on available data (Popa et al., 2022; Popa et al., 2021), it can be assumed that different feeding strategies can have as outcome a reduction in GHG emissions of 9-32 kg CO_2 eq/t of fat and protein corrected milk (FPCM), estimated by Van Middelaar et al. (2014b).

Roque et al. (2019) showed that adding 0.5-1% seaweed in the dairy cow's diet led to a 27-67% reduction in methane intensity per kg of milk produced.

All individual studies used in the meta-analysis by Almeida et al. (2021) noted the efficacy of 3-nitrooxypropanol (3-NOP) in reducing enteric CH_4 emissions. Supplementation of the diets with 3-NOP reduced ruminant CH_4 emission by 23.3% compared to the control diets, based on different studies, methane reduction ranged from 6.5% to 38.0% in lactating dairy cattle.

Cattle productivity and GHG emissions

Generally, higher milk yields result in higher emissions per animal, while lowering emissions per kg of FPCM. In their study, Zehetmeier et al. (2012) showed that GHG emissions per kg of milk decrease with the increase in milk yields from 6000 kg/lactation to 10.000 kg/lactation, from 1.06 kg CO_2 eq/kg of milk produced to 0.89 kg CO_2 eq/kg of milk produced. Similar reports by Van Middelaar et al. (2014a) showed that increasing milk yield with 698 kg/year/cow has lead to a reduction of 27 kg CO_2 eq/t of FPCM. These values correlated favourably with Bannink et al. (2011), which found that increasing the daily milk yield per cow from 17.2 kg to 22.9 kg has

decreased the enteric CH_4 emissions per unit of milk by 13%.
Alongside milk yields, the animal live weights were shown to influence GHG emissions. Niemann et al. (2011) reported that dairy cow with 650 kg live weights, 25 kg dry matter intake/day and milk yields of 40 kg/day, emitted 12 CO_2 eq/t FPCM vs. cows with 650 kg live weights, 12 kg of dry matter intake/day and having 10 kg milk/day, which emitted 30 CO_2 eq/t FPCM.
Regarding milk yields and GHG emissions, there is a growing body of studies showing that an increase in productivity, leads to a reduction of the overall emissions per kg of milk, thus throughout a better animal selection and improved herd management, GHG emissions could be indirectly reduced, synergically.

Longevity, fertility and GHG emissions
Prolonging dairy cattle productive life is regarded as one of the main alternatives to contribute to a more sustainable dairy production sector.
The longevity of dairy cows has gained increasing attention in recent years, largely due to the environmental (Bergea et al., 2016) and economic implications (Dallago et al., 2021) associated with a short longevity of 2.2-2.4 lactations.
Increasing the length of the productive life (LPL) in dairy cattle could be considered as an option to mitigate GHG emissions, as this reduces GHG emissions resulted from the rearing of replacement heifers (Bell et al., 2015), with implications in the profitability of milk production (De Vries, 2017).
Moreover, the milk production increases in dairy cows up to the 3rd-4th lactations, when it reaches the maximum yields, and starting the 5th or 6th lactations, it starts to decrease. As a result, culling dairy cattle during the first two lactations represents a significant loss, due to the overall physiological underproduction of primiparous and secundiparous cows.
Several studies, such as ones published by Humer et al. (2018) and Bell et al. (2011), demonstrated that poor fertility of dairy cows leads to increases in the GHG emissions.
One of the most noteworthy estimates regarding relationships between fertility and GHG was that of Garnsworthy (2004), which determined that improvements in the fertility of cows could reduce CH_4 emissions by 24%, throughout reducing the number of replacement heifers needed in the herd. The same author showing that in dairy farms, the GHG emissions decrease with the number of artificial inseminations (AIs) per gestations confirmed, from 0.926 kg CO_2 eq/kg of FPCM at 4 AIs/gestation to 0.915 kg CO_2 eq/kg of FPCM at 6 AIs/gestation.
Recently, Han (2023) showed that although lower subfertility culling reasons has the potential to extend dairy cattle longevity, the increase in the number of AI services could benefit more benefit more the economic net return, while mitigating GHG emissions as well. Similarly, Grandl et al. (2019) showed that increasing the length of the productive life of dairy cows reduces the climate impact per animal and improves profitability.
Authors estimating that the contribution of breeding replacement costs decreased continuously from 38% to 9% of the total costs, for cows when the length of productive life increased from 1 year to 7 years.
Furthermore, Van Middelaar et al. (2014a) reported that increasing longevity with 270 days/cow has led to a reduction of 23 kg CO_2 eq/t FPCM.
From a different perspective, Sekyere et al. (2023) found an association between farm infrastructure and farm investments, which according to the authors is strongly and positively correlated with cow longevity in Swedish dairy herds.
It is generally accepted within the dairy industry that higher dairy herd longevity is associated with higher milk yields and longer calving intervals, while prolonging age at first calving for primiparous cows reduces the productive longevity of the herd.
Grandl et al. (2019) reported that a large number of cows are removed from the herd early in lactation mainly because of metabolic health reasons.
Based on findings of recent studies, it can be affirmed that cows with an increased longevity produce less methane per kg of milk (Grandl et al., 2018), which in return improves the overall environmental sustainability (Overton & Dhuyvetter, 2020) and is indicative of good animal welfare (Barkema et al., 2015).

Metabolic disease and GHG emissions

Globally, Grace et al. (2015) estimated that livestock diseases are reducing livestock productivity by 25%. It was demonstrated that for dairy cattle, the transition period represents the time in which the risk of developing metabolic diseases is the highest. This risk during the transition from late gestation to early lactation is caused mainly by the significant metabolic and hormonal changes that occur (Dzermeikaite et al., 2024). Poor nutritional status during the transition period leads to higher incidences of metabolic diseases such as ketosis, mastitis, hypocalcaemia, retained placenta, metritis and displaced abomasum. Furthermore, subclinical ketosis (SCK), increases the risk of developing other diseases such as clinical ketosis, mastitis, metritis, displaced abomasum and lameness, all while increasing GHG emissions per kg of milk, reducing thus the production efficiency in dairy cattle herds. The implications of SCK on GHG emissions was highlighted in a recent study by Mostert et al. (2018a), which showed that the GHG emissions increased on average by 20.9 kg CO_2 eq/t FPCM per each case of SCK per cow, related to reduced milk production, discarded milk, prolonged calving interval, and removal (Table 1).

Authors reported that the increase in emissions was caused indirectly by resulting prolonged calving intervals (31%), discarded milk due to antibiotics use (30%), reduced milk production (19%), and the culling of cows (20%). Moreover, for cows which developed SCK exclusively, the GHG emissions increased by 7.9 kg CO_2 eq/t FPCM, whereas GHG emissions for cows that were culled increased by 188.2 kg CO_2 eq/t FPCM and for cows that died on-farm, the GHG emissions increased by 463.0 kg CO_2 eq/t FPCM. In a study following the effects of the udder health on GHG emissions, Hospido and Sonesson (2005) found that by reducing the subclinical mastitis incidence from 33% to 15%, and the incidence for the clinical mastitis from 25% to 18%, the GHG emissions could be decreased by 2.5%.

Similarly, in another study focused on de clinical mastitis (CM), it was shown that GHG emissions increased on average by 58 kg CO_2 eq/t FPCM per each case of clinical mastitis within the herd, related to reduced milk production, discarded milk, prolonged calving interval, removal, and avoided burden (Mostert et al., 2019).

Authors attributing the increase to causes such as animal culling (39%), discarded milk due to antibiotics treatment (38%), reduction of the milk production (17%) and to prolonged calving intervals (6%).

Same authors found that the increases in GHG emissions per case of CM varied based on parity, from 75 kg CO_2 eq/t FPCM in primiparous cows, to 34 kg CO_2 eq/t FPCM in cows in their 5th parity.

Additionally, similar findings were reported by Ozkan Gulzari et al. (2015), who found a 2% increase in GHG emission/kilogram of milk produced for subclinical mastitis cases.

In another similar study Ozkan Gulzari et al. (2018), showed the potential to reduce GHG emission intensity in dairy cattle farms by up to 3.7% throughout a reduction in the somatic cell count from 800.000 cells/mL to 50.000 cells/mL.

Next to metabolic diseases, foot lesions (digital dermatitis, white line disease and sole ulcer) were shown to increase GHG emissions in dairy cattle, on average by 14 kg CO_2 eq/t FPCM, related to prolonged calving interval, culling, and avoided burden (Mostert et al., 2018b).

The authors found a similar trend in foot lesions as in the case of clinical mastitis, with the impact on GHG emissions being lower for cows with higher parities, decreasing emissions from 17 kg CO_2 eq/t FPCM in 1st parity cows to 7 kg CO_2 eq/t FPCM in 5th parity multiparous cows.

Chen et al. (2016) estimated that generally lameness increases GHG emissions per kg of milk produced by 0.7% to 7.8%.

There is a growing body of literature and evidences showing that GHG emissions at the dairy herds level are significantly impacted by the prevalence of diseases, and some of the diseases act synergistically.

As a results, a well-managed transition period for dairy cows can lead to a reduction in the GHG emissions, while improving the overall health and welfare levels during this critical time.

Table 1. Impact of the diseases on GHG emission in dairy cows (source: Mostert et al., 2018a; 2018b; 2019)

Disease	GHG emissions (kg CO_2 eq/t FPCM*)
Subclinical ketosis and confounding effects (average/case)	+20.9
Subclinical ketosis (alone)	+7.9
Subclinical ketosis + mastitis	+63.4
Subclinical ketosis + metritis	+33.8
Subclinical ketosis + displaced abomasum	+55.8
Subclinical ketosis + lameness	+31.6
Clinical ketosis	+27
Subclinical ketosis (parity 1)	+15.1
Subclinical ketosis (parity 5)	+26.6
Subclinical ketosis which has lead to culling of the cow	+188.2
Subclinical ketosis which has lead to death of the cow	+463.0
Clinical mastitis and confounding effects (average/case)	+58
Clinical mastitis (one case/lactation)	+48
Clinical mastitis (two cases/lactation)	+69
Clinical mastitis (three cases/lactation)	+92
Clinical mastitis (parity1)	+75
Clinical mastitis (parity 5)	+34
Clinical mastitis (first case/lactation) – type of pathogens: Gram -	+65
Clinical mastitis (first case/lactation) – type of pathogens: Gram +	+54
Clinical mastitis which has lead to culling of the cow	+115
Clinical mastitis which has lead to death of the cow	+322
Foot lesions and confounding effects (average/case)	+14
Foot lesions (parity 1)	+17
Foot lesions (parity 5)	+7
Digital dermatitis	+4
White line disease	+39
White line disease (parity 1)	+59
White line disease (parity 5)	+6
Sole ulcer	+33
Sole ulcer (parity 1)	+60
Sole ulcer (parity 5)	+11

*CO_2 eq/t FPCM = CO_2 equivalents/ton of fat-and-protein-corrected milk (FPCM).

CONCLUSIONS

Preventing diseases could represent an effective strategy for farmers to reduce GHG emissions, and can contribute to the sustainable development of the dairy sector.

This review showcased existent research results on reducing GHG emissions throughout nutritional, health, management and selection approaches, which can synergistically be used to reduce GHG emissions per unit of produced milk, mitigating the impacts of livestock production on the environment.

The evidence from the reviewed literature strongly suggests that GHG emissions could be reduced by over 20% through the use of feed additives such as methane blockers, while a reduction of the most prevalent diseases (ketosis, mastitis and lameness) could contribute to a further reduction of 2-5%. Overall, the reductions in GHG emissions intensity could be achieved through the implementation of proper animal health management programs at dairy cattle farm level.

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EFFECTS OF THE DIETARY FLAXSEED AND ALFALFA ON THE ORGANOLEPTIC QUALITIES OF THE BROILERS' MEAT

Dumitru-Filip ILIESCU[1, 2], Tatiana Dumitra PANAITE[2], Dumitru DRĂGOTOIU[1], Gabriela Maria CORNESCU[2]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]National Research and Development Institute for Animal Biology and Nutrition (IBNA), 1 Calea București, Balotești, 077015, Ilfov, Romania

Corresponding author email: filip.iliescu@yahoo.com

Abstract

Due to the customers` desire to improve their healthy diet enriched in Omega-3, researchers included ingredients such as flaxseed in poultry feed. The study was conducted on a commercial farm to compare the organoleptic qualities of broilers' meat using dietary flaxseed and alfalfa. The broilers (44.000 heads) divided into two groups (C, E) were fed with compound feed containing 6% flaxseed + 2% alfalfa (growth phase) and 12% flaxseed + 2% alfalfa (finishing phase). At the end of the experiment, the organoleptic and physico-chemical (color and pH) meat parameters of breast and thigh were assessed for storage period (24, 48, 72 and 168 h). The results showed no significant differences ($p\geq0.05$) concerning the pH of the thighs and breast, but significant differences ($p\leq0.05$) were observed for all color parameters (L, a*, b*) within the storage period*group interaction. In conclusion, using combined dietary rich Omega-3 sources (flaxseed) and xanthophyll (alfalfa) contributes to meat quality enrichment by increasing the beneficial fatty acid concentration and meat color, important criteria in customer decision.*

***Key words**: alfalfa, broiler, flaxseed, meat quality, pH.*

INTRODUCTION

Numerous scientific studies demonstrate the role of n-3 polyunsaturated fatty acids (PUFA) in the normal development of the central nervous system in children, maintaining its functions in adults, regulating immune functions, and preventing and managing coronary diseases (Simopoulos, 2000; Griffin, 2012; Calder, 2013).

The benefits of PUFAs from the Omega-3 fatty acid family (n-3) are scientifically proven through studies conducted on both animals and human subjects. In human food, the intake of n-3 fatty acids comes from both plants (seeds, nuts, oils, legumes) and marine (fatty fish, seafood) sources. However, modern agro-food industry practices have led to a reduction in n-3 fatty acids in the diet of most people (Stark et al., 2016). Nevertheless, the industry is attempting to readjust and contribute to an increase in the consumption of n-3 fatty acids, especially those with long chains.

The intake of n-3 fatty acids from marine sources is limited in diets due to factors such as cost, availability, and sustainability, as well as other concerns including allergies, taste, and smell (Moghadam et al., 2017; Cherian, 2016).

The alternative would be animal-derived products such as eggs and meat, which can be enriched with n-3 fatty acids by introducing plant sources of Omega-3 into the feed of poultry. The poultry industry has quickly adapted by producing eggs enriched with n-3 fatty acids. This technology primarily utilizes feed sources rich in n-3 fatty acids to enhance the nutritional value of food products (Bourre, 2005).

Flaxseed (*Linum usitatissimum*) serves as a plant source for increasing the level of n-3 PUFA in chicken meat (thigh, breast). Chemically, flaxseeds contain approximately 32-43.6% oil, 19-33.8% fiber, 20.3-26.78% protein, 6% carbohydrates, and 4% ash, depending on the genotype (Iliescu et al., 2023).

Flaxseed is a plant source with a high content of PUFA compared to fish oil, soybean, corn, or marine algae (Jiang et al., 2021). The composition of flaxseed oil highlights its distinct fatty acid content: 57% alpha-linolenic

acid, 16% linoleic acid, 18% oleic acid, 4% stearic acid, and 5% palmitic acid (Cloutier et al., 2012).
The protein content (20-30%), metabolizable energy level (3800-3960 kcal/kg), and the alpha-linolenic acid (ALA) content in flaxseed oil make flaxseeds an ideal plant source in poultry feed (Jia et al., 2021; Moghadam & Cherian, 2017; Moghadam et al., 2020).
Although, flaxseed contains up to 50% ALA also contains anti-nutritional factors (trypsin inhibitor, phytic acid, etc.) that can affect the health and muscle development of broilers. These anti-nutritional factors are eliminated through the "extrusion" process (Wu et al., 2008), which also enhances the metabolism of other nutrients, including ALA (Anjum et al., 2013; Kumar et al., 2023;). The high degree of unsaturation, which is desired for its beneficial effects on the health of consumers, can affect the oxidative stability of meat, inevitably leading to the deterioration of its nutritional and functional value (Betti et al., 2009; Cortinas et al., 2005; Juskiewicz et al., 2017). Therefore, it is necessary that alongside sources of n-3 PUFA, there are also antioxidants to slow down lipid oxidation and add nutritional value to the meat (Bernardi et al., 2016; Moghadasian, 2008).
Products containing higher amounts of n-3 PUFA must have at least equal attractiveness to other products in the same category. Therefore, color and its preservation over time during shelf exposure are important attributes (Janisch et al., 2011).
By combining extruded flaxseeds with various antioxidants such as vitamin E and selenium, the meat oxidation can be stabilized, and a positive contribution can be made to the nutritional value of broiler meat (Parveen et al., 2016; Perez et al., 2010; Leskovec et al., 2019; Taulescu et al., 2010).
The alfalfa (*Medicago sativa*) stands as a valuable feed for livestock. Its high protein content, complemented by a well-balanced amino acids profile, vitamins, and essential minerals, contributes significantly to animal health and growth. Moreover, the presence of biologically active compounds, including beneficial saponins, enhances its value as a feed source (Soto-Zarazúa et al., 2017). Alfalfa is rich in xanthophylls and can be used in poultry feeding to add value to the final products (Yildiz et al., 2020). Alfalfa is well-known for its superior fiber quality, and serves as a beneficial dietary supplement in animal feeding, enhancing production, reproductive performance, health status, and meat quality (Ma et al. 2022).
This study evaluated the effects of including dietary sources rich in Omega-3 fatty acids (flaxseed) and xanthophyll (alfalfa) in poultry feeding on meat quality parameters as pH and color, during different storage periods. These meat quality parameters are considered crucial factors, and manipulating the broilers' diet structure represents a viable feeding strategy for meeting consumers preferences.

MATERIALS AND METHODS

The study was conducted on a commercial farm in southern Romania where broilers (44.000 birds) were divided into two groups (30.000 birds - Control group; 14,000 birds – Experimental group). The experimental group diet structure, presented in Table 1, contained 6% flaxseed + 2% alfalfa (growing phase) and 12% flaxseed + 2% alfalfa (finishing phase).

Table 1. The experimental diet structure composition during different production phases

Ingredients	Starter phase (day 0-10)	Growing phase (day 11-24)	Finishing phase (day 25-42)
Corn	34.41	40	34.09
Wheat	20	13.04	25.00
Alfalfa	-	**2.00**	**2.00**
Flaxseed	-	**6.00**	**12.00**
Soybean meal	37.98	31.82	20.30
L-lysine	0.25	0.16	0.33
DL-methionine	0.35	0.29	0.31
L-threonine	0.11	0.15	0.18
Calcium carbonate	1.28	0.59	0.94
Monocalcium Phosphate	1.24	1.24	0.85
Salt (NaCl)	0.40	0.40	0.36
Vegetable oil	3.47	3.62	2.94
Colourant	0.50	0.18	0.18
Phytase	0.01	0.01	0.01
Vitamin Premix	0.50	0.50	0.50

At the end of the experiment, the chemical-physical and organoleptic parameters of the collected meat samples for breast and thigh were evaluated for storage period (24, 48, 72, and 168 hours, respectively).
For evaluating the color of the meat samples, a portable spectrophotometer 3nh YS3020 (Shenzhen Threenh Technology Co., Ltd, Beijing, China) was used, with a wavelength

ranging from 400-700 nm. The measurements conducted with the spectrophotometer were based on the trichromatic system adopted by CIE, utilizing three primary spectral colors: red, green, and blue. The values obtained in the CIE color space were determined by reading the Cartesian parameters (L*, a*, b*). Flaxseed meal was ground using an electric grinder to pass through a 1-mm mesh.

The results were analised using general linear model (GLM) procedures of SAS (Statistical Analysis System, Minitab version 17, SAS Institute Inc., Cary, NC, USA). The storage period effects on meat parameters (pH and color) were analyzed to determine whether the factors studied (diet and storage period) influenced the pH and meat color. The data obtained were analyzed by two-way ANOVA used the Tukey test following the statistical model:

$$Yijk = \mu + \alpha i + \beta j + \alpha i\beta j + eijk$$

where Yijk = variable measured for the k^{th} observation of the ith treatment and j^{th} feeding or storage period; μ is the sample mean, αi is the effect of the ith treatment; βj is the effect of the j^{th} feeding or storage period; αiβj = interaction of i^{th} treatment and j^{th} feeding time, and εijk is the effect of error. The differences were highly significant when $p < 0.001$, significant if $p < 0.05$, and a tendency of influence was considered when $p < 0.10$.

RESULTS AND DISCUSSIONS

Various scientific studies have recommended different inclusion of dietary flaxseed percentages for broilers: 2.5% and 10% (Mridula et al., 2015); 12-15% (Shen et al., 2005; Pekel et al., 2009; Najib & Al-Yousef, 2011) increasing the Omega-3 fatty acid content of meat. The alfalfa utilization in poultry feed reduces oxidative processes (Mattioli et al., 2022) but the high fiber content of alfalfa (25-30%) is a limiting factor in monogastric diets due to its potential antinutritional properties (Wang et al., 2018).

Also recent studies showed that alfalfa inclusion have no negative impact on growth performance if is included less then 4% (Sánchez-Quinche et al., 2023).

More authors stated that an optimum inclusion level can promote the abundance of beneficial microbiota (Zhang et al., 2016).

The pH values

The processing capacity, appearance, and sensory quality of broiler meat are influenced by the pH value (Petracci et al., 2009; Allen et al., 1997; Beauclercq et al., 2016). A normal pH of chicken meat, measured 24 hours after slaughter (final pH), ranges between 5.7 and 6.0. A pH lower than 5.7 is generally associated with a pale appearance and reduced water retention capacity, similar to PSE (pale, soft, exudative) meat, whereas a pH higher than 6.0 is associated with darker, firmer, and drier meat, qualitatively poor and not recommended for storage (Petracci et al., 2014)

Table 2. The pH measured values on breast and thighs samples during different storage period

Estimated time	Group	pH breast	pH thighs
Initial (T_0)	Control	6.292^a	6.055
	Experimental	6.227^a	5.858
24 h (T_{24})	Control	5.690^b	5.938
	Experimental	5.732^b	5.923
48 h (T_{48})	Control	5.622^b	5.887
	Experimental	5.683^b	5.843
72 h (T_{72})	Control	5.568^b	5.707
	Experimental	5.603^b	5.867
168 h (T_{168})	Control	5.663^b	5.823
	Experimental	5.705^b	5.930
Multiple effects			
Group			
Control		5.767	5.882
Experimental		5.790	5.884
SEM_{group}		0.022	0.034
Period			
Initial (T_0)		6.259^a	5.957
24 h (T_{24})		5.711^b	5.931
48 h (T_{48})		5.652^b	5.865
72 h (T_{72})		5.585^b	5.787
168 h (T_{168})		5.684^b	5.877
SEM_{period}		0.034	0.053
The interaction (p-Value)			
Group		0.453	0.961
period		0.000	0.206
period*group		0.704	0.161

The average values located on the same column, with different letters as superscripts, show significant differences $p < 0.05$.

In Table 2, no significant intra-lot differences are observed (p=0.453) for the pH value measured at the level of the breast; however, distinct significant differences (p=0.000) are observed for the values recorded at different pH measurement time periods (T0 h, T24 h, T48 h, T72 h, T168 h). It is noted that the T0h pH value of the experimental group for breast is slightly above 6, but subsequently measured values are within the normal pH range, with a minimum of 5.59 (T72 h) and a maximum of 5.71 (T24 h). The measurements taken at the thigh level do not exhibit statistically significant differences ($p>0.05$) in terms of group ($p=0.961$), period ($p=0.206$), or the interaction between group and period ($p=0.161$). Other authors used flaxseed oil in feed (over 35 days) to assess its influence on quail meat and conducted pH measurements at the breast level at different time intervals (0, 7, 14, 21, 28, 35 days) without any differences noticed ($p=0.12$). (Mirshekar et al., 2021).

A correlation is noted between meat pH and various meat quality parameters, such as shelf life, water retention capacity, and color (Gratta et al., 2019). In this study, the fresh pH of broiler breast ranged from 5.89 to 6.23, and the pH of thighs ranged from 5.87 to 5.96. This range considered normal (5.00-6.00) for pH recorded in the broiler breast meat and thighs is due to a proper growth and welfare conditions for poultry (Genchev & Mihaylov, 2008).

In other studies, it is shown that there are differences between various types of poultry meat, including between species. Thus, for the breast meat from broilers, we have more white glycolytic fibers, whereas the composition of quail breast meat's red color is caused by the predominance of oxidative fibers. It has been demonstrated that the pH of the breast is positively correlated with higher yield and is due to the lower glycogen content in the muscle tissues (Berri et al., 2005).

The glycogen content and low levels of phosphocreatine result in the white color of muscles. In the red-colored muscle fibers of quail breast, there are large deposits of phosphocreatine that provide the necessary energy for ATP recycling and their high capacity to supply oxygen through their mitochondria, resulting in slower aerobic glycolysis. Rigor mortis occurs, and a lower rate will be observed, thus resulting in a higher final pH (Velleman & McFarland, 2015).

Broiler meat color

The meat color measurements, shown in Table 3, were carried out using the trichromatic system adopted by the CIE, with three primary spectral colors: red, green, and indigo.

Table 3. The evaluation of broilers' meat parameters

Parameter		Breast			Thigh		
Estimated time	Group	L*	a*	b*	L*	a*	b*
Initial (T_0)	Control	39.187^{bcd}	-0.859^{b}	4.972^{b}	44.238^{ab}	-0.956	4.719
	Experimental	37.010^{d}	0.649^{a}	11.373^{a}	44.451^{ab}	4.543	2.400
24 h (T_{24})	Control	42.828^{ab}	0.172^{ab}	3.882^{bc}	45.531^{a}	-0.610	3.679
	Experimental	41.770^{abc}	0.036^{ab}	4.882^{b}	41.281bc	0.506	4.076
48 h (T_{48})	Control	38.421^{cd}	0.277^{ab}	2.222^{c}	39.641^{c}	-0.244	1.788
	Experimental	41.541^{abc}	1.089^{a}	5.809^{b}	42.742^{abc}	0.231	2.456
72 h (T_{72})	Control	41.191^{abc}	0.235^{ab}	2.008^{c}	41.554^{bc}	-0.147	1.029
	Experimental	43.415^{a}	-0.650^{b}	3.909^{bc}	44.496^{ab}	0.198	3.937
168 h (T_{168})	Control	40.822^{abcd}	-0.805^{b}	2.177c	41.187^{bc}	-0.404	0.992
	Experimental	41.408^{abc}	-0.757^{b}	6.321b	41.344^{bc}	-0.206	4.568
Multiple - effects							
Group							
Control		40.490	-0.196	3.052^{b}	42.430	-0.472	2.442
Experimental		41.029	0.073	6.459^{a}	42.863	1.054	3.488
SEM lot		0.384	0.114	0.260	0.334	0.593	0.918
Period							
Initial (T_0)		38.099^{b}	-0.105^{bc}	8.173^{a}	44.344^{a}	1.793	3.56
24 h (T_{24})		42.299^{a}	0.104^{ab}	4.382^{b}	43.406^{a}	-0.052	3.88
48 h (T_{48})		39.981^{ab}	0.683^{a}	4.016^{b}	41.192^{b}	-0.007	2.12
72 h (T_{72})		42.303^{a}	-0.208^{bc}	2.958^{b}	43.025^{ab}	0.026	2.48
168 h (T_{168})		41.115^{a}	-0.105^{c}	4.249^{b}	41.266^{b}	-0.305	2.78
SEM period		0.604	0.179	0.408	0.527	0.937	1.45
Interaction (p-Value)							
lot		0.321	0.096	0.000	0.361	0.070	0.422
period		0.000	0.000	0.000	0.000	0.512	0.905
period*group		0.013	0.000	0.000	0.000	0.223	0.631

The average values located on the same row, with different letters as superscripts, show significant differences $p < 0.05$.

The values obtained in the CIE space were determined by reading the Cartesian parameters (L*, a*, b*). Thus, when the parameters a* and b* are positive, the color of the sample will fall within the red-orange-yellow range; when the parameter a* is negative and b* is positive, the color of the sample will fall within the yellow-yellowish-green-green range; when the parameters a* and b* are negative, the color of the sample will fall within the green-turquoise-blue range; when a* is positive and b* is negative, the color of the sample will fall within the blue-purple-red range.

The luminosity axis L* is perpendicular to the axes a* (green/red) and b* (blue/yellow) and extends from the ideal black domain (L*=0), passing through the neutral point (gray) (N) (achromatic), to the ideal white (L*=100). The parameter L* refers to the brightness of the sample, a* to the intensity of the red color of the sample, and b* to the intensity of the yellow color of the sample. Each meat sample (breast, thigh) was measured three times, and the final values were obtained by calculating the average of the three measured values.

For the luminosity parameter L* measured at different storage periods at the breast level, distinct significant differences were recorded (p=0.000). The values recorded at 24 h, 72 h, 168 h are higher compared to the initial pH measured at T0h. Additionally, a significant interaction (p=0.013) between period and group can be observed for this parameter. For the parameter a* measured at different storage periods at the breast level, distinct significant differences were recorded (p=0.000) both for the period and the period*group interaction.

Based on the luminosity values (L*) measured in the breast samples, the authors (Lee et al. 2022) classified the color shades as follows: dark (L*<56), normal (L* 56-62), and pale (L*>62). More open values were observed in the E group for the luminosity parameter L* measured in breast samples, however without statistical significance (p>0.05). Distinct statistically significant higher values for L* (p=0.000) were recorded at intervals of 24 h, 72 h, 168 h. Also, in the case of breast samples, distinct statistically significant differences (p=0.000) were recorded for the parameter a* (negative values) max. at 72 h (-0.208) and positive values of the same parameter at 48 h (0.683), giving a yellowish hue to the sample. Distinct statistically significant differences (p=0.000) were also recorded for the group*period interaction, breast sample.

For the b* color parameter, distinct statistically significant differences (p=0.000) are recorded for group, period, as well as for the interaction between group*period. Thus, the b parameter, measured at the level of the breast sample, records significantly higher positive values in batch E (6.459) compared to C group (3.052). Once again, an increased value of the b* parameter indicates a higher intensity of the yellow color. For the L* parameter measured at the level of the thigh, distinct statistically significant differences (p=0.000) are observed in terms of period and the interaction between group*period. Maximum values were recorded initially at T0 (44.344), at T24 h (43.406), and at T72 h (43.025) compared to T48 h (41.192) and T168 h (41.266). For the other two colors parameters, a* and b* measured at the thigh, no significant differences found (p > 0.05).

The color of meat is generally chromatically correlated with the quantity of compounds containing heme, such as myoglobin in all its forms and variations, especially regarding hue, which can be caused by a variety of intrinsic and extrinsic factors affecting the content of heme pigment. (Boulianne & King, 1995). Furthermore, the color of meat is determined by the degree of light scattering through the microstructure of the meat and its easy modification depending on post-mortem muscular events (Hughes et al., 2020).

In the case of poultry meat, color is influenced to a lesser extent by the content of heme pigment but is affected by numerous other intrinsic factors (age at slaughter, sex, and genotype) and extrinsic factors (feed formula, rearing system) with a greater impact (Wideman et al., 2016).

For determining meat color, the luminosity parameter (L*) can represent a very important indicator of meat quality (poultry breast) for subsequent processing (Petracci et al., 2004). Generally, an L* value between 50 and 56 indicates normal values for poultry breast, while L* < 50 and L* > 56 present a darker or paler color of the poultry breast. In the present study, L* values were within the normal range, considering all investigated variables. In the literature, a negative correlation between pH and the L* parameter measured at the level of the breast has been reported in the case of darker poultry breast color. (Le Bihan-Duval et al., 1999). In the case of meat derived from poultry breast, the parameter a* (redness) is inversely correlated with the L* parameter and directly related to the pH level in the muscle tissues, so that a higher redness with higher values is generally recorded in darker-colored meat with a higher pH (6.53). Specialized literature has reported that the value of the b* parameter (yellow color) of poultry breast decreases concurrently with the L* parameter, while the pH value and the values of the a* parameter increase (Allen et al., 1997).

This means that chicken meat with a higher pH generally has a darker and redder color. On the other hand, paler meat with a lower pH records higher values of the b* parameter (Hughes et al., 2020).

In the literature, there are presented very different values of color parameters for poultry breast measured depending on age (Bianchi et al. 2014) focused on meat chickens from the native system and observed a decrease in L*, a*, and b* values when transitioning from light to heavy breeds (Bianchi et al., 2007; Bosco et al. 2014) observed an increase in L*, a*, and b* values in measurements taken on breast samples from chickens of different genotypes (aged from 70 to 81 days).

On the other hand, (Połtowicz & Doktor, 2013) noticed an increase in the value of the L* parameter and decreasing trends in the values of the a* and b* parameters in breast samples from laying hens (aged from 35 to 42 days).

CONCLUSIONS

During the different time storage periods, variations in physicochemical and organoleptic parameters were observed between the two groups, attributed to the dietary inclusion of xanthophyll sources which mitigated the PUFA's oxidation. Importantly, the incorporation of flaxseed and alfalfa in broilers' diets did not adversely impact poultry meat quality across various storage periods.

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AFLATOXINS OCCURRENCE AND LEVELS IN MAIZE FROM A ROMANIAN FEED MILL

Dragos Mihai LĂPUŞNEANU, Cristina Gabriela RADU-RUSU, Mădălina MATEI, Silvia Ioana PETRESCU, Ioan Mircea POP

"Ion Ionescu de la Brad" Iasi University of Life Sciences,
3 Mihail Sadoveanu Alley, 700490, Iasi, Romania

Corresponding author email: popmirceais@yahoo.com

Abstract

The present study provides a two years overview of quantitative determination of total aflatoxin in maize from a Romanian feed mill. The aim of the study was to monitor the occurrence and levels of total aflatoxins in maize samples analyzed during the 2020 and 2021; maize used as raw material in the compound feed production was prelevated for analyzes both in the raw material reception stage and from the unit's stock. To determine the aflatoxins concentration of maize from the reception stage, 1034 analysis were carried out in 2020 and 1191 analyzes in 2021. In order to assess aflatoxins occurrence and levels for deposited maize, were prelevated samples from the stock of the studied unit (48 samples in 2020 and 43 in 2021. Results showed that in 2020, from 1034 samples, 77.3% (n = 800) were positive, while in 2021, from 1191 samples, 57.9% were positive. For the determination of the total aflatoxin content of maize from the units stock, the incidence of positive samples was slightly higher in 2021 (65.1%), compared to year 2020 (56.2%). It can be concluded that it is important for feed mills to establish adequate control measures for mycotoxin contamination.

Key words*: aflatoxins, feed mill, feed and food safety, maize.*

INTRODUCTION

Due to the frequent contamination of food and feed by different contaminants, food and feed safety is an major issue (Lorenzo et al., 2018; Marin et al., 2022).

Mycotoxins, a group of toxic compounds produced by spore-forming fungi, could contaminate a wide range of food products both pre- and post-harvest (Zain, 2011).

Feed and food contaminated products pose high risks to animal health and human metabolic conditions, ranging from acute symptoms of severe disease to long-term effects (Anukul et al., 2013; Pinotti et al., 2016; Matei et al., 2023).

Due to incidents of mycotoxin poisoning (Probst et al., 2007; Galarza-Seber et al., 2016), most countries have regulatory levels for the presence of mycotoxins in certain staple food or feed, and therefore testing for those specific regulated mycotoxins is required (Smit et al., 2004); to verify food safety and protect consumer health, specific and selective analytical techniques adapted are used (Pleadin et al., 2019).

Maize, one of the most important cereals produced for human and animal consumption in the European Union, is primarily grown for grain and forage; over 80% of maize grain is utilized for feed (Palumbo et al., 2020).

Aflatoxins are a class of carcinogenic mycotoxins produced by *Aspergillus* species, particularly *Aspergillus flavus* and *Aspergillus parasiticus*, and are commonly identified in staple foods such as corn, rice, peanuts, dried fruits and spices, and dairy products (Reddy et al., 2010; Ismail et al., 2018; Rushing & Selim, 2018). *Aspergillus* fungi contaminate cereal crops and may produce aflatoxins in different harvest stages (Sserumaga et al., 2020). Corn kernels can contain aflatoxins up to 400,000 ppb, therefore sampling is very important in analyzing contamination levels (Richard, 2015).

All primary conversions of aflatoxin B_1 lead to the production of hydroxyl metabolites. The most significant resultant toxin, in terms of toxicity, is aflatoxin M_1, famously dubbed the "milk toxin" due to its excretion in the milk of farm animals fed on aflatoxin B_1 (Prandini et al., 2009). According to studies, aflatoxin B_1 is genotoxic, induces genetic mutations and chromosomal changes (IARC, 2002).

During the 2000s, the European Union revamped its food safety policy, aligning with the integrative concept of "from farm to fork", ensuring a high standard of food product safety across all stages of the production chain (Lapusneanu et al., 2021; Matei & Pop, 2022); even feed mills, like food units must to have auto control programs for contaminants (Lapusneanu et al., 2020; Reg. EU No 183/2005).

The present study provides a two years overview of quantitative determination of total aflatoxin in maize, as a raw material for compound feed, in a Romanian feed mill.

Our study aimed to monitor the occurrence of total aflatoxins in maize samples collected during 2020 and 2021 from a Romanian feed mill. Maize used as raw material in compound feed production underwent analysis both upon arrival at the raw material reception stage and from the unit's stock. Investigating aflatoxin concentrations at the feed mill level was necessary to identify aflatoxin levels in maize. Therefore, information regarding aflatoxin levels in the feed mill will be crucial for ensuring feed safety.

MATERIALS AND METHODS

To ascertain the total aflatoxin content in maize content from the feed mill reception stage, 1034 analyzes were carried out in 2020, respectively 1191 analyzes in 2021; to determine the total aflatoxin of maize from the feed mill stock, 48 analyzes were carried out in 2020, respectively 43 analyzes in 2021.

To quantify total aflatoxins, we employed the laboratory kits Ridascreen®Fast Aflatoxin. Initially, 5 g of ground sample was weighed into a suitable container, to which 25 ml of methanol (70%) was added. After shaking for three minutes, the extract was filtered through filter paper. The resulting filtrate was diluted with 1 ml of distilled water, and 50 µl of this solution was used per well in the test. A sufficient number of wells were inserted into the microwell holder for all standards and samples, with their positions recorded accordingly. Next, 50 µl of standard or prepared sample was pipetted into separate wells, using a new pipette tip for each. To each well, 50 µl of enzyme conjugate and 50 µl of anti-aflatoxin antibody solution were added, followed by manual shaking of the plate and incubation for 10 min at room temperature (20- 25°C). Subsequently, the liquid was emptied from the wells into a sink, and the microwell holder was tapped upside down onto a clean filter towel to remove any remaining liquid. The wells were then filled with washing buffer (250 µl per well) using a wash bottle or multichannel pipette, and the liquid was emptied again to ensure thorough washing. This washing step was repeated twice more. Following this, 100 µl of substrate/chromogen was added to each well, and the plate was gently mixed before incubating for 5 min at room temperature in the dark. Finally, 100 µl of stop solution was added to each well, gently mixed by manual shaking, and the absorbance was measured at 450 nm. Readings were taken within 10 minutes after the addition of the stop solution (R-biopharm Ridascreen®Fast Aflatoxin).

The data obtained from the analyses underwent statistical processing and interpretation. The minimum and maximum values were determined, and position and variation estimators were calculated. Specifically, the arithmetic mean ($\bar{x}$) and the standard deviation (s) were computed for samples that yielded positive results.

The results obtained underwent comparison with the values regulated by European legislation. Interpretation of these results led to the formulation of conclusions regarding feed safety.

RESULTS AND DISCUSSIONS

The results of the analyzes were interpreted and compared with reference to the European Union legislation (Table 1) regarding the maximum permitted limits of mycotoxins in the raw materials and compound feed.

Table 1. Maximum permissible limits for aflatoxin B_1 in feed (Reg. EU No 574/2011)

Animal feed	**Maximum content (ppb)**
Feed materials	20
Complete feed, except:	10
- compound feed foryoung poultry	5
- compound feed for poultry (except young animals)	20

To determine the mycotoxins concentration of maize, analyzes were carried out to determine the total aflatoxin content of the samples from the reception stage and from the stock of the feed mill, during the years 2020 and 2021 (Table 2).

To assess the total aflatoxin content of maize from the reception stage, 1034 analyzes were carried out in 2020, and 1191 analyzes in 2021. Depending on the maize quantities received by the unit, the number of samples analyzed monthly ranged from 23 (in April) to 203 (in May) in 2020, and from 13 (in August) to 189 (in February) in 2021.

Incidence studies revealed that in 2020, out of 1034 samples analyzed, 77.3% (n = 800) tested positive. Conversely, in 2021, out of 1191 samples analyzed, 57.9% tested positive.

In both 2020 and 2021, there's a noticeable peak in aflatoxin contamination during the summer months, particularly in July. This peak is quite pronounced, with percentages reaching as high as 84.7% in 2020 and dropping slightly to 40% in 2021. While the summer months consistently show higher levels of aflatoxin contamination, there are also fluctuations throughout the rest of the year. For instance, there's a notable decrease in contamination percentages during September and October in both 2020 and 2021 (Figure 1). This could be due to factors such as changes in climate conditions, harvesting practices, or crop rotation patterns.

The average values established for each month in which analyzes were carried out varied from 1.1 ppb to 2.6 ppb in 2020, respectively from 0.6 ppb to 3.9 ppb in 2021. The maximum values identified in the reception stage were 70.4 ppb for the analyzes carried out in 2020, respectively 62.1 ppb for the analyzes carried out in 2021.

From all the analyzes carried out in 2020, for one sample (0.09% of the total) a total aflatoxin concentration above the maximum limit allowed by legislation was found; from all the analyzes carried out in 2021, for nine samples (0.7% of the total) the concentration in total aflatoxin was above the maximum allowed limit. Since the identification of exceeding the maximum allowed limit of total aflatoxin content in maize samples occurred at the reception of raw materials, the respective batches were rejected based on this parameter

In order to assess aflatoxins occurrence and levels for deposited maize, were prelevated samples from the stock of the studied unit (48 samples in 2020 and 43 in 2021). For the determination of the total aflatoxin content of maize from the stock (Table 3), the incidence of positive samples was slightly higher in 2021 (65.1%) than in 2020 (56.2%). On the other hand, the average value established was slightly lower in 2020 (0.9 ppb) compared to 2021 (1.4 ppb).

In Lăpuşneanu et al. (2023) study, they investigated the microbiological contamination of raw materials and compound feed, noting the presence of the *Aspergillus* genus in over 50% of analyzed maize samples. The total fungal count ranged from 4.0×10^2 to 4.9×10^3 cfu/g. Such contamination can contribute to the production of aflatoxins. A survey conducted in 2019 in Romania focused on the prevalence of aflatoxins in maize. In total, 95 maize samples were collected, revealing that 88 samples (92.63%) were contaminated. Among these, only one sample exceeded the limit, with a concentration of 77.59 µg/kg (Smeu et al., 2020).

Table 2. Results of total aflatoxin assessment for maize from reception stage (ppb)

Specification		2020 Year						2021 Year					
		n	Positive (%)	x̄	s	Min.	Max.	n	Positive (%)	x̄	s	Min.	Max.
Maize reception stage	Jan.	105	79	2	0	2	2	117	56.1	1.7	3.9	0.1	34.8
	Feb.	136	75.7	2	0.5	1.7	7.5	189	66.1	1.1	0.8	0.1	4
	Mar.	88	78.4	2	0.3	2	4.5	128	59.3	1	0.8	0.1	3.3
	Apr.	23	65.2	2	0	2	2	86	54.6	0.9	0.6	0.1	2.9
	May	203	82.2	2	0.2	1	4.3	79	59.4	0.9	0.6	0.1	2.7
	June	111	76.5	1.8	0.5	0.1	4.5	143	57.3	0.8	0.6	0.1	4
	July	46	84.7	1.5	1.4	0.1	5.2	30	40	0.8	0.4	0.2	1.9
	Aug.	0	-	-	-	-	-	13	61.6	0.6	0.5	0.1	1.3
	Sept.	105	56.1	2.6	8.9	0.3	70.4	80	62.5	1.5	4.4	0.1	30.1
	Oct.	71	61.9	1.3	1	0.01	4.1	80	37.5	3.9	11.7	0.1	62.1
	Nov.	58	87.9	1.5	1	0.1	3.8	129	59.5	2	6	0.1	36.9
	Dec.	88	86.3	1.1	0.7	0.12	2.9	117	51.2	3	9.6	0.03	60.7

n - number of samples analysed; x̄ - mean; s - standard deviation; Min. - minimum value identified; Max. - maximum value identified.

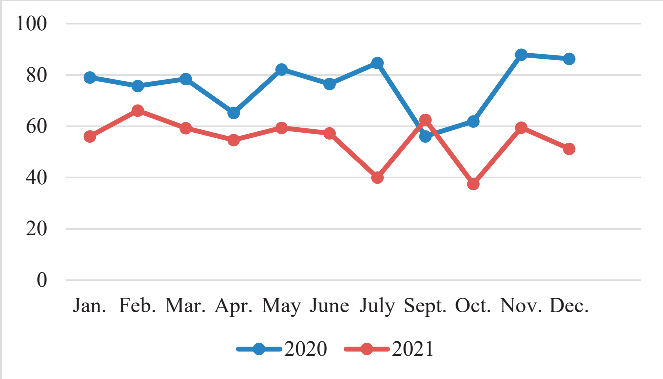

Figure 1. Overview of total aflatoxin positive maize samples depending on seasonality (%)

Table 3. Results of total aflatoxin assessment for maize from stock (ppb)

2020 Year						2021 Year					
n	**Positive (%)**	**x̄**	**s**	**Min.**	**Max.**	**n**	**Positive (%)**	**x̄**	**s**	**Min.**	**Max.**
48	56.2	1.4	0.7	0.14	2.5	43	65.1	0.9	0.5	0.09	2.32

CONCLUSIONS

The present study underscores maize as a potential source of aflatoxin exposure, both in maize itself and in compound feed. Our outcomes revealed that only a small fraction of the analyzed samples contained unsafe levels of aflatoxins. However, the percentage of total aflatoxins exceeding the threshold set by European regulations was 0.09% in 2020 (maximum 70.5 ppb) and 0.7% in 2021 (maximum 62.1 ppb).

It is important for feed mills to establish adequate control measures for mycotoxin contamination of the raw materials, in order to guarantee feed and food safety; considering that in our study, exceeding the limits of the allowed content in mycotoxins were identified at the reception, the maize batches were rejected and did not enter in production.

Understanding the seasonality of aflatoxin contamination is crucial for implementing effective mitigation strategies and ensuring food safety throughout the year. By identifying peak contamination seasons and addressing the underlying factors contributing to contamination, stakeholders can work towards minimizing the risk of aflatoxin exposure and protecting public health.

The application of every measure capable of preventing feed and food contamination, along with the potential adverse effects of toxins on human and animal health, as well as economic losses, is paramount for ensuring feed and food safety. Therefore, further research is imperative for feed mills to investigate aflatoxin and other mycotoxin contamination in maize.

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EFFECT OF DRIED PROBIOTIC ON LIVER GLYCOGENOLYSIS PATHWAY THE END OF THE FINISHER PHASE OF HEAT STRESSED LAYING HENS

Adriani LOVITA[1], Monica MARIN[2], Roostita BALIA[3], Tuti WIDJASTUTI[1], Andi MUSHAWWIR[1], Roxana STEFAN (VASILIU)[2]

[1]Animal Science Faculty, University of Padjadjaran, West Java, Indonesia
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[3]Veterinary Medicine Department, Faculty of Medicine, University of Padjadjaran, Indonesia

Corresponding author email: lovita@unpad.ac.id

Abstract

Homeothermic livestock such as laying hens have been widely developed and cultivated throughout Indonesia. This group of animals physiologically has a system that is able to maintain a normal body temperature range of 40-42°C. One of the strategies used to reduce the impact of heat stress is the provision of natural feed additives, namely probiotics containing a consortium of bacteria. An experiment was conducted on forty laying hens, which were kept in cages with heat stress. Probiotic feeding was done by dividing four groups of hens each. A probiotic level of 4% is the most effective level. This means that heat stress in laying hens can be overcome by giving probiotics. Probiotics have an important role in preventing changes in the osmotic pressure of body fluids, so that overall, they can overcome metabolic changes associated with heat stress.

Key words: *heat stress, laying hens, probiotics.*

INTRODUCTION

Indonesia is one of the countries with the highest layer population and egg production in ASEAN. In addition, the high population causes the demand for eggs to increase every year. The biggest challenge for layer productivity in Indonesia is the high ambient temperature (average from 28 to 34°C). Laying hens are able to express their genetic potential in a comfortable environmental zone or commonly called the *thermoneutral zone*, which is 21-25°C.

The impact of temperature has been widely reported by previous researchers. An increase in temperature up to 10°C from the normal threshold causes thermoregulation and hyperthermia in laying yams, characterized by an increase in muscle creatine and HSP expression, up to a 50% decrease in production and a decrease in shell thickness and an increase in the proportion of shell cracks (Mushawwir & Latipudin, 2011; Amin et al., 2014). Furthermore, it was also reported that this situation makes homeostasis very difficult to achieve, even with the provision of vitamins and low energy rations. It is more difficult to achieve optimum production, because in this situation, more energy is supplied to meet the needs of homeostasis processes (Al-Haidary et al., 2001). Many researchers have shown excessive concern in addressing the issue of energy balance under heat stress. The provision of energy through feed intake to help with thermoregulation, on the other hand, has an impact on the incidence of heat increment.

On the other hand, an enhanced risk of DNA mutation and denaturation of body proteins including hormone peptides, cell transporters, and enzymes, among others, is likely to be induced by excessive heat exposure. Other studies have shown that increased free radical production can be induced by heat stress and ultimately result in protein translational failure (Slimen et al., 2016; Mushawwir et al., 2021, 2024). This condition is directly related to metabolism, especially to the shock of energy production in mitochondria, as well as excessive redox activity.

This situation, described in the previous paragraph, causes metabolic regulation to meet energy, to undergo major changes and even accompanied by energy requirements to maintain these alternative mechanisms. The basic mechanism that animals can utilize is carbohydrate catabolism, through the breakdown of glycogen into glucose by the phosphorylation pathway. This catabolic reaction is known as glycogenolysis. Activation of adenylyl cyclase leads to the induction of protein kinase in catalyzing glycogen into glucose and pyruvate (Peace et al., 2013; Mushawwir et al., 2021) either with the help of oxygen (aerobic glycolysis), or without oxygen (anaerobic glycolysis).

An approach presented in this paper to reduce the impact of heat stress is to provide *feed additive*, namely probiotics containing bacterial consortia (Adriani et al., 2023; 2024). Probiotics are non-pathogenic bacteria that have active peptides and active compounds (Adriani et al., 2015; 2020; 2021; Antosiewicz et al., 2006; Tanuwiria et al., 2022). Probiotics in the ration are expected to be able to create better ecological conditions for digestion, especially in the ileum. The microbial consortium should be able to contribute positively to the physiological and biochemical conditions, both of the villi and metabolism as a whole. Improvements in the condition of the villi and metabolism should be able to have a positive impact on the ability of layers in the final phase of production to be able to cope with the heat exposure experienced by reducing the rate of glycogenolysis.

MATERIALS AND METHODS

Animal Samples

In this study, a sample of 70-week-old laying hens, totaling 40 layers, was used. Experiments with the administration of dry probiotics at the Poultry Experimental Center, Faculty of Animal Science, University of Padjadjaran. Chicken samples were placed in individual boxes with battery cages, respectively. These battery cages were placed in a semi-close house system. Each chicken sample received basal feed, with a crude protein content of 20.15%, metabolic energy of 2931 Kcal/kg feed. The microclimate of the housing was recorded during the study with the average temperature and humidity ranging from 37-38.5°C and 82-87%, respectively.

Experimental Design

A completely randomized design (CRD) was used in this experiment. The experimental chickens were distributed into four experimental groups (each consisting of ten sample chickens), namely without and with the administration of dry probiotics. Each experimental unit consisted of 1 laying hen. Treatment was applied from the start of the research. The four treatments applied were P0: Basal diet (BR) and no dry probiotics, and no heat stress; P1: BR contains dry probiotics (DP) 2% of the total ration, with a housing temperature of 38°C; P2: BR contains DP 3% of the total ration, with a cage temperature of 38°C; P3: BR contains DP 4% of the total ration, with a cage temperature of 38°C.

Experiment Protocols

Blood Sample Collected

Glycogenolysis rate analysis was performed by measuring metabolites in blood plasma. Blood samples were collected after 40 days of dry probiotic administration. A sterile thermo-branded syringe was used to draw 3 ml of blood through the flank vein (external pectoralis vein). The blood sample was placed in a sterile thermo-branded EDTA tube, with a gentle swing, the EDTA tube was shaken for approximately 30 seconds. The whole sample was placed in a cool box to prevent damage and blood clotting.

Sample Analysis

The external pectoralis vein is a vein that has been used as a vascular source of blood. Forty 3 mL syringes and 3 mL EDTA-coagulated venojecte tubes, respectively, were used to collect blood samples. The entire process was done carefully and sterile. Blood plasma was obtained by centrifuging each sample at 3500 rpm for 15 minutes. Plasma was removed from the blood cell solids, and then each sample was put into a 3 mL append of tube.

Plasma was used for the analysis of glycogen level and enzyme concentration. Spectrophotometric methods with various types of equipment have been used to examine blood samples. All analyzes were carried out based on analytical techniques based on the instructions in KIT Biolabo and KIT Rendox, following previous research protocols.

Statistical Analysis

Regarding the statistical analysis of the experimental results, the ANOVA (one-way analysis of variance) technique based on a one-way model with high homogeneity, and a significance degree of 95%, was applied in this study to evaluate the effect of probiotics.

All analyses were conducted following the analysis protocol by General Linear Models (GLM) of SAS Version 8.216, for a completely randomized design.

Differences between experimental groups were justified based on Duncan's test (SAS Institute, 2001).

RESULTS AND DISCUSSIONS

The impact of probiotics in the diet on the rate of glycogenolysis of laying hens reared in microclimatic conditions of cages in comfort zone bags, based on the current experiment is shown in Table 1. The average glycogen level of the experimental group of chickens without probiotics and placed in cages with temperatures above the comfort zone was 0.82 mg/dL (Table 1). Glycogen levels in this group appeared to be lower ($P<0.05$) compared to the experimental group of chickens given probiotics with the same heat exposure.

Table 1. The impact of probiotic administration on metabolite concentrations related to glycogenolysis in laying hens

Metabolites	Dried Probiotics			
	PO	P1	P2	P3
Glycogen (mg/dL)	0.82±0.12[a]	1.02±0.11[a]	1.29±0.13[b]	1.41±0.12[c]
Glycogen Phosphorylase (IU/dL)	0.22±0,01[ab]	0.21±0,01[b]	0.17±0,01[bc]	0.16±0.01[c]
Phosphoglucomutase (IU/dL)	0.39±0.02[a]	0.18±0.03[b]	0.10±0.02[c]	0.05±0.02[d]
Glucose 6-Phosphate (mg/dL)	0.27±0.01[a]	0.23±0.01[a]	0.13±0.01[b]	0.08±0.01[b]
Glucose 1-Phosphate (IU/dL)	0.37±0,01[b]	0.19±0.01[b]	0.14±0.02[c]	0.07±0.01[d]
Glucose 6-Phosphate (IU/dL)	0.27±0.01[a]	0.17±0.01[a]	0.11±0.02[b]	0,07±0.01[b]

Glycogen levels were higher with increasing levels of probiotics in groups P1, P2 and P3, at 1.02 mg/dL; 1.29 and 1.41 mg/dL, respectively. Investigations of laying hens maintained in temperature conditions above their comfort zone resulted in an increased energy requirement.

In an effort to reduce this impact, glycogen catabolism is a good alternative. The effectiveness of probiotic administration was seen in the group of chickens given probiotics as much as 4%. The decrease in plasma glycogen levels in this study, illustrates an indication that the heat stress exposed to the research chickens, urges the need for alternative energy availability for homeostasis (Al-Haidary et al., 2001; Latipudin & Mushawwir, 2010; 2011), as well as maintaining physiological stability (Slimen et al., 2016), increased glucose synthesis (Amin et al., 2014; Ao et al., 2010; Mushawwir et al., 2011; Rahmania et al., 2022).

Based on the results shown in Table 1, it can be explained that heat stress increases the metabolic rate mainly to provide energy related to the homeostasis process. This condition is characterized by enzyme activity related to the glycogenolysis pathway that increases in the treatment group without probiotics, then the effect of heat decreases on the activity of glycogenolysis with the administration of probiotics.

Homeostasis increases under heat stress (Aritonang et al., 2024), aiming to maintain biochemical and physiological processes for survival (Muhammad et al., 2023; Kharazi et al., 2022) and reproduction (Nelson et al., 2008; Loyau et al., 2014). Heat radiation to the internal milieu of livestock instinctively causes livestock to reduce their *feed intake* or ration consumption. This decrease aims to prevent heat from food digestion (*heat increment*) in the gut (Kumalasari et al., 2023; Ao et al., 2010) as well as metabolic heat (Mushawwir et al., 2018; 2020) and increased free radicals (Royer et al., 2016; Tanuwiria et al., 2011; Mushawwir et al., 2010; Mushawwir et al., 2011; Mushawwir et al., 2020). Animal activate the glucogenolysis mechanism to compensate for the decrease in feed intake. This is triggered by an increase in neural stimulants through neurotransmitters (Royer et al., 2016; Adriani et al., 2015), thus increasing levels of the hormone epinephrine (Slimen et al., 2016).

Probiotics were effective in reducing the rate of glycogenolysis under heat stress. The results (Table 1) showed that probiotics decreased ($P<0.05$) the intermediate metabolite compounds in the breakdown of glycogen to glucose, as well as the enzymes that catalyze the breakdown reactions.

The effectiveness of probiotic utilization appeared to be optimum at 4% (P3). The optimization of the role of probiotics in reducing the rate of glycogenolysis is shown by the glycogen levels of laying hens under heat stress conditions without probiotics, significantly different ($P < 0.05$) from the glycogen levels of laying hens experiencing heat stress but given 2-4% probiotics (P1-P3). This optimization was also shown by the levels of catalyzing enzymes and intermediate metabolite compounds, which showed differences with the experimental hen groups in heat stress, without and with probiotic feeding.

Overall, probiotics are effective in reducing the rate of glycogenolysis, suggesting that probiotics can improve metabolic balance (Aritonang et al., 2024; Al-Haidary et al., 2001; Mushawwir et al., 2018) related to heat regulation and homeostasis (Slimen et al., 2016; 2016). Continuous heat stress in high conditions causes *panting* behavior to evaporate body heat. *Panting* is the behavior of releasing heat through breathing by panting (breathing fast and short) or hyperventilating. This results in an increase in blood pH (Mushawwir et el., 2010; Adriani et al., 2021; 2023) compared to quail that are not exposed to excessive heat.

One important factor that plays a role in blood acidity is environmental temperature. The effect of high ambient temperature causes *panting* behavior. Through this behavior, the release of H_2O and CO_2 compounds becomes excessive (Mushawwir & Latipudin, 2011; Royer et al., 2016), causing the formation of bicarbonate (H_2CO_3) to decrease (Adriani et al., 2024; Mushawwir et al., 2024). Bicarbonate is an H^+ proton donor and forms carbonic acid (HCO_3^-). The ability of active peptides in probiotics with a feeding level of 2-4% to mitigate the effects of heat on the metabolic system of laying hens, confirms that probiotics have the ability to bind to proteins especially at the H atom of proteins, causing reduced protein denaturation (Pearce et al., 2013; Ao et al., 2010; Kharazi et al., 2022; Rahmania et al., 2022). This means reducing cell death and maintaining protein function (Royer et al., 2016; Adriani et al., 2024; Mushawwir et al., 2024). Both positive effects are simultaneously able to maintain proteins in the erythropoesis system and blood cell proteins (erythrocytes and leukocytes). The results of research by Pearce et al. (2013), showed the role of probiotic active compounds in maintaining blood precursor proteins from damage due to reactive compounds (ROS) and cellular level energy stress.

The role of active peptides in probiotics in controlling and overcoming heat stress is related to their ability to increase reaction kinetics with H_2O. The high binding energy of active peptides with H_2O makes it difficult to be evaporated and excreted through the kidneys, resulting in a decrease in body fluid loss. In addition, biomolecules present in blood plasma that are amphiphilic in nature lead to the formation of very favorable interactions with active compound carrying charged S and O atoms. This increases the pattern of electrostatic interactions. Mushawwir et al. (2018) state that these electrostatic interactions are able to maintain the structure of proteins, carbohydrates, and lipids to which they bind. Indirectly, it reduces the risk of heat-induced damage to biomolecules, and increases the capability of body fluids to retain heat.

In addition, the ability of the active peptides in these probiotics, either by enhancing the reaction kinetics with H_2O or by their electrostatic interaction patterns, both have an impact on heat stress management through enhanced body fluid adaptation (Royer et al., 2016). It directly impacts on increasing the retention of body fluid cations, especially Na^+ so as to maintain the osmotic pressure of body fluids. Good body fluid adaptation also maintains water retention so that extracellular fluid volume can be maintained.

CONCLUSIONS

The probiotic level of 4% was the most effective, so this means that heat stress in laying hens can be overcome by probiotics. Probiotics have an important role in preventing changes in the osmotic pressure of body fluids, so that overall it is able to cope with metabolic changes related to heat stress.

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ASSESSING OF THE IMPACT OF HEMPSEED *JUBILEU* VARIETY ON PIG GROWTH PERFORMANCE, N METABOLISM, AND N_2O PREDICTION

Gabriel MIHĂILĂ[1], Mihaela HĂBEANU[2], Anca GHEORGHE[2], Nicoleta LEFTER[3], Livia VIDU[1], Carmen Georgeta NICOLAE[1], Monica MARIN[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]Research Station for Sericulture Baneasa, Bucharest, 013685, Romania
[3]Animal Nutrition and Biotechnology Department, National Research-Development Institute for Biology and Animal Nutrition, Calea Bucuresti No. 1, Balotesti, 077015, Ilfov, Romania

Corresponding author email: mihaela.habeanu@scsbaneasa.ro

Abstract

Hempseed has an excellent antioxidant content and is a rich source of essential amino acids and n-3 fatty acids. The objective of this paper consists on evaluating the hempseed Jubileu variety effects on performance, nitrogen (N) balance. The prediction of nitrous oxide (N_2O) emissions was done as well. For 3 weeks of trial, a total of 15 pigs (39 kg ± 0.32) were randomly allocated individually in digestibility cages. Following a 7-day adaption period, there were two balancing periods where urine and feces were quantitatively collected. Two types of diet were used: Control group received a classical diet and Experimental group which received the same diet, supplemented with 5% hempseed. The growth parameter and carcass quality were not significantly altered by hempseed addition; however, a slight increased of ADG and feed conversion was noticed. Due to the significantly decreased of N concentration in the urine, TNO value was lower as well ($P<0.05$). A decline of N digestibility was noticed for HS group diet ($P<0.001$). Average value of N2O estimated was lower in HS fed group ($P = 0.03$). In conclusion, hempseed is a proteo-oleaginous source, which can be added in pigs' diet with beneficial effect on performance, N metabolism indicators and N_2O emissions.

Key words: *digestibility, hempseed, nitrogen (N), nitrous oxide (N_2O), pigs.*

INTRODUCTION

A vital nutrient for pigs is nitrogen (N), required in very large quantities throughout the pig's life cycle, mainly for the synthesis of nucleotides, but also as a structural element of proteins. N is involved in many metabolic processes, but it is one of the most expensive nutrients for pigs and can have a negative impact on the environment. A large amount of ingested nitrogen is eliminated through excretion (2/3 of ingested N) (Millet et al., 2018, Hăbeanu et al., 2020), in the form of nitrous oxide (N_2O) and ammonia (NH_3), compounds associated with a negative impact on the environment. The global warming potential of N_2O is 298 times higher than that of carbon dioxide (CO_2). N_2O is formed as a result of nitrification and denitrification processes.

As a result of drought and other less controllable phenomena, the accessibility and price of some fodder resources changes, and implicitly there is the need to evaluate the nutritional, productive potential and the impact on the environment of any plant resources and/or by-products available (Dmytrotsa et al., 2023).

Current approaches have suggested that oil-rich forages and their by-products containing poly-unsaturated fatty acids (PUFA) in a high concentration could decrease enteric CH_4 generation in ruminants (Ebeid et al., 2020) and monogastrics (Hăbeanu et al., 2022). However, adding oil to feed also reduced dry matter intake, although this strategy can be relatively expensive. Nutritional solutions have been identified and developed for the effective utilization of some less studied plant resources to complement or replace part of the classic pig resources (corn and soybean meal). For example, in pigs, mustard cakes associated with those from grape seeds were tested (Hăbeanu et al., 2022), peas, guar flour (Mihăilă et al., 2023; Hăbeanu et al. 2020) They were established as potential predictors of CH_4 enteric volatile fatty acids that are in close correlation with the fiber level of the food and related to it with the microbial population (Hăbeanu et al., 2022). An increased level of fiber through the addition of a mixture of grape cakes and mustard, can lead to a slight decline in performance, but at the environmental level the effects are positive, in the sense that the amount of excreted nitrogen is reduced (by approx. 6% per the background of the higher level of fiber and its fractions). The same trend was observed regarding N_2O which decreases by approx. 5.5% in animals fed with mustard cakes (Hăbeanu et al., 2021). In the same species, although peas and linseed have valuable nutritional potential and can replace part of soybean meal, due to a higher intake of some carbohydrate fractions, a slight decline in growth performance is reached. However, the mixture between peas and flax seeds contributed to its cancellation (Hăbeanu et al., 2020). Based on the above, it can be hypothesized that feed with the addition of oleo-proteins and by-products with a different structure can represent an effective method of reducing excreted nitrogen with implicit effects on the environment.

Prohibited for a long time, but re-included According to the EFSA Regulation (2015) in the list of feed raw materials that can be used in animal feed, hemp returns to the center of attention of specialists due to its nutritional composition of interest for animal husbandry. Data in the scientific literature are lacking or incomplete regarding the impact hemp seed has on pig performance and emissions.

Hemp is one of the oldest industrial plants. The *Jubileu* hemp variety was approved in 2012. Hemp seeds contain approximately 30% oil, and 80% of this oil is made up of unsaturated essential fatty acids, which are not synthesized by the body, and 31% easily digestible proteins that can supplement or replace other protein sources. Hemp seed protein contains all 21 known amino acids, including the 8 essential amino acids that the body cannot produce, namely leucine, lysine, methionine, phenylalanine, tryptophan, valine and threonine. According to Bälter et al. (2017), feed ingredients of animal origin emit more GHGs than raw materials of plant origin, it seems that sows have an even greater impact on GHG amounts, especially enteric CH_4 (E-CH4) and from manure M-CH_4 and N_2O (House, 2010; Hăbeanu et al., 2021).

Reducing GHG emissions through pig feeding and manure management remains a major issue for intensive pig farmers, as the microbial process of N cycling and manure C content, as well as storage and treatment time, determine the amount of N_2O emitted during storage (House, 2010; Bälter, 2017).

This study was conducted on pigs to evaluate the effects of *Jubileu* hemp seed on performance and nitrogen metabolism and N_2O prediction based on indicators derived from nitrogen balance experiments.

MATERIALS AND METHODS

1. Ethical procedure

The balance experiment of N was carried out at INCDBNA Balotești, and was carried out in accordance with the experimental protocol authorized by the Institute's Ethics Committee and in compliance with the provisions of Law no. 199/2018 and from EU Directive 2010/63/EU on animal research. *Jubileu* hemp seeds were supplied by SCDA Secuieni and are characterized by a high nutritional value and a similar cost compared to other protein sources.

2. Experimental scheme and treatment

The nitrogen balance experiment was carried out for 3 weeks (1 week of acclimatization and 2 weeks of balance) on a number of 10 Topigs hybrid male pigs [♀ Large White x Hybride (Large White x Pietrain) ♂ × Talent, mainly Duroc] growing - fattening. The mean initial weight was 39.65 kg ± 0.53. Animals were randomly assigned to two groups (Control and experimental), each with five repetitions. The weight of the animals was recorded at the beginning and at the end of the experiment. Individual metabolism cages (1.2 × 1.5 × 1 m) were used. The temperature during the experiment was 22 ± 1°C.

Feed was given ad libitum twice a day (08.00 h and 15.00 h) and water *ad libitum*.

The hemp, the *Jubileu* variety, was supplied by SCD Secuieni. To evaluate growth performance, nitrogen balance and N_2O prediction, *Jubileu* hemp seeds were subjected to laboratory analysis for the determination of crude protein (CP), ether extract (EE), fiber (CF), amino acids and minerals. The composition and nutritional characteristics of the *Jubileu* hemp seeds used in compound feed recipes administered to pigs are shown in Table 1.

Table 1. Nutritional characteristics of *Jubileu* hemp seeds

Jubileu* hemp seeds	**(%)**
C18:3n-3	17.06
Ratio n-6:n-3	3.20
Dry matter (DM)	89.67
Protein (CP)	21.26
Ether extract (EE)	27.70
Fiber	28.82

*C18:3n-3 – α-linolenic polyunsaturated fatty acid

The composition and nutritional characteristics of the compound feed recipes used in the feed of the control group and the experimental group with an intake of 5% *Jubileu* hemp seeds (HS) are presented in Table 2.

Table 2. Structure of compound feed recipes for grow - fattening pigs (%)

Ingredients (%)	Control group (C)	Experimental group (HS)
Maize	62.9	60.02
Rice flour	10	10
Hemp *(Jubileu)*	0	5
Soybean meal (44%)	15	12
Sunflower meal	8	9
DL-methionine	0.01	0.01
L-Lysine-HCl	0.28	0.32
Calcium carbonate	1.65	1.7
Monocalcium phosphate	0.65	0.45
Salt	0.4	0.4
Choline premix	0.1	0.1
Vitamin and trace mineral premix (P3+4)**	1	1
Nutritional characteristics (g x kg^{-1} as feed bases)		
DM	87.80	87.99
Metabolizable energy (ME, Mj x kg^{-1}))	13.64	13.78
Crude protein (CP)	15.21	15.12
Ether extract (EE)	3.46	4.78
Fiber	4.05	4.72
Lys	0.91	0.91
Lys d	0.81	0.82
Met +Cys	0.57	0.57
Met +Cys d	0.51	0.51
Calcium (Ca)	0.89	0.87
Phosphorus (P)	0.51	0.50

*Abbreviation: dry matter (DM), lysine (Lys), methionine + cysteine (Met + Cys), digestible (d).
**The vitamin-mineral premix introduced in the compound feed is specific for the age and weight category and provided per kg of feed: 6000 IU vitamin A; 800 IU vitamin D3; 20 IU vitamin E; 1 mg vitamin K3; 1 mg vitamin B1; 3.04 mg vitamin B2; 10 mg vitamin B3; 6.3 mg vitamin B5; 1.5 mg vitamin B6; 0.03 mg vitamin B7; 0.3 mg vitamin B9; 0.02 mg vitamin B12; 30 mg Mn; 80 mg Fe; 25 mg Cu; 100 mg Zn; 0.22 mg I; 0.22 mg Se; 0.3 mg Co; 60 mg antioxidant.

The compound feed recipes contain the energy and protein requirements related to the growth category. *Jubileu* hemp seeds come with an increased intake of CP. To obtain the same level of CP, the level of sunflower meal was slightly increased and the level of soybean meal decreased. To ensure an optimal level of ME, maize adjustments were made at different levels. The crystalline amino acids DL-methionine and L-lysine-HCl were included in the developed recipes to cover the requirements for the two recipes, as well as calcium carbonate and monocalcium phosphate to provide Ca and P requirements.
The level of fiber was higher in the diet of the experimental group (HS) compared to the diet of the control group (C). Food was provided twice a day at 08:00 and 14:00 and water was available *ad libitum*.

3. Measurement and sampling

3.1. Growth performance and carcass quality

The body weight (BW) of the pigs was recorded on an electronic scale at the beginning and end of the digestibility test. Average daily gain (ADG) and feed conversion ratio (FCR) were calculated based on BW (Broucek, 2017), equations used to calculate Kleiber ratio (KR), relative growth rate (RGR, %) based on ADG and Metabolic BW ($MBW^{0.75}$) on the one hand and BW and age on the other.
Carcass characteristics (backfat thickness, *Longissimus dorsi* area and lean meat percentage) were determined on the left side using the PIGLOG 105 ultrasound machine (SFK-Technology, Denmark) equipped with a meat percentage evaluation formula: Y= 64.39-0.28 Fat-1+0.14 LD muscle thickness-0.55 Fat-2, where LD is the *Longissimus dorsi* muscle. Fat-1 was measured 7 cm laterally behind the last rib from the middle dorsal line, while fat-2 was measured 10 cm from the last rib to the cranial section and 7 cm lateral to the middle dorsal line.

3.2. Nitrogen balance

During the two periods of nitrogen balance (4 days per week), urine and faeces samples were collected, at 08.00 h-08.30 h. Fecal samples collected from each animal were mixed and homogenized for each batch, and 10% of the amount obtained was frozen at -18°C for analysis (according to the experimental protocol described by Hăbeanu et al. (2019). The volume of urine was recorded daily and 10% was separated and stored at -18°C for analysis. To lower the pH and conserve nitrogen, H_2SO_4 25% concentration was used in each urine container. Pig faecal samples of 0.4 g were weighed with an accuracy of $\pm 2 \times 10^{-4}$ g.

3.3. Chemical analyses

The chemical composition of *Jubileu* hemp seeds, of NC administered during the experiment, was determined in the Chemistry Laboratory of IBNA Balotești. The analyzed samples were collected in duplicate, for determinations of DM, CP, EE, crude fiber (CF), in accordance with the recommendations of Commission Regulation (EC) no. 152 (JOUE, 2009). The traditional semi-automatic Kjeldahl technique using Kjeltek auto 1030 – Tecator (Standard SR EN ISO 5983-2:2009 AOAC 2001.11) was used for CP determination. CF extraction was performed using an intermediate filtration method (Standard SR EN ISO 6865:2002).

The semi-automatic Kjeldahl method (Kjeltec Auto 1030 Analyzer, Hillerod, Denmark) was used to determine the N content of the excreta. The samples were subjected to distillation and titration. Class A glassware was used for decanting, dilution and storage. All reagents used were supplied by Merck (Darmstadt, Germany). Stock solutions traceable to the National Institute of Standards and Technology (NIST) standard reference material (SRM) were used for calibration. Nitrogen retained (NR), total nitrogen output (TNO) and nitrogen digestibility were determined by measuring N intake (based on DM) and N excretion according to the methods described by Hăbeanu et al. (2019), using the equations described by Hlatini et al. (2020).

Determinations of fatty acids were performed from the fodder samples: CI4:0 (myristic); CI6:0 (palmitic); CI8:0 (stearic); CI8:1 cis-9 (oleic); CI8:2n-6 (linoleic); CI8:3n-3 (α-linoleic); CI8:4n-3 (octadecatetraenoic); C2O:4n-6 (arachidonic) and C22:I-9 (uric);

All determinations were performed in duplicate by high-performance liquid chromatography using a Surveyor Plus Thermo Electron HPLC (Waltham, MA, USA) equipped with a HyperSil BDS C18 silica column (Thermo Electron, Waltham, MA, USA). Dimensions: 250 mm × 4.6 mm × 5 µm, according to the methods described in the research papers by Vărzaru et al. (2013), Hăbeanu et al. (2019), and Gheorghe et al. (2020).

3.4. Calculations

3.4.1. The coefficient of total tract apparent (CTTAD), coefficient of apparent metabolizability (CAM), biological value of protein (BVP) and net protein utilization (NPU) were calculated using the following equations:

CTTAD = (N intake – faecal N output)/N intake (1)

CAM = N intake − N fecal output − N urinary output/N intake (2)

BVP = N retained/N digested (3)

NPU = N retained/ Nintake (4)

3.4.2. Prediction of N_2O emission

Equations based on input data (recorded at the beginning of the experiment) and output data recorded at the end of the experiment were used to predict N_2O. The equations have been established in IPCC (2006, 2019) and earlier work. The TNO assessed in our study was integrated into the N_2O prediction equation given by Philippe & Nick (2014): N_2O = TNO x 0.2/100 x 44/28. The conversion factor of excreted N was 0.2 to N_2O (IPCC, 2006), for manure storage spaces under animal housing and 44/28 is the ratio between the molar mass of N_2O and that of N. The N_2O value of 298 - times greater global warming potential than CO_2 was shown in Eq-CO_2.

3.4.3. Statistical analyses

IBM SPSS (2011, version 20) is the statistical program for the analysis of recorded experimental data. Animals were assigned to two experimental groups in a completely randomized experimental design.

ANOVA test was used to check the significance of the data. The impact of diet is considered statistically significant if the P value is less than 0.05. Repetition values were not included in the study if the P value was greater than 0.05. The Pearson test was used to determine and measure correlation.

EvaPig® software, version 2.0.3.2 (2020), created by the French National Institute of Agricultural Research, Metex Nvistago and the

French Association of Animal Husbandry was used to develop compound feed recipes.

RESULTS AND DISCUSSIONS

1. Chemical composition of the analyzed feed ingredients

Following the laboratory determinations regarding the chemical composition of the feed ingredients analyzed (Table 3), *Jubileu* hemp seeds stand out for their high protein content - CP analyzed (21.26%), representing almost half the value of soybean meal, EE (27.70%) was higher than in the meal due to the fact that the fat is extracted from the meal, and the concentration in essential amino acids for pigs was 0.959% Lyz., 0.832% Met., 0.347% Cys.

Although lysine is at a lower level compared to that of soybean meal, which determined the addition of synthetic lysine to the feed at a level 1.14 times higher than in the control group, Met + Cys have close average values in the two ingredients.

However, the level of lysine, but also of Met + Cys, in *Jubileu* hemp is higher than that reported by House et al. (2010), respectively an average of 0.860 for Lys and 0.97 for Met + Cys.

Table 3. Comparative chemical composition of soybean meal and hemp seed

Specification (%)	Soybean meal	*Jubileu* hemp
Composition		
DM	87.74	89.67
CP	44.0	21.26
EE	1.69	27.70
Fiber	6.29	28.82
Amino acids (AA)		
Lysine	2.75	0.959
Methionine	0.64	0.832
Cysteine	0.67	0.347
Met. + Cyst.	1.31	1.179
Minerals		
Ca	0.20	0.09
P	0.60	0.93

2. Growth performance and carcass quality

The mean values and standard error of the mean (SEM) of the results obtained by determinations on animals regarding growth performance are presented in Table 4, and carcass quality is presented in Figure 1.

Table 4. Mean and SEM of consumption and performance for growing-fattening pigs

Specification*	Control group	Experimental group	SEM	P**
Consumption of compound feed (kg/day)	2.77	2.51	0.06	0.30
Consumption of DM (kg/day)	2.43	2.21	0.06	0.30
IBW (kg)	40.10	37.30	0.24	0.60
FBW (kg)	67.0	65.6	0.78	0.16
Average daily gain (ADG) (kg/day)	1.05	1.12	0.03	0.10
Kleiber ratio	5.28	5.78	0.09	0.08
Feed conversion ratio (FCR)	0.38	0.45	0.01	2.77
CAM	0.46	0.44	0	0.9
CTTD	0.88	0.86	0	0.88

*Abbreviation: dry matter (DM); initial body weight (IBW); final body weight (FBW); coefficient of apparent metabolizability (CAM); coefficient of total tract apparent (CTTAD). Feed conversion ratio was calculated as the ratio of ADG to the daily consumption of compound feed.
**P>0.05 insignificant differences.

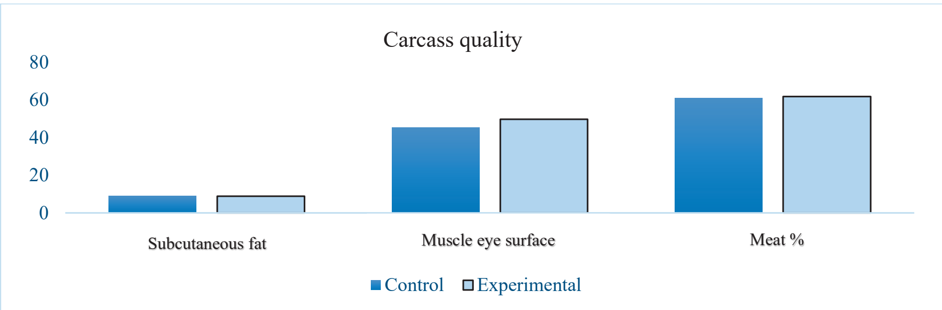

Figure 1. Means and SEM of carcass parameters as effect of 5% hemp seed addition; P>0.05 non-significant differences between batches. To determine carcass traits, measurements were performed on live animals with the Pig log 105 apparatus

As the obtained data show, body weight, average daily gain, food consumption and feed conversion ratio were not significantly affected in the experimental group, which received the addition of hemp seeds in the food. On the contrary, the average daily gain was 6% higher in the case of the experimental group, and the feed conversion increased by 18.42%. Coefficient of apparent metabolizability (CAM) and coefficient of total tract apparent (CTTD) were similar (P>0.05).

Subcutaneous fat in the experimental group was 3.27% lower compared to the control group, and the muscle eye surface in the experimental group was 9.73% higher compared to the control group, but without the differences between the means being statistically significantly different. The same aspect was observed in the case of the percentage of meat, i.e. a higher share of it by 1.49% in the experimental group.

3. Nitrogen balance and N_2O prediction

3.1. Nitrogen metabolism

Table 5 shows the mean experimental data and SEM for the nitrogen balance indicators, as well as the value estimated by equations for predicting the amount of N_2O.

A significant decrease in urinary nitrogen by 12.49% was observed, which led to a decrease in total nitrogen output (TNO) content by 10.86% for pigs fed with the addition of 5% *Jubileu* hemp seeds in the feed (P<0.05). There was a decrease in the percentage of N retained (NR) by 10.12% (P = 0.016), due to a lower N digestibility coefficient by 1.39% in pigs from the experimental group. The net protein utilization (NPU) value was similar.

As a result of the correlation between the balance parameters and N_2O, a significant reduction of the latter was recorded by 10.81%, although, if we refer to ingested N, ingested DM and the average daily gain register an insignificant increase.

3.2. Correlations

To establish the influence of various dietary factors on N metabolism parameters, the Pearson correlation shown in Table 6 was estimated. As expected, DM and N consumption have a close positive correlation with TNO, NR and implicitly with N_2O (P<0.0001). Average daily gain is not correlated with N and its derivatives resulting from metabolic processes.

Table 5. Mean and SEM values for nitrogen balance indicators and N_2O prediction

Specification*	Control group	Experimental group	SEM	P**
N balance				
Urinary N (g/day)	28.45	25.29	0.70	0.02
Fecal N (g/day)	7.63	7.27	0.12	0.13
TNO (g/day)	36.08	32.56	0.82	0.03
NR (g/day)	31.22	28.17	0.64	0.016
N digestibility (%)	88.58	87.37	0.16	<0.001
NPU (g/day)	46.45	46.46	0	0.9
N_2O prediction				
N_2O (g CO_2 Eq/day)	33.80	30.50	0.76	0.031
N_2O / ingested N (g CO_2 Eq/day)	0.59	0.64	0.01	0.11
N_2O /DMI (g CO_2 Eq/day)	16.49	17.71	0.46	0.14
N_2O/ ADG (g CO_2 Eq/day)	38.59	35.02	0.10	0.10

*TNO - total nitrogen output; nitrogen retained (NR), NPU - net protein utilization
**P>0.05 insignificant differences; P<0.001 very significant differences

Table 6. Pearson correlation between input data and indicators of nitrogen metabolism

Specification*		DM consumption (kg/day)	N consumption (g/head/day)	AVG (g/day)	N_2O (g CO_2 Eq/day)
TNO	R	1	1	-0.002	0.632
	P	<0.0001	<0.0001	0.990	<0.0001
NR	R	0.99	1	-0.011	0.620
	P	<0.0001	<0.0001	0.941	<0.0001
N_2O	R	0.632	0.631	0.119	1
	P	<0.0001	<0.0001	0.409	

*r= correlation
P>0.05 insignificant differences; P<0.001 very significant differences.

Discussions

Nutritional options are evaluated as tools to effectively reduce pollutant emissions. Respecting the nutritional requirements of the animals, using balanced feed recipes against the background of the use of lesser-known feed resources, can contribute to maintaining performance. Through this study, we aimed to deepen the knowledge regarding the effects of the addition of *Jubileu* hemp seed improvement in the feed of growing-fattening pigs on growth performance and nitrogen metabolism.

Hemp is a variety of *Cannabis sativa*, attested since ancient times on the territory of our country. Hemp culture is profitable because it is exploited in multiple ways (textiles, seeds and meals, oils). Hemp seeds contain approximately 30% oil, characterized by a valuable profile in n-3 FA, antioxidant properties and 31% easily digestible proteins (Hăbeanu et al., 2018). Hemp protein has high biological value due to its excellent amino acid profile.

As mentioned by Chen et al. (2023), hemp seed protein is becoming an important alternative source of vegetable proteins in food and as nutraceuticals in industry for its high nutritional values, processing ability and as a homologue with other medicinal plants. With changing dietary habits and increasing demand for natural food ingredients, the need to use hemp seed protein is expected to grow rapidly. However, to fully utilize the competitive advantages of hemp seed protein a number of key issues must be resolved for its promotion, namely: availability and price, primary and secondary effects on animal and human health, anti-nutritional factors, also active biocompounds present in this resource that could diversify the possibilities of use and, last but not least, the quantification of the effects on the environment through its use in animal feed.

Jubileu hemp is an improved variety obtained at SCDA Secuieni, Romania, monoecious, approved for seed and oil.

In the literature, to the best of our knowledge, there is no information on the effects of hemp seeds on N_2O emission. The high level of essential fatty acids for health, especially n-3, was an argument used in most studies to evaluate the influence of hemp (seeds or meal, oil) on meat quality or immune response and oxidative stress (Vodolazska & Lauridsen, 2020; Hăbeanu et al., 2018a,b; Palade et al., 2019; Li et al., 2022). Our previous studies considered the impact on N and its derivatives by using as feed a mixture between mustard cakes and grape cakes (Hăbeanu et al., 2021), or *Tudor* pea grains compared with *Lirina* flax and soybean meal (Hăbeanu et al., 2020) or of guar flour (Mihăilă et al., 2023). In 2010, House and in 2011, Presto et al. determined the digestibility of hemp protein and amino acids, demonstrating that hemp seed protein has highly digestible value either in its native form or as hemp seed meal, which these are also confirmed in our study.

According to the studies conducted by Ahmed et al. (2022), hemp proves competence in the search for new sustainable resources because it is naturally resistant to diseases and pests, conserves water, degrades quickly and produces environmentally friendly industrial products such as biodiesel, bio-composite, paper, textile, and so on. Hemp biofuel could be an excellent alternative to petroleum-based fuel to produce heat and power for transportation and industrial sectors. Hemp would break new ground by harnessing it in the paper industry using its advantage of higher yield and more recyclability of hemp paper than wood.

Lanzoni et al. (2024) showed that the inclusion of hemp-based products in the diet of monogastric animals led to different results depending on the species. The use of these products requires further investigation, particularly in pigs and broilers, due to limited studies. However, for laying hens, for example, the greater number of scientific studies have made it possible to identify and confirm hemp as an effective and safe source, capable of positively modulating animal health and performance, simultaneously enhancing the nutritional and functional profile of eggs. However, the inclusion of hemp in food requires further evaluation. Practically, in the literature there were no data regarding the impact of hemp on GHG emissions, with an emphasis on N_2O.

In 2018, GHG emissions from agriculture amounted to 17.5 million tonnes of CO_2 equivalent, representing 3.9% of the total EU GHG emissions from agriculture. Total GHG emissions generated by agriculture decreased between 1990 and 2018 by 44.3% (-29.3% in the EU) and ammonia emissions decreased (by

4%36) in the same period, this justifies due to the reduction of livestock numbers, but also due to investments in new technologies used in animal husbandry.

In 2016, methane (CH_4) and N_2O emissions reported per hectare (exploited agricultural area) were 1.29 kilotons CO_2 equivalent/1 000 ha, being among the lowest in the EU. Nitrogen management is an important part of the activities carried out on livestock farms and an important part of the process of estimating and reducing pollutant emissions.

In the present study, hemp seed did not affect growth performance and carcass quality in growing-fattening pigs, although a slight increase in ADG and feed conversion was observed. Our data are comparable to those obtained in 2018 by Hăbeanu et al.

Due to the significant decrease in the N concentration in the urine, the TNO value was also lower (P<0.05). A decrease in N digestibility was observed for the hemp seed-based feed group (P<0.001). In our study, the mean value of estimated N_2O was lower in the group fed with hemp (P = 0.03).

The N_2O level was higher than that reported by Hăbeanu et al. (2020) using soybean meal compared to peas and flax, or in 2021 by Hăbeanu et al., when they used mustard cakes and grape cakes in growing pigs. The consumption of DM and implicitly of N is correlated with indicators of nitrogen metabolism and implicitly with the emission of N_2O. Although the combined feed recipes were balanced, the mechanisms underlying the recorded impact differences should be sought in the depth of the physiological processes.

CONCLUSIONS

Hemp seeds are a proteo-oleaginous source that can be added to pig diets with a beneficial effect on productive performance, causing a slight increase in average daily gain and feed conversion.

The addition of hemp seeds in the compound feed recipes administered to pigs determined a decrease in the concentration of nitrogen in the urine and a low value of N_2O emissions.

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PRELIMINARY STUDY REGARDING THE BIOLOGIC ACTIVITY OF SPICES

Maria-Luiza MIRCEA, Daniela-Mihaela GRIGORE, Ștefania Coman, Elena Narcisa POGURSCHI

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: daniela-mihaela.grigore@usamv.ro

Abstract

Cinnamon, cloves, and turmeric have long been valued as aromatics and components in oils and fragrances, and have been used as spices and remedies since ancient times. Ground spice is crucial in the food business for seasoning baked goods such as cakes, buns, biscuits, cookies, steaming puddings, pies, sweets, chewing gum, and desserts. However, the food business can benefit from the antioxidant activity of essential oils, for functional food production, with potential health claims. The paper aimed to evaluate the biologic activity (total phenolic content, the antioxidant activity and the antimicrobial effect) for the grounded and oil form of cinnamon, cloves, and turmeric. Among the evaluated spices, the grounded clove indicates the highest antioxidant activity ($p<0.05$), when compared with grounded cinnamon and turmeric. The value of the total phenolic content present in the grounded cloves oils was the highest (284.42 ± 32.98 mg GAE/100 mL), when compared with cinnamon oil (100.43 ± 9.85 mg GAE/100 mL), and turmeric oil (166.50 ± 12.76 mg GAE/100 mL). The biologic activity of the spices suggests that in addition to imparting flavour to the food, they possess high potential as health promoters by their antioxidant effect, phenolic content and might be suitable for antimicrobial activity.

Key words: *evolution, milk production, NW Region, Romania, trends.*

INTRODUCTION

Free radicals are formed both as a result of natural processes that take place in the body (Chaudhary et al., 2023), and under the action of external factors, like oxidative stress (Sharma & Mehdi, 2023), thermal stress of dietary fats (Mircea & Mititelu, 2023), unhealthy diet (Mustafa, 2023), sedentary lifestyle, cigarette smoke (Seo et al., 2023), pollution, drugs, radiation (Liu et al., 2023).

The body's cells (DNA, cell membrane) can be affected by their existence, because they are characterized by increased reactivity.

Although the human body has the ability to produce antioxidants, with a role in neutralizing free radicals by giving up spare electrons, in some cases the number of free radicals formed can be greater than the antioxidants that can neutralize them (Parcheta et al., 2021).

In this context, an intake of antioxidants (food, spices) is essential. Antioxidants present in food determine numerous benefits for the food product: they slow down the formation of oxidation production with toxic potential, control the development of the rancidity process, protect lipids and oils against oxidative food degradation (Akbari et al., 2022), contribute to the preservation of the nutritional characteristics of food and the extension of the shelf life (Munekata et al., 2020).

Turmeric (curcuma) is a herbaceous plant that belongs to the genus *Curcuma longa*, the ginger family, and is recognized for its powerful anti-inflammatory and antioxidant properties (Srivastava et al., 2022).

The main active ingredient, curcumin, can be extracted from the turmeric rhizome and is found at 2.5 to 4% in turmeric powder (Van Nong et al., 2016).

To benefit from the benefits of turmeric, it is recommended to take supplements in which curcumin is bound to phosphatidylcholine (Began et al., 1999), for better absorption and bioavailability.

Studies carried out on the properties of curcumin on the body have shown that it also contributes to the stimulation of the neurotrophic factor derived from the brain (Hosseinzadeh et al., 2013; Tizabi et al., 2014).

Cinnamon (*Cinnamomum verum*) is one of the oldest spices, being well-known for its characteristic, intense aroma. The high content in polyphenols, as well as the characteristic active principles (cinnamaldehydes, alcohol and cinnamyl acetate) determine antioxidant properties (Al-Mijalli et al., 2023), with a role in protecting cells against degradation. Cinnamon essential oil contains 90% cinnamaldehyde, which works to improve blood circulation, regulate blood pressure, and reduce platelet viscosity. Among its many benefits, cinnamon is a neurological tonic, with effects on stimulating brain activity, improving attention and memory (Tabassum et al., 2012). According to some studies, satisfactory results can also be obtained in the regulation of blood glucose, cholesterol and triglyceride levels (Ayati et al., 2021). Cinnamon can also be administered to combat bacterial and fungal infections (Ebani & Mancianti, 2020), being effective in inhibiting the development of some bacteria - *Listeria* sp. (Somrani et al., 2020), and *Salmonella* sp.

Cloves (dried buds of the flowers of the clove tree - *Syzygium aromaticum*) belong to the category of the most valuable spices and have been used since ancient times, both for flavouring and preserving food, and for therapeutic purposes. The rich content of volatile compounds gives cloves their characteristic aroma, and eugenol, which is the majority component (70-90%) in clove oil determines the anti-inflammatory properties for which it is recognized, especially in toothaches. Also, clove oil has antibacterial and antifungal effects, being effective in treating infections caused by Candida. The high antioxidant content of clove essential oil helps to significantly reduce oxidative stress.

This study aims to investigate the biologic activity (total phenolic content, the antioxidant activity and the antimicrobial effect) for the grounded and oil form of cinnamon, cloves, and turmeric.

MATERIALS AND METHODS

Materials

Three beneficial bivalent (food and medicinal) spices (cinnamon, cloves and turmeric) powders and oils were represented by commercial formula products (powder and oils), purchased from Romanian trade markets. The vegetal materials were scavenged for total phenolic content and total antioxidant activity, and *in vitro* determination for antimicrobial effects.

Analytical grade reagents and standards were represented by: Folin-Ciocalteu (Merck, Germany), methanol, sodium carbonate, gallic acid, and 2,2-diphenyl-1-1picrylhydrazyl, purchased all from Sigma-Aldirch, Missouri, USA.

Total phenolic content

For evaluating the total phenolic content, the Folin-Ciocalteu method was adapted for laboratory conditions, using a spectrophotometer (PG instruments® T60, UV-Vis spectrometer, Tokyo, Japan), for measuring the absorption at fixed wavelength, 765 nm. Individual spices samples were weighed (5 ± 0.57 g) an analytical balance (KERN ABJ 220-4M), and each oil sample was measured (1 mL) using the automatic pipette. The samples (powders and oils) were transferred to balloon flask 100 ml, and vortexed for 5 minutes (IKA®Vortex 3, Werke GmbH & Co. KG., Germany) with 70% methanolic solution, preheated at 70°C (30:70, ultrapure water: methanol). The incubation time for the samples was 24 h at 70°C. The samples mixture was separated using a centrifuge (Boeco C28-A, Germany), at 5000 rpm, 10 min. The resulted spices supernatants were collected (1 mL) and mixed with 5 mL Folin-Ciocalteu (1:1, F-C: ultrapure water), 1 mL ultrapure water and 4 mL Na_2CO_3. The samples stand for 30 minutes at room temperature 20°C, in dark conditions.

Blank sample was prepared in a similar way to the standard curve without adding the gallic acid. The calibration curve, having different gallic acid concentrations (10, 20, 30, 40, and 50 μg/mL) was done in order to calculate the total phenolic content, expressed as milligrams of gallic acid equivalents per g of extract (mg GAE/g).

Antioxidant activity

By using 2,2-diphenyl-1-1picrylhydrazyl (DPPH), the antioxidant activity was measured, for cinnamon, cloves, and turmeric. The powders extracts were individually prepared weighing 5 ± 0.65 g and mixing with 20 mL 80% methanolic aqueous solution, and incubated at 40°C, during 30 minutes. After separation (5000 rpm, 10 minutes), the supernatant was collected, and the volume of 500 µL of extracted sample was homogenised with 500 µL 80% methanolic aqueous solution, and 5 mL DPPH, and left to rest in dark conditions, during 30 minutes. By using the PG instruments® T60, UV-Vis's spectrometer (Tokyo, Japan), the absorbance of the test samples was measured at 517 nm fixed wavelength, and antioxidant activity results were represented as a percentage:

$$\text{Antioxidant effect } \% = \frac{X_c - X_s}{X_c} \, x \, 100,$$

X_c - Absorbance of control;
X_s - Absorbance of tested sample.

Antimicrobial effect

The disk infusion assay (Figure 1) was employed to determine the antimicrobial activity of spices (n = 3, powder). Different individual concentrations were prepared as soaked disks, in water (concentration ranged 50, 100, and 150 mg/dL) and alcohol 96% ethanol (concentration ranged 50, 100, and 150 mg/dL). The control was represented by antibiotic active substances specific action (Amoxicillin, Sulfamethoxazole, Streptomycin, purchased from Oxoid™ LTD, Ontario, Canada). Individual 1 cm^3 x 10^9CFU (0.5 McFarland standard) of *Enterococcus faecium* ATCC 51559, *Escherichia coli* ATCC 8739, and *Staphylococcus aureus* subsp. *aureus* ATCC 25923 (ATCC, Virginia, USA), were inoculated by flooding technique, in 9 mm diameter Petri dish, on TSA nutritive media (Tryptic Soy Agar, Merk), incubated for 24 h (LLG-uniINCU 28 COOL, Precisa, Germany), at 37°C. For evaluation, the presence of inhibition was taken into consideration, inclusively the diameter of the inhibition area, in mm.

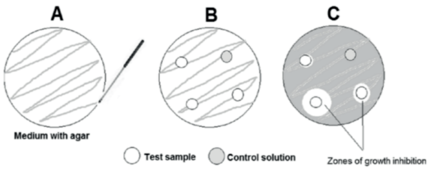

Figure 1. Graphic representation of evaluation of antibacterial activity, by using the disk diffusion test: (A) Petri dish containing culture medium on agar nutritive media; (B) Paper disks soaked with control solution or different concentrations placement; (C) Results after incubation bacterial colonies and the zones of growth/ inhibition observed in the sample exerted of the antibacterial effect of tested samples (after Pavia et al., 2013)

Statistical analyses

Data were analysed employing the ANOVA procedure with SPSS software (version 20 Inc. Chicago, IL, USA). When comparing group means, Post hoc Tukey's test was carried out to evaluate the significant differences for the measured parameters. Statistical differences were considered significant at a level of $p \leq 0.05$.

RESULTS AND DISCUSSIONS

Total phenolic content

The total phenolic content in plants exhibit high redox characteristics, participating in the antioxidant activity (Aryal et al., 2019). Hydroxyl groups within plant extracts aid in free radical scavenging (Lalhminghlui & Jagetia, 2018). The results for the total phenolic content are displayed in the Table 1.

Table 1. Total phenolic content of spices

Sample	Mean*	SD	CV
Cinnamon powder[1]	17.65	2.44	13.82
Cloves powder[1]	299.54[a]	12.34	4.11
Turmeric powder[1]	198.28[b]	27.89	14.07
Cinnamon oil[2]	100.43	9.85	9.81
Cloves oil[2]	284.42[a]	32.98	11.59
Turmeric oil[2]	166.50[b]	12.76	7.67

*Mean of n = 3 tested samples; the results were expressed as equivalent of gallic acid, in mg /100 g product; [a, b]different superscript highlights the statistical significances at the level of interest $p<0.05$; [1]mg/100 g product; [2]mg/100 mL product.

The 70% methanol is an efficient solvent for extracting antioxidant compounds, due to its polarity and the capacity to solubilize and recover phenolics from plant matrices, according to previous literature reviews (Li et al., 2008; Sultana et al., 2014; Zhang et al., 2023). All our tested spices, in oil and powder form expressed total phenolic content. Results on this study indicate that cloves oils and powder have a significant greater amount of total phenolic content, 284.42 ± 32.98 mg GAE/100 mL, respectively 299.54 ± 12.34 mg GAE/100 g, when compared with cinnamon and turmeric powder and oils. Turmeric powder and oil had significant amounts of total phenols, 198.28 ± 27.89 mg GAE/100 g, and 166.50 ± 12.76 mg GAE/100 mL, when compared with the cinnamon powder and oil formula. Similar to our results (Ereifej et al., 2016; Hossain et al., 2023; Sellami et al., 2013) found that methanolic extraction of cloves had highest amounts of total phenolic content.

Antioxidant activity

The antioxidant activity of spice's (powder and oils) measured by the DPPH assay followed the same trend with the total phenolic content assay (Figure 2).

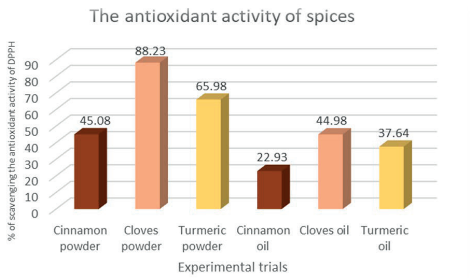

Figure 2. The total antioxidant activity of spices

As previously studied (Grigore et al., 2023; Saranya et al., 2017), the antioxidant capacity was highly dependent on the constituent composition within the extractor capacities. However, there are other assays for scavenging the antioxidant capacity of vegetal matrices, such as 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) (De-Montijo-Prieto & Razola, 2021). For evaluating the ability of a molecule to scavenge or eliminate the free radicals, we consider that DPPH stands out due to the fact that some chemical compounds (polyphenols, carotenoids and pigments) may interfere within the ABTS assay due to their absorbance at the wavelength used for measurement (Yehmed et al., 2023).

The highest antioxidant activity was obtained when testing the cloves powder and cloves oil, 88.23%, and 44.98%, when compared with turmeric and cinnamon (powders and oils). Cloves demonstrate significant antioxidant activity attributed to their substantial levels of phenolic compounds (Frohlich et al., 2023), flavonoids (Kiki, 2023), and essential oils (Manzoor et al., 2023). The presence of these bioactive constituents enables cloves to effectively neutralize free radicals (Aldabaan et al., 2024), thereby mitigating oxidative stress and potentially mitigating the incidence of developing chronic diseases (Rani et al., 2023) linked to free radical-induced damage. Introducing cloves into dietary regimens or employing clove extracts may serve as a strategy to bolster the body's antioxidant defenses and counteract oxidative stress-related health concerns.

In accordance to our results, others studies regarding the antioxidant activity showed similarities regarding the cloves (Frohlich et al., 2023; Kiki, 2023), turmeric (Ballester et al., 2023; Tüzün & Saraç, 2023), and cinnamon (Pagliari et al., 2023).

Antimicrobial effect

The antimicrobial activity was evaluated by employing the principal disk diffusion method, for water and alcoholic extracts of spices (Table 2).

Table 2. Antimicrobial effect of spices[1]

Sample (mg/dL)	*E. faecium*		*E. coli*		*S. aureus*	
	H_2O	C_2H_5OH	H_2O	C_2H_5OH	H_2O	C_2H_5OH
Cinnamon 50	1.74	2.33	0.41	1.52	0.34	1.74
Cinnamon 100	2.55	3.62	1.08	4.75	0.71	8.87
Cinnamon 150	3.98	8.22	2.41	6.23	2.31	10.33
Cloves 50	2.06	3.76	1.18	3.21	4.44	12.89
Cloves 100	2.73	5.53	3.44	8.79	6.44	13.39
Cloves 150	4.12	8.77	5.37	12.11	8.56	15.77
Turmeric 50	1.96	2.60	1.03	2.74	0.72	5.98
Turmeric 100	3.42	6.01	2.84	6.21	2.78	8.21
Turmeric 150	5.29	9.02	6.09	8.76	5.61	8.99
Control	Amoxicilin		Sulfamethoxazole		Streptomycin	
10 μg/mL	0.00		0.00		0.00	

[1]The results are expressed as means of the measured diameters (n = 3, mm) of the inhibition zones formed.

The control was represented by different commercial antibiotic active substances, well known for their inhibitory effect spectrum (Amoxicillin, Sulfamethoxazole, Streptomycin) in the concentration equal to 10 μg/mL. The control plates shown inhibition of cellular development, from the start of the experimental trials.

The largest diameter of the spice's inhibition zone was shown at a concentration of 150 mg/dL of cloves alcoholic extract, measuring 15.77 mm, which was tested against *Staphylococcus aureus* ATCC 25923, and the following measuring 13.39 mm, in the same biologic conditions, the concentration equal to 100 mg/dL cloves alcoholic extract. By increasing the concentration of cloves extract is directly proportional accompanied by the diameter of the inhibition zone, for this small range of concentrations, which is increasingly various compounds.

Same trends were observed for turmeric, who had best results inhibiting the *Enterococcus faecium* strain ATCC 51559, average value of the measuring diameters was equal to 9.02 mm, when concentrated at 150 mg/dL, in alcoholic extraction. Moreover, when comparing the antimicrobial activity of the current three spices, we could highlight the fact that, the smallest activity concerning the antimicrobial effect, was observed for the cinnamon water extract, having the smallest mean values measured for the concentration 50 mg/dL, against the *Escherichia coli* strain ATCC 8739.

Nevertheless, similar results were published (Parisa et al., 2019; Rayess et al., 2023), when evaluating spices for their antimicrobial effects.

CONCLUSIONS

The tested spice that showed the highest antioxidant activity was represented by the cloves. The food formulates obtained oils from aromatic plants showed high antioxidant activity and sufficient amounts of total phenols. The strong antimicrobial activity of cloves powder and the large spectrum they have shown to provide evidence that they might represent a potential solution prolonging the shelf life of food products, developing functional foods, and for protecting plants and crops, and of course they can be an important raw material for the bio-therapeutic cosmetics, for anti-aging and antioxidant effects. Future researches are needed in order to evaluate the mechanisms throughout the antioxidant and anti-aging effects and the interactions between the nutritional effects of the antioxidant plant materials.

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THE IDENTIFICATION OF THE EXTERNAL FACTORS THAT HAVE INFLUENCED THE PROGRESS OF A WEIGHT MANAGEMENT PROGRAMME IN A POPULATION OF DOGS

Silvia Ioana PETRESCU, Cristina Gabriela RADU-RUSU, Mădălina MATEI, Anamaria Hortensia STRICHEA, Dragoș Mihai LĂPUȘNEANU, Ioan Mircea POP

"Ion Ionescu de la Brad" Iași University of Life Sciences, 3 Mihail Sadoveanu Alley, Iași, Romania

Corresponding author email: mircea.pop@iuls.ro

Abstract

Raising awareness and educating dog owners about weight gain and the risk of obesity in their animals has become a priority for veterinarians and nutritionists. The prevalence of obesity in dogs in the United States and some European countries has been estimated to range from 30% to 70%. In the current research, external factors related to the animal, the owner and the family members of the investigated dogs were observed in relation to the implementation of nutritional programmes for excess weight management. Exogenous factors discussed included the physical activity level, environment and lifestyle of the animal, and the influence and behaviour of the owners and family members involved in the dog's weight loss programme. The research highlights the importance of obese dog owners' awareness and acceptance of the need to implement and run nutritional weight management programmes to improve the health and well-being of the animal.

Key words: *dogs, nutritional programmes, obesity, weight loss.*

INTRODUCTION

The official recognition of obesity as a disease by the American Medical Association was achieved in 2013 and marked a turning point in the understanding and management of this public health problem (Kyle et al., 2016).
In parallel with this recognition among humans, major animal welfare organizations, including the BSAVA, have adopted the same perspective on obesity in pets. It has also been pointed out that neglecting the problems associated with overweight pets can be a violation of pet welfare and may be contrary to the terms of the Animal Welfare Act 2006.
In a study conducted in the UK in 2016 by applying a questionnaire to owners it was concluded that overweight and obese canine patients showed a decrease in quality of life caused by altered health status (Yam et al., 2016).
To gain a detailed insight into the prevalence of obesity or overweight among dogs, data collected in 2022 revealed significant numbers in both the United States and European countries. According to the US Association for Obesity Prevention, approximately 59% of the country's canine population is overweight. In parallel, in European countries, a comprehensive study estimated that the prevalence of obesity among dogs ranges from 31.3% to 69.4% (Muñoz-Prieto et al., 2018), highlighting a wide range of the problem in European countries as well.
This integrated approach to obesity in both humans and animals highlights the importance of coordinated action and ongoing education in these areas.

MATERIALS AND METHODS

Medical and nutritional studies highlight the contribution of both internal and external factors in the development of overweight in dogs. In the literature, internal or endogenous factors mentioned include age, gender, hormonal status, presence of hormonal abnormalities and genetic predisposition.
The current study cohort consists of 29 dogs that were brought to a private veterinary clinic in Iasi between 2020 and 2023. These dogs were diagnosed as overweight, forming the basis of the biological material for the analysis and research conducted in this study.
In the current research, external factors contributing to weight gain in dogs were

investigated and considered as part of an extensive nutritional monitoring programme.
The external factors discussed included the level of physical activity, environment and lifestyle of the animal, and the influence and behaviour of owners and family members involved in the dog's weight loss programme. Therefore, both pet and owner behaviour, along with the implementation of healthy habits, became goals in achieving and maintaining a healthy body weight in pets.

RESULTS AND DISCUSSIONS

The causal agents that induced obesity but also influenced the animal's weight loss programme were divided into two categories in order to highlight as concretely as possible the less positive contribution of each party in the development of the nutritional programme, as can be seen in Table 1.

The factors most often mentioned were categorised as animal factors with a behavioural explanation and human factors related to the environment and the owner's responsibility towards the dog.
The seven external factors followed in dogs have been systematized in Table 1, three of these factors are behavioral factors related to the animal and have been defined as follows: sedentary dog that does not play and prefers to sleep, dog with increased appetite, greedy that steals or asks for food and uncooperative dog that aggressively asks for food or becomes aggressive when food is restricted. Furthermore, the factors that concerned the owner as well as the family of the monitored dog were also addressed, and in the table the four factors were defined as follows: owner who was uncooperative or made infrequent visits to the veterinary practice or did not keep appointments at the veterinary practice, owner who had periods of non-compliance with the nutritional plan, owner who was sedentary or limited the activity time of the animal and family members who were uncooperative or did not follow the weight loss program of the monitored animal.
Both the influence of factors related to the dog and those related to the dog's owner or family members have been presented graphically in Figure 1. Therefore from the first chart the more obvious influence of the owner in the progress of the dog's nutritional weight loss plan is observed.

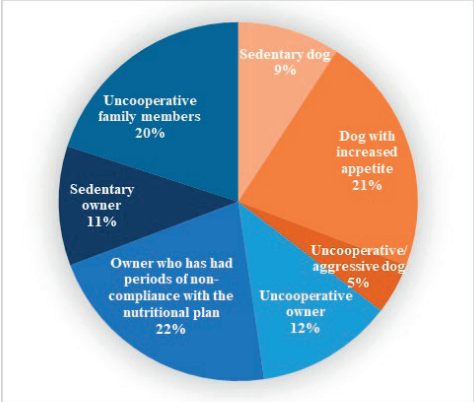

Figure 1. Proportion of each external factor that influenced the dogs' weight loss programme

The evolution of the canine patients enrolled in the study was influenced by external factors in an individual manner, therefore variations in external factors were recorded both in quantitative sense and in the type of majority factors (predominantly animal dependent/ predominantly owner dependent/equal proportion). Quantitatively, the dogs in the study were influenced by a number ranging from 0 to 5 external factors, none of them recorded the maximum possible number (7) of external factors involved. The percentage distribution of the number of patients according to the number of factors that were involved in the evolution of the weight management programme is plotted in Figure 2.

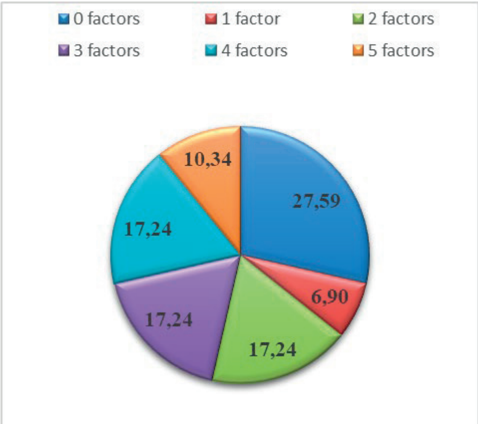

Figure 2. Percent of the dogs reported to the number of external factors involved during the weight loss program

Table 1. External factors influencing weight loss in dogs

No.	Name	External factors - behavioural - related to the animal			External factors related to the owner				Total number of influencing factors
	External factors	Sedentary dog	Dog with increased appetite	Uncooperative/ aggressive dog	Uncooperati ve owner	Owner who has had periods of non-compliance with the nutritional plan	Sedentary owner	Uncooperati ve family members	
1	ZIGGY								0
2	BELLA 1		1			1		1	3
3	BELLA 2								0
4	ZENY		1					1	2
5	KYRA		1		1			1	3
6	INDY					1			1
7	KIKI								0
8	BETY	1	1		1	1		1	5
9	FOXIE		1			1	1		3
10	SASHA								0
11	NEGRUTA	1	1			1	1	1	5
12	LIZUCA	1	1			1	1	1	5
13	CORA			1		1		1	3
14	JIMINA		1			1		1	3
15	PUGGY		1	1	1			1	4
16	OLI								0
17	BENI				1				1
18	ALFIE		1		1			1	3
19	DANTE	1				1	1	1	4
20	TOTO	1	1		1	1			4
21	TAZ					1	1		2
22	BERNIE								0
23	FIDO		1			1			2
24	HAPPY		1	1	1			1	4
25	OSCAR		1			1			2
26	JACK								0
27	BOBO	1				1	1	1	4
28	PATRICK								0
29	DIXY				1		1		2
Total (number)		6	14	3	8	14	7	13	65
Total (%)		9.2%	21.5%	4.6%	12.3%	21.5%	10.8%	20.0%	100%

Monitoring each overweight or obese patient involved an initial nutritional consultation that required the owner to follow a new feeding protocol for the pet (Petrescu, 2022).
The consultation questionnaire involved questions related to the pet's voluntary activity time manifested by its need to go for walks or to do activities such as playtime with the owner, family members or other animals in the household.
The communication with the owner during the implementation of the weight loss programme included questions about possible pathological feeding behaviours of the animal such as seeking food in inappropriate places, obtaining food from other animals in the household or showing aggression when food was restricted.
The observations made above are consistent with research on canine physiological behavior. Hence researchers have observed that similar to wolves, dogs also over-consume food or eat too quickly if fed in the presence of other dogs in the household (Case, 2005) which may cause pathological behaviour manifested by over-consumption or consumption in excess of the dog's nutritional needs.
The success of a weight loss programme is also a matter of the owner's willingness and responsibility towards his pet. Hence, in the current research, the influence factors manifested by the owners and the family of the animal studied were also analysed, such as: compliance with the recommended visits to the vet's clinic, compliance with the recommended nutritional plan and with the activity or play time with the pet included in the weight loss programme, as well as the cooperation of the family members in the correct implementation of the weight loss plan.
Maintaining communication with the owners of the monitored dogs was achieved by recommending and scheduling visits to the veterinary practice by sending notifications for nutritional recheck throughout the DigiTail veterinary software. As user-friendly methods for changing the nutritional program or for body weight updates for monitored dogs, email and WhatsApp were chosen as communication methods.
A complete nutritional program requires 3 to 6 months of adherence to see results. At the start of the pet's weight management programme, the dog owners receive an explanation regarding the difficulties they will face during the 3 to 6 months of their pet's diet depending on the correctness and compliance with the protocol that was imposed if they wanted their pet to lose weight. Behavioural changes that will occur in the dog following the nutritional weight management programme were explained.
Exogenous influencing factors were counted in the table and so a score of 0 meant that no factor had an influence and the nutritional programme was successful, and a score of 7 would have meant that all the factors presented would have influenced the nutritional programme and then the chances of the animal losing weight would have decreased drastically. Nevertheless, owners were most often unaware of all the changes that occurred when limiting the animal's food intake. As in the case of the animal's behaviour, the reasons given by the owners for their own behaviour or the reasons that emerged when the feeding programme was carried out were also revealed.
The investigation of the external behavioural factors related to pets that took part in the weight reduction nutritional programme shows in Figure 3 an increased tendency for dogs to show their desire for food by demanding food, showing cravings for their owners' or family members' food or even having a tendency to seek food in inappropriate places.

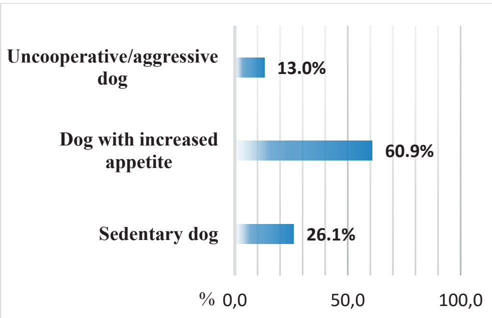

Figure 3. External factors (behavioural-animal-related) that influenced the implementation of the weight loss programme in dogs

Manifestation of scavenging behaviour has also been found in similar studies; German et al. (2012) discussed a challenge for pet owners

adhering to a weight loss program for their dog, namely energy restriction and its potential to cause hunger, which can then lead to more prominent manifestation of foraging behavior.
The level of voluntary activity was the second most common cause cited by owners with regard to their dog in 26% of cases, explaining that the pet is sedentary, prefers to sleep and is not interested in walking or playing.
According to studies by Zoran (2010), German et al. (2012), Salt (2019), nutritionally unbalanced food, excessive consumption of rewards and a sedentary lifestyle are risk factors leading to obesity in pets.
In the case of the dogs, the restriction of food resulted in behaviour considered by their owners to be ' bothering' or more aggressive, in 13% of cases the dogs became uncooperative, leading to a change in the initial feeding programme.
Training dogs to wait before being fed can be beneficial, increasing the predictability of the environment and reducing anxiety levels. However, studies on canine behaviour show that the expectation from owners that dogs should wait for long periods of time for food can exacerbate frustration in dogs and can have negative effects on their physiological and emotional state (Heath, 2013) which would result in an aggressive reaction when attempting to limit food portions.
Exogenous factors related to pet owners that influenced the implementation of the nutritional weight loss program were observed and compared with the results obtained in peer reviewed studies.
Therefore, it was observed that dog owners did not strictly follow the recommended nutritional program, as can be seen in Figure 4, having periods when they offered treats or more food to the pet indicating several reasons such as: they were away from home for a longer time and the pet stayed with another person, they did not have time to responsibly take care of the pet.
A recent, globally conducted peer-reviewed study investigated the success of a weight-loss plan in dogs, therefore the researchers of the study state that the major challenge for nutritionists and veterinarians who monitored and guided the dog weight-loss program was maintaining owner compliance with their pet's weight-loss program.
Therefore, in the research conducted by Flanagan et al. (2017) it was found that 37% of dogs initially enrolled in the weight loss program did not complete the study program mainly due to owners and noncompliance with the individualized nutritional program or failure to attend scheduled or recommended reweaning visits.
At the beginning of the nutritional weight management program in dogs, dog owners are asked that only they feed the dog and no other family member should be involved in the process, as this attempts to minimize the contribution of external influencing factors on the animal's weight loss plan.

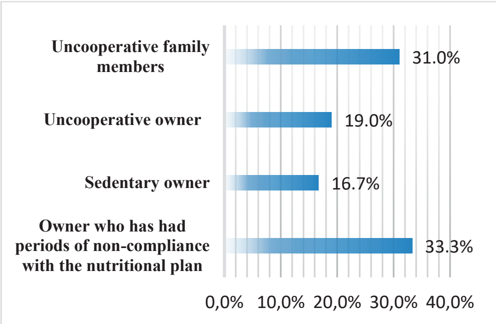

Figure 4. External factors (owner-related) that influenced the implementation of the weight loss programme in dogs

By observing both the behaviour of the dog's owner and its family members, misbehaviour was more often observed in the behaviour of family members, explained by a manifestation of the members' affection towards the dog. Therefore, it was found that in 31% of the cases, family members were not willing to cooperate and comply with the dog's weight loss program given reasons such as pity towards the animal (asking for food or not getting enough food), not being able to offer home-cooked food or table scraps because the dog is greedy.

CONCLUSIONS

The present study reveals that the success of a nutritional weight loss plan for pets is strictly correlated with limiting as much as possible the interference of external factors. Rigorously maintaining a nutritional weight loss plan for a canine patient is an activity that is the responsibility of the owner and is based on knowledge and training of their pet. External factors related to the owner had a decisive influence on the patient's evolution during the implementation of the nutritional programme, as inconsistency of the owner and their family were the main external factors identified.

The role of the nutritionist and the veterinarian in the development of the nutritional weight management program is to provide solutions to situations encountered by both owners and pet dogs with inappropriate behaviours. For example, in the case of dogs, limiting food-seeking behaviour could be done by controlled stimulation (calculated according to the recommended daily energy requirement) of satiety through the improvement of the individualised nutritional programme.

In terms of limiting the contribution of the human factor in weight management in dogs, this could be addressed by encouraging the whole family to attend nutritional consultations so that all members of the dog's family become aware of the severity of a problem such as obesity in their pet.

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THE INCIDENCE OF RABIES IN ANIMALS IN THE REPUBLIC OF MOLDOVA DEPENDING ON THE VACCINATION OF FOXES IN THE SYLVATIC ENVIRONMENT

Sergiu BALACCI[1], Ion BALAN[2], Vladimir BUZAN[1], Nicolae ROȘCA[1], Ion MEREUȚA[1], Vlada FURDUI[1], Galina OSIPCIUC[1], Roman CREȚU[1], Gheorghe BACU[1], Parascovia ȚURCANU[1]

[1]Moldova State University, Institute of Physiology and Sanocreatology, 1 Academiei Street, MD-2028, Chișinău, Republic of Moldova
[2]Technical University of Moldova, 168 Stefan cel Mare Blvd, MD-2004, Chișinău, Republic of Moldova

Corresponding author email: vladimirbuzan@yahoo.com

Abstract

The study presents the results of the research on the epidemiological situation of the rabies virus following the vaccination campaign of the fox population in the sylvatic environment in the last 5 years on the territory of the Republic of Moldova. Both in Europe and in the Republic of Moldova, Vulpes vulpes represents in the sylvatic environment the main vector of the spread of rabies in wild and domestic animal populations. In 2019-2023 in the Republic of Moldova were registered 12 species of animals that reacted positively to the rabies virus. The most frequent cases of rabies in animals were registered in cattle, dogs, foxes, cats and constituted 93.72% of the total number of registered cases (239). Following the start in 2020 of the fox vaccination campaign, there was observed a decrease in the incidence of rabies in 2021 by 74.73%, in 2022 by 85.71% and in 2023 by 76.92% compared to 2019. The obtained data demonstrate that the application of vaccine baits by air and land has led to the immunization of wild animals and the reduction of the sick animals number.

Key words: *red fox, rabies, vaccination, animal biodiversity.*

INTRODUCTION

Rabies is spread all over the globe and, without being influenced by climate or season, can have a sporadic or even epizootic character. Rabies is a disease with a natural focus. The rabies virus is preserved only in the organism of infected animals, which constitute the natural reservoirs of infection. According to Mânzat (Mânzat, 2005), two types of rabies virus reservoirs are distinguished: "domestic" or "urban" - made up of domestic animals (dogs, cats and less often cattle, horses, pigs) and "sylvatic" or "wild" - made up of numerous wild animals (foxes, wolves, badgers, otters, jackals, hyenas, rats, mice, rabbits et al.). Between the two reservoirs there are reciprocal relations. For example, in most cases of "urban" rabies in Europe, the dog intervenes, and in "sylvatic" rabies, the main role is played by the fox. It is known that the incidence of rabies varies over time and differs from one country to another, it is conditioned by the geographical position, the dynamics of the population development of the animal species, the state of their immunity and the veterinary sanitary measures undertaken. In the world annually more than 60,000 cases of rabies in animals are registered. In Europe, the red fox represents the natural reservoir of rabies and the main vector of its spread in wild and domestic animal populations. (OIE, 2021; WHO, 2018; WHO, 2021; Mânzat, 2005).

In the Republic of Moldova, a relatively large number of rabies cases are registered annually in relation to the surface of the country's territory. According to the study carried out in the years 2012-2021 there were registered 1156 cases of rabies in domestic and wild animals, which constitutes an average of 115.6 cases per year (Balacci et al., 2022). The incidence of rabies in various animal species is similar to the incidence of rabies in animals in European Union (EU) countries. Thus, in the sylvatic environment the most affected animal species is the red fox and it constitutes 16.26% of the total number of animals affected in the period 2012-2021 (Balacci & Balan, 2022).

The beginning of the spread of sylvatic rabies in Europe was recorded during the Second World War, when the first cases were discovered at the former Russian-Polish border (Bourhy et al., 1999). The disease spread progressively from the north-east to the center and south-west of Europe (Aubert et al., 2004; Pastoret & Brochier, 1999). The first country to launch a massive oral rabies vaccination (ORV) campaign using attenuated vaccines was Switzerland in 1978 (Wandeler et al., 1988). In EU countries, large-scale rabies eradication programs in wildlife using ORV started in 1989 due to funding specified for this purpose by the EU (Müller et al., 2015). It has been established and demonstrated that the vaccination campaign represents the only effective means of controlling rabies in livestock (Müller & Freuling, 2018; OIE, 2022).

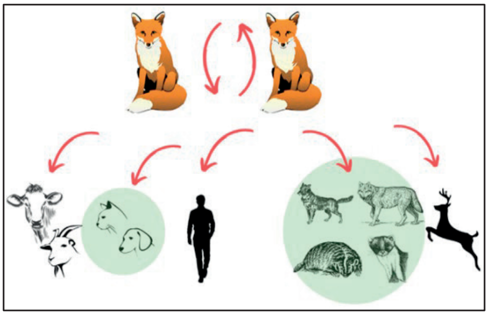

Figure 1. Transmission and maintenance of RABV among the population of the primary carnivorous reservoir host (red fox) (Müller et al., 2015)

In the current epidemiological context of the evolution of rabies both on the national territory and in the neighboring countries, it is necessary to establish measures to prevent the occurrence of cases of the disease in the Republic of Moldova. To reduce the number of rabies cases, the Republic of Moldova joined the OIE, WHO and FAO initiative to combat rabies. Thus, in 2019, he approved the "Plan of measures for surveillance, control and eradication of rabies in foxes in the Republic of Moldova for the years 2019-2023" which provides for the vaccination of foxes in the forest environment (HG 185/2019, 2019).

MATERIALS AND METHODS

The start of the anti-rabies vaccination campaign for foxes through the distribution of vaccine baits and related activities began in 2020. In 2020, the vaccination campaign was carried out 2 times (the spring campaign and the autumn campaign) and covered the entire accessible area for the distribution of vaccine baits on the territory of the Republic of Moldova. Both campaigns were carried out within 15 calendar days. For the implementation of the Action Plan were distributed rabies vaccine baits for oral immunization "Lysvulpen" produced by Bioveta, a.s., Czech Republic (Figure 2).

Figure 2. Vaccine baits against rabies "Lysvulpen" (own source)

The spring and autumn distribution of vaccine baits was carried out by air and by land, taking into account and in accordance with the physiological and biological states of the red fox organism, characteristic for these seasons, as well as with the environmental conditions and abiotic factors (Anderson et al., 1981; Smith & Wilkinson, 2003). For the distribution of vaccine baits by air and cover the entire area planned for vaccination, which constituted 21725.42 km^2, four specially equipped aircraft were used for this purpose (Figure 3).

Figure 3. Aircraft used to distribute of vaccine baits "Lysvulpen" (own source)

The distribution of baits was made automated by means of a "vaccine thrower" with a frequency of 25 vaccine baits per 1 km^2 (EC, 2002; AHAW, 2015) and at a distance between the flight lines of 500 meters. According to the scheme drawn up for the distribution of vaccine baits, the aircraft flew at low altitudes, up to 300m from the ground level and had a flight speed adapted accordingly to the homogeneous distribution of vaccine baits.

The land-based vaccination was carried out by the authorized staff of the territorial subdivisions of the National Agency for Food Safety and by the staff of the Moldsilva Agency and the Society of Hunters and Fishermen of the Republic of Moldova through the manual distribution of the vaccine baits at each entrance to the burrow, on the fox's trails and in the immediate vicinity of the burrows (Figure 4).

Figure 4. Manual distribution of vaccine baits at burrow entrance (own source)

After the distribution of the vaccine baits, the free movement of dogs and cats was restricted as far as possible, since these animals could disrupt the proper progress of the vaccination campaign by the fact that they could have consumed the distributed baits. Also, in the regions where the vaccine baits were distributed, hunting was prohibited for a period of 14 days from their distribution.

The number of vaccine baits distributed on the national territory according to the "Plan of measures for surveillance, control and eradication of rabies in foxes in the Republic of Moldova for the years 2019-2023" is represented in Table 1.

Table 1. The number of vaccine baits distributed on the national territory in 2020-2021 (pcs.)

Year of distribution of baits	Distribution of baits				Total number of baits distributed (pcs.)
	By air (pcs.)		By land (pcs.)		
	Spring campaign	Autumn campaign	Spring campaign	Autumn campaign	
2019	0	0	0	0	0
2020	515 746 july	570 526 november	73 800 july	73 800 november	1 233 872
2021	0	606 465 october	0	52 000 october	658 465
2022	0	0	0	0	0
2023	0	0	0	0	0

The costs foreseen for the implementation of the Plan of measures for surveillance, control and eradication of rabies in animals for the years 2020-2023 are presented in Table 2.

Table 2. Costs foreseen for the implementation of the Plan of measures for surveillance, control and eradication of rabies in animals for the years 2020-2023 (mdl)

No.	Proposed action	Planned costs per year (mdl)	Actual costs incurred according to the years (mdl)				
			2019	2020	2021	2022	2023
1	Aerial distribution of vaccine baits and related activities	23 351 689	0	22 723 048	12 330 957	0	0
2	Control of virus concentration on cell cultures from immunological veterinary medicinal products	0	0	23 760	15 290	0	0
3	Establishing post-vaccination efficacy (active and passive rabies surveillance)	1 403 154	0	644 686	689 400	0	0
4	Elaboration and distribution of leaflets intended to inform the population	35 000	0	24810	17 466	0	0
Total		24 789 843	0	23 416 304	13 053 113	0	0

RESULTS AND DISCUSSIONS

Rabies is characterized by a cyclical evolution of the disease. Under natural conditions, direct contact between sick and sensitive animals is necessary for the development of a rabies epizootic, and each diseased animal must infect more than one susceptible animal, otherwise the epizootic will die out.

The start of the works of the anti-rabies vaccination campaign for foxes through the distribution of vaccine baits and related activities was planned for 2019, but for organizational reasons the campaign started only in 2020, which was directly reflected proportionally to the large number of diseases of various species of animals with the rabies virus. Animal rabies disease according to animal species is presented in table 3.

Table 3. The number of rabies cases in the Republic of Moldova registered by animal species for the years 2023-2019

Animal species	Number of cases					Total for 5 years	%
	2023	2022	2021	2020	2019		
Bovine	3	7	8	39	34	91	38.07
Dog	9	3	8	20	22	62	25.52
Fox	4	1	3	15	16	39	16.31
Cat	4	1	3	12	12	32	13.39
Goat	0	0	1	0	1	2	0.84
Marten	1	0	0	0	1	2	0.84
Polecat	0	0	0	1	2	3	1.25
Horse	0	0	0	2	0	2	0.84
Swine	0	0	0	0	1	1	0.42
Wild cat	0	0	0	0	1	1	0.42
Jackal	0	1	0	2	0	3	1.25
Badger	0	0	0	0	1	1	0.42
Total per year	21	13	23	91	91	239	100

The data presented in Table 3 show that in the Republic of Moldova in 2019 were registered 91 cases of animal rabies. In 2020, although the process of implementing the red fox vaccination program started by distributing 1 233 872 vaccine baits, were also registered 91 cases of rabies. That is, no reductions in rabies cases are noted, resulting from the campaign to vaccinate the herd of wild animals. In this context, it is worth mentioning, that the high number of registered rabies cases is basically due to the fact that the organism of animals subject to vaccination in 2020 did not have sufficient time or adequate conditions to develop an integral specific immunity.

The data of the table show that in 2021 there is a significant decrease of 74.72% of cases of animals rabies compared to 2020. Subsequently, in 2022 there were 13 cases of rabies in animals, which represents a decrease of 85.71% compared to 2020 and 43.47% compared to 2021. At the same time, there is also a change in the number of rabies cases depending on the animal species. Thus, if in 2020 the number of cases of rabies in foxes from the total species of sick animals constituted 16.48%, in 2021 it was significantly reduced and constituted 13.04%, and in 2022 it constituted 7.69% of cases.

A similar trend was also observed in cattle where the number of rabies cases during this period decreased from 39 cases in 2020 to 8 cases in 2021, to 7 cases in 2022 and to 3 cases in 2023.

In this context, it can be mentioned that with the implementation of the oral anti-rabies vaccination program for foxes, there was a decrease of rabies cases among them. As an unwanted result of this campaign, a significant increase in cases of rabies in dogs was identified. Thus, in 2020 the number of sick dogs constituted 21.98% (20 cases out of 91), and in 2023-42.86% (9 cases out of 21) of the total number of sick animals. According to the data obtained it can be mentioned that, there was a redistribution of the rabies virus by decreasing it in the wild fauna and increasing it in the urban area. Therefore, in this case it is necessary to implement a more rigorous vaccination campaign for pets and especially dogs.

We further mention that in the year 2023 there is 1.62 times increase in the number of animals sick with the rabies virus compared to the year 2022. We believe that this result is caused by the fact that in the years 2022 and 2023 the vaccination program was not implemented; thus, wild animals were not vaccinated with vaccine baits. As a consequence, the specific immunity obtained previously in the years 2020 and 2021 was not sufficient to protect the animals against the rabies virus in the year 2023 as well.

Next, the number of rabies cases was investigated according to the season of the year. Data on the number of animal diseases according to the months of the year are presented in Table 4.

Table 4. The number of rabies cases in the Republic of Moldova registered by month during the years 2023-2019

Month of registration of illness	Year of registration of illness, heads					Total illnesses for 5 years	
	2023	2022	2021	2020	2019	heads	%
January	2	0	4	9	2	17	7.12
February	3	0	3	8	7	21	8.78
March	4	2	3	11	5	25	10.46
April	1	1	2	6	2	12	5.02
May	1	0	5	2	2	10	4.18
June	3	0	2	4	6	15	6.28
July	0	1	1	6	5	13	5.44
August	0	1	2	11	12	26	10.88
September	4	3	0	12	8	27	11.29
October	0	2	1	14	16	33	13.81
November	0	1	0	6	17	24	10.04
December	3	2	0	2	9	16	6.69
Total per year	21	13	23	91	91	239	100

From the data of table 4 it can be seen that in the period before the vaccination period (2019) the rabies virus in animals is recorded practically throughout the year. With the consumption of vaccine baits and the formation in animals of specific resistance against the rabies virus, cases of illness were not recorded in all months of the year. Thus, in 2021, rabies cases were recorded in 9 months of the year, in 2022 - in 8 months of the year and in 2023, also - in 8 months of the year. At the same time, it is worth mentioning that there is no obvious correlation between the number of animal diseases and the season of the year. Therefore, practically, the incidence of rabies in animals in the researches carried out depends, on the one hand, to a greater extent on their physiological needs, such as acquiring food, caring for offspring, the reproduction process, the expression of territorial behavior (marking and defending the territory et al.), migration and others, and on the other hand, to a lesser extent depends on abiotic environmental factors.

Next, the geographical distribution of rabies cases on the national territory was studied. Thus, it was determined that this distribution is uneven. The major number of disease cases in all animal species was recorded in the Central and Northern Region (Slobozia, Sângerei, Edineț and Briceni), and the minimum number of diseases was recorded in the Southern Region of the country.

CONCLUSIONS

In the 21st century, rabies remains one of the most feared and important threats to public health. The evolution of rabies in the Republic of Moldova was and continues to remain endemic, thus constituting a vital danger for humans and animals and a barrier in the intra-community circulation of animal products, agricultural animals, including pets. Oral vaccination of wild animals is the only effective tool to eradicate rabies in wildlife. From the data presented in this research, it can be mentioned that the rabies virus in animals on the territory of the Republic of Moldova evolves sporadically. The fox represents one of the natural reservoirs of rabies and the main vector of the spread of rabies in wild and domestic animal populations, including among the human population. Thus, it is necessary to strengthen the veterinary sanitary activity for the purpose of surveillance, control, prophylaxis and eradication of rabies in animals from the sylvatic environment. At the same time, it is necessary for the long-term continuous implementation of the "Plan of measures for surveillance, control and eradication of rabies in foxes in the Republic of Moldova for the years 2019-2023" and it is also appropriate to make a constant political and financial commitment from on the part of the state and close cooperation between neighboring countries in order to jointly eradication of the disease.

Previously in the Republic of Moldova during the period of 2012-2021 there were registered 1156 (on average 115.6 cases per year) cases of rabies, which included 20 species of animals from all districts of the country. During the research period between the years 2021-2023 there were registered 57 cases of illness with the rabies virus (on average 19 cases per year) which included 7 species of animals.

Therefore, in order to fortify the results obtained from the vaccination of foxes with vaccine baits (in the medium and long term) and in order to prevent the danger of the emergence of new cases of disease as a result of the epidemiological situation in neighboring countries, it is necessary to continue the anti-rabies vaccination program of foxes, dogs and in some cases of agricultural animals, approved by the Government of the Republic of Moldova in accordance with the approval of similar acts by the Governments of neighboring countries and the taking of concomitant measures to combat against rabies. In support of this action is also the following argument, that in the year 2021 only one vaccination campaign was carried out, and in the years 2022 and 2023 none, a fact that led to the increase of registered cases of rabies in animals in the years 2022 and 2023. In this vein, we mention that the missed vaccination campaigns will in the near future have a devastating impact on the rabies eradication programs and a considerable increase in the cases of animal diseases with the rabies virus and the existence of an obvious danger for *homo sapiens*.

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THE INFLUENCE OF POLYPHENOLS OF NETTLE EXTRACT (*Urtica dioica*) ON THE ANTIOXIDANT ACTIVITY IN THE BLOOD SERUM OF ROOSTERS

Ion BALAN[1], Nicolae ROȘCA[2], Vladimir BUZAN[2], Ion MEREUȚA[2], Sergiu BALACCI[2], Roman CREȚU[2], Gheorghe BACU[2], Vlad TEMCIUC[2], Artiom FILIPPOV[2], Ecaterina VÎHRIST[2]

[1]Technical University of Moldova, 168, Stefan cel Mare Blvd, MD-2004, Chișinău, Republic of Moldova
[2]Moldova State University, Institute of Physiology and Sanocreatology, 1 Academiei Street, MD-2028, Chișinău, Republic of Moldova

Corresponding author email: vladimirbuzan@yahoo.com

Abstract

In conditions of intensification of growth and improvement of farm animals and birds, in order to maintain the immune status of the organism it is necessary to monitor the state of the body`s antioxidant system. The concentration of free radicals in the cells of the body can reach levels, at which the own antioxidant system is not able to deactivate the damaging agents, as a result of which oxidative stress can occur. Different remedies are used to correct oxidative stress, including natural and synthetic of various chemical nature, possessing antioxidant activity (AOA). The biochemical structure of vegetable origin remedies is close to the structure of metabolites of living organisms, which is conditioned by adaptation through evolution and correspondingly these remedies are more easily submit to the influence of fermentative systems, compared to synthetic analogues. In this way, the problem of researching and studying new opportunities and phytoprotective sources of natural antioxidants is currently being pursued. In this paper will be elucidated results about the influence of polyphenols extracted from nettle on the antioxidant activity in the blood serum of roosters.

Key words*: antioxidant activity, oxidative stress, polyphenols.*

INTRODUCTION

Oxygen free radicals (OFR) have important functions in maintaining the body's homeostasis and regulatory processes. It is certain the involvement of OFR in apoptosis, induction of genes responsible for immunological protection, participation in the degradation of phagocytosed compounds, recruitment of leukocytes to inflammation sites, activation of ion transport systems, renewal of biological membranes, activate as second messengers etc. (Jones, 2008). The intensification of peroxidic oxidation of lipids (LPO) in various pathological processes and diseases is accompanied by the accumulation in tissues and biological fluids of LPO products, such as lipid hydroperoxides, diene conjugates, carbonyl compounds and malonic dialdehyde. The significant increase of LPO products has been established in various pathologies, including, with disruption of the physiological mechanisms of living organisms (Cabre et al., 2000).

Currently, special attention is paid to the research of the imbalance in the lipid peroxidation (LPO) - antioxidant defense (AOD) system, as a factor of chronicization of the pathological process. The relevance of studying disorders of the LPO-AOD system is determined by their connection with inflammation and destruction of membranes, the negative impact of peroxidation products on various structures (inactivation of key enzymes of glycolysis), participation in the immune conflict, and also the possibility of developing other pathologies. Tissues and organ systems have different sensitivities to the effects of reactive oxygen species (ROS). This is probably due to the dynamic perception of the expression of antioxidant enzymes and the specificity of the metabolism of different tissues, which is ensured by the intracellular redox potential (redox potential), which is a

derivative of all the biochemical reactions of the cell.
The physiological state of the organism can be assessed by analyzing the imbalance between the LPO processes and the antioxidant system (Uglanova et al., 2010).
The state of lipid peroxidation is assessed by the content of lipid peroxidation intermediate products, which may include: lipid hydroperoxides, aldehydes, ketones and a number of low molecular weight acids (formic, acetic, butyric). These products are toxic agents that disrupt the functionality of membranes and the integrity of metabolism. In this case, the synthesis of conjugated diene compounds, peroxide radicals, malondialdehyde, etc. takes place. The synthesis of conjugated dienes reflects the initiation of oxidation steps.
Conjugated dienes, which are the primary products of lipid peroxidation, are toxic metabolites that have a detrimental effect on lipoproteins, proteins, enzymes and nucleic acids (Kudaeva & Masnavieva, 2015).
Free radicals cause oxidative damage to lipids, proteins and nucleic acids. Reactive oxygen species attack or damage all cellular components and, primarily, membrane lipids. Lipids (cholesterol, polyunsaturated fatty acids), the main component of cell membranes, located in close proximity to mitochondria, are the main target of oxidative attack with the formation and accumulation of lipid peroxidation products (LPO), especially hydroperoxides, oxysterols and endoperoxides (Barrera, 2012). Reactive oxygen species and LPO products alter the permeability and fluidity of the lipid membrane and significantly damage membrane function and cell integrity (Barrera, 2012).
Oxidative stress caused by increased levels of thyroid hormones is accompanied by aggravation and decreased quality of reproductive material, especially decreased in the mobility of male reproductive cells. A series of disturbances, which occur in living organisms and their systems, a series of medicinal remedies used in various pathologies of the animal organism, especially of reproductive males, can reduce the active potential of the reproductive system. With age, there are changes in the metabolism, which lead to a decrease in the amount of antioxidants, both enzymatic and non-enzymatic, which intensifies the increase in the level of oxidative stress, including in the testicles, which causes a decrease in the quality and quantity of male reproductive material (Bajenov & Filippova, 2018).
Oxidative stress primarily affects the membrane, which contains large amounts of polyunsaturated fatty acids, which leads to a decrease in sperm mobility. The inclusion of antioxidants in the food ration of breeding males or in the freezing-thawing mediums primarily influences the mobility and concentration of spermatozoa and increases the percentage of pregnant females with natural or artificial pregnancy (Ovcinnicok, 2022).
The inclusion of antioxidants in the food ration is recommended for breeding males but also in freezing-thawing mediums for a more efficient success of assisted reproduction. Reactive oxygen species (ROS) present in the ejaculate under natural conditions can cause oxidative stress when using assisted reproduction methods and technologies: an increase in ROS was recorded during cryopreservation and thawing of ejaculate and exposure to environmental factors and medium for freezing and thawing of reproductive material. The oxygen concentration in the medium for freezing and thawing reproductive material is much higher than that in the female reproductive tract (Bajenov & Filippova, 2018; Efremov et al., 2017).
Direct-acting antioxidants have immediate antiradical properties that can be detected in *in vitro* tests. Indirect antioxidants are understood as all compounds that reduce the development of oxidative stress *in vivo* (Shchulkin, 2018). A distinctive feature of this class of antioxidants is the ability to inhibit the processes of oxidation of free radicals, exclusively in biological objects (from cellular components to the whole organism).
The antioxidant protection of cells is a very effective system organized on several levels, and therefore the points of action of this system can be different. The most significant are direct endogenous antioxidants. Among them are α- and γ-tocopherols (vitamin E), which possess maximum activity. Retinols and β-carotene are of the second level as the most important endogenous fat-soluble antioxidants. The most

widely used in the practical field, oral antioxidant complexes have been obtained, which can be incorporated into the food ration (combined feeds), because they ensure the synergy of the components, due to the different mechanisms and levels of action, and at the same time ensure the greatest total antioxidant effect (Cekman et al., 2014; Shchulkin, 2018).

It should be noted that male reproductive cells have almost no antioxidant system of their own in the cytoplasm. But seminal plasma contains antioxidant defense components. Deficiency of these substances is associated with structural damage of reproductive material, such as membrane lipid peroxidation, protein denaturation and DNA fragmentation. Existing data indicate that ROS levels in seminal plasma are significantly higher in individuals with idiopathic infertility than in fertile breeders. The effectiveness of oral antioxidant complexes is due to increased protection against ROS, mainly in seminal plasma.

On the other hand, antioxidant enzymes (superoxide dismutase, catalase, peroxidase) have great activity and specificity, in relation to which they are expressed. Direct antioxidants have been suggested to have limited pharmacological capabilities. Indirect methods, such as normalization of mitochondrial function, are much more promising (Shchulkin, 2018). In this sense, the search for mitochondrial endogenous antioxidants is relevant.

Urtica dioica (stinging nettle) is a very widespread plant and present approximately all over the globe, whose benefits for maintaining the health of living organisms are of major importance. This plant, apart from the properties of maintaining the body's homeostasis through the ROS neutralization mechanisms, also possesses nutritional properties.

Chemical investigations revealed the presence of polyphenol carboxylic acids (caffeoylmalic acid, chlorogenic acid, ferulic acid and neochlorogenic acid) of flavonoids (rutin, isoquercitrin, astragalin), pelargonidin, epigallocatechin gallate, coumarins (scopoletin), sterols, carotenoids (β-Carotene, lycopene, lutein, neoxanthin, luteoxanthin) and lectins (Grevsen et al., 2008).

The presence of reactive oxygen species is related to various pathologies, including nutritional ones (metabolic pathologies). Free radicals act mainly by attacking the unsaturated fatty acids in the biological membrane which extend to the peroxidation of membrane lipids and finally to the cell inactivation or death. The mechanism of antioxidants is mainly the neutralization and elimination of free radicals (Kataki et al., 2012).

Nettle contains protein substances, amino acids, substances of carbohydrate nature, amines, sterols, ketones, volatile oil, fatty substances, formic, acetic, pantothenic and folic acid, vitamins A, B2, C, K, chlorophyll, mineral salts such as magnesium, calcium, iron, potassium, copper and silicon. The vesicant substance (for the skin) in the fresh plant consists of formic acid, an enzyme, and also a toxalbumin, responsible for the urticaria effect on the skin.

The nettle helps to detoxify the body, favors the transport of uric acid from the tissues into the blood circulation, thus increasing the elimination of uric acid from the body, especially of birds, which is related to the digestive and metabolic peculiarities of these animals from a physiological point of view, in order to prevent gout. It also stimulates the activity of metabolism, activates blood circulation, strengthens the immune system, and is used in the treatment of avitaminosis.

MATERIALS AND METHODS

The researches were carried out on 10 roosters, maintained *in vivarium* conditions in accordance with the prescriptions of the Regulation for the maintenance of laboratory animals. The roosters were distributed in cells of one individual each and were adapted to the maintenance conditions. The animals were divided into 2 groups: control group and experimental group. The animals from the experimental group were administered hydroalcoholic extract of polyphenols, obtained from nettle (*Urtica dioica*). The roosters in the experimental group were administered orally a dose of 1 ml per animal of hydroalcoholic extract from nettles, with a total polyphenol content of 33.2 mg gallic acid equivalent (GAE)/100 g. To exclude the excitation of the digestive tract, the extract was diluted with distilled water in a ratio of 1:4. The administration was carried out with the automatic device for oral administration of medicinal remedies to animals.

The nettle was harvested from purely ecological areas and dried according to the requirements in force. For extraction, the dried nettle was ground with a coffee grinder. The separation of soluble polyphenolic compounds from plants can be achieved by diffusion of solid material (plant tissue) using a solvent that is selective for the target groups of compounds. Each plant material has certain specifics that can significantly influence the extraction of polyphenolic compounds (physico-chemical properties of target bioactive compounds, sensitivity to heat, light, enzymatic and non-enzymatic decomposition, oxygen concentration, etc.). Therefore, it is important to develop optimal extraction methods for the quantification and identification of polyphenols (Singleton et al., 1999). In order to obtain a hydroalcoholic extract with a high extraction efficiency of polyphenols, for experimental purposes, the ratio was constituted by 1:10 in hydroalcoholic solution with an alcohol concentration of 60%.

The determination of total phenolic compounds, expressed as gallic acid equivalent, was performed according to the Folin-Ciocalteu method. The Folin-Ciocalteu method was used to determine the total content of polyphenols in the extracts (Singleton et al., 1999). For this, 0.5 ml of the investigated solution was transferred into a 25 ml volumetric flask containing 10 ml of distilled water, where after that 0.5 ml of Folin-Ciocalteu reagent was added. After 5 min of rest, 8 ml of 7.5% sodium carbonate solution was added and thoroughly mixed. The volume of the flask was brought up to the mark with distilled water. After 2 hours, the absorbance was measured at the wavelength λ=765 nm. The total polyphenol content was estimated using the gallic acid calibration curve.

The total antioxidant activity (TAA) by the ABTS test is based on the ability of antioxidant compounds to annihilate the ABTS cationic radical and reduce the radical to the colorless neutral form. This test was performed according to Re's method (Re et al., 1999).

RESULTS AND DISCUSSIONS

In these researches, the antioxidant activity of polyphenols from the hydroalcoholic extract of nettle (*Urtica dioica* L.) was evaluated. The research results are presented in Table 1.

Table 1. Antioxidant activity of polyphenols from the hydroalcoholic extract of nettle (*Urtica dioica* L.) on biochemical indices in the blood serum of roosters

Group	Total protein g/L	TAA by ABTS, mM/L	TAA CUPRAC, mM/L	SOD, c.u. (min/L)	Catalase, μM/L
Control	54.64±0.68	396.31±0.56	1.01±0.16	55.62±0.61	11.72±0.53
Experimental	61.10±0.80	453.39±0.41	1.84±0.41	62.54±0.53	15.63±0.54

The role of proteins is very varied and consists in maintaining and ensuring the normal metabolism of the body but also in the maintenance of various biosynthetic pathways. They participate in the formation of immunity, provide intensive growth according to the species and breed. Proteins belonging to the Morpho-functional structures of living organisms, participate in biological processes, through the supply of amino acids that are used as plastic material for the growth of the locomotor system, the production of enzymes, the production of hormones, participate in immuno-reactive reactions, producing a series of antibodies, which fight against various infections of different nature. In the given research it is observed that the polyphenols extracted from the nettle influence the metabolism of proteins increasing them up to 61.10±0.80 g/L for the experimental group, where they can more certainly determine the capacity to meet the metabolic needs for amino acids and nitrogen, and for the control group constituting a value of 54.64±0.68 g/L, which will influence the protein metabolic needs of the body by reducing them.

To evaluate the total antioxidant activity of phenolic compounds, different methods are used, which involve the use of various mechanisms for determining the antioxidant activity. In this case we used the $ABTS^{•+}$ test (radical cation 2,2′-azinobis-(3-ethyl-6-sulfonate benzothiazoline) and the CUPRAC total antioxidant activity test, based on the ability to reduce the Cu ion by capturing the hydroxyl radical. As a result of our own research, we notice that the total antioxidant activity in the blood serum of roosters changes, obtaining an index of 453.39±0.41 mM/L in the experimental group, compared to the control group, which demonstrates a value of 396.31±0.56 mM/L, which proves to us that the polyphenols extracted from the nettle have a beneficial influence not only on the total antioxidant activity determined by the ABTS method, but also by the CUPRAC (Cupric Reducing Antioxidant Capacity) method, based on the capacity to reduce the Cu ion by capturing the hydroxyl radical, indicating an amount of 1.01±0.16 mM/L for the control group and a value of 1.84±0.41 for the experimental group.

As is known, oxygen is an indispensable element for all important vital processes in living organisms, especially for cellular and tissue respiration. However, oxygen metabolism can generate reactive elements called free radicals, especially the superoxide ion (O_2^-) and the hydroxyl ion (OH^-). These chemically unstable compounds carry free electrons that react with other molecules, destabilizing them in turn and thus inducing a chain reaction. In particular, free radicals damage DNA, essential cellular proteins and membrane lipids (lipid peroxidation), which can lead to cell death. Under physiological conditions there is a balance between the production of free radicals and endogenous mechanisms of antioxidant defense. These mechanisms mainly involve specific enzymes (superoxide dismutase, catalase and/or Gpx (glutathione peroxidase), as well as radical inhibitors such as vitamins with antioxidant properties, thiols, etc. The antioxidant system being also made up of hydrophilic antioxidant compounds, present in the cytoplasm of cells and blood serum, as well as hydrophobic compounds, which are localized in biological membranes, and strengthens its antioxidant capacities through the presence of superoxide dismutase (SOD) and catalase (CAT), being enzymatic antioxidants in the serum and cell cytoplasm, have a special contribution in the neutralization reactions of free radicals. As is known, when antioxidant defense systems are overloaded, oxidative stress occurs. This can ultimately contribute to the development of inflammatory diseases and other proliferative or degenerative biological dysfunctions. From the specialized literature it is proven that the superoxide ion (O_2^-) is the starting point in the chain production of free radicals. At this early stage, superoxide dismutase inactivates the superoxide ion by converting it to hydrogen peroxide (H_2O_2). The latter were then rapidly catabolized by catalase and peroxidases into Bio-Oxygen (O_2) and water (H_2O). Various studies have confirmed that the production under the action of SOD is the trigger of natural antioxidant defense mechanisms. Therefore, SOD is the enzyme in the natural defense against free radicals. The results obtained and presented in the table confirm a stimulating influence of superoxide dismutase activity, in the experimental group, compared to the control group, correspondingly indicating the following values, 62.54±0.53 and 55.62±0.61 c.u. (min/L). These enzymes act together, catalytically, which means that they are continuously regenerated.

Catalase is an important antioxidant enzyme responsible for the degradation of reactive oxygen species and hydrogen peroxide. It is present in all types of cells that contain the cytochrome system. Catalase catalyzes the decomposition of hydrogen peroxide into water and oxygen. Hydrogen peroxide (H_2O_2) being a by-product of various oxidase and superoxide dismutase reactions that occur inside the cell and is a normal product of cellular metabolism. The accumulation of H_2O_2 is extremely harmful to the cell and can lead to the oxidation of cellular proteins, lipids and DNA. This oxidative damage can eventually lead to DNA mutagenesis and cell death. To prevent this damage and survive, the cell relies on various antioxidants, including catalase. This enzyme has an important-vital function in cells, because it catalyzes the decomposition of hydrogen peroxide into water and oxygen, always requiring the binding of two molecules of H_2O_2 at the active site to initiate this reaction. In the conducted research, a difference of catalase activity is observed in both groups, as follows for the control group a mathematical value of 11.72±0.53 μM/L was obtained, and for the experimental group it was a value of 15.63±0,54 μM/L, which demonstrates an obvious influence of polyphenols from nettle extract (*Urtica dioica*) on the antioxidant activity in the blood serum of roosters.

CONCLUSIONS

In this way, against the background of the appearance of various pathologies, the development of oxidative stress is observed, which is manifested by increasing the level of lipoperoxidation products and endogenous intoxication. In connection with this, it is necessary to evaluate in the complex the parameters of endotoxicosis and lipoperoxidation, including the level of conjugated dienes and trienes.

Apart from polyphenols and low molecular weight pigments, further research could also include other beneficial compounds present in nettle, such as oligomers and polymers, as well as sterols, for a complete insight into the bioactive and pharmaceutical potential of nettle.

The increased total antioxidant activity and the increase in the concentration of total proteins, which have an antioxidant role through different mechanisms, demonstrate the activation of the antioxidant system against oxidative stress.

The obtained results can be used to optimize the procedures for maintaining the body's homeostasis within the normal limits of the functioning of organs and organ systems in bio-objects.

SOD induces the activation of the endogenous antioxidant defense system. As a result, it is an enzyme that, fighting against overload with free radicals, and especially when the body's own natural protective forces are diminished under the influence of chronic stress, overwork, etc. By protecting and preventing chronic disorders, which involve oxidative stress, it stops their evolution, thus favoring the living conditions of bioobjects.

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OPTIMIZING ENVIRONMENTAL INTELLIGENCE IN AN INTERNET OF THINGS SYSTEM FOR SUSTAINABLE HEALTH MONITORING

Ana-Maria COMEAGĂ, Iuliana MARIN

National University of Science and Technology POLITEHNICA Bucharest,
313 Splaiul Independenţei, District 6, 060042, Bucharest, Romania

Corresponding author email: marin.iulliana25@gmail.com

Abstract

The transformative influence of the Internet and the expansive growth of the Internet of Things (IoT) have become integral components of contemporary life. This paper delves into the intersection of IoT systems and environmental health, emphasizing the challenges posed by memory constraints in low-end IoT devices. As these devices play a role in monitoring and managing environmental parameters, the effective utilization of resources through robust memory management becomes paramount. With focus on design, configuration, scalability, and performance in scene management, this study explores the critical role of memory management in ensuring optimal functionality of IoT systems. In the context of environmental health, the paper sheds light on the intricate dynamics of memory allocation, scene execution, memory reduction, and system scalability. The study highlights the role of efficient memory management in facilitating seamless and adaptive IoT experiences in environmental monitoring applications. In conclusion, the paper underscores the need for memory management strategies as the IoT ecosystem continues to evolve. This comprehensive exploration contributes to the integral role that effective memory management plays in advancing both IoT technologies and environmental health initiatives.

Key words: *environmental health, Internet of Things, IoT applications, memory management, operating systems, resource optimization.*

INTRODUCTION

In an era dominated by interconnected technologies, the fusion of the Internet and the Internet of Things (IoT) has emerged as a driving force shaping our daily lives. From smart homes to industrial applications, the widespread adoption of IoT systems has fundamentally altered our perception and interaction with the world. At the core of this technological revolution lies a critical juncture, the convergence of IoT systems and environmental health (Elgazzar et al., 2022).

The ever-expanding landscape of the Internet of Things (IoT) has appeared in a new era of interconnected devices, revolutionizing the way we interact with our surroundings. At the heart of this digital transformation lies the critical nexus of memory management, operating systems, and environmental health, a convergence that is pivotal for the seamless operation of IoT applications (Akhigbe et al., 2021). Memory management becomes paramount in low-end IoT devices tasked with monitoring and managing environmental parameters. Operating systems play an important role in orchestrating data exchange, while the growing concern for environmental health amplifies the significance of robust IoT applications (Abid et al., 2022).

This paper tests a created IoT system, with a specific emphasis on the challenges posed by memory constraints in low-end IoT devices. As the backbone of IoT functionality, memory management ensures the smooth operation of devices, particularly in the context of environmental monitoring and management.

The study underscores the pivotal role that IoT devices play in monitoring and managing environmental parameters. From air quality sensors to presence detectors, these devices are tasked with collecting, processing, and transmitting data that forms the foundation of environmental health initiatives. However, the omnipresent challenge of memory constraints in low-end IoT devices necessitates an understanding of memory management strategies to optimize their performance.

With a focus on design, configuration, scalability, and performance in scene

management, this study delves into the role of memory management in ensuring the optimal functionality of IoT systems. The exploration extends to the multifaceted memory allocation, scene execution, memory reduction, and system scalability, providing insights into the management of resources within the proposed IoT ecosystem.
In the context of environmental health, this paper sheds light on the relationship between efficient memory management and the facilitation of seamless and adaptive IoT experiences. As environmental monitoring applications continue to evolve, the study emphasizes the need for tailored memory management strategies to navigate the changing landscape of IoT technologies. In the next section are presented the materials and methods used for a complete analysis of the proposed IoT system, followed by the outlines of results and discussion. The last section describes the conclusions and the future work.

MATERIALS AND METHODS

In addition to memory management, the study considered the influence of communication protocols on the performance of IoT systems. Various protocols, such as MQTT, CoAP, and HTTP, were evaluated for their impact on data transmission efficiency, latency, and adaptability to resource-constrained environments (Silva et al., 2021). The choice of an appropriate communication protocol is integral to the seamless operation of IoT devices, especially in applications related to environmental health where timely and reliable data transmission is important.
The lightweight and efficient publish/subscribe mechanism of MQTT proved crucial for minimizing the overhead associated with data exchange (Amanlou et al., 2021). Memory management strategies were intricately examined to ensure that MQTT, as a protocol, could operate seamlessly in resource-constrained environments. Emphasis was placed on streamlining message processing and minimizing memory footprint to enhance the protocol's adaptability, particularly in scenarios demanding low-latency communication, such as real-time environmental parameter monitoring (Donta et al., 2022).
CoAP emerged as a focal point in the evaluation of protocols due to its designed suitability for constrained devices and low-power networks (Alhaidari & Alqahtani, 2020). Memory management practices were refined to align with the specific demands of CoAP, emphasizing the need for representations of data structures (Mniszewski et al., 2021). The objective was to ensure that CoAP could operate optimally within environments where memory constraints are pronounced, maintaining responsiveness without compromising on the integrity of transmitted data (Bansal & Kumar, 2020).
As a foundational protocol for web communication, HTTP was examined through a lens that considered its implications within the IoT ecosystem. Memory management strategies were tailored to address the potentially high memory overhead associated with HTTP, ensuring that IoT devices utilizing this protocol could operate effectively without compromising on responsiveness (Abu Bakar & Kijsirikul, 2023). This scrutiny was relevant in applications where existing HTTP infrastructure was leveraged for IoT connectivity (Concha Salor & Monzon Baeza, 2023; Chen et al., 2023).
A consideration in the evaluation of these protocols was their impact on data transmission efficiency. Memory management practices were fine-tuned to facilitate streamlined data exchange, reducing overhead and optimizing the use of available resources (Saqib et al., 2023; Ajani et al., 2021).
Latency, a critical factor in real-time IoT applications, was examined for these protocols (Kondoro et al., 2021). Memory management strategies were tailored to mitigate latency challenges, ensuring that data transmission occurred with minimal delays (Ma et al., 2019).
Recognizing the diversity of IoT deployments, a study placed emphasis on the adaptability of these protocols to resource-constrained environments (Tsigkanos et al., 2019; Imteaj et al., 2021).
Memory management frameworks were designed to adapt to constraints imposed by low-end IoT devices (Samaila et al., 2020; Heidari & Jabraeli Jamali, 2022), striking a balance between protocol efficiency and memory optimization.

The current paper outlines the approach used to design and set up an IoT system. The software is built using Python as the backend and Home Assistant Community Store (HACS) for the graphical user interface (GUI). It acts as a user interface, streamlining the creation of personalized monitoring and assistance solutions. Home Assistant, a powerful and open-source home automation platform, serves as the orchestrator for the seamless integration of IoT protocols such as MQTT, CoAP, and HTTP (Fortino et al., 2022). This strategic amalgamation enhances the functionality and user experience of the IoT system, providing a centralized hub for smart home control and automation.

Home Assistant's compatibility with MQTT, a lightweight and efficient messaging protocol, brings advantages to the IoT ecosystem. MQTT seamlessly integrates into Home Assistant, enabling real-time communication between devices. This integration is instrumental in creating a responsive and interconnected IoT network, where sensors and actuators communicate. The publish/subscribe mechanism of MQTT aligns with Home Assistant's event-driven architecture, allowing for instant updates and actions based on changes in the IoT environment.

The integration of Constrained Application Protocol (CoAP) further extends the versatility of Home Assistant. CoAP, designed for constrained devices and low-power networks, aligns with Home Assistant's commitment to resource-efficient operation. This integration facilitates efficient communication between devices with reduced overhead, making it ideal for scenarios where memory constraints and energy efficiency are paramount. CoAP's ability to handle constrained environments finds a natural home within the Home Assistant ecosystem, enhancing the adaptability of the IoT system.

Home Assistant's integration with HTTP adds a layer of accessibility and compatibility to the IoT system. While HTTP is a standard protocol for web communication, its integration within Home Assistant ensures seamless interaction with a wide range of web-based services and applications. This inclusion allows users to leverage existing web infrastructure, providing a familiar and accessible means of communication. Whether interacting with external APIs, cloud services, or web-based applications, Home Assistant's HTTP integration facilitates a comprehensive and interconnected IoT experience.

For a new location, a name must be added, then you must choose the exact position on the interactive map or to introduce the values for latitude and longitude, as shown in Figure 1. If the interactive map is used, the values are introduced automatically by the program. These zones can be used for automations, so that it is known if the monitored person has left his home and moved to a new zone that exists in the system.

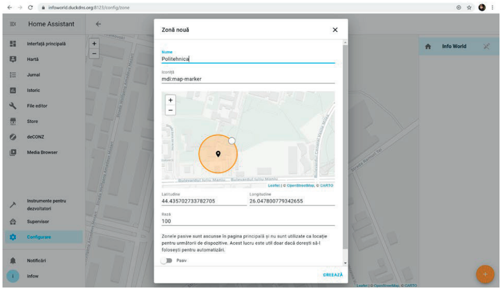

Figure 1. Adding a New Location (own source)

After authentication, the user will have access to the main interface of the system, such as the one presented in Figure 2. With this, the user can verify the status of multiple IoT devices, such as indoor lightning illumination, along with the presence of movement, CO, smoke, humidity, and door opening.

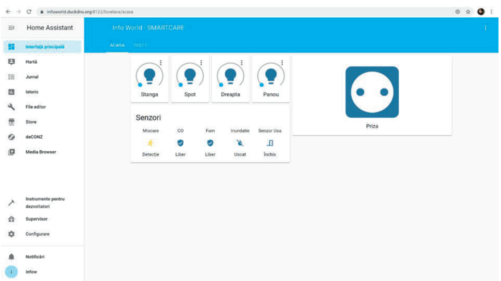

Figure 2. Main User Interface after Login (own source)

The colour and the intensity of the light sensors can be changed by the user from the graphical interface, and after this action is performed it can be observed that the specific sensor’s logo already has the chosen colour. This procedure is shown in Figure 3 and is applied to observe the changes in production when the sensors intensity is modified.

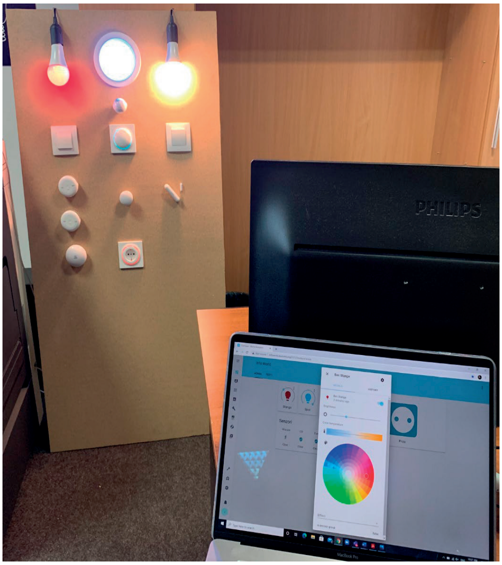

Figure 3. Changes made to the Left Bulb (own source)

The integrations made to operate the tested system involved the use of deConz software for Zigbee gateways, HACS, Meteorologisk Institutt web service to retrieve weather data, Mobile App to access the application on the mobile phone, Raspberry Pi Power Supply Checker to allow checking the power source of the Raspberry Pi which sends the data from the test board to the IoT system and the Z-Ware protocol.

deConz is a system that communicates with the ConBee and RaspBee Zigbee gateways and exposes Zigbee devices. HACS simplifies the discovery, installation, update, and removal of monitoring devices from the IoT system, customized for overseeing person activities.

The Z-Wave protocol is a technology of the wireless communication, based on radio frequency, specially designed for controlling, monitoring, and reading the states of smart devices.

Integrations are managed from the dashboard, by choosing the option called Configuration, as in Figure 4. From here, the options of each entity can be modified to reach the expectations of the user.

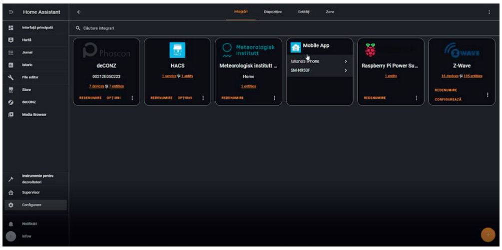

Figure 4. User Configurations for the IoT System (own source)

After entering the Devices option, the installed devices are shown to the user, like in Figure 5. In the displayed table, the installed devices can be seen, with their producer, model, assigned area, integration, and battery charge value.

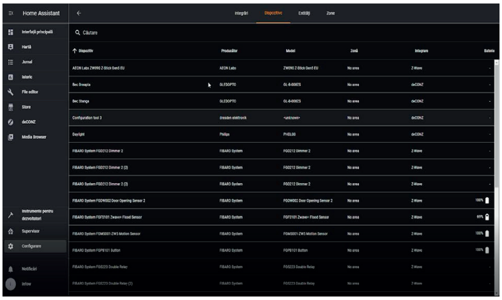

Figure 5. List of Managed Devices (own source)

In the tab named Entities appear devices that are characterized by the associated name for the action, the entity identifier, containing the name of the automation and the action, as presented in Figure 6.

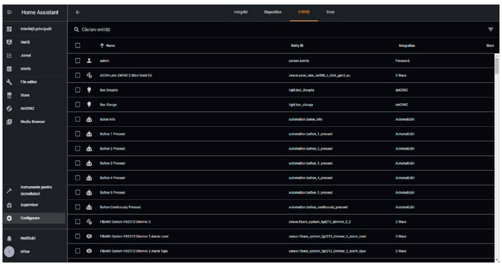

Figure 6. System Entities (own source)

Entities are individual parts of a device, such as temperature sensor, light bulb, and motion sensor. A specific device can have more entities. For example, a light bulb that monitors the indoor temperature has as device exactly the light bulb which contains the circuits and the light, and the entities are a temperature sensor and a light bulb.

Another type of example can be a button pressed in case of an alert, where the device is the button and the entities are the button's information, how many times it was pressed, namely once, to show the data for the last hour, twice, for obtaining the summary of data in the last 12 hours.

To organize better the devices, as the project grows, zones can be added for a further sorting by on them, as presented in Figure 7. In this way, the environmental factors can be observed considering the specific factors of each area, like the temperature, humidity, or CO, and see how individuals react to the specific conditions.

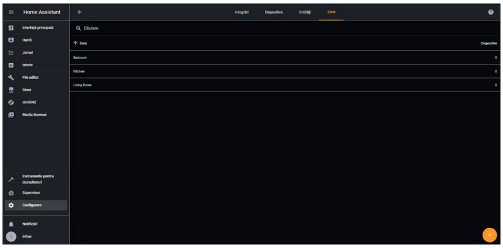

Figure 7. System Zones (own source)

The interface of the mobile application for the IoT system that allows the administration of the devices on the panel is shown in Figure 8.

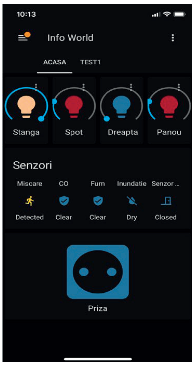

Figure 8. Mobile Application for the Management of Devices placed on the Panel (own source)

With this mobile application, the user can monitor the entire activity within a monitored zone, while being everywhere and at any time. The mobile interface is very useful, especially for elder users who live alone.

To be located within the home, the user can create a map using an editing application, such as Magicplan (Figure 9).

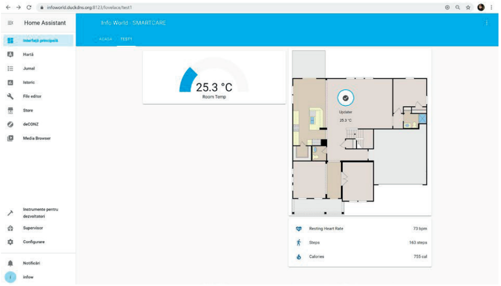

Figure 9. 2D House Plan Creation using Magicplan (own source)

The status of an entity belonging to a device can be seen by selecting the Developer Tools button from the interface, followed by the Status tab. Depending on the entity, the state can be a Boolean value (for a button, motion sensor) or a numerical one, as in the case of the temperature sensor (Figure 10). Depending on the entity, various attributes of it appear on the GUI. The status of an entity can be changed using the Set Status button.

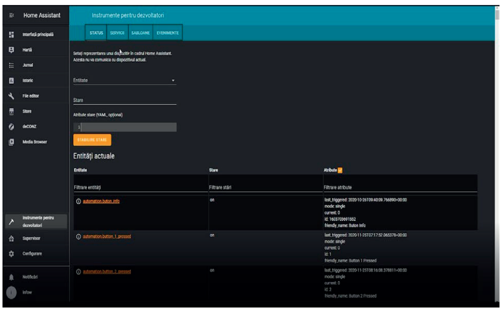

Figure 10. Entity Status (own source)

The next tab called Services is dedicated to the functionality part of the program to be able to manage the platform, in the case of reloading a web page, or physically, to turn on the light, as shown in Figure 11. A service can be called on this page to see if it works. The services are called automatically, using a button placed on the graphical interface or which can be found physically in the user's house. A service may accept parameters such as light intensity or colour.

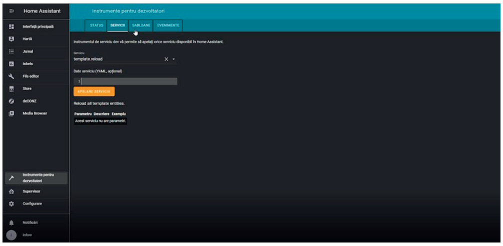

Figure 11. Platform Services (own source)

The tab called Templates is used for adding personalized panels into the graphical interface of the user, like in Figure 12. For example, the user can add a panel in which CO, humidity, person movement, and average temperature are shown. The web template engine used for implementing them is Jinja2.

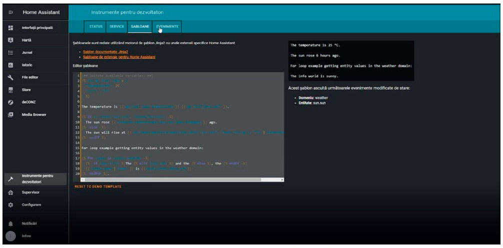

Figure 12. Platform Templates (own source)

Events can be automatically called from the platform to test their behaviour by pressing the Trigger Event button, as shown in Figure 13. You can press a button and then observe what happens with all the analysed factors and this brings some improvements to the entire smart system for managing the environmental health.

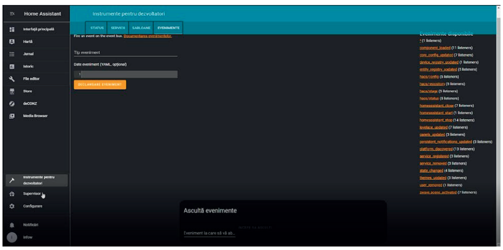

Figure 13. Event Triggers (own source)

An automation can be activated manually from the Automations menu by pressing the Trigger button. Triggering an automation in this way does not consider the conditions or the reason for the trigger. This action is only performed to verify that the automation result is what a user wanted, and then to test the conditions and triggers without pressing the Trigger button.
An automation has the following steps:
• Automation is triggered by an event, such as when a button is pressed, or a motion sensor activates. An automation can have multiple triggers. Some types of automation include the detection of a person moving, a temperature drop, or a high presence of CO.
• Automation conditions are checked. An automation can have several conditions. For example, the temperature is higher than a certain value, or the CO is higher than the average value of the past 7 days.
• If the conditions are met, a service is called, such as turn on light or open the door.
Editing of the Button 1 Pressed automation can be done by pressing the button with a pencil, and the graphical interface displayed will be the one shown in Figure 14. The type of action and the dates of the event are established in the graphical interface.

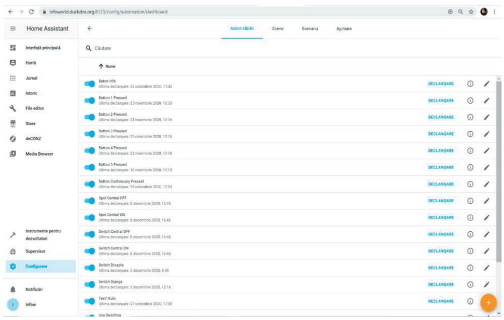

Figure 14. Automations View (own source)

RESULTS AND DISCUSSIONS

The user can view reports from the application menu. The Log button is used to generate a report containing the type of event (motion detection, light bulb/panel off, call hang up for mobile devices associated with the application) and the entity/device it is linked to. You can select a period of time for which you want to view the events, as in Figure 15. This is very helpful for the user to monitor the activity and the measures on a specific time interval and can keep the reports for further analysis and observe how the entities modify during a month or a year. With this report the user easily manages the perturbing factors and improves the general quality of the home's environment.

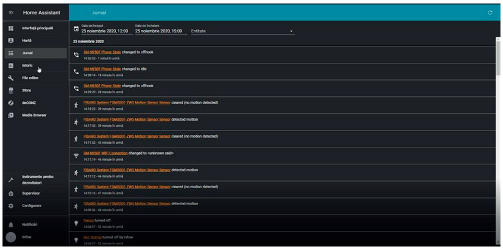

Figure 15. Event Report (own source)

Another report is the device status report. It is accessed from the main menu by clicking on the History button. Like the system event report, the period for which the report is to be generated can be selected. In green, the on state appears, and in red, the closed or off states appears. Figure 16 illustrates the state of the devices placed on the panel from Figure 3. This report is useful to analyse the state of the devices in a certain period of time and to see which of them are essential for the system, what we need to improve or eliminate to make it more efficient in terms of memory management, power consumption or time of execution.

Figure 16. Device Status Report (own source)

In the pursuit of optimizing memory management for IoT applications in environmental health, the current paper delved into the intricate dynamics of memory allocation, scene execution, memory reduction, and system scalability.
As illustrated in Table 1, the allocation of memory resources across diverse environmental sensors, namely monitoring indoor illumination, movement detection, CO levels, smoke presence, humidity levels, and door status forms the backbone of accurate and timely data collection.

Table 1. Memory Allocation Breakdown for IoT Sensors in Environmental Health Monitoring

Sensor	Memory Allocation (KB)	Purpose and Insight
Indoor Illumination	120	Ensures precise monitoring of lighting conditions, crucial for environmental assessments.
Movement Detection	80	Facilitates the rapid identification of spatial changes, enabling real-time responses to dynamic environmental conditions.
CO Levels	150	Substantial allocation for robust and accurate assessment of air quality, a cornerstone in environmental health initiatives.
Smoke Presence	100	Allocated memory to promptly detect and respond to potential fire hazards, contributing to safety and environmental well-being.
Humidity Levels	90	Dedicated memory for meticulous examination of moisture content, pivotal in assessing environmental conditions and potential health impacts.
Door Status	60	Judicious allocation to monitor door status in real-time, contributing to both security and environmental health considerations.

The interface of the IoT system serves as a central hub, allowing users to promptly verify the real-time status of multiple IoT devices. There is a direct correlation between effective memory management and the responsiveness of scene execution that consists in the system's ability to swiftly retrieve and present environmental data to users.
In our quest to enhance the adaptability of the IoT system, we delved into memory reduction strategies, as depicted in Table 2.

Table 2. Impact of Memory Reduction Strategies on Memory Consumption

Scenario	Original Memory Consumption (KB)	Reduced Memory Consumption (KB)	Reduction Percentage
Peak Usage	500	350	30%
Sudden Data Fluctuations	600	420	30%
Intensive Computational Processing	700	490	30%
Burst Data Transmissions	550	385	30%

The table illustrates the outcomes of employing memory reduction strategies during diverse scenarios, showcasing the effectiveness of these techniques in optimizing memory consumption. The scenarios include peak usage, sudden data fluctuations, intensive computational processing, and burst data transmissions.

Under peak usage conditions, the original memory consumption of 500 KB is efficiently reduced to 350 KB, resulting in a 30% reduction. This reduction enhances the system's ability to manage heightened demand without compromising responsiveness.

In scenarios where environmental parameters exhibit sudden fluctuations, the system adapts. The original memory consumption of 600 KB experiences a 30% reduction, bringing it down to 420 KB. This demonstrates the resilience of memory reduction strategies in handling abrupt changes in data patterns.

During periods of intensive computational processing, the system showcases its ability to optimize memory usage. The original consumption of 700 KB is effectively reduced to 490 KB, emphasizing the versatility of memory reduction techniques in scenarios demanding elevated computational resources.

In situations requiring rapid data transmissions, the system excels in memory optimization. The original memory consumption of 550 KB undergoes a 30% reduction, reaching 385 KB. This underlines the significance of memory reduction strategies in maintaining efficiency during bursts of data activity.

The effectiveness showcased in Table 2 is attributed to targeted memory reduction techniques, specifically optimized data compression and intelligent flushing of non-essential data. These techniques prove instrumental in sustaining system responsiveness, particularly when confronted with abrupt changes in environmental parameters.

The optimized data compression technique involves compressing data in a way that reduces its size while preserving essential information. By implementing optimized data compression, the system minimizes the memory footprint of stored information, ensuring efficient utilization of resources during peak demands and sudden data fluctuations.

The system employs intelligent algorithms to identify and flush non-essential data, prioritizing critical information for real-time processing. This targeted flushing of unnecessary data ensures that the memory space is utilized for relevant and timely information, contributing significantly to the system's adaptability during scenarios involving burst data transmissions or computational intensity.

In essence, the combination of optimized data compression and intelligent flushing of non-essential data reflects a proactive approach to memory management. These techniques not only optimize memory consumption but also enhance the system's ability to navigate and respond effectively to dynamic environmental conditions, aligning seamlessly with the goals of environmental health monitoring applications.

Our study emphasizes that efficient memory management functions for ensuring system scalability. The adaptive allocation of resources positions the IoT system to seamlessly integrate additional sensors, thus expanding its capabilities organically without compromising performance, as in Table 3.

Table 3. System Scalability and Resource Adaptation

Number of Sensors	Original System Capacity	Scalability Achieved	Key Observations and Insights
5	Low	Achieved	Even with a minimal number of devices, the system demonstrated successful scalability, showcasing adaptability to a small-scale deployment.
10	Moderate	Achieved	The system continued to scale efficiently as devices increased, indicating versatility in handling a growing network.
15	Moderate-High	Achieved	With a slight rise in devices, the system maintained high performance, highlighting its robust scalability and resource allocation capabilities.
20	High	Achieved	The system consistently adapted to increased device counts, affirming its capability to handle a diverse and growing network.

The system showcased scalability even with a minimal number of devices, proving its adaptability to smaller-scale deployments. As the number of devices increased, the system demonstrated scalability, ensuring that the architecture could handle growing workloads effectively. The system efficiently allocated resources even at lower device counts, indicating a balanced approach to resource management. The ability to scale down effectively suggests that the system is versatile and can be adapted for deployments with varying device requirements, providing flexibility in environmental monitoring applications.

CONCLUSIONS

In conclusion, our exploration into the realm of IoT systems and environmental health has unravelled the dynamics of memory management, unveiling its role in ensuring the optimal functionality and adaptability of the system. The paper outlined an approach to design and set up the IoT system, utilizing Python as the backend and Home Assistant Community Store (HACS) for the graphical user interface.

The memory management strategies discussed, such as memory allocation breakdown, memory reduction techniques, and system scalability assessments, collectively contribute to the robustness of the IoT system. The optimized data compression and flushing of non-essential data emerged as instrumental techniques in maintaining responsiveness, particularly in the face of changes in environmental parameters.

The practical implementation of the IoT system provides insights into its user-friendly interface, mobile application, and various integrations with protocols and devices. The system's ability to create zones, manage devices, and offer detailed reports provides support for environmental health monitoring. The presented results and discussions underscore the successful application of memory management strategies, making the IoT system adaptive, scalable, and resource efficient.

Looking ahead, future research in the domain of memory management for IoT systems in environmental health monitoring holds space for exploration and enhancement. Optimizing memory reduction strategies by delving into advanced compression algorithms and sophisticated intelligent flushing mechanisms presents an opportunity for more efficient memory utilization in resource constrained IoT devices. Additionally, the integration of edge computing could be investigated to reduce latency and enhance real-time responsiveness. Implementing machine learning algorithms for dynamic memory allocation based on changing environmental conditions and user preferences is another area that could enhance adaptability. Strengthening security measures through advanced encryption techniques and secure communication protocols is important for applications related to environmental health. Exploring the scalability of the IoT system in larger deployments, user-centric enhancements for a more intuitive interface, and integration with wearable devices for continuous health monitoring represent key directions for future work. Furthermore, the exploration of new environmental sensors and their seamless integration into the IoT system, as well as investigating personalized features and feedback mechanisms, will contribute to the ongoing evolution of IoT ecosystems in environmental health monitoring.

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EFFECT OF SELECTION IN LIVE WEIGHT ON REPRODUCTIVE TRAITS OF LOCAL GERZE CHICKENS

Ezgi İLKILINÇ, Elif CİLAVDAROĞLU, Umut Sami YAMAK

Ondokuz Mayis University, Agricultural Faculty, Department of Animal Science, Samsun, Turkey

Corresponding author email: usyamak@omu.edu.tr

Abstract

This study was executed to determine the effect of selection in live weight on reproduction of chickens. A total 300 Turkish local Gerze chickens were used in the study. All chickens were individually weighed at 8 weeks of age. Heaviest 75% (225 chickens) were chosen and separated from the flock. The mean live weight of chosen birds was 404.34 gram at 8 weeks and significantly higher than unselected birds (359.29 gram; P<0.05). At 18 weeks of age, the chickens were weighed again. The selected group had reached 1068.92 grams while unselected chickens 1059.69 grams and the difference between them was insignificant. Age at first egg was 182 days for selected hens while 177.5 days for unselected hens. The mean egg weights of eggs produced until 40 weeks of age were 47.11 g and 46.76 g, respectively for selected and unselected groups.

Key words: *egg weight, Gerze Chicken, live weight, selection.*

INTRODUCTION

Gerze chicken is one of two indigenous chicken breeds of Turkey. Mature body weight is 1400 g for females and 1600 g for males at one year old age. Total egg production at this age is between 85-100 per hen (Arslan et al., 2023). These lower production levels causes Gerze chickens not to be preferred for village flocks. As a result, Gerze chickens threatened with extinction. Therefore, a project started to increase live weight of Gerze chickens to make more preferable for back yard flocks. Measuring body weight is easy and there are high correlations with other traits. Therefore, selection for body weight is important for genetic improvement (Abd El-Ghany, 2005, Kosba et al., 2006; Ramadan et al., 2014). Body weight in poultry is known to be moderately to lightly heritable and hence the selection of heavier individuals in a population should result in genetic improvement of the trait (Oke et al., 2004).

While increasing body weight by selection, egg production has to be also stayed constant, because main factor influencing egg size and feed intake is body weight (Summer & Leeson, 1983).

Egg production depends of many characters and is the yield of overall performance of a bird concerning many variables such as body weight, egg weight, egg number, age at sexual maturity, egg quality, these variables are correlated with body weight and with each other in the positive or negative trends (Saleh et al., 2006; 2008; Younis et al., 2014).

On the other hand, selection for the live weight gain in laying hens showed that egg production decreased and egg weight and feed consumption increased as body weight increased because heavy birds consume more feed and lay larger eggs with large egg yolk than light hens (Lacin et al., 2008). Although egg size can be manipulated by nutrition, some other factors such as age and body weight of the hen can influence egg size (Iqbal et al., 2016).

The reproductive performance of Gerze chickens which were selected on body weight or not was investigated in the study. The effects of increased body weight on egg production, egg weight and fertility were examined.

MATERIALS AND METHODS

This study was conducted at the Experimental Farm of Ondokuz Mayis University Agricultural Faculty. Eggs collected from the Gerze chicken flock at the farm. After incubation, a total of 800 male-female mixed Gerze chickens were used in the study. 600 of chicks were wing-banded and grew on a litter system. At 8 weeks of age, all chickens were individually weighed and the heaviest 75% of females (225 chickens) and heaviest 10% of males (30 chickens) were separated as the selected group. Selected birds were individually weighed at 18 weeks of age. For the control group; 200 male-female chickens were wing-banded and reared on a litter system without selection. Control group was also individually weighed at the ages of 8 and 18 weeks. At 18 weeks of age, selected birds were divided into 6 groups each containing 37 females and 4 males. Control group was divided into two groups each containing 40 females and 4 males. These chickens were randomly chosen. Feed and water were given *ad libitium*. The nutritional values of feed given during all periods of production are given in Table 1. Lighting provided 8 hours daily until 18 weeks, gradually increased by one hour weekly, reached 13 hours at 23 weeks, and stayed constant.

Production ended at 52 weeks of age. Egg production was daily recorded. Eggs were daily collected and weighed. Sexual maturity was determined when hens reached 50% egg production and chickens were weighed at that age.

SPSS software used for statistical analyses. the Results were compared with one-way analysis of variance and the Duncan multiple comparison test was used to determine the difference between the groups.

Table 1. Nutritional values of feed given at different ages

	Ages (weeks)	Crude Protein (%)	ME (Kcal/kg)	Calcium (%)	P (%)
Egg type chick feed	0-8	19	2800	0.8-1.2	0.35
Egg type chicken development feed	9-20	16	2800	1.0-1.5	0.33
Layer feed	21-52	15	2750	3.5-4.5	0.32

RESULTS AND DISCUSSIONS

The weight of chickens at 8 and 18 weeks of age; the ages at first egg and sexual maturity ages and total egg production at 52 weeks of age are given in Table 2. The chickens were selected according to weights at 8 weeks of age. The heaviest 75% of the chickens were chosen for selected group and the mean live weight of them at 8 weeks of age was 404.34 g while the mean live weight for control was 359.29 g (P<0.05). The birds were reared until 18 weeks of age and weighed again. It’s seen that the difference between the body weights disappeared and the mean weights were determined as 1068.92 grams and 1059.69 for selected and control groups, respectively. It’s thought that this is related to variations of the flock. In both groups, pullets reached their mature weight which their genetic potential allows. The effects of selection in live weight could be seen in the next generations.

The onset of laying was earlier in the control group than in selected hens (P>0.05). The first egg was taken at 177.5 days in the control group whereas; the selected group hens laid the first egg at 182 days. Similarly, age at 50% egg production which was commented as sexual maturity age was earlier in the control group than selected hens. Control group hens reached sexual maturity at 222 days while selected group hens reached 227.67 days. In both traits, there were five-day delays for the selected group, but the differences were not significant. The mean body weights at sexual maturity were 1387.1 g and 1469.5 g for selected and control groups, respectively (P<0.05). Although the control group had significantly higher sexual maturity body weight, both selected and control group hens' body weights were around 1400 g; this weight is evaluated as a limit sexual mature weight for indigenous chicken genotypes (Tang et al., 2009). This means that both selected and control groups reached their adult body weight at sexual maturity ages. It has been reported that there exists a threshold body weight for each strain of bird (Dunnington, & Siegel, 1984). Yuan et al. (1994) reported that underweight pullets have a delayed onset of egg production and overweight ones have started egg production earlier. This notice is contrary to our findings. But it is mostly about the uniformity of birds in the same flocks. In our study, selected birds and the control group were separately reared. So, there was no competition between the birds of the same flocks which had different weight groups. Similar to our results, Bish et al. (1985) reported that body weight was not an influencing factor on age at 50% production.

Total egg production until 52 weeks of age was 52.49 and 44.0 for selected and control group hens. These values are lower than previous reports (Arslan et al., 2023). We thought that this could be related to management of the flocks. Deviation from the body weight threshold of hens can decrease the efficiency of egg production. Therefore sexual maturity weight one of the most important factors affecting egg productivity. Body weight at onset of egg production and throughout the production year influences the efficiency of egg production (Bish et al., 1985).

Table 2. Some productive traits of selected and control group chickens

	Weight at 8 weeks (g)	Weight at 18 weeks (g)	Age at first egg (days)	Age at 50% egg production (days)	Sexual maturity weight (g)	Total egg Production at 52 weeks of age	Mean egg weight (g)
Selected	404.34	1068.92	182.00	227.67	1387.1	52.49	47.11
Control	359.29	1059.69	177.50	222.00	1469.5	44.00	46.76
P	<0.05	0.684	0.159	0.230	0.001	0.299	0.418

The relationship between egg production, egg weight and mature body weight follows the same pattern as observed in the body weight at sexual maturity (Ayorinde et al., 1988; Oke et al., 2004). The sexual maturity weight of the hen is one the most significant factors affecting egg weight, at the beginning of the laying period (Robinson & Sheridan, 1982; Summers & Leeson, 1983). The mean weight of eggs produced by the control group hens until 40 weeks of age was 46.76 g, while the mean weight for selected hens at 40 weeks was 47.11 g. Although the sexual maturity weight of the control group was higher than the selected hens, the mean egg weight of the control group was lighter than selection groups' eggs. This result is contrary to the findings given above about the relationship between sexual maturity weight and egg weight. It's thought that this could be related to ages at first and sexual maturity ages of the groups. The age at the first egg was five days earlier than selection group. Similarly control group hens reached to sexual maturity age five days than selection hens. It's well known that hens begin to lay small eggs at the onset of production and eggs get bigger in the following weeks (Abiola et al., 2008). Therefore, the control group had more eggs that were laid earlier, and these eggs caused the mean weight to be lower than the selection group. Disregarding, the egg weights of both groups are in the ranges which reported that egg size within the intermediate range of 45-56 g would hatch better than small eggs (Asuquo and Okon, 1993). A positive correlation was reported between egg weight and hatchability (Senapati et al., 1996). Therefore, higher egg weight in the selection group was one of the aims of project.

CONCLUSIONS

As a conclusion, selecting local birds for live weight at immature ages did not significantly affect mature body weight at 18 weeks of age. But selected chickens. But, selected birds reached sexual maturity afterwards control group hens. Otherwise, cumulative egg production at 52 weeks of age was higher in selection group. Also, mean weight of eggs produced until 40 weeks of age was higher in selection group. This is an important point for the selection programs aiming body weight increase in the offsprings.

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CHARACTERISTICS OF HOOF GROWTH AND WEAR OF BULGARIAN RHODOPEAN CATTLE RAISED ON MANURE AND PASTURE IN THE TROYAN REGION IN THE CENTRAL BALKAN MOUNTAIN

Nikolay MARKOV, Tsvetan MARKOV, Miroslav HRISTOV, Tsvetelina DIMITROVA

Agricultural Academy - Research Institute of Mountain Stockbreeding and Agriculture, 281 Vasil Levski Str., 5600, Troyan, Bulgaria

Corresponding author email: ncm64@mail.bg

Abstract

The study examined two groups of Bulgarian Rhodopean Cattle with differing genotypes, focusing on their hoof horn qualities and analyzing the effects of both internal and external factors on these traits. Employing a blend of visual, metric, anatomical, and topographical techniques, the research measured various aspects of the cows' hooves, including length, width, overall width, height, and the angle of the hooves on both the front and back legs. These measurements were taken during periods when the cows were housed in barns and while grazing in pastures. The study also calculated the hoof's weight-bearing surface in square centimeters and the ratio of the cow's live weight per unit area of this weight-bearing surface. Results indicated that for both genotypes, cows grazing in pastures had a higher weight-bearing area relative to their live weight. The research highlights the importance of cow mobility for their health, productivity, and longevity. A key finding is that the even growth of the hoof horn, which is crucial for the animal's well-being, depends on how the body weight is distributed across the limbs. Additionally, variations in hoof growth throughout different seasons were noted.

***Key words**: angle, breed, growth, hoof horn, length, width.*

INTRODUCTION

The Bulgarian Rhodopean Cattle (BRC) is a domestic breed developed through the sophisticated reproductive crossbreeding of Rhodopean, Grey, Brown Alpine, and Jersey cattle. This breed is known for its substantial milk production, averaging between 2800-3500 kg with a high milk fat content of 5.12-5.59% and protein content of 3.6-3.7%. The BRC is characterized by its small size, pronounced mammary glands, high fertility, efficient fodder utilization, and strong adaptive capacity. Predominantly located in the mountainous regions of Bulgaria such as Kardzhali, Smolyan, Blagoevgrad, Rila, Troyan, Targovishte, and Strazhitsa, the breed was officially recognized in 1981. It is currently listed as at risk of extinction by the FAO (Gergovska & Panayotova, 2016).

The growth of the hoof horn in cattle is influenced by a range of external and internal factors, including breed, sex, management practices, dietary sufficiency, seasonal changes, and physiological state (Mohamadia & Khaglani, 2013; Zemlyanukhina, 2016; Lomonov & Skorkina, 2020). The condition of the hoof horn is an important indicator of the constitutional strength of cattle in adapting to new conditions of rearing and nutrition (Ulimbashev & Adigirova, 2016).

The use of concrete floors in farming operations is associated with accelerated growth of the hoof horn and a notable decrease in hoof angle due to increased wear. This highlights the importance for farmers to be highly aware of the environmental conditions in which livestock are raised and the growing need to ensure comfort for the cows. Investing in hoof maintenance and providing a balanced diet can yield significant long-term benefits (Marinov, 2016; Fedoseeva et al., 2016; Longova et al., 2020).

Bystrova (2008) argues that the hooves of cattle are a critical part of their exterior and that their formation is an important process that begins during their individual development.

Biomechanical principles are universal across species. In the context of cattle, it is crucial to recognize that the body weight is evenly distributed across all limbs, which is essential for understanding the biomechanics of cattle

hooves (Hernandez-Mendo et al., 2007; Homin et al., 2017; Hamzaev, 2019).
This research aimed to examine the growth and wear of the hoof horn among two groups of Bulgarian Rhodopean Cattle during the barn and pasture phases in the region of Troyan in the Central Balkan Mountain.

MATERIALS AND METHODS

The experimental study spanned from late May to late November 2023 and was conducted at two distinct locations: the farms of the Experimental Base of RIMSA-Troyan and those owned by Ivan Dimitrov, an agricultural producer in the village of Shipkovo. The research involved forming two groups, each consisting of seven Bulgarian Rhodopean Cattle (BRC), designated as Group I and Group II. The selection process for these groups was meticulously carried out using the method of analogues.
During the winter months, the cows were housed in barns that allowed for free movement, with each animal being allocated its own bed. Additionally, an outdoor courtyard was made available for the cows to roam and exercise during the colder season. The dietary regimen for the cows throughout the duration of the experiment was uniform and carefully rationed to ensure consistency.
The study focused on recording and analyzing various physical dimensions of the cows, specifically targeting the thoracic and pelvic limbs. Measurements taken included length, width, total width, height, and hoof angle. These measurements were conducted using precise instruments such as a retractable tape measure, measuring tape, plumb line, and angle ruler, adhering to the Pesechtkin methodology for assessing hoof characteristics. All collected data were meticulously documented.
Furthermore, the live weight of each cow was measured with high precision using an electronic scale accurate to 0.01 kg. Calculations were also performed to determine the weight-bearing area in square centimeters and the ratio of live weight (in kilograms) to this area.
The study employed a diverse array of methods including visual, metric, anatomical, and topographical techniques to ensure comprehensive data collection. The data were then statistically analyzed using the Statistika software (version 2010) and the results were systematically organized into tabular forms for clearer presentation and analysis. This biometrical processing utilized variational statistical methods to ensure the reliability and accuracy of the findings.

RESULTS AND DISCUSSIONS

Cattle have their peculiarities of anatomical-orthopedic features. The hoof represents an adaptation of the limb's skin, encasing the third and fourth toes in a slipper-like structure. Its lateral wall is comparatively thicker than the medial wall. The wall is about 7 mm thick in the interdigital cleft, and about 5 mm in the heel part (Marinov, 2016; Black & Krawczel, 2016; Lomonov & Skorkina, 2020).
Growth and wear of the horn layer characterize hoof horn condition in cows (Kennedy et al., 2007; Cook et al., 2009). The hoof horn colour of the experimental cows was deep black with various shades. Studies indicate that the most significant acceleration in the growth of hoof horn is observed during the grazing season, reaching up to 0.62 cm. Depending on the experimental groups, the growth of hoof horn ranges between 0.2 cm and 0.62 cm (Table 1).
Table 1 shows the data from the study of Bulgarian Rhodopean Cattle, cows from both genetic groups exhibited increased hoof horn growth rates at the conclusion of both pasture and barn periods. Specifically, in Group I (first genotype) cows raised on pasture, thoracic limb hoof measurements increased: length by 0.3 cm, width by 0.2 cm, and total width by 0.3 cm. When housed in a barn, these increases were slightly less: length by 0.2 cm, width by 0.1 cm, and total width by 0.11 cm. For pasture-raised cows in Group II (second genotype), increases were more pronounced: length by 0.62 cm, width by 0.31 cm, total width by 0.36 cm, and height by 0.1 cm. In contrast, the barn-raised counterparts showed increases of: length by 0.57 cm, width by 0.17 cm, total width by 0.38 cm, and height by 0.17 cm, with a statistical significance ($P<0.05$).
During the comprehensive study of Bulgarian Rhodopean Cattle, it was observed that cows from both genetic groups demonstrated

enhanced hoof horn growth by the end of their respective pasture and barn periods. More specifically, for Group I, which comprises the first genotype, cows that were grazed in pasture environments showed noticeable increases in their thoracic limb hoof dimensions: length grew by 0.3 cm, width by 0.2 cm, and total width also by 0.3 cm. When these same cows were housed in barn settings, the increments in their hoof measurements were somewhat less extensive, with length increasing by 0.2 cm, width by 0.1 cm, and total width by 0.11 cm. In Group II, which represents the second genotype, the pasture-raised cows exhibited more significant growth in their hoof dimensions: the length increased by 0.62 cm, width by 0.31 cm, total width by 0.36 cm, and height by 0.1 cm. Meanwhile, their barn-raised counterparts also showed noticeable growth, but with slightly different metrics: length increased by 0.57 cm, width by 0.17 cm, total width by 0.38 cm, and height by 0.17 cm. These changes in hoof dimensions were found to be statistically significant with a $P < 0.05$, highlighting the impact of rearing conditions on the hoof growth of Bulgarian Rhodopean Cattle.

Table 1. Characteristics of hoof horn, in groups (genotypes), cm

Sizes, cm	Period			
	Spring/Pasture/		Autumn/Barn/	
	Beginning of experiment	End of experiment	Beginning of experiment	End of experiment
Bulgarian Rhodopean Cattle (I group)				
Front hooves of thoracic limbs				
Length, cm	10.1±0.05	10.4±0.05**	10.51±0.01***	10.71±0.05
Width, cm	4.9±0.05	5.1±0.05	5.4±0.05	5.5±0.05
Total width, cm	13.2±0.05	13.5±.0.05**	5.43±0.05	5.54±0.05
Height, cm	5.9±0.05	6.0±0.06	6.1±0.05***	6.23±0.05
Angle of the interdigital cleft	45±0.25*	45±0.30*	45±0.30	45±0.30
Rear hooves of the pelvic limbs				
Length, cm	10.12±0.04	10.56±0.07**	10.71±0.23	10.4±0.03
Width, cm	5.1±0.05*	5.34±0.05	5.89±0.04	5.57±0.04
Total width, cm	13.65±.0.04	13.88±0.05	13.72±0.05***	13.97±0.02
Height, cm	5.8±0.04	6.0±0.02***	6.2±0.01***	6.3±0.03
Angle of the interdigital cleft	53±0.30	53±0.30*	53±0.05	53±0.04*
Bulgaria Rhodopean Cattle (II group)				
Front hooves of thoracic limbs				
Length, cm	11.02±0.03**	11.64±0.05	11.31±0.02**	11.78±0.04
Width	5.5±-0.03	5.81±0.04	6.5±0.04*	6.64±0.03*
Total width, cm	13.87±0.02*	14.23±0.03**	14.0±0.05*	14.38±0.04*
Height, cm	6.4±0.02**	6.5±0.03*	6.6±0.03**	6.77±0.04
Angle of the interdigital cleft	45±0.01**	45±0.02**	45±0.02**	45±0.04**
Rear hooves of the pelvic limbs				
Length, cm	11.62±0.03**	11.64±0.04	12.33±0.04	1278±0.02
Width, cm	6.14±0.03	6.21±0.04**	7.15±0.03	7.34±0.02**
Total width, cm	13.99±0.04	14.89±0.03*	14.24±0.04	14.53±-0.02
Height, cm	6.5±0.05**	6.8±0.06	6.6±0.03**	6.9±0.04
Angle of the interdigital cleft	54±0.4*	54±0.4***	55±0.5	55±0.4*

P<0.05*, P<0.01**, P<0.001***

In the experiment, the impact of different rearing environments on the hoof growth of cows was assessed. In Group I, cows that were housed on premises with concrete brick and cement flooring exhibited relatively minor growth in the dimensions of their pelvic limb hooves. Specifically, there was an increase in length of 0.02 cm, width by 0.07 cm, and total width by 0.90 cm. In contrast, cows raised in barn conditions demonstrated more substantial growth, with increases in hoof length by 0.55 cm, width by 0.19 cm, and total width by 0.29 cm.
Similarly, in Group II, the hoof growth patterns also varied depending on the rearing conditions. Cows that were raised in pasture settings showed similar minimal increases in hoof measurements as the concrete-raised cows in Group I: length by 0.02 cm, width by 0.07 cm, and total width by 0.90 cm. Conversely, cows that were raised in barn conditions exhibited more notable increases in their pelvic limb hoof measurements: length by 0.55 cm, width by 0.19 cm, and total width by 0.39 cm. These observations suggest that the type of flooring and the environment significantly affect the growth rates of hoof dimensions in cows.
This intensive growth of the hoof horn in cattle is influenced by various factors including breed, sex, technology, feed quality and quantity, seasonal variations, physiological state, and others. (Kvochko et al., 2010; Zemlyanukhina, 2016; Lomonov & Skorkina, 2020).

Table 2. Live weight, weight-bearing area, and the ratio between them of dairy cows of different genotypes of the Bulgarian Rhodopean Cattle breed

Raising period	Number (n)	Live weight, kg	Weight-bearing area, cm^2	Weight-bearing area/live weight ratio, kg/cm^2
Bulgarian Rhodopean Cattle (I group)				
Pasture	7	417.43±0.67*	218.86	1.9073
Barn	7	421.67±0.94*	231.24	1.8235
Bulgarian Rhodopean Cattle (II group)				
Pasture	7	423.62±1.13*	291.70	1.4522
Barn	7	434.46±1.95	363.92	1.1938

(P<0.05*)

The hoof height varied from 5.9 cm to 6.3 cm in the cows of group I, and from 6.4 cm to 6.9 cm in the cows of II group, and the angle of the interdigital cleft in both genotypes showed acceptable values of 45 cm for thoracic limbs and 53-55 cm for pelvic limbs ($P<0.05$).
The visual assessment, measurements, and calculations of the weight-bearing area of both groups of examined cows give a real description of their limbs and hooves (Muliug & Creenough, 2007; Rauibar et al., 2016; Longova et al., 2020). From Table 2, there was a higher coefficient (ratio) per unit of weight-bearing area in pasture-raised cows in both genotypes, compared to live weight, respectively 1.9073 points for I group, or by 9.6% and 1.4522 points for Group II, or a difference of 8.2%.
The data obtained in the present study are close to the results obtained by Homin et al. (2017), Longova et al. (2020), and Lomonov & Skorkina (2020), and in some indicators they complement them.

CONCLUSIONS

Several factors influence the growth of hoof horn in cattle, including the animal's innate resistance to disease and stress, the load distributed per unit area on the hoof, and varying environmental conditions. Observations from the study indicate that pigmented hoof horn, present in both groups examined, exhibits a slower rate of wear compared to its non-pigmented counterpart. Additionally, the growth of the hoof horn demonstrates noticeable seasonal variations: it tends to decelerate during the colder winter months and accelerates in the warmer summer months.
A crucial aspect of hoof health is the even growth of the hoof horn, which relies heavily on the uniform distribution of the cow's body

weight across its distal limbs. This balanced distribution is vital for maintaining the structural integrity and functionality of the hoof, which in turn reflects the overall condition of the hoof horn.

The health and maintenance of the hoof are not merely aspects of animal welfare but are integral to a cow's mobility. Effective mobility is essential for the animal's health, productivity, and longevity, which directly impact its economic value to the farming operation. Therefore, managing hoof health is a critical component of agricultural technology and practices, emphasizing the need for regular and thorough care and maintenance protocols to ensure the optimal well-being and productivity of cattle.

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INFLUENCE OF WEATHER CONDITIONS IN THE COLD PERIOD OF YEAR ON THE MICROCLIMATE IN COWSHEDS AND MILK PRODUCTIVITY OF COWS

Volodymyr SHABLIA[1], Victoria KUNETS[2], Tetiana DANILOVA[1], Petro SHABLIA[2]

[1]State Biotechnological University, 44 Alchevskikh Str., Kharkiv, Ukraine
[2]Institute of Pig Breeding and Agricultural Production of the National Academy of Agrarian Sciences of Ukraine, 1 Swedish Grave Str., Poltava, Ukraine

Corresponding author email: shabliavladimir@gmail.com

Abstract

The influence of weather conditions during the cold period of year on microclimate parameters of cowsheds and on the milk productivity of cows kept in traditional tethered conditions in typical brick cowsheds with natural ventilation was determined. It was established that in the conditions of Ukraine the temperature in cowsheds during the cold period of the year significantly depends on: the number of cows in them (r = 0.509; p = 0.018); the number of livestock per unit volume of the cowshed (r = 0.68; p = 0.001); the area of ventilation holes (r = 0.745; p<0.001). The humidity inside the cowsheds is most dependent on the outdoors air humidity (r = 0.514; p = 0.017), as well as on the number of cows per unit volume of the cowshed (r = 0.533; p = 0.013). In the cold period of the year the most problematic from the point of view of comfort for animals are periods of severe frosts. When cows were kept on a tether in typical brick cowsheds with natural ventilation and walking areas, the correlation coefficients between gross milk yield and: the average temperature outdoors were in the range of r = 0.625...0.636 (p<0.001); atmospheric pressure – r = –0.237...–0.276 (p≤0.001). A significant synchronicity of fluctuations in time of outdoor air temperature indicators and average milk yield per cow was established at periods of severe frosts: the decrease in daily milk yield due to frost reached 0.8 ... 2.2 kg per day (up to 21%). Cluster analysis established that for the synchronous distribution of days of the cold period into groups (clusters) based on the average outdoors night air temperature (tv) and gross milk yield (valmol), it is expedient to distribute the sample into 3 gradations.

Key words: *cow, cowshed, cold weather, microclimate, milk yield.*

INTRODUCTION

The influence of microclimate indicators on the condition of animals and their productivity is different (Kucevic et al., 2013).
The practice of animal husbandry in Ukraine has shown that of all microclimate indicators, air temperature has the greatest effect on animal productivity (Shablia et al., 2015).
Despite the fact that the thermoneutral zone for cattle is quite wide, for a highly productive herd creating an optimal environment including air temperature is very important.
This is due to the fact that intensive exploitation of animals requires maximum body tension. And this affects their resistance, health and productivity. This applies to both warm (Michael et al., 2023; Polsky & von Keyserlingk, 2017) and cold (Song et al., 2023; Angrecka et al., 2020; Ahmed et al., 2022) periods of the year.
Next in importance for maintaining an optimal microclimate are indicators of humidity (Xiong Yan et al., 2017; Havelka et al., 2022) and the content of harmful gases in the air (Mazur et al., 2021).
Regulation of the microclimate in livestock premises is one of the important links in the technology of industrial milk production. This is possible if the construction solutions of livestock premises involve the use of effective ventilation systems and building materials that correspond to the climatic zones (Picuno, 2016).
Farmers must provide such zoohygienic parameters of the microclimate that would fully meet the physiological needs of the body and ensure good health of animals (Assatbayeva et al., 2022).
A comfortable indoor microclimate promotes effective heat exchange, quick adaptation, and prevention of animal diseases. It makes it possible to increase natural resistance, ensure

high reproductive capacity, reduce feed and energy costs, as well as obtain a genetically determined amount of high-quality products from animals.

According to Samarin (2000), the productivity of animals is determined by 10-30% by the microclimate in livestock premises and the conditions of keeping. In particular, the deviation of the microclimate parameters from the optimal limits can lead to a decrease in milk yield by 10-20%, a decrease in live weight gain - by 20-35%, an increase in the death of young animals - by 5-40%, a decrease in the resistance of animals to diseases.

Creating and maintaining an optimal micro-climate is also associated with extending the service life of buildings and the equipment installed in them (Skliar et al., 2023), decrease in the cost of additional feed as well as positive affects service personnel.

The analysis of literary sources shows that cowshed ventilation systems with natural excitation of air movement are currently the most widely used in cattle breeding (Kavolelis et al., 2008; Wójcik et al., 2017; Ogink et al., 2013; Nieckarz et al., 2023). This is explained by the fact that such systems are independent of energy sources and have high reliability.

However, under such ventilation system the microclimate and therefore the dairy productivity of cows can be largely determined by the influence of weather factors (Hill & Wall, 2015; Głuskia et al., 2014).

The purpose of the research is to determine the influence of the weather conditions in the cold period of the year on the parameters of microclimate in typical brick cowsheds with natural ventilation and on the milk productivity of cows kept in traditional tethered housing.

MATERIALS AND METHODS

The material for the research was information on the main characteristics of the weather that took place in the conditions of the farm "Gontarivka" (Kharkiv region, Ukraine) in the cold period of the year (the period from October to April lasting 195 days), when the average daily air temperature outside was in the range of +10ºC to –26ºC).

Thus, air temperature, relative air humidity, speed of air movement outdoors, atmospheric pressure, wind direction, cloud cover, and amount of precipitation were taken into account. These meteorological indicators can potentially affect the indoor microclimate and animal comfort, and therefore milk production.

At the same time, the collection of information on the volumetric and planning characteristics of 6 cowsheds of different sizes was carried out. In particular, the length, width, height of the cowsheds (m), their volume (m^3), the total area of open ventilation holes, the number of cows and other indicators were measured. The features of cowsheds and applied technologies are determined.

All cowsheds were built of brick and had a natural ventilation system. Cows were kept on a leash in all cowsheds. From 100 to 200 cows were kept in various cowsheds.

The parameters of the microclimate inside the cowsheds were recorded, as well as the dynamics of milk yield, fat and protein content in milk during the same period in two branches of the farm "Gontarivka" – "Central" (3 cowsheds) and "Profintern" (3 cowsheds).

Databases have been created, which include the above indicators characterizing milk productivity, microclimate of cowsheds, technology characteristics and weather.

The relationship between the main indicators of weather conditions, the microclimate of cowsheds and milk productivity were evaluated by correlation analysis. Regularities of influence of weather conditions on milk productivity were additionally studied using cluster analysis.

RESULTS AND DISCUSSIONS

The results of the conducted research show that the microclimate of cowsheds in the cold period of the year is formed to a large extent under the influence of weather conditions. It was established that the correlations between the air temperature outdoors and the temperature inside different cowsheds was at the level of $r = 0.80 ... 0.94$ ($p = 0.029 ... 0.002$).

Among other studied factors, the temperature in the cowsheds depended most on: the number of cows kept in the cowshed ($r = 0.509$; $p = 0.018$); the number of cows per unit volume of the cowshed ($r = 0.680$; $p = 0.001$); the area of open ventilation holes ($r = 0.745$; $p<0.001$).

The relative humidity of the air inside the cowsheds was most dependent on the outdoor air humidity ($r = 0.514$; $p = 0.017$), as well as on the number of cows per unit volume of the cowshed ($r = 0.533$; $p = 0.013$). Thus, our research supports the claims of Głuskia et al. (2014) and Hill & Wall (2015) about the significant direct influence of weather conditions on microclimate parameters (such as temperature and air humidity inside cowsheds when using natural ventilation). In our studies, this influence refers to the cold period of the year.

As a result of the listed factors, the winter temperature in the cowshed with the lowest number (density) of cows per unit volume of the cowshed periodically dropped below 0ºC. This happened mostly when the air temperature outside was below –12ºC. The result was freezing of the water in drinking troughs, uncomfortable conditions for animals and workers (temperature outside the comfort zone).

In the autumn and spring periods, the temperature indicators were closer to the optimal level, which is largely due to more accepted indicators of the outdoors temperature and greater possibilities of regulating the intensity of ventilation. Humidity indicators during these seasons slightly exceeded the normative due to high humidity values outside.

It was established that in the cold period of the year (lasting 195 days), the correlation coefficients (Tables 1, 2) between the gross milk yield on the farm and: the average day and night air temperature outdoors were within $r = 0.625 ... 0.636$ ($p < 0.001$); atmospheric pressure - $r = –0.237 ... –0.276$ ($p \leq 0.001$). The significant correlation coefficients obtained by us testify to the correctness of the conclusions of Angrecka et al. (2020), Ahmed et al. (2022) and Song et al. (2023) regarding the considerable influence of the microclimate in the cold period of the year on animal productivity. And the data on the maximum correlations of milk productivity with air temperature are consistent with the statement of Shablia et al. (2015) on air temperature as the most influential indicator of microclimate.

Table 1. Correlation coefficients between the characteristics of night weather conditions in the cold period of the year (lasting 195 days) and indicators of morning milk productivity of cows at the "Profintern" branch

Characteristics	Gross milk yield, kg	Milk fat content, %	Milk protein content, %	Average air temperature, ºC (tv)	Minimum air temperature, ºC	Maximum air temperature, ºC	Atmospheric pressure, mm Hg	Relative air humidity, %	Speed of air movement, m/s	Cloudiness, %	Amount of precipitation, mm
Gross milk yield, kg	1	0.479**	0.107	0.636**	0.609**	0.711**	-0.276**	-0.178*	-0.070	-0.016	0.085
Milk fat content, %	0.479**	1	0.195**	0.205**	0.217**	0.282**	-0.079	-0.199**	-0.031	-0.124	0.043
Milk protein content, %	0.107	0.195**	1	0.064	0.046	0.098	-0.164*	-0.198**	-0.006	0.067	0.052
Average air temperature, ºC (tv)	0.636**	0.205**	0.064	1	0.992**	0.970**	-0.471**	0.228**	-0.105	0.277**	0.162*
Minimum air temperature, ºC	0.609**	0.217**	0.046	0.992**	1	0.950**	-0.460**	0.268**	-0.091	0.327**	0.166*
Maximum air temperature, ºC	0.711**	0.282**	0.098	0.970**	0.950**	1	-0.450**	0.133	-0.117	0.180*	0.156*
Atmospheric pressure, mm Hg	-0.276**	-0.079	-0.164*	-0.471**	-0.460**	-0.450**	1	-0.126	-0.256**	-0.271**	-0.369**
Relative air humidity, %	-0.178*	-0.199**	-0.198**	0.228**	0.268**	0.133	-0.126	1	-0.146*	0.308**	0.185**
Speed of air movement, m/s	-0.070	-0.031	-0.006	-0.105	-0.091	-0.117	-0.256**	-0.146*	1	0.086	0.141*
Cloudiness, %	-0.016	-0.124	0.067	0.277**	0.327**	0.180*	-0.271**	0.308**	0.086	1	0.112
Amount of precipitation, mm	0.085	0.043	0.052	0.162*	0.166*	0.156*	-0.369**	0.185**	0.141*	0.112	1

Notes. * – significance level of correlation coefficient P≤0.05; ** – significance level of correlation coefficient P≤0.01.

In the coldest period of winter which took place in January-February 2014 (lasting 31 days), correlations between indicators of milk productivity and weather were mostly slightly smaller than when taking into account the entire cold period, but mostly statistically significant.
Extreme deviations of the air temperature from the norm in the coldest period have a particularly strong effect on the productivity of animals. Thus, the decrease in milk yield due to severe frosts (with unchanged feeding) was of the order of 0.8 - 2.2 kg per cow per day or up to 21% (Figures 1, 2). The detected level of decrease in milk productivity of cows generally corresponds to the 10-20% decrease in milk yield indicated by Samarin (2000). The reason of both is the deterioration of the microclimate.
It should be noted that after the warming there was no recovery of milk productivity to the level of indicators that occurred before severe frosts at the branches of the farm "Gontarivka".

Table 2. Correlation coefficients between the characteristics of night weather conditions in the cold period of the year (lasting 195 days) and indicators of morning milk productivity of cows at the “Central” branch

Characteristics	Gross milk yield, kg	Milk fat content, %	Milk protein content, %	Average air temperature, °C (tv)	Minimum air temperature, °C	Maximum air temperature, °C	Atmospheric pressure, mm Hg	Relative air humidity, %	Speed of air movement, m/s	Cloudiness, %	Amount of precipitation, mm
Gross milk yield, kg	1	-0.388*	0.087	0.401*	0.369*	0.430*	-0.575**	0.470**	-0.146	0.349	0.279
Milk fat content, %	-0.388*	1	0.037	-0.288	-0.273	-0.291	0.374*	-0.343	0.236	-0.024	-0.080
Milk protein content, %	0.087	0.037	1	-0.597**	-0.591**	-0.599**	0.179	-0.524**	0.490**	-0.013	-0.048
Average air temperature, °C (tv)	0.401*	-0.288	-0.597**	1	0.996**	0.995**	-0.777**	0.865**	-0.293	0.390*	0.387*
Minimum air temperature, °C	0.369*	-0.273	-0.591**	0.996**	1	0.983**	-0.771**	0.854**	-0.255	0.387*	0.412*
Maximum air temperature, °C	0.430*	-0.291	-0.599**	0.995**	0.983**	1	-0.783**	0.877**	-0.323	0.398*	0.366*
Atmospheric pressure, mm Hg	-0.575**	0.374*	0.179	-0.777**	-0.771**	-0.783**	1	-0.819**	0.082	-0.509**	-0.356*
Relative air humidity, %	0.470**	-0.343	-0.524**	0.865**	0.854**	0.877**	-0.819**	1	-0.401*	0.287	0.359*
Speed of air movement, m/s	-0.146	0.236	0.490**	-0.293	-0.255	-0.323	0.082	-0.401*	1	0.083	0.128
Cloudiness, %	0.349	-0.024	-0.013	0.390*	0.387*	0.398*	-0.509**	0.287	0.083	1	0.025
Amount of precipitation, mm	0.279	-0.080	-0.048	0.387*	0.412*	0.366*	-0.356*	0.359*	0.128	0.025	1

Notes. * – significance level of correlation coefficient P≤0.05; ** – significance level of correlation coefficient P≤0.01.

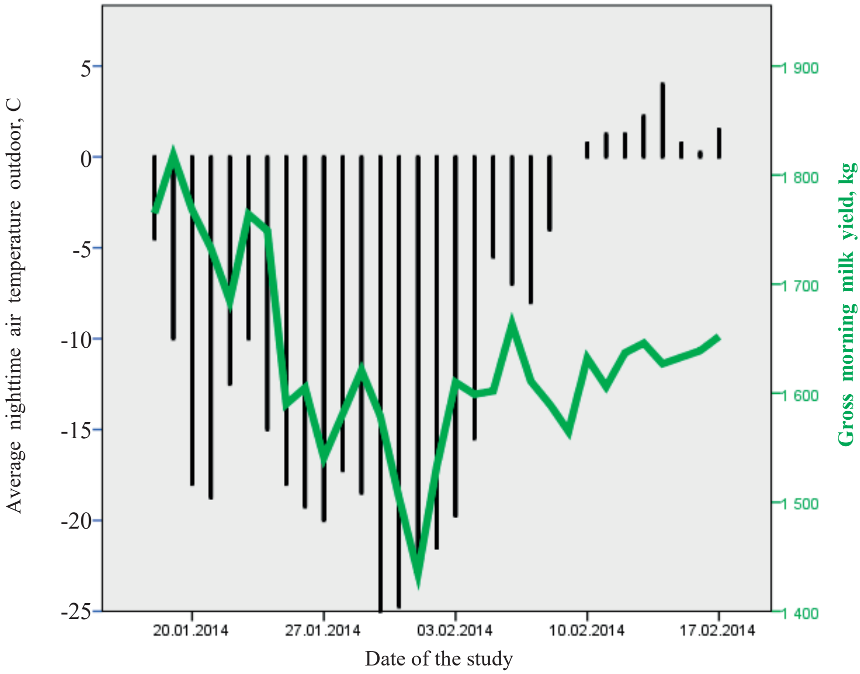

Figure 1. Dynamics of changes in time of the average nighttime temperature outdoor (left scale, columns) and gross morning milk yield (right scale, curve) during the coldest period of the year at the "Profintern" branch

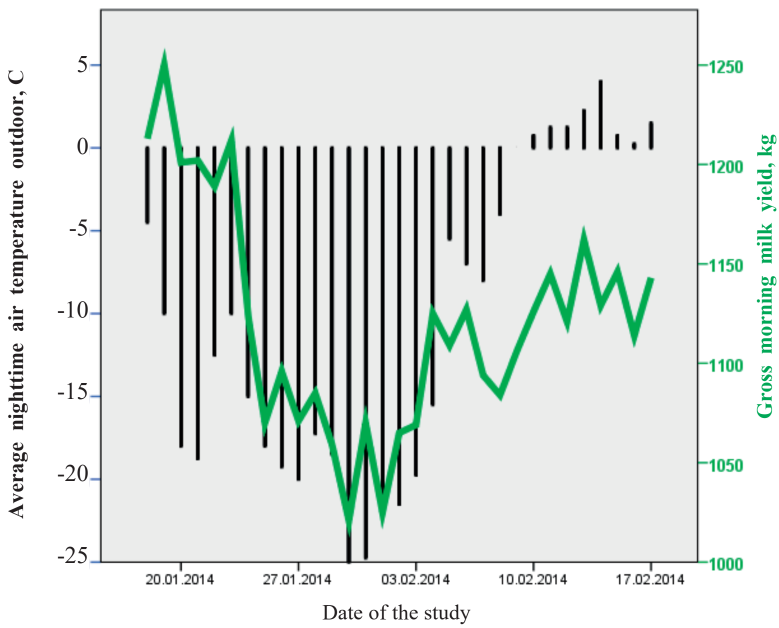

Figure 2. Dynamics of changes in time of the average nighttime temperature outdoor (left scale, columns) and gross morning milk yield (right scale, curve) in the coldest period of the year at the "Central" branch

Cluster analysis established that in order to synchronously distribute the days of the cold period of the year into groups (clusters) based on the average outdoor night air temperature (tv) and gross milk yield at the branch (valmol), it is expedient to distribute the sample into 2-3 gradations.

In particular, at the "Profintern" branch (Figure 3), the centers of three temperature gradations were: tv_1 = +6.11°C, tv_2 = –0.55°C and tv_3 = –19.07°C; and the corresponding average gross milk yields were: $valmol_1$ = 2276 kg, $valmol_2$ = 1883 kg and $valmol_3$ = 1609 kg. At the "Central" branch, the centers of the two temperature gradations were: tv_1 = 2.38°C and tv_2 = –16.50°C; and the corresponding average gross milk yield were: $valmol_1$ = 1478 kg and $valmol_2$ = 1150 kg. The differences between the gross milk yields of the groups (clusters) extreme in terms of outdoor temperature (t_1 and t_2 for the "Central" branch; t_1 and t_3 for the "Profintern" branch) were 328–667 kg ($p<0.001$). These results confirm the correctness of the statement of Shablia et al. (2015) that the negative influence of microclimate parameters on milk production of cows is observed during periods of long maximum deviations of these parameters from optimal values.

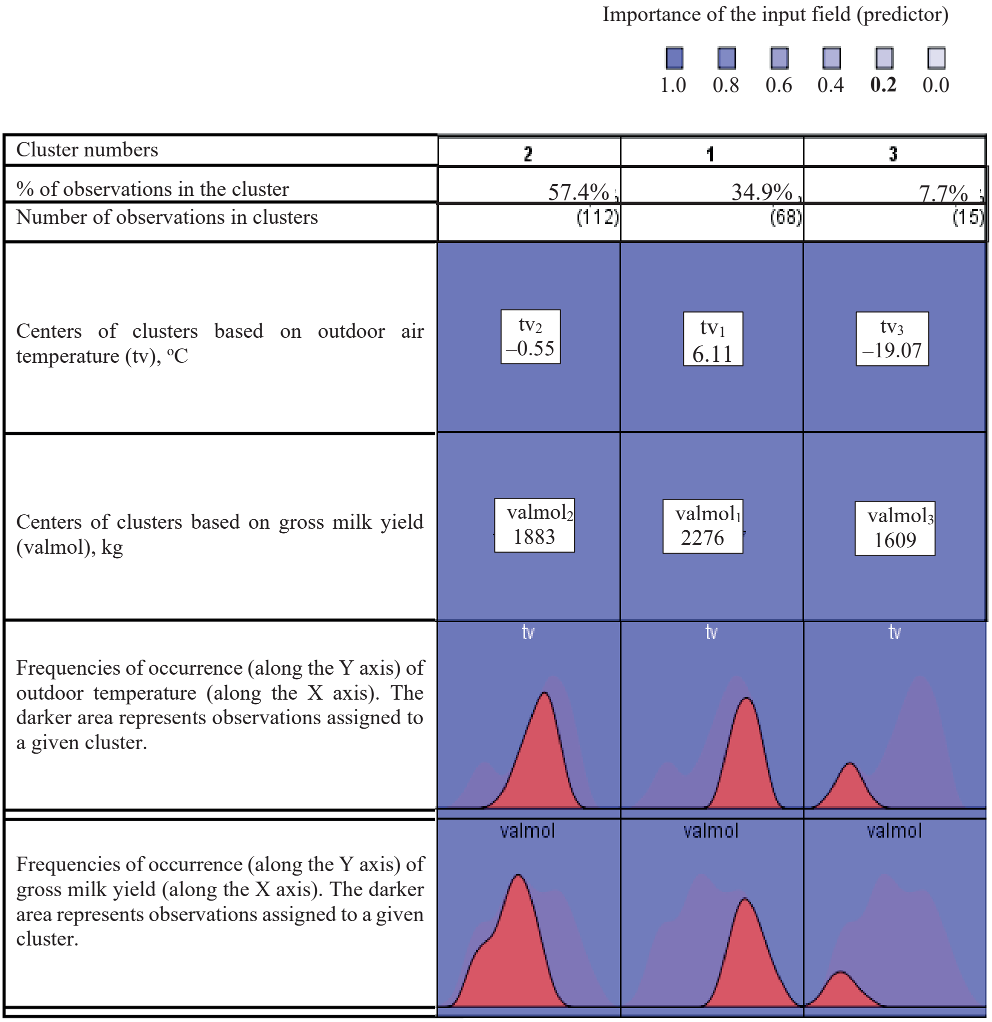

Figure 3. Results of cluster analysis of average nighttime outdoor temperature (tv) and gross milk yield (valmol) during the cold period at the "Profintern" branch

CONCLUSIONS

1. The temperature in the cowsheds during the cold period of the year significantly depends on: the number of cows in it ($r = 0.509$; $p = 0.018$); the number of cows per unit volume of the cowsheds ($r = 0.68$; $p = 0.001$); the area of ventilation holes ($r = 0.745$; $p<0.001$).
2. The humidity inside the cowsheds is mostly dependent on the outdoors air humidity ($r = 0.514$; $p = 0.017$), as well as on the number of cows per unit volume of the cowshed ($r = 0.533$; $p = 0.013$).
3. In the cold period of the year, the most problematic from the point of view of comfort for animals are periods of severe frosts. When cows were kept on a tether in typical brick cowsheds with natural ventilation and walking areas, the correlation coefficients between gross milk yield and: the average temperature outside were in the range of $r = 0.625$ ... 0.636 ($p<0.001$); atmospheric pressure – $r = –0.237$ ... $–0.276$ ($p≤0.001$).
4. A significant synchronicity of fluctuations in time of outdoor air temperature indicators and average milk yield per cow was established at periods of severe frosts: the decrease in daily milk yield due to severe frost reached 0.8 ... 2.2 kg per day (up to 21%).
5. Cluster analysis established that for the synchronous distribution of days in the cold period into groups (clusters) at the same time according to the average outdoors night air temperature (tv) and gross milk yield (valmol), it is more expedient to distribute the sample into 3 gradations. The centers of temperature gradations are tv1 = +6.11ºC, tv2 = –0.55ºC and tv3 = –19.07ºC. And the corresponding average gross milk yield are valmol1 = 2276 kg, valmol2 = 1883 kg and valmol3 = 1609 kg . The difference between extreme groups by outdoors temperature were 667 kg of gross milk yields ($p < 0.001$).

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MORPHOLOGICAL INDICATORS OF VISCERAL ORGANS OF THE SNAILS *Helix pomatia* AND *Helix aspersa* Muller UNDER THE ABIOTIC AND BIOTIC FACTORS` INFLUENCE

Olena ZHIGALOVA, Mykola KUSHCH, Olena BYRKA, Viktoriia YURCHENKO, Liubov LIAKHOVICH, Andriy ZAKHARIEV, Sofia SHILINA

State Biotechnological University, 44 Alchevskikh Str., Kharkiv, Ukraine

Corresponding author email: histology@ukr.net

Abstract

The microstructure of the stomach, intestines, hepatopancreas, kidneys, lungs, reproductive organs, and protein gland of two species of snails (Helix pomatia and Helix aspersa Muller) under the influence of abiotic (climatic) and biotic (parasite) factors was studied. In snails of the first group, the visceral organs had a typical structure. The parenchyma of the hepatopancreas, as the most vulnerable organ, is represented by glandular tubules and the system of excretory ducts that open into the intestinal cavity. Structural changes in the hepatopancreas and gonads were observed in snails of the second group, which were in the state of anabiosis under the influence of dry, hot weather. The helminthic invasion in the snails of the third experimental group caused necrosis of the hepatopancreas and replacement of the parenchyma of the gland with loose connective tissue.

***Key words**: hepatopancreas, microstructure, snails.*

INTRODUCTION

Snails of the genus *Helix* belong to the family *Helicidae* of the order *Pulmonata*, class *Gastropoda* (El-Khayat et al., 2015; Bouchet et al., 2005). Snails of the species *Helix pomatia* and *Helix aspersa* are common components of the biocenosis in all climatic and geographical zones of the world (Kotsakiozi et al., 2012; Ligaszewski, 2009). The deepening of research on this class of invertebrates is due not only to their unique biological properties, but also to the growing demand for them as food. In recent years, snail farming as a business sector has been developing intensively. Technologies for snail breeding and processing are being developed and improved (Tluste & Birkhofer, 2023; Rygało-Galewska et al., 2022; Zubar & Onyshchuk, 2020; Carbone & Faggio, 2019; Cilia & Fratini, 2018; Ligaszewski et al., 2016, Zymantiene et al., 2008; S.-Rózsa, 2002).

In Ukraine, snail business entities such as Snailgroup company, the "Agroravlyk" Farm, the Ukrainian Association of Snail Producers, and others are increasing their production capacity (DSTU 7353:2013. Meat. Method of histological determination of freshness and degree of ripening. Valid from 2014-01-01. Kyiv, Ukrainian). In December 2021, the first all-Ukrainian conference was organised on the basis of the "Agroravlyk" Farm, which aims to promote snail farming, as well as to improve the level and quality of its technological processes (First All-Ukrainian conference of snail breeders, "Agroravlyk" Farm, Kyiv, Ukraine, 2021; Paska et al., 2020; Zubar & Onyshchuk, 2020; Carbone & Faggio, 2019; Zymantiene et al., 2008).

In the European Union, legal regulations are mainly focused on the control of the microbiological quality of cooked snail meat and hygienic conditions during their cultivation (Rygało-Galewska et al., 2022; Ligaszewski et al., 2016; Charrier et al., 2006). Regulation № 2073/2005 of the European Union Commission defines requirements for *Salmonella* in snail meat and for *E. coli* and coagulase-positive *Staphylococci* in the hygiene of their cultivation (Paszkiewicz et al., 2014; Regulation № 2073/2005 of the European Union Commission on microbiological criteria for foodstuffs, Brussels, Belgium, 2005).

When analysing scientific studies on the biological characteristics of snails, attention has been drawn to the very limited speed of their movement (0.047 km/h), which is why all generations of a single snail population exist in a certain territory, i.e. they are almost incapable of migration (Becker et al., 2021; Habib et al., 2018; Lőw et al., 2016; Nowakowska et al., 2006; S.-Rózsa, 2002). The life expectancy of a snail in artificial conditions can reach 20 years, but in the "wild", due to the influence of unfavourable factors, it does not exceed 8 years (Peña et al., 2017; Kotsakiozi et al., 2012). Since snails are predominantly herbivorous organisms, plants are one of the main links in the cycle of ecosystem pollutants (Tluste & Birkhofer, 2023; Amal & Abdel-Rahman, 2020; Lobo-da-Cunha, 2019; Otitoloju et al., 2009; Hamed et al., 2007; Rabitsch, 1996).

Ecological pollutants, both organic and inorganic, have cumulative properties in relation to biotic (plants, animals) and abiotic (soil, water, air) ecosystems. In this sense, first of all, the question arises of the safety of consuming products of "spontaneous" snail farming, which is often used by collecting gastropods in the "wild" (Tluste & Birkhofer, 2023; Rygało-Galewska et al., 2022; Ali et al., 2019; Rota et al., 2016; Köse et al., 2015; Hamlet et al., 2012; Sherifa et al., 2007; Charrier et al., 2006; Gomot & Pihan, 1997).

It is known that the animal body reacts with appropriate structural and functional changes to the accumulation of exotoxins in it. Therefore, attention has been drawn to the possibility of using snails as environmental bioindicators and biological test systems for organic and inorganic pollutants not only in their habitat but also in feed and livestock products obtained from these areas (Yasmeen S. M. Abd El Mageed et al., 2023; Carbone & Faggio, 2019; Parvate & Thayi, 2017; Rota et al., 2016; Sharaf et al., 2015; El-Khayat et al., 2015; Mohammadein et al., 2013; Almedros & Porcel, 1992; Janssen & Dallinger, 1991).

MATERIALS AND METHODS

The goal of the study is to determine the structural parameters in the organs of the visceral part of the body of the snails *Helix* under the influence of habitat conditions and pathogenic factors (helminthiasis).

To achieve this goal, the following objectives have been set:

1. To collect the snails *Helix pomatia* living in different natural environmental conditions in the temperate continental climate zone of Europe.
2. To study the anatomical features of the body structure of the snails *Helix pomatia* and to select material for histological examination.
3. To development the technique of making histological preparations from the visceral part of the snail body and their microscopy with the identification of organs.
4. To compare the histological structure of the organs of the visceral part of the body of the snails *Helix pomatia* collected under different natural environmental conditions.
5. Coproscopic examination of the snails *Helix aspersa* to detect helminthic invasion and determine the state of the microstructure of the visceral organs of the infested snails.

To conduct the planned research, 3 groups of snails of the same age, 10 individuals each, have been used.

The *first group* (*control group*) includes the snails *Helix pomatia*, which have been collected during their life activity in May in cool and moderately humid weather on the territory of a household plot where chemical plant protection products were not used during the spring and summer season.

The *second group* includes the snails *Helix pomatia* collected from the same household plot, but in hot weather – in August. The snails were found in a state of anabiosis, fixed mainly to the bark of tree trunks.

The *third group* includes the snails *Helix aspersa* Muller from a specialised farm collected in May under weather conditions similar to those of the first group. Snails with low motor activity and reduced feed consumption have been used.

Snails were dissected according to the method described in *Atlas of Animal Anatomy and Histology* (Lőw et al., 2016) (Figure 1).

Figure 1. Preparation for dissection
(Own source)

The visceral part of the snail's body was separated from the foot with scissors along the lower surface of the mantle edge, starting from the pneumostome.
Samples for histological examination were fixed during the week in a neutral aqueous formalin solution, with the first day in 5% followed by re-fixation in 10% solution. According to the classical method of making paraffin blocks, the sampled material was passed through a battery of alcohols of increasing concentration and embedded in paraffin. Histological sections of 7-10 µm thick were stained with haematoxylin and eosin (Horalskyi et al., 2019). Histological preparations from the visceral part of the snail's body were examined using *Jenamed-2* light microscope.
Coproscopic examination for parasitosis has been performed using the native smear and Fülllebom methods. A native smear was prepared on a slide, a drop of distilled water was added, in which a lump of snail secretions was thoroughly stirred and covered with a cover slip. According to the Fülllebom method, the snail secretions were stirred in a beaker with a cooled saturated solution of sodium chloride, filtered through a metal strainer and extracted for 40 minutes. Three drops were taken from the surface of the sample with a metal loop onto a degreased slide and covered with a cover slip. Microscopy in both cases was performed at low microscope magnification (MBS, objective – 10^x, eyepiece – $12,5^x$) (Halat et al., 2004).
The experiment was conducted in accordance with generally accepted principles of humane treatment of animals (Law of Ukraine "On the Protection of Animals from Cruelty Treatment", No. 3447-IV as of 21.02.2006, Kyiv; European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, Strasbourg, 1986). The work was done at the Department of Normal and Pathological Morphology of the State Biotechnological University (Kharkiv, Ukraine).

RESULTS AND DISCUSSIONS

When studying the anatomical structure of the snails, it has been revealed that the body of the snail is firmly connected to the calcareous shell and consists of a head, body and foot. The visceral part of the body is located in the distal whorls (Figures 2, 3).

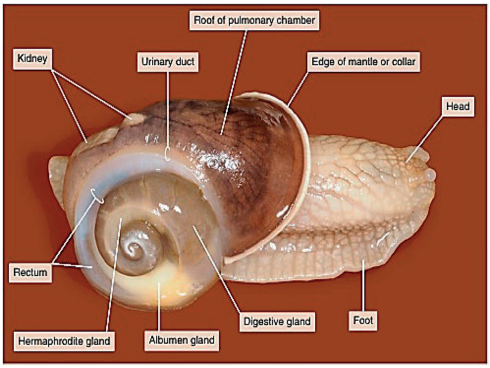

Figure 2. Structure of the snail's body after shell removal (Lőw et al., 2016)

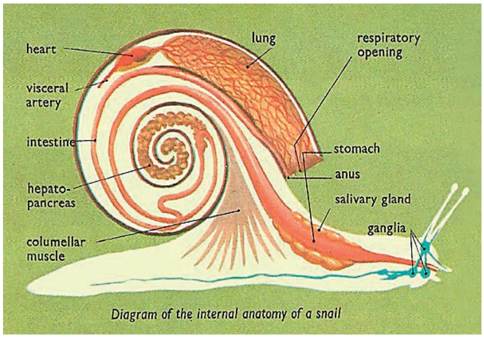

Figure 3. Diagram of the body structure of a snail. Retrieved from https://www.daviddarling.info/encyclopedia/S/snail.html

It has been determined that the snail *Helix pomatia* (Grape snail) belongs to the pulmonary snails and is a hermaphrodite. Under unfavorable conditions (too high or low air temperature, drought), snails retract their bodies into the shell and close its outer opening with an epiphragm formed by congealed mucus with minerals, which makes it strong. During this period, snails are in a state of anabiosis, which can last for 3-5 months (Noothuan et al., 2021; Kotsakiozi et al., 2012; Pirger et al., 2004).
Histological studies have shown that the section covers the visceral part of the snail's body and allows to identify almost the entire complex of internal organs: stomach, intestines, hepatopancreas, and gonad. A special feature of the digestive system of a snail is the presence of an organ called the digestive gland, or hepatopancreas, which combines the functions

of the liver and pancreas and performs an excretory function (Figures 2, 3).

In snails, there is no separation of visceral organs by serous membranes due to the very limited size of the secondary body cavity – coelom (Figure 4). Only the pericardial cavity is formed.

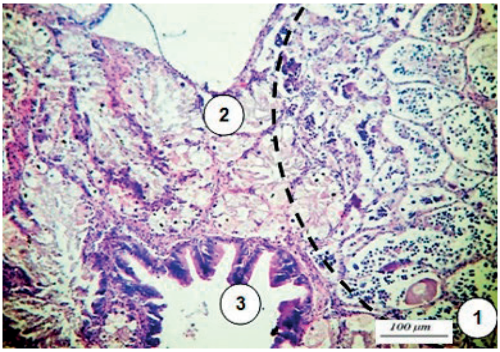

Figure 4. Fragment of the visceral part of the body of the snail *Helix pomatia* of the first group: 1 - hermaphroditic gonad; 2 - hepatopancreas; 3 - intestine. H & E (Own source)

On the side of the mantle cavity, there is a separating barrier formed by a single-layer single-row low prismatic epithelium (Figure 5).

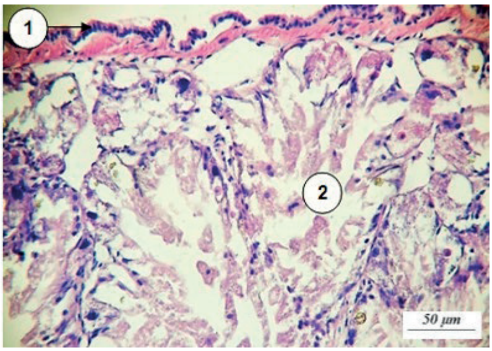

Figure 5. Fragment of the visceral part of the body of the snail *Helix pomatia* of the first group: 1 - single-layer epithelium from the side of the mantle cavity; 2 - hepatopancreas. H & E (Own source)

According to the results of our study, the hepatopancreas of the snails of each group showed peculiarities of a microscopic structure. The hepatopancreas of the snails of the first group has a typical structure. Its parenchyma is represented by glandular tubes and a system of excretory ducts that open into the intestinal cavity (Figure 6).

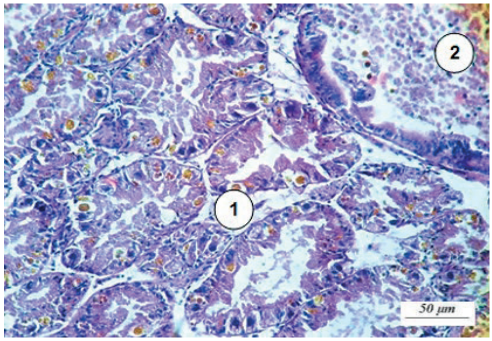

Figure 6. Fragment of the hepatopancreas of the snail *Helix pomatia* of the first group: 1 - hepatopancreas; 2 - stomach cavity. H & E (Own source)

Glandular tubes contain 4 types of cells: digestive cells, excretory cells, calcium cells and thin cells. The cytoplasm of digestive cells contains numerous secretory granules and sometimes green granules of various sizes. Excretory cells are distinguished by the presence of a vacuole with a large yellow granule in the cytoplasm. Calcium cells are predominantly triangular in shape compared to other cell types, smaller in height, with large nuclei and optically dense cytoplasm. Sometimes spherical calcium granules – spherules – are found in their cytoplasm. The granules are also present in the lumen of the excretory ducts and the gastric cavity (Figure 7). Thin cells are undifferentiated cambial cells (Lobo-da-Cunha, 2019; Lőw et al., 2016).

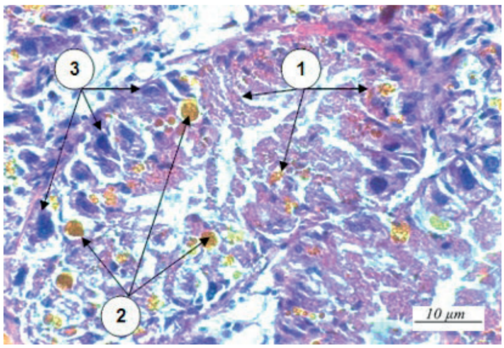

Figure 7. Cellular composition of the hepatopancreas of the snail *Helix pomatia* of the first group: 1 - digestive cells; 2 - excretory cells; 3 - calcium cells. H & E (Own source)

According to the literature, the hepatopancreas is the organ that to the greatest extent deposits toxic substances and ensures their detoxification, which is why it is highly responsive to environmental factors (Yasmeen S. M. Abd El Mageed et al., 2023; Carbone & Faggio, 2019; Parvate & Thayi, 2017; Rota et al., 2016; Sharaf et al., 2015; El-Khayat et al., 2015; Mohammadein et al., 2013; Almedros & Porcel, 1992; Janssen & Dallinger, 1991).

In snails of the second group, which were in a state of anabiosis, significant structural changes occurred in the digestive, excretory and genital organs. Due to the closure of the shell opening by the epiphragm and the absence of communication between the organism and the external environment, it is impossible for the excretory organs to remove metabolic products. This situation leads to a change in the function of the hepatopancreas. The hepatopancreatic glandular cells transform and differentiate into excretory cells. The glandular tubes are dominated by cells with vacuoles with yellow granules in the cytoplasm. The excretion products accumulate in the lumen of the glandular tubes and the hepatopancreatic ducts, as well as in the stomach and intestinal cavity. The colour of the excretory products suggested that they are in a colloidal state, possibly as a result of water reabsorption (Figures 8, 9).

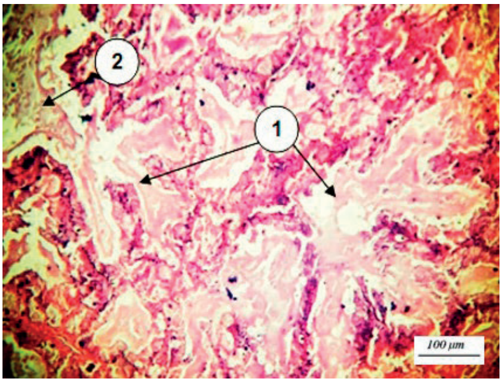

Figure 8. Hepatopancreas of the snail *Helix pomatia* of the second group: 1 - glandular tubes filled with compacted excretory products; 2 - excretory duct with excretory products. H & E (Own source)

Snails, like many invertebrate hermaphroditic organisms, have a sexual organ, the spermatheca, for the accumulation and storage of male reproductive products (Rogers & Reeder, 1987).

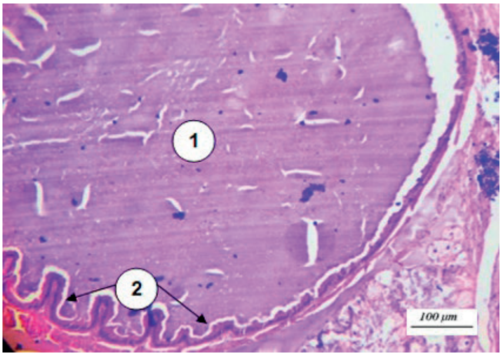

Figure 9. The stomach of a snail Helix *pomatia* of the second group: 1 - excretory products in a compacted state in the stomach cavity; 2 - stomach wall. H & E (Own source)

In snails of the second group, lysis of sperms in the spermatheca is observed, which is evidence of the limited viability of sperm and its autonomous disposal during a period of prolonged absence of sexual activity (Figure 10).

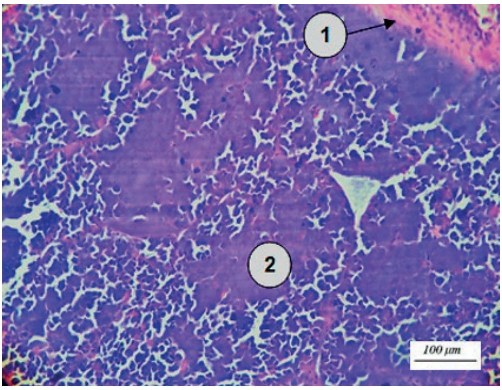

Figure 10. Spermatheca of the snail *Helix pomatia* of the second group: 1 - wall of the spermatheca; 2 - lysis of sperms in the spermatheca. H & E (Own source)

At the same time, the hermaphroditic gonad shows no destruction of ova and spermatogonia, which indicates their resistance to hypoxia and other unfavourable conditions of the internal environment cellular metabolism during hibernation.

In the snails *Helix aspersa* Muller of the third group, according to the results of coproscopic studies, in native smears and by the Füllleborn method, larval and imaginal stages of nematodes of the order *Strongylata,* family *Strongylidae*, have been detected (Figure 11).

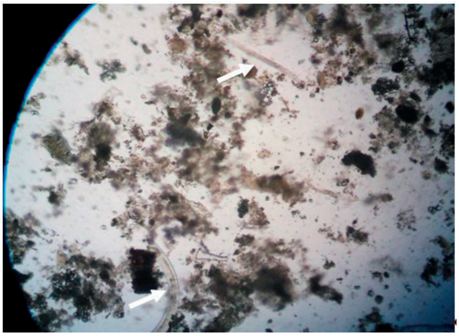

Figure 11. Helminths of the *Strongylidae* family in the native faecal smear of the snails *Helix aspersa* of the third group. Native preparation, magnification x125 (Own source)

Morphological analysis of the histopreparations from the visceral organs of the snails *Helix aspersa* enables to determine the effect of one of the biotic factors on their organism. The digestive gland of the snails retained its typical structure, but in some places, glandular tubes of the hepatopancreas are represented by a structureless mass in which individual swollen epithelial cells with nuclei in the stage of pyknosis, rexis and lysis are visible, indicating a process of necrosis.

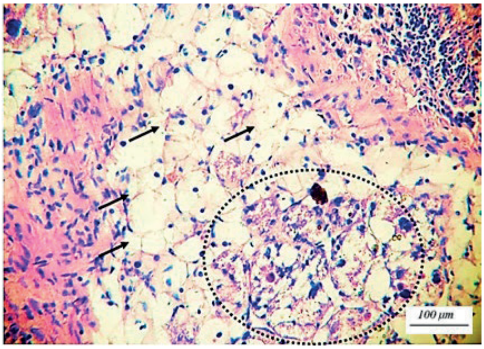

Figure 12. A fragment of the hepatopancreas of the snail *Helix aspersa* of the third group: the area of necrosis is marked with a circle; arrows indicate the replacement of parenchyma by the connective tissue. H & E (Own source)

These morphological manifestations should be considered as changes in hepatopancreatic tissues to the destructive effects of metabolic products of the detected nematodes. Destroyed areas of the hepatopancreatic parenchyma are replaced by the connective tissue (Figure 12). Connective tissue layers are also increased between microscopically intact glandular tubes. Such structural changes are caused by prolonged exposure to the metabolic products of the detected nematodes and lead to the development of hepatopancreatic cirrhosis.

CONCLUSIONS

Based on the results of our research and analysis of the literature, we have come to the following conclusions:

1. The organism of the snails *Helix* reacts with clear structural changes in visceral organs to the action of adverse environmental factors.
2. In the snails *Helix pomatia*, which were in a state of anabiosis, transformation and differentiation of glandular cells of the parenchyma into excretory cells takes place in the hepatopancreas. Excretory substances in a compacted state accumulate in the digestive organs. Lysis of sperm is observed in the spermatheca.
3. In the snails *Helix aspersa*, which have been exposed to prolonged helminthic invasion, foci of necrosis are found in the hepatopancreas, which are replaced by the connective tissue, causing cirrhosis of the organ.

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THE ASSESSMENT OF DAIRY COWS WELFARE IN A FARM FROM THE NORTH-EAST OF ROMANIA

Gabriela AMARIȚII[1] , Andra-Sabina NECULAI-VĂLEANU[2], Vasile MACIUC[1]

[1]University of Life Sciences "Ion Ionescu de la Brad" Iasi, 3 Mihail Sadoveanu Alley, 700490, Iasi, Romania
[2]Research and Development Station for Cattle Breeding Dancu, 9 Soseaua Iasi-Ungheni, 707252, Holboca, Iasi, Romania

Corresponding author email: amaritiigabriela@yahoo.com

Abstract

The objective of this study is to present the findings of a welfare assessment conducted on dairy cows reared in an intensive farming system, in a farm from the north-east of Romania. The assessment utilized well-being indices outlined in the TIERWOHL-CHECK programe, which was designed for German farmers. The results of the assessment, particularly focusing on the body condition score (BCS) of RM and late-gestation period female cows, indicate elevated percentages of overweight cows, ranging from 30.77% to 37.5%. Additionally, the prevalence of lame animals was notably high (28%, surpassing the recommended threshold of 10%). These welfare indices serve as indicators of the extent to which optimal rearing conditions are maintained, and the findings underscore the necessity for prompt interventions to enhance various aspects such as the comfort of lying spaces, the quality of bedding, and certain housing conditions. These measures are imperative for improving the overall welfare of the animals in question.

Key words: *animal welfare, dairy cows, indices.*

INTRODUCTION

The welfare of dairy cows is now one of the major concerns of society (Sirovica et al., 2022), the consumers willing to pay more for products obtained from animals whose welfare has not been compromised.
Animal welfare is a concept that considers a holistic approach to all factors related to animal husbandry management and technology. (Freigang et al., 2023; Gieseke, 2022; Nica et al., 2023).
In the European Union strategy named Farm Fork are include provisions on the welfare of dairy cows, The traceability of animal origin products will also include the assessment of farms from this point of view. In order to improve the legislation that protects farm animals, the European Commission advised the European Food Safety Authority (EFSA) to develop assessment indicators for animal welfare based on scientific studies that identify the dangers presented by farming systems. The assessment covers the animal husbandry system through the welfare consequences. Indicators based mainly on animal measurements are used for their relevance, as they are based on the direct response of organisms to environmental conditions. (EFSA Panel Animal Health & Animal Welfare, 2023).
The concept of animal welfare has developed over time in the sense that if it originally included the freedom of animals to experience a life free from major threats that determine their survival (hunger, thirst, disease, etc.), nowadays it includes notions of emotional states of the animal that it reflects the subjective experience of the animal in relation to lived experiences. Animal welfare is now considered optimal when the balance between positive and negative affective states is generally positive, and several assessment methodologies have been developed based on the concept that the living environment influences animals' affective states and subsequent cognitive biases by conditioning individuals' propensity to experience positive and negative events (Nica et al., 2023; Russell et al., 2023).
Ensuring animal welfare is primarily reflected in the health status of animals and has direct economic implications (Magrin et al., 2023; Owusu-Sekyere et al., 2023; Thomsen & Houe, 2023).

MATERIALS AND METHODS

The herd of dairy cows for which the welfare assessment was made is raised in free standing stables, on compact concrete floors, in stables without paddocks. The rest area is individual (bunk beds) and the bedding is a special carpeted mattress. The waiting area and the movement area are shared. The feed is made from stock and the ration is represented by a mixture of fodder and is of the monodiet type. Feeding is differentiated according to productive performance and physiological state. Watering cans for cows have a constant level and the water is supplied from the network.
The herd of cows (lactating and weaning) for which the welfare assessment was carried out in July 2023 and on the basis of which the animal sample was established, was as follows: 309 lactating cows, 51 cows in advanced gestation and 37 cows in the weaning period. In January 2024, the cow herd had the following structure: 308 lactating cows, 51 cows in advanced pregnancy and 21 cows in the weaning period. The percentage applied to establish the sample was 20% applied to all categories of animals evaluated. The evaluation of the avoidance distance was performed for 10 heads from each group in the stable as recommended in the procedure and in our case, it was performed for 54 animals.
Regarding the evaluation of the herd of cows for foot problems, this was done for all lactating cows and for a percentage of 20% in the case of maternity cows. The herd average in 2022 was 358 cows and 363 heads in 2023.
The welfare assessment was carried out by applying the German assessment system implemented for farmers in order to self-assess the animals and the herd on their own farm. The analysis is based on the collection of information in the stable, on indicators measured on animals but also on data from farm or official registers.
In the case of the herd of milk cows evaluated, the data obtained by applying the animal-based indicators and those registered in the AfiMilk application of the farm, in the official production control and from the Bullfighting Association were processed. The obtained values are annual averages obtained based on the results calculated following the two assessment sessions.

RESULTS AND DISCUSSIONS

The results presented in the tables are the average values obtained from the evaluations by animal category in July 2023 and in January 2024. They are also the presentation and the warning and target values.

Table 1. Evaluation of the avoidance distance and body condition in the herd

INDEX	Qualifying	Lactating cows %	Weaned cows %	Cows in advanced gestation %	Medium value %	Warning value	Target value
Avoidance distance	*0*	40.85	60,71	65.71	*55.76*	-	-
	1	42.44	39.28	25.71	*35.81*	-	-
	2	16.71	0	8.57	*8.43*	-	-
Body condition	*0*	73.89	52.68	45.33	*57.30*		
	1	9.92	0	0.00	*3.31*	≥ 10%	≤5%
	2	16.19	47.32	54.67	*39.39*	≥ 12%	≤ 5%

The distance to which the animal accepts human proximity is an indicator of how they are treated and handled by their caretakers (Table 1). Small avoidance distances are associated with a good human-animal relationship. The results in the table show that approximately half of the animals (44.24%) had an avoidance behavior when approaching them was attempted.
If we refer to the body condition of dairy cows, it can be said that although it is generally a good one, still the percentage of 39.39% of animals that are fat is above the warning value limit of

12%. The most fat females are those in the mammary rest period and in advanced pregnancy, the percentages being 47.32% and respectively 54.67% for these categories. These females are prone to difficult calvings, metabolic disorders (ketosis, acidosis) and fertility problems, in which case it is recommended to reformulate the rations administered to these categories
.

Table 2. Evaluation of soiling and injuries

INDEX		Qualifying	Lactating cows	Weaned cows	Cows in advanced gestation %	Medium value %	Warning value	Target value
Soilling	back leg (superior part)	0	25.48	38,39	14.84	26.23		
		1	74.52	61,61	81.59	**72.57**	≥ 55%	≤10%
	back leg (inferior part)	0	22.62	44.64	18.96	28.74		
		1	77,38	55.35	77.47	**70.07**	≥ 40%	≤ 10%
	udder	0	43.10	73.21	77.20	64.50		
		1	56.90	26.78	19.23	**34,31**	≥ 20%	≤ 5%
Injuries to the skin	hock	0	44.37	58.93	74.18	59.16		
		1	55.63	41.07	25.82	40.84		
	knee	0	72.14	81.25	70.05	74.48		
		1	27.86	18.75	29.95	25.52		
	neck	0	99.29	87.5	100.00	95.60		
		1	0.71	12.5	0.00	4.40		
	animals with at least one lesion		57.94	52.68	26,37	**45,66**	≥ 10%	≤ 4%
Tail damage		0	93.25	100	96.15	96.47		
		1	6.75	0	3.85	3.53	6%	0%

The degree of soiling of the animals gives clues about the extent to which the walking/access surfaces are cleaned, but also about the ration administered which determines the consistency of the faeces (Table 2). The presence of dirt on the skin has an irritating effect on the skin of animals and can cause the appearance of local infections. In the case of the herd of cows analyzed, the degree of soiling exceeds the alert limit for all three body segments (upper and lower part of the hind limb and udder).

The injuries that appear on the animal's body are the result of the interaction between the animal and various factors related to the maintenance system. Neck damage is usually due to the height of the feed fence not being adapted to the size of the herd. Most injuries occur at the hock, knee and carpal joint, giving clues to the quality of the resting surface (insufficiently soft, deformable, clean and dry), the walking surface or the height of the feeding fence.

If we do an analysis by category of cows, in the case of lactating cows the percentage of animals showing at least one injury to the limbs is very high, 57.94% if we refer to the maximum value of 10% of the warning threshold. The highest frequency is injuries at the hock level, which is 40.84%. For females in advanced gestation, the percentage of those with at least one lesion is lower, at 26.37%, although it is double compared to the warning value. The reduction in the incidence of injuries in this category of cows can be explained by the fact that in the calving pens where the bedding is straw, some of the determining factors are no longer active, which leads to wound healing. The percentage of neck injuries were found in the assessment in July, when some categories of animals were maintained during the summer period, injuries that occurred due to the fact that during feeding, the height of one of the bars of the feeding front is not arranged at the height corresponding to.

Broken, injured or shortened tails can be the result of mechanical impact with brushes or doors, or in the milking parlor but also as a result of the brutal handling of cows by those who care for them. Although it is desirable not to exist in the herd, the percentage of 3.53% of affected animals falls below the warning level of 6%.

The welfare and productive performance of cows is negatively influenced by poor hoof condition. Although it also has genetic determinism (the quality of the horn), the most frequent problems appear due to the influence of environmental factors (lack of movement, wet and dirty floors, the proliferation of pathogens, etc.) but also if the ongloos are not adjusted in time and professionally. The problems that arise determine the deterioration of the general state of health of the animal, which can lead to its removal from production. The percentage average on the farm is 24.65% of animals with improper condition of the hooves, above the warning value, the most common being the hooves that have a defective shape at the tip (Table 3)

Table 3. Assessment of hoof condition and lameness

INDEX	Qualifying	Lactating cows	Weaned cows	Cows in advanced gestation %	Medium value %	Warning value	Target value
The condition of the hooves	*0*	71.51	80.35	74.18	*75.35*		
	1	28,49	19.64	25.82	***24.65***	≥ 15%	≤ 5%
Lameness	*0*	78.04	66.96	77.75	*74.25*		
	1	15.65	26.78	18.41	*20,28*		
	2	6.31	6.25	3.85	*5.47*	≥ 3%	0%
total clinically lame animals		21.96	33.03	22.25	***25.75***	≥ 10%	≤ 5%

The improper condition of the hooves results in visible changes in the gait over time. Lameness is determined by factors such as diseases of the hoof, infections in the lower segment of the limb, but also by the inadequate condition of the walking surfaces, overcrowding and the quality of the resting beds. The warning threshold for the percentage of severely lame animals is a maximum of 3% and is exceeded at least twice in the case of lactating cows and those in udder rest. For the entire herd, the percentage value of 25.75% of mildly and severely lame cows can be considered high. In order to keep this aspect of animal health under control, it is recommended that a monthly assessment of the herd be made in terms of walking behavior and those that affected the walking pattern be noted separately and investigated.

Cows exhibit synchronicity in behavior especially when eating and when resting, so sufficient beds and places at the feeding front must be provided. In addition, the fact that the beds are not optimally designed leads to decreased rest times which affects the welfare of the cows and can promote the occurrence of hoof and limb diseases, with a negative effect on milk production. The evaluation was carried out 3 hours after feeding, when they feed and the results are presented in Table 4.

The behavior of animals that do not rest after feeding, synchronously with the herd, is determined by the quality of the bedding and the design of the beds. The percentage of 12.16% of animals standing inside the bed reveals that they are not comfortable. Those that are outside the sleeping area are the cows that rest on the floor of the movement or waiting areas as a result of their non-adaptation to the rearing technology. Also, the fluency of movements during lifting from the stand informs about the quality of the bedding in the first place and the comfort provided by the bed. Following the evaluation, 44.58% do not have correct lifting behavior, the target value of this indicator being 50%.

Table 4. Assessment of animal comfort

Index	Description	% Annual average	Warning value	Target value
Use of the bunk	Actively involved in drinking and eating	13.46		
	Fully stretched out on the sleeping area	64.24		
	Lying incompletely on the sleeping area	0		
	Which sit with 2 or 4 legs in the bunk	12,16		
	Located outside the sleeping area	5.07	≤ 1%	≥ 3%
Bunk utilization Index		68.98	≤ 50%	≥ 66%
Cow comfort index - CCI		75.79	≤ 70%	≥ 80%
Lifting Behavior (number of animals that do NOT rise fluently)		44.58	≥75%	≤ 50%
Water supply		6 water/100 heads	-	6 water/100 heads

The bunk utilization and cow comfort indices have values of 68.98% and 75.79%, respectively, close to the recommended optimum.

In the evaluation of the health status of the cows, the number of somatic cells and the coefficient of fat and protein are estimated (Table 5). The latter has relevance in terms of feeding management of animals in the last part of gestation and those in the first days of lactation. A fat-protein (G/P) ratio of < 1.0 is an indication of a rumen fermentation disorder, which may be caused by an insufficient supply of crude fiber (acidosis) and values greater than 1.5 may be indicative of animals suspected of ketosis after calving

Table 5. Herd health and slaughter evaluation

Index	Description	Annual average %	Warning value	Target value
Mastitis	NCS ≤ 100,000 cells/ml milk	59.16%	≤ 50%	≥ 75%
	NCS > 400,000 cells/ml milk	17.85%	≥ 15%	≤ 5%
	Infection rate of HEifers	3.44%	≥ 30%	≤ 15%
G/P ratio in milk	suspected of ketosis (FEQ ≥ 1.5)	17.27%	≤ 15% of cows	
	suspected acidosis (FEQ < 1.0)	30.63%	≥ 15%	≤ 5%
Duration of economic life		32 months	≤ 30 months	≥ 48 months
Outputs from cash	total	36.34%	≥ 40%	≤ 25%
	the first 100 days of lactation	16.5%	≥ 10%	≤ 5%
Mortality of cows	2022 and 2023	1.25%	≥ 5%	≤ 2%

The known factors that contribute to the increase in the incidence of mastitis in the herd are related to the hygiene of the stable, to the technology and hygiene of milking, to the weakening of the defense of the cows' body through metabolic disorders, etc. The percentage of animals with mastitis is 17.85% just above the warning threshold, which requires disease management measures. The infection rate of primiparous females is low, which means that hygiene measures in the maternity ward are adequate.

Lactating cows on the farm, the G/P percentage is higher than the recommended warning thresholds, so 17.27% of the cows can be suspected of subclinical ketosis and 30.63% of acidosis, which again requires measures of feeding management of females in these physiological periods.

The reasons why animals are culled are very different and are often conscious management decisions or unplanned losses. The most common causes of herd exit include: fertility disorders, mammary gland diseases and limb and hoof diseases. The number of animals removed from the herd in 2022 and 2023 was 262 heads with a monthly average of 11 heads and the percentage approaches the warning value, which represents 36.34% of the average herd. The 1.25% fatality rate is below the recommended 2%.

From an economic point of view, it is not desirable for cows to be slaughtered before 100 days of lactation. From the analysis of the results included in Table 4, it can be seen that the percentage of cow slaughters in this category is high compared to the warning value.

CONCLUSIONS

Ensuring the well-being of animals is an integral part of farm management, all the more so since, under the conditions of sustainability of animal breeding, it has economic implications, cost reduction and easy access to the consumer market.

In the evaluated population, based on the results obtained, it can be concluded:

-following the evaluation of the avoidance distance, only 8.43% showed an avoidance behavior from the approach distance of 2 m;

- with regard to body cleanliness, the warning percentages are exceeded in all cases, which means that additional measures must be taken to clean the stables, but also to rethink the administered ration that influences the consistency of feces:
- the comfort provided by the bedding on which the cows rest, its softness most influences the percentage of animals that have at least one injury, which in the case of the evaluated cows is 45.66%, very high if we compare it with the warning value of 10%;
- in 24.65% of the cows the condition of the hooves is inadequate and 25.75% of the total of the evaluated cows shows clinical signs of lameness;
- regarding the comfort provided by the beds, the use index of bunks has an optimal value of 68.98%, which means that it is provide the comfort during rest;
- the percentage of animals that can compromise the quality of the delivered milk because they have NCS above the maximum allowed is 17.85%, which recommends taking measures to keep the disease under control in the herd;
- the high values of the percentages of fat animals in females in the last period of gestation and those of 17.27% and 30.63%, respectively, in the case of cows in the first part of lactation and which can be suspected of the subclinical manifestation of metabolic diseases, directs in the sense of taking some management measures of the feeding of females in these physiological stages.
- the post-partum period is a critical one for cows, the percentage of slaughter being higher for females that have not reached the productive maximum of the current lactation.

The management of the rearing of dairy cows on the farm must be aimed at decreasing the incidence of mastitis and podological problems in the herd and also at reducing the percentage of slaughtering of females at the beginning of lactation. It is also very important to re-evaluate the feeding management of cows during the mammary rest period.

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IMPACT OF WEATHER CHANGES ON BEE FAMILIES DEVELOPMENT AND PRODUCTION WITH SPECIAL REFERENCE TO TRANSYLVANIA AREA

Otilia BOBIŞ, Victoriţa BONTA, Adela Ramona MOISE, Claudia PAŞCA, Alexandru Ioan GIURGIU, Daniel Severus DEZMIREAN

University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, 3-5 Manastru Street, Cluj-Napoca, Romania

Corresponding author email: alexandru.giurgiu@usamvcluj.ro

Abstract

Lately, there have been increasing efforts to stimulate the strategic role of the bees in biodiversity protection, in agriculture & horticulture. European Union (EU) has invested in the beekeeping sector in the last years by funding Farm to Fork strategy. The aim of this strategy is to protect them and preserve their contribution in sustainable environmental models. Biodiversity is decreasing by using intensive agriculture, chimization and automatization and consequently a decline in ecosystem services in many parts of the world, most representatives being pollination. Over the past three decades, there was an increasing in atmospheric temperature. Because of changes in plant flowering times patterns, the interaction between pollinators and their food sources is also affected. The challenges facing bee health have multiple sources, including poor nutrition due to less nectar source, heat stress resulting from global warming, agrochemicals used in agriculture, and pathogens becoming increasingly resistant to conventional treatments. However, thermal stress can negatively affect hive activity, which can affect foraging activity, immunocompetence, reproductive capacity, and the growth and development of bees. This, in turn, will affect pollination services and hive production overall.

***Key words**: apicultural production, bees, bee development, climate change, Transylvania.*

INTRODUCTION

In the last years, there was an intensive effort made to stimulate the crucial role of bees in agriculture and horticulture by pollination and protecting biodiversity. These small and wonderful creatures pollinate more than two-thirds of the crops and wild plants found in the European landscape. It is estimated that bees contribute at least €22 billion to European agriculture every year. European Union (EU) is investing in bees by the known strategy From Farm to Fork. The aim is to protect the bees and preserve their contribution to sustainable environmental models. To do this, European Union spends 80 million euros annually to finance specific programs designated to beekeeping half of them from EU funds, half of them paid by EU members themselves in their countries.

One of the primary culprits behind the decline of biodiversity is the intensive use of agricultural land, which results in the reduction of natural habitats (Newbold et al., 2015). This form of agriculture is responsible for a significant loss of biodiversity (de Heer et al., 2005), leading to a decline in ecosystem services, including pollination, in many regions across the globe (Kremen et al., 2002; Kremen et al., 2007; Potts et al., 2016). According to Rivera-Gomis et al. (2019), modern beekeeping in Europe is confronted with a multitude of opportunities and threats, primarily linked to the environment, but also to economic and social aspects. Today, good beekeeping practices (BPA) encompass activities that promote animal, environmental, and human health. Ecological beekeeping, one of the modern beekeeping practices, is a testament to these elements, with its numerous beneficial effects.

MATERIALS AND METHODS

Different approaches were studied in the present work, taking into consideration the global climate change and how this problem affect the bees in general and beekeeping as important sector of animal science.

Using specific key words such as climate change, beekeeping, heat stress, bee monitoring, bee health and development and heat stress, scientific literature was studied. Relevant literature was gathered and processed from the most used databases (Web of Science, Science Direct or Google Scholar) where the keywords were found. In addition, studies of our research group were found and compared with other available literature findings. Our research gathered several articles focusing mainly on the research progress in our geographical region, where the impact of climate changes applies to the health and development of bee families. In the end, some data of weather monitoring in the University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca was used, to highlight the periods of the beekeeping season where different measures to protect the bee families must be taken into consideration in order to have strong and healthy bee colonies.

RESULTS AND DISCUSSIONS

Climate change and beekeeping

As per a European Commission (EC) report (Causes of Climate Change, 2018), the current global average temperature on Earth has surged by a staggering 0.85°C since the end of the 19th century. This means that each of the past three decades has been warmer than the previous one, a trend that should raise serious concerns. Another European source (Consequences of Climate Change, 2018) points out that climate change is already impacting human health, with the most affected economic sectors being agriculture, forestry, energy, and tourism.

A Technical Report by Greenpeace Laboratories (2013) states that: "climate change, such as rising temperatures, changes in precipitation patterns and many other irregular or extreme weather events, will impact pollinator populations. Some of these climate changes could affect pollinators at individual level but consequently also their communities, reflected in higher rates of extinction of these pollinator species". For example, in Poland, bees have been observed to react to climate change by performing their first cleaning flight (in spring) earlier than expected, a behaviour that coincides with the phenomenon known as "seasonal change", which refers to the shift in seasons due to climate change. This early cleanup flight occurred one month earlier compared to the average of the last 25 years of the observation period, a phenomenon attributed to the increase in global temperature (Sparks et al., 2010).

Climate change is leading to changes in all flowering patterns, changing implicitly the flowering period of melliferous plants, indispensable source of food for bees, or seasonal changes, in which case the flowering period no longer corresponds to the moment when bees "wake up" in the spring (Kremen et al., 2007). Because of changes in plant flowering times and patterns, climate change also affects the interaction between pollinators and their food sources. Thus, some studies (Memmott et al., 2007) demonstrate the reduction of the number of floral resources available, up to 50% of all pollinator species, depending on the climate change model that causes the modification of plant flowering patterns. The authors anticipate that the predicted outcome of these perturbations is the extermination of pollinators, plants, and interactions between them. In the bibliographic sources mentioned above, in addition to the general conclusions formulated by the authors, concrete information about the influence of climate changes on the evolution of the morpho-productive characters of bee families is not found. Of course, similar studies must be made in order to draw a clear conclusion in this respect.

Bees and heat stress

Bees are affected by heat stress in many aspects: their growth may be affected, the development of the family, bee physiology, and foraging activity, directly related to pollination services and not lately their reproduction (Alqarni, 2020; Bordier et al., 2017; Greenop et al., 2020; Medina et al., 2018). Also, thermal stress may trigger different malformations of bee anatomy (legs, wings, stinger, the proboscis) (Groh et al., 2004).

Because we are talking of global heating, abnormally high temperatures will definitively change the bees normal physiological activities by affecting their immune system. There are not enough studies to state that the changes that occur in time in the immune system of the bees exposed to heat stress are due solely to heat stress, so the conclusions remain unclear. However, lately, some scientific studies have demonstrated the impact of thermal stress on the immune system of social insects (*A. cerana* and *A. mellifera*), such as the study of Li et al. (2022). Three genes responsible for antimicrobial peptide production (Abaecin, Defensin and Hymenoptaecin) were analyzed at different temperatures and treatment times, and their expression patterns were registered. The expression of these genes was affected by high temperature and treatment time. Under stressful conditions (heat stress), immunity is activated, and as a result, the infection may be prevented. A basis for determining the mechanisms by which the bees' immune system adapts to high temperatures was initiated by these results.

One recent scientific study demonstrates climate change's impact on bee families' vital activities (Cebotari et al., 2019). This work aimed to determine the correlation between the values of the average monthly atmospheric air temperature in different periods of the year and the evolution of the morpho-productive value characters of bee families, thus elucidating the impact of climate change on the vital activity of *A. mellifera* bee colonies. In order to study the impact of climate change on bee families' activity, the data recorded during eight years (2010-2017) from the closest hydro-meteorological station to the apiary were used. During this period, different coefficients were calculated between the monthly average of the atmospheric air temperature and the average values on the hive for each of the six main morpho-productive characters of bee families: queen prolificacy, family strength, colony resistance to wintering, disease resistance, brood viability and honey production of bee colonies. The research results showed that the phenomenon of global warming was also manifested in the area where the experiment was conducted.

Moreover, in this sector, the effects of global warming were more evident than in the EC Report (2018) data. It was found that the annual average temperature is increasing from 10.4°C in 2010 to 10.9°C in 2017, i.e. by 0.5°C. The average air temperature in the first three years (2010-2012) was 9.6°C, compared to the last three years (2015-2017), it can be observed an increasing with 1.5°C (to 11.1°C), a significant increase which is very much and also worrying. The high temperatures and drought periods recorded harmed the flora and fauna of the ecosystem, and, of course, the effects were present on the agriculture in this area. We can assume that if the air warming continues at this rate, we will witness various transformations in a few decades, leading to the change to an arid and desolate area. Research has shown one of the most important climatic factors' influencing the vital activity of *A. mellifera* bee families is temperature.

Between this factor and the morpho-productive evolution of bee colonies, there are different sizes of correlative links. The impact of air temperature on the vital activity of bee families was found to be determined by the average monthly temperatures in some periods of the year and by the average annual temperatures (Cebotari et al., 2019). Another study was conducted to determine the influence of temperature on nectar collection and its storage in the hive in some of the main collections (acacia, linden and sunflower) (Eremia et al., 2017). Nectar production in honey plants is closely related to air temperature, sunlight level, air and soil humidity, plant age and density. If, for example, cold or dry winds blow or there are torrential rains during the flowering of the linden, in these cases, the nectar collection and honey production are interrupted, and the heavy rains destroy the flowers or unopened flowers of the linden. Nectar secretion is influenced by several factors, which include soil factors (soil moisture and fertility, use of fertilizers, agrotechnical factors), sunlight, temperature (below 10°C and above 35-38°C plants do not secrete nectar, the optimum temperature being 16-26°C), meteorological conditions, winds and prolonged droughts (Eremia, 2009). The different harvests are influenced, as mentioned, mainly by the climatic conditions in specific periods of the year.

Both climatic and geographical factors play an essential role in the biogeographical distribution of bee species (Coroian et al., 2014). The study was conducted at the University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca (USAMV Cluj). The Carpathian Mountains are considered a natural geographical division between two subspecies of bees in our country, *Apis mellifera carnica (A. m. carnica)* and *Apis mellifera macedonica (A. m. macedonica)*. To evaluate the role of the Carpathian Mountains on the biogeography of

European *A. mellifera* bees, the bee samples collected from the Transylvanian plateau offer an excellent sampling model because they are almost entirely isolated from the Carpathian Mountains. This population was not only geographically isolated, but it also knew extensive historical isolation (Foti et al., 1965; Ruttner, 1988; Mărghitaș et al., 2009) and the comparison with the adjacent *A. m. carnica* and *A. m. macedonica* would be highly informative about the separating role of the Carpathians. The Transylvanian Plateau with it's over 25,000 km² spreading, would have supported a sufficiently large endemic bee population during the last glaciations (surrounded by an ice belt), is still detectable today. Few years back reports on the Transylvanian ecotypes seem to support such a view (Căuia et al., 2008; Mărghitaș et al., 2009). The experiment was carried out by collecting samples of bees from the entire territory of the country. Thus, samples were taken from 138 traditional beehives inside the Carpathian arc and outside, up to the Danube Delta. The mitochondrial COX2 tRNA intergenic region was sequenced for all samples and genotyped at 12 microsatellite loci. The Carpathian Mountains had a limited impact on biogeography because both subspecies were frequently found on both sides of the mountains. Instead, the differentiation of subspecies was strongly correlated with the different temperature zones in Romania. The conclusion of the study was that *A. m. carnica* is more common in regions with average temperatures below 9°C, while *A. m. macedonica* is more common in regions with average temperatures above 9°C. This selection may impact future biogeography in light of anticipated global climate change.

Weather monitoring and possible impact on bee colony development

Taking the data from weather station of USAMV Cluj from the last two years (up to and including September), registering different parameters that could influence the development of bee families, the following were found: in 2022 (Figure 1), during the period June - September, much higher values of the global solar radiation (the chart is presenting the average values recorded in every month because very high values were registered as maximum in some specific days) were recorded, compared to 2023 (Figure 2), the other recorded parameters remaining relatively constant. This parameter can significantly influence the development of bee families and can influence the quantity of nectar and pollen in melliferous plants and crops. Among the other important parameters for the development of bee families and also for nectar production are precipitations and relative humidity of the air.

More linear average relative humidity in 2023 was recorded compared to 2022. These parameters may be correlated with the development of the bee families and honey production.

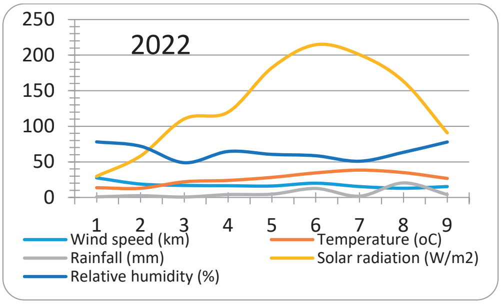

Figure 1. Meteorological records in 2022 at the Meteo Station of USAMV Cluj

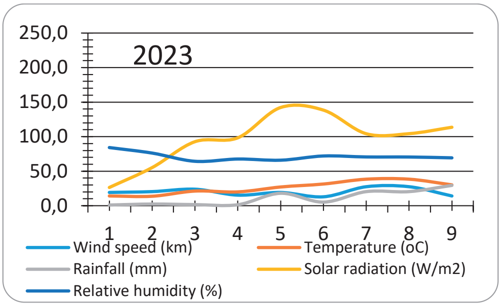

Figure 2. Meteorological records in 2023 at the Meteo Station of USAMV Cluj

If we strictly consider the summer period (USAMV Cluj weather station records), we can observe a decrease in global solar radiation values over the last three years (Figure 3), which can be an encouraging factor for honey production in the central area of Transylvania and the development of bee families, including correlation with all other parameters that determine the development or decline of bee families.

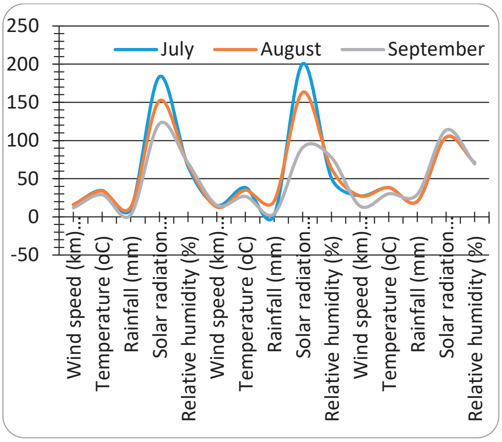

Figure 3. Highlighting the parameters recorded at the weather station of the USAMV Cluj in the summer (July-September) between 2021 and 2023

CONCLUSIONS

Different parameters such as their growth, family development and foraging activity are affected by heat, and conclusively, this may threaten their entire survival.

For this reason, it is very important to follow the weather conditions during a beekeeping year. In addition to the data collected by meteorological stations, different electronic devices are available to measure the honeybee colony development in relation to the weather conditions.

In order to provide quality information on climate changes and bee family development, electronic devices must be placed in the apiary to register the evolution of the harvest, predict the production, and monitor the health of the bees, all in relation to weather changes.

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EFFECT OF DIFFERENT COLOR LED LIGHTING IN INCUBATION ON HATCHING PERFORMANCE

Elif CILAVDAROĞLU[1], Umut Sami YAMAK[1], Uğur ŞEN[2], Hasan ÖNDER[1]

[1]Ondokuz Mayis University, Agricultural Faculty, Department of Animal Science, Samsun, Turkey
[2]Ondokuz Mayis University, Agricultural Faculty, Department of Agricultural Biotechnology, Samsun, Turkey

Corresponding author email: elif.cilavdaroglu@omu.edu.tr

Abstract

Three different LED light colors were applied during Gerze local Turkish chicken egg incubation. Red, green, and white colors were used each containing 160 eggs for 18 days. Eggs were individually weighed at the onset of incubation and during transfer to the hatchery. Lighting was not applied in the hatchery and chicks were hatched in darkness. Total egg weight loss of incubation was 10.45%, 9.43% and 8.90% for red, green, and white led groups and the differences were significant (P<0.05). Fertility between light-color groups was insignificant. The red light group had the highest hatchability (82.64%) in fertile eggs, while the green and white groups had 81.72% and 67.67% respectively. While the egg weight differences between light groups were insignificant; the highest chick weight was obtained in the green light group (P<0.05). The white LED color delayed the hatching time of the chicks. All hatched chicks were scored according to Pasgar Scoring. The scores of chicks were 9.68, 9.62, and 9.71 for the red, green, and white lighting groups' chicks.

Key words: *Gerze chicken, incubation, LED lighting, Pasgar Score.*

INTRODUCTION

The incubation is one the most important steps of poultry production and the main goal of incubators is to get healthy and strong chicks (Scripnic & Dreahlo, 2016). The environment during incubation has a permanent effect on the health and welfare of chickens after hatching. (Archer et al., 2009). Temperature, humidity, air composition in the cabin and turning of the eggs are major environment components of the incubation. Also, other factors like, the age and nutrition of breeders, eggshell thickness (Yamak et al., 2015), and storage condition of eggs (Yamak et al., 2023) could affect hatching traits. Light is an important exogenous factor for controlling many physiological and behavioural processes in animals (Li et al., 2021). It is also reported that providing light during incubation could affect hatchability. Hatchability (Archer et al., 2017), embryonic growth (Zhang et al., 2016), hatching time (Shafey & Almohsen, 2002), feed conversion (Zhang et al., 2012) and 1behaviour after hatching (Archer et al., 2017) are affected by lighting during incubation. It is reported that lighting during incubation was helpful for chicks to adapt to the new environment after hatching (Ozkan et al., 2012). The hatch window is the duration between the first hatched and last hatched chicks in incubation. The aim of the hatcheries is to shorten the hatch window (Calik et al., 2023). The effect of providing lighting during incubation on the hatch window has not been investigated widely before. The effect of hatching time on chick quality is determined by scoring chicks at the end of hatching. Using lighting during the incubation had some difficulties like increasing the inside temperature of the cabins. However new lighting techniques i.e., light-emitting diode (LED), made the use of light sources more practical, without affecting inside temperature (Yameen et al., 2020). In this study, red, green, and white LED lighting was applied during incubation of Turkish native Gerze chicken eggs. Hatching traits and chick quality of the eggs according to lighting colors were investigated in the study.

MATERIALS AND METHODS

This study was conducted at the Ondokuz Mayis University Experimental Farm Hatchery Unit. A total of 480 eggs were collected from Gerze flock of the farm for 4 days. All eggs were kept in a storage machine set at 18°C and 70% relative humidity. After storage; eggs were individually numbered, weighed and divided into three groups each containing 160 eggs. Each group had two replicates with trays 80 eggs. The incubation machine had 10 tiers. The LED lightings were placed in three different tiers by separating each from the others by black curtains to prevent light transmittance.

Incubation

Eggs were placed in the incubator, which was set to 37.7°C and 60% relative humidity. On day 18 (432 hours) eggs were transferred to hatching machine which was set to 37.5°C and 70% relative humidity. At transfer, all eggs were individually weighed to calculate egg weight loss during incubation. No lighting provided in hatching machine. 24 hours after egg transfer (456 hours) all hatching baskets were checked to count hatched chicks at 6 hour intervals. All unhatched eggs were individually broken to determine fertility and embryonic mortality after incubation was completed. The fertility, hatchability of fertile eggs, and hatchability were calculated as follows:

Fertility: number of fertile eggs/total number of eggs

Hatchability of fertile eggs: number of hatched chicks/number of fertile eggs

Hatchability: number of hatched chicks/total number of eggs

Chick quality

The Pasgar©Score method was used to determine chick quality. After hatching, all chicks were scored individually according to the method described by Boerjan (2006). The Pasgar©Score is based on the chicks' reflex as a measure of activity and the appearance of the navel, legs, beak and absorption of the yolk sac. A top quality keet has a score of 10, with 1 point being subtracted for each abnormality recorded in one of the five aforementioned criteria.

Statistical Analyses

The normal distribution of the data was analyzed with the Shapiro-Wilk test. The results indicate that the data were normally distributed ($P > 0.05$). The homogeneity of variances was analyzed using the Levene test. The result showed that the variances were homogeneous ($P > 0.05$). Data analysis was performed with the one-way analysis of variance (ANOVA), while the comparison of means was analyzed using Duncan's multiple comparison test. SPSS 21 software was used with an OMU license.

RESULTS AND DISCUSSIONS

The egg weight, egg weight loss ratios, fertility, hatchability of fertile eggs, total hatchability, chick weight and Pasgar scores of chick for red, green and white lighting groups were given in Table 1. The weights of eggs in lighting groups were 47.46 g, 47.49 g and 47.04 g for red, green and white colors, respectively. The weight of eggs was in parallel with the findings of Arslan et al. (2023) who reported the egg weight 48.32 g for Gerze chickens. The differences between egg weights were insignificant. This was an expected result of collecting the eggs from same flock. The egg weight loss ratios were significantly differed between lighting groups ($P<0.05$). Highest egg weight loss occurred in red lighting group (10.45%), while green and white light provided groups lost 9.43% and 8.90% of weights, respectively. Eggs have to lost 11.5-12% of their weights during incubation (Tona et al., 2001). This is also important for chick quality and success of the incubation. Similar to our findings, Li et al. (2021) determined relatively higher egg weight loss in the eggs provided red lighting during incubation, but the differences were not significant. Hatchability of fertile eggs was significantly higher in red and green lighted eggs than white lighted eggs. It is previously reported that lighted incubation positively affects hatchability of eggs. Ghatpande et al. (1995) attributed this increase to acceleration of embryonic development by light. Hluchý et al. (2012) had similar findings to this present study with red light increasing hatchability. Contrary to our results, Archer (2017) reported that white light and monochromatic red light increased the hatchability when compared with the dark incubated eggs. Also, Archer (2015) remarked the red light is the success key of hatchability for eggs. The lower hatchability of white lighted group could be related to egg shell colours of Gerze chickens. They lay white eggs. Huth & Archer (2015) reported that increased hatchability could be related to the light spectrum passes through the eggshell. Also, it has been defined that only 1% of the light reaches the embryo (Shafey et al., 2005). We thought that the ratio could be less in white light-white shell match-up. The total hatchability of eggs had similar results to the hatchability of fertile eggs. This is mostly about the fertility-hatchability relation. While the differences between the fertility of lighting groups were not significant, the effect of lower hatchability of fertile eggs caused lower total hatchability in the white lighted egg group.

Table 1. Hatching traits of eggs incubated with different LED lighting colours

Specification	Red	Green	White
Fertility (%)	93.75	95.00	91.87
Hatchability of fertile eggs (%)	82.64[a]	81.72[a]	67.67[b]
Total hatchability (%)	77.50[a]	77.50[a]	61.88[b]
Egg weight loss (%)	10.45[a]	9.43[b]	8.90[c]
Egg weight (grams)	47.46	47.49	47.04
Chick weight (grams)	29.93[b]	32.16[a]	29.35[b]
Pasgar Scores of chicks	9.68	9.62	9.71

a, b, c: Differences in the same lines are significant; $P<0.05$

The mean chick weight at hatch was significantly higher in green lighted group eggs. When light exposure was continuous an increased overall embryo weight as well as embryo muscle weight was also found by Wang et al. (2014). But Li et al. (2021) reported that muscle growth may depend on light exposure, but was not only associated with circadian rhythms entrained by photoperiod. Besides light exposure, light wavelength and intensity can influence the amount of light that can pass through the eggshell and reach the embryo (Li et al., 2021). Similar to our findings, Zhang et al. (2014) showed that using green light during incubation results in higher chick weight at hatch by enhancing proliferation and differentiation of skeletal muscle satellite cells in the late embryonic stage and newly hatched chicks. Yu et al. (2018) found green LED light at low intensity (50 lux) stimulated embryo growth during incubation, but no improvement in the embryo when the green light was set to 150 or 300 lux. These results indicate that light intensity could have a strong effect on embryo development.

Hatching distributions of the chicks are shown in Figure 1. Hatching started at 456 hours of incubation and ended at 522 hours in the eggs incubated with red lighting. Hatch window was calculated as 66 hours. In green lighted group, hatching started at 462 hours and completed at 522 hours. Hatch window of green lighted group was calculated as 60 hours.

Conversely to red and green lighted group, eggs provided white light during incubation had a delayed hatching performance. The hatching of the eggs started at 546 hours of incubation and completed at 576 hours with 30 hours hatch window. The reason of this delaying is unclear. Although Tang et al. (2023) reported a postponement in the hatching of eggs incubated with green and white lights, the delay in our study is much longer and could not explained. Another reason for the delay could be related to temperature fluctuations, particularly in the hatching machine. Further studies need to investigate the effects of higher light intensities on hatching time. Chick quality is determined by Pasgar scoring in the current study. There was not a significant difference between the scores of lighting groups.

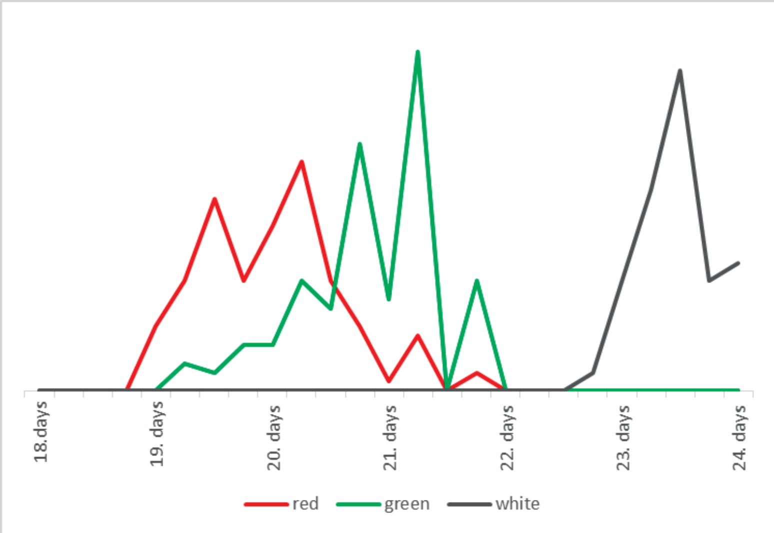

Figure 1. Hatching spread of chicks according to lighting groups

CONCLUSIONS

In conclusion, providing red and green LED lighting during incubation improved the hatchability of eggs while causing a delay in white lighting which resulted in significantly lower hatchability in this group. The chick weight in the green lighted group was significantly higher.

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RESEARCH ON THE USE OF MULBERRY VITROPLANTS IN ORDER TO PRACTICE A SUSTAINABLE AGRICULTURE

Georgeta DINIȚĂ[1], Mihaela HĂBEANU[2], Cristiana DIACONESCU[1], Anca GHEORGHE[2], Teodor MIHALCEA[2]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]Research Station for Sericulture Băneasa, 013685, Bucharest, Romania

Corresponding author email: mihaela.habeanu@scsbaneasa.ro

Abstract

The conservation of sericultural vegetative genetic resources is a permanent concern in the sericulture field, being known that the mulberry leaf is the only source of food for the silkworm Bombyx mori L. These sericultural vegetal genetic resources are mainly used to obtain planting material used for establishment the mulberry plantations, but also for the mulberry improvement program and the establishing of the phytoremediation potential of mulberry plants. The research carried out for the preliminary testing in laboratory of in vitro multiplication potential of some mulberry varieties have highlighted the possibility of using the biotechnologies of tissue cultures, meristems and organs for testing the phytoremediation potential of mulberry plants with sericultural destination. Thus, a planting material was produced, consisting of mulberry vitroplants colonized with vesicular-arbuscular endomycorrhizae, which keep the genetic characteristics of parental forms, but present superior bioproductive parameters and physiological tolerance on soils contaminated with lead.

***Key words**: mulberry, silkworm, vesicular-arbuscular mycorrhizae.*

INTRODUCTION

Land degradation processes by human activities are very varied, sometimes producing the soil damage or even the total anihilation of its functions into ecosystems (Jie et al., 2002; Nkonya et al., 2016; Sutton et al., 2016; Zhang et al., 2023).

Due to soil contamination, the quality of the harvest is depreciated and the agricultural production is reduced or compromised, the consequences being felt in the entire chain: soil - micro-organisms - plants - animals - human (Dotaniya et al., 2020; Angon et al., 2024).

The cause of the heavy metals accumulation in soil may be of *geogenic* nature (as a result of the geochemical processes of rock and minerals alteration) or of *anthropogenic* nature (as a result of human activities: fertilizers, amendments, pesticides, gases or dusts from the atmosphere from various industries or from combustions) (Haque et al., 2009; Pečiulyté et al., 2009; Kumar et al., 2015). The degradation of agricultural land also takes place through processes of contamination/pollution with heavy metals: iron, manganese, copper, zinc, lead, cadmium, chromium, cobalt, nickel (Alengebawy et al., 2021; Rashid et al., 2023).

The research and development in the field of technologies of physical, chemical and biological remediation of soil pollution with heavy metals have evolved significantly, facilitating their currently use in order to counter this major environment problem (Fan et al., 1013; Lan et al., 2020; Srivastava et al., 2022).

Adopting the sustainable agricultural practices is important for the conservation of sericultural vegetative genetic resources considering that the mulberry leaf (*Morus* spp.) is the only source of food for the silkworm *Bombyx mori* L., but also for maintaining the ecological balance in ecosystems with mulberry plantations (Rohela et al., 2020; Hăbeanu et al., 2023).

The processes of bioremediation in situ are important for pollution control, the study and implementation of such processes being able to be conceived by investigations on natural mechanisms of absorption, biotransformation, bioaccumulation and toxicity of pollutants in plants and micro-organisms (Liu et al., 2018; Diaconu et al., 2020).

The silkworms having a high sensitivity of the organism can be used as bio-indicator to detect the environment pollution. Thus, the high content of heavy metals influences the development of biological characteristics, mostly the silk filament length and weight (Nikolova, 2019).
One of the main research directions in the sericultural field consists in the reducing of the dependence on chemical fertilizers through the implementation of ecological agricultural practices (Vinod et al., 2020; Chakraborty et al., 2016; Saqib et al., 2024).
The phytoremediation is recognized as being an ecological and cost-effective approach to remediate the risks of soil pollution (Lan et al., 2020; Xianghong He, 2023; Deng et al., 2024).
Considering that the pollution produces un-balance in the mycorrhizae associations it can be proceed at phytoremediation of land contaminated by using plants from *Morus* genus (Baqual et al., 2017; Ma et al., 2022).
Among these practices stands out the biofertilizers using, such as vesicular-arbuscular mycorrhizae (VAM), in the form of commercial products obtained by biotechnology, together with extraradicular fertilizing (Rajaram et al., 2014; Berruti et al., 2016; Diniță et al., 2023). This approach promotes greater sustainability in the sericultural production, contributing to the environment protection and to the maintaining of ecological balance of agricultural ecosystems (George et al., 2023; Sun et al., 2023).
The obtaining of planting material from the valuable mulberry varieties is made exclusively by vegetative means, by usual methods (cutting, marking, grafting) or by *in vitro* regeneration biotechnologies (Gecer et al., 2016, Choudhary et al., 2023). In the sericultural field the conservation of vegetal genetic is also achieved by utilizing the vegetative cloning, due to the fact that the mulberry being a monoecious or dioecious plant with unisex flowers, it presents a pronounced heterozygous character of the descendants (Tikader et al., 2009).
The *in vitro* cloning consists the only vegetative multiplying method of the mulberry varieties reluctant to cutting and marking, the graft being used to obtain mulberry varieties destined for establishing mulberry plantations with forms of tall bushes, half-trunk and tall trunk, because the production cuttings consist in completely cutting the annual herbaceous shoots (Taha et al., 2020). The biotechnologies of mulberry plants multiplication refer to vitroplants obtaining by tissues culture, meristems and by cells culture (Vijayan et al., 2014; Saha et al., 2016; Litwińnczuk et al., 2020).
The using of *in vitro* multiplying biotechnologies offers the possibility to reduce the time necessary to highlight the accumulation of different substances in various plants' organs (Choudhary et al., 2015).
The researches aimed to apply the work protocol elaborated to obtain mulberry vitroplants and to establish the optimum conditions for *in vitro* multiplication of some valuable genotypes, located in the collection of mulberry varieties in Research Station for Sericulture Baneasa Bucharest.
To establish the phytoremediation potential of the mulberry plants, the researches aimed to highlight the impact of lead contamination of mulberry plants in the biological processes of their germination and development.

MATERIALS AND METHODS

The vegetative propagation *in vitro* is based on the meristems' formation and multiplication, which represents the tissues with the greatest genetic stability. The *in vitro* micro-propagation can have as starting point the pre-existent meristems (the embryonic apex, the apex of the main shoot or the axillary ones), or adventive meristems, induced by the technique of *in vitro* culture.
The design of an experimental *in vitro* model to obtain mulberry microplants was made taking into account the type of explants used, respectively herbaceous shoots developed in laboratory conditions by dormant buds' forcing from the branches of the mature mulberries, belonging to genotypes resistant to cutting – China 98 and China 99 (*Morus multicaulis*) and to mulberry plants obtained from seeds (hybrids) in aseptic conditions.
The work protocol includes the following steps:
- *obtaining the source of explants* is made by forcing in laboratory conditions the lignified shoots harvested in the period November – December from the mulberry plantation (vegetative rest period). The forcing is done by simulating budding conditions, placing these

shoots in vessels with tap water and ensuring an ambient temperature of 24-25°C. After 60 days, from the dormant buds will develop apically the herbaceous shoots, which will constitute the source of explants;

- *the sterilizing of the vegetal material* consists in an operation of washing with normal tap water (pre-sterilizing) necessary to remove mechanically some contaminants. The herbaceous shoots are cut in pieces of about 1 cm, each containing one axillary bud, this becoming a nodal explant. The effective sterilization was made with $HgCl_2$ in concentration of 0.03% for 30 minutes, the nodal explants being continuously mixed in this solution, after which they have been washed with distilled water for 10 minutes of three rounds. The sterilization can also be done using a solution of 3-5% with potassium hypochlorite;
- *the initiation of primary culture (inoculation)* takes place in aseptic conditions at the laminated flow hood. The culture environment used is of Murashige-Skoog type with 0.8% agar distributed in culture vessels of "baby food" type, the culture medium being supplemented with the following hormone variants:

V1 – 1 mg/l BAP (Benzylaminopurine)
V2 – 1 mg/l K (Kinetin)
V3 – 2 mg/l BAP
V4 – 2 mg/l K
V5 – 1 mg/l BAP + 1 mg/l K
V6 – 2 mg/l BAP + 2 mg/l K
V7 – 2 mg/l BAP + 1 mg/l K
V8 – 1mg/l BAP + 2 mg/l K

After inoculation (placing the nodal explants in culture vessels sterilized on the culture medium with respect of normal polarity) the culture vessels are covered with parafilm;

- *the incubation of initial culture* – the multiplication consists in transferring into the incubation room (for growing) of the culture vessels for 15 days at the temperature of 25-28°C and with artificial lighting, with a photoperiod of 16:8 hours at a 3,000 lux intensity;
- *subculturing* is necessary to obtain clones from the same apex in the conditions of caulinar apexes using, by transferring from the initial culture of some explants in culture vessels with fresh medium;
- *rooting and regeneration of autonomous plants* is made when the shoots developed in the culture vessels reach the length of 1.5-2 cm and has as its purpose the rootlets obtaining by changing the culture medium, where the formula remains unchanged, except the agar which is no longer added, the culture medium being liquid. The maintaining of vitroplants in the liquid culture medium is made by utilizing some bridges of filter paper, the rooting being ensured by supplementing the culture medium with IBA (Indole-3-Butyric-Acid) hormone in concentration of 1 mg/l. The environment conditions of temperature and light are kept at the same parameters. The first root primordia appear at an interval of 10-15 days after passing on the liquid culture medium;
- acclimatization is the stage in which the plants are transferred in culture vessels, after they have been well washed beforehand from the remains of the culture medium. For the *in vitro* multiplied plants to be transferred in the culture medium itself, it is necessary the gradual adaptation of the root and leaf system of plants. In this acclimatization stage was made the inoculation with vesicular-arbuscular mycorrhizae of the vitroplants utilizing the product Endorize SOL in dose of 15 mg/plant.

During the researches there were carried out laboratory tests concerning *in vitro* multiplication of some mulberry varieties with organogenetic regeneration potential.

To highlight the impact of Pb contamination of mulberry plants, the planting material used was obtained from mulberry seed, the mulberry plants being unisexual dioecious from the botanic point of view and heterozygous, hybrids in first generation, from genetic point of view.

The experiment was carried out in *ex situ* conditions.

The experiment concerning the lead contaminants impact on the germination process of the mulberry seeds used the following work variants:

- V_0 Control represented by 10 mulberry seeds germinated on filter paper with tap water in Petri vessels;
- V_1 Variant represented by 10 mulberry seeds germinated on filter paper with solution of $Pb(NO_3)_2$ in concentration of 0.4 mg Pb% ml solution;
- V_2 Variant represented by 10 mulberry seeds germinated on filter paper with solution of Pb

$(NO_3)_2$ in concentration of 0.04 mg Pb% ml solution.

Each variant was made in two repetitions, the solutions being completed how many times their level was decreasing. At an interval of 7 days from the date of starting the simulation, the first observations were made concerning the seed swelling, the hypocotyl appearance, its elongation, the photosynthesis appearance by coloring the hypocotyl green.

The experiment concerning the lead contaminants impact on the replanting process of the mulberry plants was made in the conditions of utilizing some identical concentrations mentioned in the germination case (water and Pb $(NO_3)_2$). The mulberry microplants have been obtained by sowing a quantity of 30 g mulberry seeds in a substrate consisting of flower soil sterilized in oven at 105˚C for 8 hours. Then, the obtained plants have been replanted into a culture vessel with the same substrate, when their high was of 10 cm minimum and at which the lignification process at the parcel level was noticed.

The observations concerning the lead contamination of the substrate were made weekly until the end of the work stage.

RESULTS AND DISCUSSIONS

Results concerning the *in vitro* multiplication potential of some mulberry varieties

The research concerning the *in vitro* multiplication of some mulberry varieties highlighted their organogenetic regeneration potential.

Regarding the morphogenetic capacity of the sampled apexes from the mulberry varieties taken into study, it was found that the meristematic apexes aseptic inoculated on the eight variants of experimented nutritive mediums have developed shoots in variable number, in accordance with the genotype and the hormonal supplement administrated (Tables 1, 2, 3). It is worth noting the accentuation of morphogenetic capacity in time, after 3-4 subculturing, under the effect of some balanced combinations of BAP (Benzylaminopurine) and Kinetin when there have been developed multiple shoots from every apex. The varieties China 98 and China 99 and the mulberry hybrids come from seed can be multiplied by *in vitro* cloning biotechnology in compliance with the work protocol which utilizes 2 mg/l BAP + 1 mg/l K or 1 mg/l BAP + 2 mg/l K, that determined maximum values for the length of the shoots developed from meristematic apexes. The research on inducing rootedness and regeneration of autonomous plants aimed the effect of auxins IBA (Indole-3-butyric-acid) and 2.4-D (Dichlorophenoxyacetic acid) on the developing of adventitious roots at the base of shoots elongated at 2-5 cm, in the morphogenetic cultures of *Morus* sp. genotypes studied. The rooting of vitroplants presented maximum values of 70-100% in the contidions of utilizing IBA (Indole-3-Butyric-Acid) - 1 mg/l and 2.4-D (Dichlorophenoxyacetic acid) - 1 mg/l (Table 4).

The experimental data concerning the mycorrhizae influence on the mulberry microplants obtained by *in vitro* cultures from the varieties China 98, China 99 and mulberry hybrids highlight greater values for this biofertilization. The mulberry hybrids Eforie presented a seedling length of 65.04 cm and a number of 24 leaves/seedling in the case of mycorrhizae utilization, while in the variant of chemical fertilization NPK, they had a number of 20 leaves/seedling with a seedling length of 64.28 cm (Table 5).

Results concerning the testing of phytoremediation potential of mulberry plants with sericulture destination

The biological processes studied in conditions of lead contamination have been represented by seed germination and plants replanting.

The experimental results concerning the lead contaminants impact on the germination process of mulberry seeds highlighted the following aspects:

- the process of mulberry seeds swelling was observed to all work variants;
- the process of hypocotyl appearance and development was observed in variants and V_0 and V_2;
- the process of hypocotyl appearance is blocked in the case of variant V_1, that constitutes the maximum limit of lead contamination of the germination substrate against which is considered the tolerance to germination of the mulberry seeds;

- the process of photosynthesis appearance is observed in both variants mentioned before, but the development pace is higher in variant V_0.

The results concerning the viability of mulberry seeds in the conditions of simulating lead contamination are presented in Table 6.

In *ex situ* conditions, the accumulation of lead contaminants in mulberry plants influences the germination process under its blocking aspect in concentrations higher than 0.4 mg Pb% ml solution, the nitrate inhibiting the enzymatic biochemical processes. The concentration interval 0.0-0.04 mg Pb% ml solution doesn't influence the germination, the mulberry leaves being tolerant to lead contaminants in the form of salts, respectively lead nitrate.

In the conditions of *ex situ* lead contamination of the transplanted seedlings, the results of this biological testing highlight the fact that the transplanted mulberry plants do not present visible phenotypic modification in V_0 and V_2, the tolerance interval remaining the same both in the germination process and in the development process of the mulberry plants, including the radicular system.

In the two variants do not exist significant differences concerning the high of the transplanted seedlings (Table 7).

Table 1. The effect of the hormonal supplement on the shoots developing in *in vitro* conditions - mulberry variety China 98

Experimental variant	Apexes started growing (%)	Medium number of shoots/explant	Medium length of shoots (cm)
1 - 1 mg/l BAP*	50	2	1.7
2 - 1 mg/l K**	30	3	2.2
3 - 2 mg/l BAP	75	3	2.5
4 - 2 mg/l K	45	2	1.8
5 - 1 mg/l BAP + 1 mg/l K	85	3	2.7
6 - 2 mg/l BAP + 2 mg/l K	70	3	2.5
7 - 2 mg/l BAP + 1 mg/l K	100	4	3.7
8 - 1 mg/l BAP + 2 mg/l K	100	3.5	3.1

*Benzylaminopurine; **Kinetin

Table 2. The effect of the hormonal supplement on the shoots developing in *in* vitro conditions - mulberry variety China 99

Experimental variant	Apexes started growing (%)	Medium number of shoots/explant	Medium length of shoots (cm)
1 - 1 mg/l BAP*	50	2	1.5
2 - 1 mg/l K**	35	2.5	2.4
3 - 2 mg/l BAP	80	2.5	2.3
4 - 2 mg/l K	50	2	1.9
5 - 1 mg/l BAP + 1 mg/l K	90	3	2.5
6 - 2 mg/l BAP + 2 mg/l K	75	3.5	2.3
7 - 2 mg/l BAP + 1 mg/l K	100	4.5	2.8
8 - 1 mg/l BAP + 2 mg/l K	100	4	2.5

*Benzylaminopurine; **Kinetin

Table 3. The effect of the hormonal supplement on the shoots developing in *in vitro* conditions - mulberry hybrids

Experimental variant	Apexes started growing (%)	Medium number of shoots/explant
1 - 1 mg/l BAP*	50	6
2 - 1 mg/l K**	35	7
3 - 2 mg/l BAP	80	8
4 - 2 mg/l K	50	8
5 - 1 mg/l BAP + 1 mg/l K	90	9
6 - 2 mg/l BAP + 2 mg/l K	75	10
7 - 2 mg/l BAP + 1 mg/l K	100	11
8 - 1 mg/l BAP + 2 mg/l K	100	15

*Benzylaminopurine; **Kinetin

Table 4. The percent of rooted shoots depending on the type and concentration of auxin

Type and concentration of auxin (mg/l)	Mulberry genotype – shoots rooting (%)					
	Hybrids Kokuso 21	Hybrids Ichinose	Hybrids Eforie	Hybrids Olteni	China 98	China 99
IBA - 1 mg/l	70	75	85	98	80	80
2.4-D - 1 mg/l	60	70	80	100	70	75

Table 5. The mycorrhizae influence on the growing of mulberry microplants

Somatometric character	Fertilization type	Mulberry genotype			
		China 98	China 99	Hybrids Kokuso 21	Hybrids Eforie
Seedlings length (cm)	Mycorrhizae	29.12	29.40	64.44	65.04
	NPK	27.64	19.00	64.68	64.28
Number of leaves	Mycorrhizae	22	16	14	24
	NPK	21	12	13	20

Table 6. The viability of seeds in conditions of lead contamination

Variant	Concentration (mg Pb % ml solution)	Viability (%)
V_0 Uncontaminated control	0	100
V_1 Lead contamination	0.4	0
V_2 Lead contamination	0.04	50

Table 7. The height variation of transplanted seedlings with the lead concentration applied to the germination substrate

Variant	Concentration (mg Pb % ml solution)	Viability (%)
V_0 Uncontaminated control	0	22.0
V_1 Lead contamination	0.4	0
V_2 Lead contamination	0.04	19.8

Figure 1. Changes concerning the appearance and consistency of mulberry leaves in the case of variant V_1 (0.4 mg Pb% ml lead nitrate) (Own source)

The presented data highlight the interval of tolerance and growing of the mulberry plants in the value margin of 0,0-0.04 mg Pb% solution. The transplanted seedlings in the frame of V_1 variant presented after a time interval of 15 days, the first signs of changes in the leaves aspect and consistency by the appearance of some insular discolorations similar to mycosis attack, the basal leaves drying up completely and the apical growth leaves twisting and having an embossed appearance (Figure 1). Until the end of the stage, the mulberry plants are still viable, but with strong appearance changes and with unfavorable prognosis.

As a result, in the conditions of *ex situ* lead contamination of the transplanted mulberry seedlings, they presented tolerance to the pollutant up to the concentration of 0.04% and at the concentration of 0.4% the lead contamination of the transplanted mulberry plants they present visible phenotypic changes with unfavorable prognosis.

CONCLUSIONS

The preliminary laboratory testing of the *in vitro* multiplication potential of some mulberry varieties highlighted the possibility of utilizing biotechnologies of tissues, meristems and organs cultures in order to test the phytoremediation potential of mulberry plants.

In *ex situ* conditions the accumulation of lead contaminants in mulberry plants influences the germination process of its blocking aspect in concentrations higher than 0.4 mg Pb/100 ml solution. The concentration interval 0.0-0.04 mg Pb% ml solution does not influence the germination, the mulberry plants being tolerant to lead contaminant in the form of salts, respectively lead nitrate.

In the conditions of *ex situ* lead contamination of transplanted mulberry seedlings, they manifest tolerance to pollutant up to the concentration of 0.04 mg Pb % ml solution, the developing process being carried out without significant differences compared to the normal conditions of developing. At the concentration 0.4 mg Pb% ml lead nitrate, the transplanted mulberry plants present visible phenotypic changes, with unfavorable prognosis.

The lead contaminants from the land that can be cultivated with mulberry hybrids do not have negative effect on the replanting and developing processes of the mulberry plants, these being tolerant in the interval 0.0-0.04 mg Pb% ml lead nitrate.

In these conditions it can be considered as a viable technology the phytoremediation of lead contaminated soils by utilizing the mulberry plants with sericulture destination, non-food activity. Over the concentration of 0.4 mg Pb% ml lead nitrate the plants are no longer viable.

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REVIEW ON GENOMIC TESTING OF DAIRY COWS, IMPORTANCE AND ADVANTAGES

Dănuț Nicolae ENEA[1], Gheorghe Emil MĂRGINEAN[1], Aurelia DEFTA (OSMAN)[1], Nicoleta DEFTA[1], Andrada Elena MOISE[1], Hippolyte MEKUIKO WATSOP[2], Livia VIDU[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]University of Ngaoundere Cameroon, School of Veterinary Medicine and Science, P.O Box 454 Ngaoundere, Camerron

Corresponding author email: liviavidu@gmail.com

Abstract

One of the main livestock productions is represented by the dairy sector. The progress made over time being significant, but constrained by a limiting factor, the interval between generations (5 years). An excellent tool that helps make progress by reducing the time period until performance is manifested is genomic testing. This provided the necessary means to constantly improve genetics in production, fitness and conformation of dairy cows. Through its implementation, important changes are occurring in this branch of animal husbandry. This paper aims to review the most important aspects regarding the genomic testing by analyzing a significant number of works on this topic Based on genomic testing, the farmer can take effective decisions about herd improvement, to verify parentage or to identify bacterial strains associated with particular disease outbreaks.

Key words*: dairy cows, genomic testing, milk production.*

INTRODUCTION

There is a high demand for animal products at the moment and dairy sector is one of the main suppliers. This sector is growing fast, world milk production is expected to increase by 177 million tons by 2025 (FAO, 2016). At this moment, this production is composed of: 81% cow, 15% buffalo, and 4% for goat, sheep and camels combined (OECD | FAO, 2023). Milk is a complex food consisting of proteins, fats, lactose, vitamins, and minerals (Nicklas et al., 2009; Vidu et al., 2010; Čuboň et al., 2012; Keresteš, 2016; Mihai et al., 2023). Dairy proteins have been suggested to help reduce adipose tissue (visceral fat) and body weight (Mirmiran, 2005; Teegarden, 2005; Zemel, 2005; Vergnaud, 2008). These effects have been observed in both healthy individuals and those who are overweight (Rosell, 2006; Faghih, 2011; Josse, 2011; Abargouei, 2012; Sanders, 2012) or have diabetes (Liu, 2006; Shahar, 2007). In addition to casein, whey proteins appear to be particularly effective (Pal, 2010; Sousa, 2012) with effects mediated by mechanisms such as increased satiety and reduced appetite (Sousa, 2012). In accordance with global trends, milk and dairy products are frequently consumed food products, in Romania (Defta et al., 2023). Our country having some traditional products made from milk (Șuler et al., 2021).

To produce the amount of milk needed for human consumption, dairy farmers are constantly striving to do more. However, the situation is more complex than it appears at first glance. Total milk production can be increased by either increasing the number of cows or improving individual production through genetic progress. The most convenient option appears to be the first one, as it provides results in a relatively short amount of time. However, it has the undesirable effects, increasing gas emissions (emission = emission factor × number of cattle) (Wójcik-Gront, 2020), respectively the demand for more feed to sustain them. Farmers often rely on increasing genetic progress, the disadvantage being time, as cows have long intervals between generations, more than 5 years in dairy cattle (Jonas and de Koning, 2015). Genomic testing has been introduced to facilitate the reduction of the generation interval in dairy cows. In 2008, the first genomic evaluation of

dairy cows was conducted by the USDA (United States Department of Agriculture), however, the service only became officially available a year later for the Holstein, Jersey and Brown breeds (Wiggans, 2011). Since then, genomic testing has been in use in many countries in order to determinate the genetic value (Loberg and Dürr, 2009). In the Netherlands, the Illumina chip was developed on the basis of SNPs (De Ross et al., 2009), and the following year, BovineSNP50 (Interbull, 2010). Canada collaborated with the USA in the development of the genomic evaluation system, based on BovineSNP50 (Wiggans et al., 2009a), the programme was officially launched in 2009 (Van Doormaal et al., 2009). In the same year, a genomic evaluation system was implamented in Germany. (Reinhardt et al., 2009). New Zealand has also adopted the BovineSNP50 chip and subsequently promoted its use for obtaining bulls that have been genomically tested. In 2012, in Lincoln, UK was developed *GeneSeek Genomic Profiler* (GGP), for dairy cattle (Wiggans et al., 2012). France has been using a marker-assisted selection programme since 2001, in 2008 started to use a small number of SNPs for evaluation, but in an unofficial setting (Ducrocq et al., 2009). The fact that many states embraced genomic testing has been extremely beneficial, a large reference population is needed to have a high predictive value (Goddard and Hayes, 2008; Hayes et al., 2009a). Accurate prediction based on genomic testing was possible in large populations such as Holsteins, but not in smaller populations such as Danish Jerseys.
Tissue samples, blood or hair follicles must be taken from the animals to be tested in order to perform genomic testing.

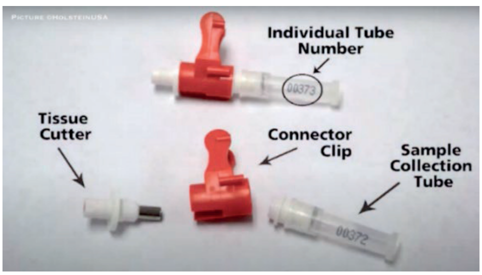

Figure 1. Tissue sampling unit and its component parts (Source: https://www.holsteinusa.com/)

As can be seen in the previous Figure 1, the sampling unit is not at all complicated, with only three component parts. It is attached to a pair of pliers similar to those used for earwigs and a single puncture is made in the ear to collect a sample of ear tissue.
The information provided by genomic testing also enables:

- ranking of the animals according to their productive/reproductive performance;
- verification of the genealogy;
- avoidance of inbreeding;
- establish of nominated pairs;
- the opportunity to use the highest quality sexed semen on the most valuable females identified through genomic testing.

MATERIALS AND METHODS

In order to carry out the present paper 41 bibliographic sources were consulted, all from specialized literature. The studies utilised for this review have been accessed from databases such as Google Academics, Journal of Dairy Science, Frontiers, Science Direct, Springer.
In this paper, the research methods used were the observation, analysis, graphical interpretation of data regarding the advantages obtained by farmers in case of genomic testing of the herd and how the genomic testing favors genetic progress.

RESULTS AND DISCUSSIONS

In the breeding of dairy cattle, selection programmes are of overwhelming interest. While improving the quality and quantity of milk, researchers have also studied the possibility of reducing the interval between generations. A study on this topic was also carried out by Jonas and de Koning in 2015. It is a well known fact that sires play a crucial role in the genetic enhancement of a population. They are having more offspring that a cow, especially if they are used in artificial insemination (AI), situation when their impact on population is even stronger (Gerrits et al., 2005; Funk, 2006).
The main disadvantage that is staying in front of genetic progress in dairy cows sector, as we mention before is the length of the interval between generations. To make an accurate

estimate, time is needed for phenotypes to manifest and be evaluated (Schefers & Weigel, 2012). In addition to the extended duration, farmers also face the expenses of maintaining a large herd over an extended period (Figure 2).

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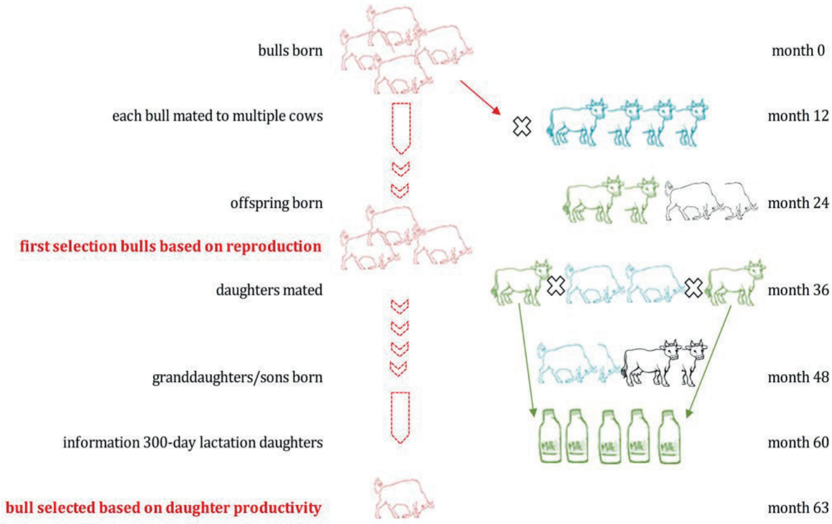

Figure 2. Selection candidates in traditional breeding at dairy cattle (Source: Jonas & de Koning, 2015)

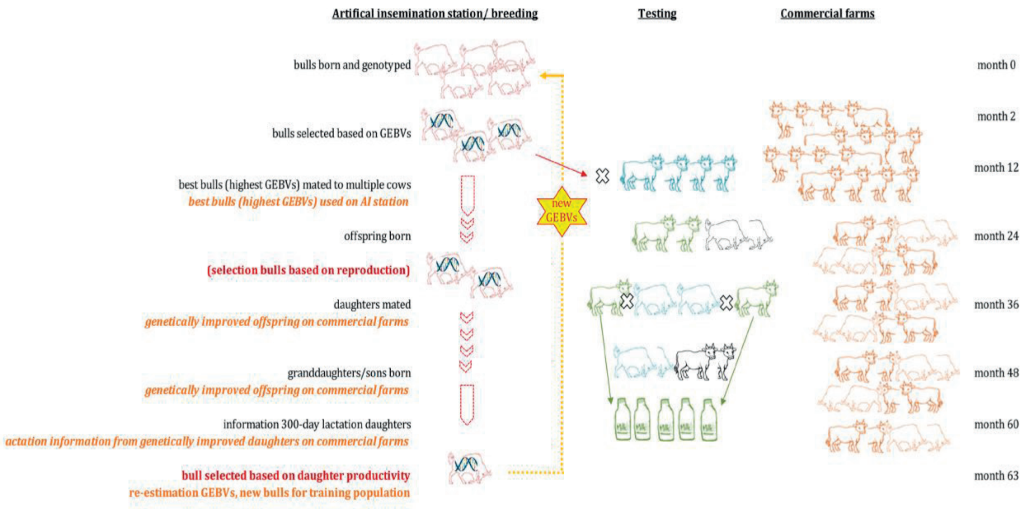

Figure 3. Genomic Selection in dairy cattle (Source: Jonas & de Koning, 2015)

As demonstrated in the previous figure, the bulls - selection candidate (born at month 0) are mated around the age of 12 months with multiples cows. After that, another 48 months must pass for daughters to record the first information regarding the milk yield (to reach puberty, to be mate and to finish the first lactation). Three months later, we obtain the estimated breeding value (EBV) of the bulls, thus obtaining a sire tested on the offsprings.

After performing genomic tests, the effects of each marker are estimated and summarised into genomic estimated breeding values (GEBV). These values can be used to rank the animals and to make a selection. The main advantage is that GEBVs are available from the month 0, in a training population. The bulls selected based on GEBV (with the highest values) are mated in order to multiple cows, in commercial farms. This action, correlated with artificial insemination favours genetic progress. As can be observed in Figure 3, the genomic selection reduces the generation interval from 5.5 years to less than 2 years, a significant reduction, if the selection is realised according to the figure.

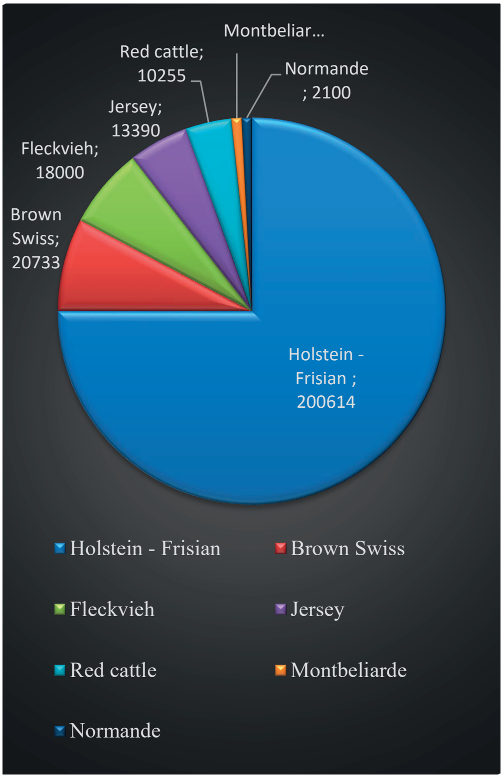

Figure 4. Numbers of bulls active in genomic selection breeding programs (Source: Thomasen, 2013)

According to Figure 4, the largest number of bulls active in genomic selection are Holstein - Frisian, followed by Brown Swiss and Fleckvieh.

To enhance genetic progress, genomic testing is useful not only in reducing the interval between generations but also by increasing reliability. The formula of genetic progress is:

$$\Delta_g = \frac{i \times r \times \sigma_g}{L}$$

Δ_g – genetic progress;

i – selection intensity;

r – reliability (accuracy of selection);

σ_g – genetic standard deviation;

L – generation interval.

Through genomic testing, companies such as Neogen Genomics are confirming the parentage. If we do not have this crucial information, we will miss the opportunity to enhance the accuracy of herd's breeding values and we will not be able to accelerate the genetic gain. If the exact parentage of an animal is unknown, data on its individual values can only be obtained by recording the performance of animals by measurements throughout its lifetime.

According to Neogen Genomics, the reliability varies as follows:

- a calf with parentage known is having a *parentage average* (PA) of reliability 27% (per trait different);
- a calf with unknow sire has a PA <21% reliability;
- a calf with incorrect parentage has a PA of 0% reliability;
- PA + own production records is approximative 50%;
- an animal genomic tested without genotyped parentage has < 70% reliability;
- an animal genomic tested with correct parentage has > 70% reliability;
- a daughter proven bull with thousands of offsprings has a reliability > 90-99%.

In order to increase the reliability in genomic testing are combined also information regarding the siblings, progenity.

The benefits of using genomic testing do not stop there; the same company, Neogen, also provides information on fat production, protein production, somatic cell score, pregnancy rate. As can be observed in the previous table, the key traits are providing a lot of data, only based on them the farmer can easily take a decision regarding if an animal is eligible for selection. Among the essential information (milk yield, fat yield, protein yield), mention before we can notice that data regarding the profit that an animal is estimated to make it, along with a prediction of its capability to survive and the most important reproduction traits.

Table 1. Health traits resulted from genomic test (Source: https://www.neogen.com/en-gb/dairy-genomics)

No.	Trait	Description
1	Cow Livability (LIV)	animals that leave the heard due to death
2	Displaced Abomasum (DAB)	the expected resistance of an animal's offspring to displaced abomasum
3	Hypocalcemia (MFV)	the expected resistance of an animal's offspring to hypocalcemia
4	Ketosis (KET)	the expected resistance of an animal's offspring to ketosis
5	Mastitis (MAS)	the expected resistance of an animal's offspring to clinical mastitis
6	Metritis (MET)	the expected resistance of an animal's offspring to metritis
7	Retained Placenta (RPL)	the expected resistance of an animal's offspring to retained placenta

Table 2. Health traits resulted from genomic test (Source: https://www.neogen.com/en-gb/dairy-genomics/)

No.	Trait	Description
1	Sire Calving Ease (SCE)	the ability of calves of a particular sire to have an unassisted birth
2	Heifer Conception Rate (HCR)	the percentage of inseminated heifers that become pregnant at each service
3	Cow Conception Rate (CCR)	the percentage of cows that become pregnant at each service
4	Daughter Stillbirth	the ability of a cow, or daughters of a bull, to have a live calf that survives past 48 h
5	Sire Stillbirth	the ability of calves from a particular sire to be born live and survive past 48 h
6	Gestation Length (GL)	measures the difference in length of gestation

From Tables 1 and 2, it is evident that genomic testing provides to the farmers a wide range of valuable information. However, the dairy breeder also receives data on conformation traits. This includes stature, feet and legs, rump, udder attachment, udder cleft etc. Additional information, such as A2 beta casein or Bovine Viral Diarrhea Virus, can be provided. A2 beta casein is a protein less common than A1, there are some studies that consider it more beneficial to the human body than A1 so in consequence this milk is having a higher price and more selected by the processors. A2 beta casein is a co-dominant trait, meaning both variants are fully expressed. Nevertheless, a herd can be selected for A2 milk rapidly, even if the cows are all A1/A1 and we will use sires only A2/A2:

- in the first generation all animals will be A1/A2;
- in the second generation approximately 50% of the animals would be A2/A2;
- in the third generation
- approximately 75% of the animals would be A2/A2 (Figure 5).

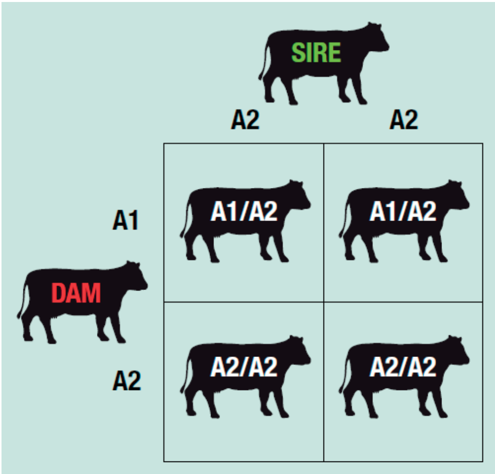

Figure 5. Selection for A2 milk

Bovine Viral Diarrhea Virus (BVDV) represents one of the most significant diseases dairy farmers face, that can cause reproductive disorders and increase mortality. So, the detection of persistently infected animals should be carried out as early as possible in order to eliminate them from the herd, which can be achieved by genomic testing.

Appears, of course, the question if the young genomic bulls are not inferior compared to the proven bulls. In order to answer to this question, de Roos and his collaborators made an study in 2011, entitled "*Effects of genomic selection on genetic improvement, inbreeding, and merit of young versus proven bulls*". The study shows that genomic selection will increase the rate of genetic gain and will not adversely affect the rate of inbreeding per generation. More precisely, using young bulls without their own performance or progeny, as parents doubled the rate of genetic progress, while the percentage of inbreeding per generation remained the same as for a traditional BLUP scheme (de Roos et al., 2011).

A similar study was conducted by Pryce et al., 2010, they analysed various selection models incorporating genomic selection, in order to determine population response through genetic progress. the conclusions recorded by them are the following:

- a higher response rate was recorded in all tested breeding schemes compared to conventional schemes;
- where GEBV were available from foreign countries, nucleus schemes and “worldwide” schemes registered the highest responses to selection;
- when juvenile females were used greater responses in reproductive technologies were achieved;
- the greatest gain in genomic selection (in dairy cows sector) is achieved by reducing the generation interval;
- in some genomic selection schemes, the inbreeding level decreased by 50%;
- the cost of efficiencies using genomic selection schemes are considerably higher than progeny-testing schemes.

One year before, in 2009, Koning et al., made an economical evaluation of genomic breeding programs. They concluded that by replacing conventional testing with genome – wide selection economic efficiency and an annual increased genetic gain is possible. In the study various breeding scenarios for German Holstein population were realised and showed that a doubling of discounted profit is possible by using genomic selection compared to traditional selection scheme.

Research on the benefits of genomic evaluations extends beyond the current scope, with studies exploring their potential future contributions. Recent studies, such as the one undertaken by McWhorter et. al., in 2023, have addressed the selection of heat stress-resistant dairy cows, specifically Jersey and Holstein, based on GEBV. The study showed that genomic predictions can be a useful tool for selecting high - yielding animals in high - temperature and high - humidity environments.

CONCLUSIONS

Summarizing the data of all the articles consulted for the present review, the following conclusions were summarized:

1. The bulls selected based on GEBV favours genetic progress;
2. Genomic selection results in a noteworthy decrease in the generation interval;
3. The confirmation of parentage is possible through genomic testing, fact that improves the accuracy of selection for a given trait;
4. The farmer can receive data regarding production, reproduction and health of animals, so that management decisions can be made more easily;
5. The genomic selection will not affect the inbreeding level, on the contrary, there are studies that prove the opposite and will increase the genetic progress;
6. Breeding programmes that utilise genomic selection are more cost-effective than traditional breeding programmes;
7. Studies on the benefits of genomic testing continue to be conducted, with new opportunities for its use being discovered.

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TOOLS FOR CARBON FOOTPRINT ESTIMATION OF ANIMAL PRODUCTION WITH APPLICABILITY IN RUMINANTS: REVIEW

Mihail Alexandru GRAS, Catalin DRAGOMIR

National Research - Development Institute for Animal Biology and Nutrition (INCDBNA-IBNA Balotesti), Calea Bucuresti no. 1, Balotesti, Ilfov, 077015, Romania

Corresponding author email: gras_mihai@yahoo.com

Abstract

The evolutionary adaptation of the ruminant to convert pasture to animal products may have been successful, but ruminant production has an unwanted by-product (greenhouse gases), that is detrimental to the environment. The greenhouse effect is a term used to highlight the contribution of certain emitted gases to the warming of the Earth`s atmosphere. The gases responsible for the greenhouse effect are: water, carbon dioxide, methane, ozone. Of the total GHG emissions in 2021, 10.7% were emitted by the agricultural sector. In developed countries, numerous research projects have been funded over time by which emission factors (of greenhouse gases) associated with various activities carried out at the level of a farm (e.g., feeding, manure management) or various influencing factors of them (e.g., the average temperature of the area). The present study aims to analyze characteristics of 19 carbon footprint estimation tools, developed and used all around the world, and to set the most suitable system for estimation on the ruminant farms level.

Key words*: carbon footprint, emission factors, estimation tool, greenhouses gases, ruminants.*

INTRODUCTION

The term "greenhouse effect" was popularized in the early 20th century when it was recognized that certain gases in the atmosphere, including CO_2 and water vapor, can trap heat and contribute to the warming of the Earth's surface. These gases allow sunlight to pass through the atmosphere and warm the planet, but they hinder the escape of heat back into space, resulting in a warming effect like a greenhouse. It is worth noting that while the greenhouse effect is a natural process necessary for sustaining life on Earth, human activities since the Industrial Revolution have significantly increased the concentration of greenhouse gases, leading to an enhancement of the greenhouse effect and global warming.

The agricultural sector is responsible for a significant amount of greenhouse gas emissions. These emissions primarily come from various agricultural practices and processes. Some key sources of agricultural greenhouse gas emissions include: enteric fermentation, animal waste management, fertilization, burning of agricultural residues, etc.

Estimating the carbon footprint of farms involves considering various factors such as energy use, livestock emissions, land management practices, and inputs like fertilizers. Here are a few approaches and tools commonly used for estimating the carbon footprints of agricultural operations:

Carbon Footprint Models: Various carbon footprint models and calculators are available specifically for agricultural operations. These models consider factors such as livestock emissions (enteric fermentation, manure management), energy use (electricity, fuel), synthetic fertilizer use, and land-use change.

Life Cycle Assessment (LCA): LCA is a comprehensive approach that evaluates the environmental impact of a product or system throughout its entire life cycle. LCA can be used to estimate the carbon footprint of agricultural products by considering all stages, from the production of inputs to cultivation, processing, transportation, and end use. It considers emissions associated with inputs, machinery use, energy consumption, and waste management.

National Carbon Footprint Inventories*:* Some countries have developed national inventories or guidelines specifically for estimating the carbon footprints of different sectors, including agriculture. These inventories provide methodologies, emission factors, and guidelines to estimate emissions from farming activities, allowing farmers to assess and reduce their carbon footprints.

It is important to note that farm-specific characteristics, such as location, type of farming system (crop farming, livestock production, etc.), scale, and management practices, can significantly influence the carbon footprint.

This review aims to present the online platforms available to farmers and stakeholders for estimating carbon footprint of ruminant farms and made possible mitigation measure implementation at operation and industry level.

MATERIALS AND METHODS

This paper is based on scientific literature published in the English language and collected from Web of Science, Scopus, Google, Tools webpages, and FAO webpage sources. A total of 60 references, covering 2006-2022 period were selected. The selection criterion was usage of GHG emission estimation tools at ruminant operation level.

RESULTS AND DISCUSSIONS

Climate change

Climate change is undeniably one of the pressing challenges that humanity faces today and will continue to confront soon. The frequent occurrence of droughts, floods, rising temperatures, and melting glaciers serves as evident indicators of the reality of climate change. Human activities have significantly contributed to the intensified "greenhouse effect" by increasing concentrations of greenhouse gases in the atmosphere, leading to a rise in Earth's temperature. Europe has experienced a temperature increase of over 1°C over the past century, which is faster than the global average, with the most rapid changes occurring in the last 50 years. While this may not seem dramatic, it has had significant consequences for various physical and biological systems, such as water resources, habitats, and human health, which are becoming increasingly vulnerable. Global warming is primarily a result of human activities, leading to two major challenges for humanity:

Mitigating Greenhouse Gas Emissions: It is crucial to take substantial measures to reduce greenhouse gas emissions so, we can stabilize their concentration in the atmosphere at an acceptable level. This action is important to prevent further anthropogenic influence on the climate system and allow natural ecosystems to adapt.

Adapting to Climate Change Effects: Climate change impacts are already evident and will persist due to the inertia of the climate system. Regardless of efforts to reduce emissions, adaptation to the effects of climate change is essential. This entails implementing strategies and measures to adjust to changing environmental conditions and minimize vulnerabilities.

The European Commission is currently prioritizing the development of a European Ecological Pact. This pact aims to introduce more ambitious measures in addressing the climate crisis and biodiversity loss. European policies have been dedicated to combating environmental degradation and climate change, with both successful and unsuccessful outcomes thus far.

Under the European project INTERREG IIIB CADSES: ACRETe, Romania participated through the National Meteorological Administration. Within this project, a document titled "Code of Attitudes for reducing the impact of climate change in agriculture" was developed. The document provides recommendations on adapting agricultural technologies and practices to the effects of climate change. It also includes examples of good practices that effectively decrease greenhouse gas emissions within the agricultural production process.

The impact of greenhouse gases on the delicate balance of natural ecosystems is a growing concern for climate and sustainability-focused forums. The inventory and ongoing monitoring of these gases have revealed that zootechnical activities contribute significantly to their emissions.

Until recently, the environmental impact of the natural byproducts originating from the internal and external fermentation in animals was considered neutral. This was because these byproducts play a vital role in the regeneration and production of biomass throughout various stages.

These components are integral to the natural cycle of matter in the environment. They are both the result and the catalyst of a complex array of chemical and biochemical processes. These processes include photosynthesis, anatomical and physiological transformations within food chains, and a range of combination and decomposition processes.
The situation has become more complex since it was discovered that the natural reintegration time of greenhouse gases into inert or useful food and non-food compounds exceeds the conventional threshold values. This prolonged reintegration time contributes to the accumulation of these gases, leading to various transfer processes such as mass transfer, heat transfer, and even momentum transfer. Consequently, it is important to find solutions that aim to reduce greenhouse gas emissions, even from these natural processes in which they occur. The Food and Agriculture Organization (FAO) of the United Nations highlights that reduction of up to 30% is achievable in this regard, representing a win-win approach for addressing these emissions (FAO 2022).

Greenhouse Gases (GHG)

Modern and post-modern society relies on the utilization and conversion of energy resources, which are essential in various domains of human activity. However, in processes involving chemical and biochemical reactions, the release of gases into the atmosphere occurs. These emissions originate from different sources within facilities associated with raw material processing and energy generation. Some instances include:
Emission of gases from transportation sources (such as road, rail, air, and maritime vehicles).
Emission at the site of production, encompassing both industrial and domestic installations.
Controlled release through dispersion systems, like tall stacks typically found in thermal power plants (with heights ranging from 100 to 200 meters).
Indeed, the issue of conventional emissions was closely tied to the use of conventional resources such as coal, crude oil, and natural gas. The adverse environmental impacts of these resources prompted the establishment of regulations, norms, and standards to address their emissions. Efforts were made to identify solutions that involved phasing out conventional energy installations and replacing them with alternative sources, with nuclear energy being one of the prominent alternatives explored.
By shifting towards alternative energy sources, world societies aimed to reduce the emissions associated with conventional resource consumption and mitigate the environmental repercussions. This transition sought to address the concerns related to climate change, air pollution, and the sustainability of energy resources.
After 1992, the concept of sustainable development gained prominence and provided a framework for the exploitation of renewable resources. Initially, it was believed that renewable resources were inherently non-polluting. However, over time, it became evident that even renewable sources can contribute to pollution through gaseous emissions. This realization expanded the scope of addressing pollution from gaseous emissions to include all sectors, including industry, energy, and agriculture.
The greenhouse effect is a term used to highlight the contribution of certain naturally or artificially emitted gases to the warming of the Earth's atmosphere. In 1824, the French researcher Joseph Fourier made a significant discovery and provided a detailed description of it. Fourier was a mathematician and physicist who made important contributions to the study of heat transfer and the mathematics of waves. In 1824, he published a work called "Théorie analytique de la chaleur" (Analytical Theory of Heat), which laid the foundation for the mathematical understanding of heat conduction.
The gases that contribute to the greenhouse effect include water (36-72%), carbon dioxide (9-26%), methane (4-9%), and ozone (3-7%).
CO_2 is primarily generated through human activities such as the burning of fossil fuels (e.g., coal, oil, and gas) for energy production, industrial processes, and deforestation. These activities contribute to the increase in atmospheric CO_2 levels, leading to the enhanced greenhouse effect and global warming.
Methane is produced by both natural processes and human activities. Natural sources of methane include wetlands, termites, and the digestive systems of ruminant animals. Human

activities that contribute to methane emissions include livestock farming, landfills, rice cultivation, and the extraction and transport of fossil fuels.

Water, in the form of water vapor, is an integral part of the Earth's hydrological cycle. It evaporates from bodies of water, condenses into clouds, and falls back to the earth as precipitation. While human activities can affect the hydrological cycle through deforestation, land use changes, and pollution, the overall amount of water in the atmosphere is primarily determined by natural processes.

Ozone (O_3) is a form of oxygen that exists both naturally in the upper layers of the atmosphere (stratospheric ozone) and near the Earth's surface (tropospheric or ground-level ozone). Stratospheric ozone is important as it absorbs harmful ultraviolet (UV) radiation. Ground-level ozone, on the other hand, is primarily formed through chemical reactions involving nitrogen oxides (NOx) and volatile organic compounds (VOCs) in the presence of sunlight. NOx is mainly released into the atmosphere through human activities such as combustion processes (e.g., vehicle emissions, power plants) and industrial activities.

The factors on which the manifestation of the greenhouse effect depends:

Table 1. Specific molar heat of gases (kJ/kmol grd)

Air	N_2	O_2	CO_2	CH_4	NO	CO	NH_3	N_2O	H_2S	Water
29.07	29.12	29.27	35.85	34.74	29.98	29.12	35.0	37.45	33.79	34.5

a. Thermal storage capacity. According to Table 1, all greenhouse gases have higher molar specific heat than air. As a result, they produce the greenhouse effect.

b. The quality of the earth's surface upon impact with caloric radiation. For the planetary ocean, water acts as a thermal buffer and can be considered constant (in global terms). It is locally influenced by latitude and the presence of clouds. For the dry side, things are much more complex. This is where the relief comes in, and especially the vegetation: forests, meadows, crops. Photosynthesis is an endothermic process and stores significant amounts of heat energy. Deforestation, fires, grassland degradation and the nature of crops influence the greenhouse effect, increasing the negative impact.

c. Natural emissions of greenhouse gases. Volcanic eruptions, mineral springs are considered. An example is the eruption of the Krakatau volcano in Indonesia in 1883. Such an event undoes decades of efforts to control the global climate balance.

d. Anthropogenic emissions of gases from conventional resources. Conventionally, since 1775 the steam engine, then the internal combustion engine and the Diesel engine have continuously amplified carbon dioxide emissions. The amount of CO_2 increased from 280 ppm in 1750 to 405 ppm in 2017.

e. Anthropogenic gas emissions from renewable resources. Agriculture is considered a regenerative environment in terms of greenhouse gases. However, the contribution of this sector to the global balance is 22% of total emissions, and of these 80% represents the contribution from animal husbandry.

The global energy balance highlighted the significant role that managing renewable resources plays in reducing emissions. By implementing measures to reduce polluting gas emissions from renewable sources, it becomes possible to lower emissions to levels favourable for environmental protection. This approach aims to strike a balance between reducing emissions and ensuring the continued operation of conventional energy sources that cannot be immediately phased out.

The management of this problem follows a cyclical approach, where impact assessments are constantly reviewed, and new measures and solutions are continuously identified to further reduce the quantities of polluting gases. This ongoing process ensures a dynamic response to evolving environmental challenges and facilitates the adoption of improved practices to minimize emissions.

GHG emission in agriculture

In agriculture, greenhouse gas emissions occur in various contexts, particularly through biochemical and microbiological processes in the soil during the integration of plant mass after a cycle of vegetation and fruiting. These degradation processes tend to occur in three distinct periods:

After Harvesting: Following the harvest, the plant residues left in the fields undergo decomposition under the influence of autumn rains. During this period, microbial activity breaks down the organic matter, releasing greenhouse gases such as carbon dioxide and nitrous oxide.

Early Spring: In regions with snow cover, the decomposition of plant residues occurs beneath the snow during the earlier part of spring. The cold temperatures do not entirely halt microbial activity, and as the snow melts, the organic matter continues to decompose, releasing greenhouse gases.

Start of the New Vegetation Cycle: At the onset of the new vegetation cycle, the decomposition and incorporation of the previous plant mass coincide with the emergence of fresh, vigorous plant growth. This transition leads to the release of greenhouse gases as the old plant matter is replaced by the new one.

Greenhouse gas emissions generated by animals and birds in households and farms mainly occur in the following ways:

Along the Digestive Tract: In animals, particularly ruminants, microbial fermentation takes place within the first segment of the tract, which includes the rumen. This fermentation process leads to the generation of gases such as methane and carbon dioxide. Similarly, in the segment of the large intestine, undigested and indigestible components undergo fermentation and homogenization, resulting in the release of additional greenhouse gases.

Through the Excretory System: Animals excrete urine, which contains organic compounds resulting from the partial degradation of substances taken in through their diets. This urine can contribute to greenhouse gas emissions as it contains organic compounds that can decompose and release gases.

Handling Manure: Manure is a significant source of greenhouse gas emissions on farms. It can be stored in liquid or solid form in pools or platforms, respectively. During storage, the manure undergoes anaerobic decomposition, releasing gases such as methane and nitrous oxide. Additionally, when manure is spread on fields or added to the soil, it can further contribute to greenhouse gas emissions as it undergoes decomposition.

The sources of greenhouse gas emissions in agricultural contexts can be categorized as follows:

Soil with Organic Matter: Soils containing vegetable or organic matter can release greenhouse gases such as methane, carbon dioxide, and nitrogen oxides. Microbial activity in the soil leads to the decomposition of organic matter, resulting in the production of these gases.

Vegetation in Different States: Depending on how vegetation is managed, it can contribute to greenhouse gas emissions. If vegetation is mowed and exposed to rain before being collected, decomposition occurs, releasing gases. Similarly, if vegetation is stored in rows exposed to weather conditions throughout the year, it undergoes decomposition, resulting in gas emissions. Additionally, if vegetation is not mowed for a year and prevents new vegetation from establishing, it can contribute to greenhouse gas emissions through various processes.

Ruminant Mouth: Ruminants emit gases, including carbon dioxide and methane, through belching during the process of rumination. These gases are generated in the rumen because of microbial fermentation.

Animal Rectum and Manure: Animals that produce dung release gases through the rectum, resulting in gaseous emissions (meteorism). The dung itself also emits gases during the fermentation process, primarily carbon dioxide and methane, along with smaller amounts of ammonia and nitrogen oxides.

Excretory System: The excretory system of all animals produces urine, which mainly contains ammonia along with small amounts of methane and nitrogen oxide. These gases are released during the excretion process.

The sources or emission points can be further categorized as follows:

Internal Sources: These sources primarily involve gas production within the body, often in an anaerobic environment. Gases are generated as byproducts of biological processes and are subsequently eliminated. Examples include gases produced in the digestive system of animals, such as belching and flatulence, which can contain methane, carbon dioxide, and other gases.

External Sources: These sources are a combination of aerobic and anaerobic processes and involve the fermentation of manure outside of the animal's body. Manure can be stored in various structures like warehouses, platforms, tanks, or pools. During storage, the manure undergoes decomposition, leading to the release

of gases such as methane, carbon dioxide, and other gases.
According to a study by Steinfeld et al. in 2006, it is estimated that the livestock sector is responsible for approximately 18% of all human-induced greenhouse gas (GHG) emissions globally (as cited in Philippe, 2015). The literature has established a clear relationship between the livestock sector, including cattle, sheep, pig, and poultry farming, and GHG emissions.
To better understand the contribution of the livestock sector to global GHG emissions, it is important to consider the global warming potential (GWP) of different gases. Using a GWP of 25 for methane (CH_4) and 298 for nitrous oxide (N_2O) (as cited in Philippe, 2015), Table 2 (which is not provided in the given instructions) provides an overview of their corresponding contributions to the total greenhouse gas amounts.

Table 2. Contribution of livestock species to global greenhouse gas emissions (Source: Phillipe, 2015 adapted from Steinfeld et al., 2006)

GHG (mil. tons CO_2 eq/y)	CO_2 Emisions	CH_4 Emisions	N_2O Emisions	Total emisions
Cattle	1166.2 (61%)	2072.8 (81%)	661.6 (60%)	3900.6 (70%)
Small ruminants	69.9 (4%)	244.5 (10%)	202.6 (18%)	517.0 (9%)
Suine	338.9 (18%)	237.3 (9%)	131,1 (12%)	707.3 (13%)
Poultry	332.2 (17%)	-	107.3 (10%)	439,5 (8%)
TOTAL	1907.2 (100%)	2554.5 (100%)	1102.6 (100%)	5564.3 (100%)

It is estimated that globally, the livestock sector is responsible for 18% of all anthropogenic GHG emissions (Steinfeld et al., 2006 cited in Philippe, 2015). The literature indicates that there is a link between the livestock sector (cattle, sheep, pig, and poultry farming) and GHG emissions. Their contribution to global amounts of greenhouse gases is (considering the global warming potential) of 25 for CH_4 and 298 for N_2O (Philippe, 2015) (Table 2).

GHG sources in ruminant operations

Animal husbandry is an important link in the food supply chain. This source is vital to the existence of mankind. This is also the reason why animal husbandry has acquired an industrial character. Moreover, the polluting effects (greenhouse gases) are intensely manifested in large and very large farms.

a. Nutrition

Ruminants have a four-chamber stomach: rumen, reticulum, omasum, abomasum. In the rumen, a complex process of preparing the food bowl takes place with the help of symbiotic bacteria. Carbohydrates are degraded in the rumen and become volatile fatty acids, AGV (acetic, propionic, butyric acid). And the starch is brought to the form of glucose and then converted into volatile fatty acids. Proteins are degraded to amino acids, but it goes even further to ammonia, carbon dioxide. Lipids are hydrolysed to volatile fatty acids.

<u>Ruminal methanogenesis</u>

The process of rumen methanogenesis is important in achieving a strategy to reduce methane gas emissions. In general, methanogenesis is the biological process of producing methane gas by methanogens. These microorganisms produce most of the methane gas. Other microorganisms that produce methane gas are some Eubacteria, but only methanogens can couple the generation of methane gas with the energy of the animal body.

b. Grazing

Although it is a seasonal activity, grazing introduces many variables beyond technical control that favour greenhouse gas emissions: the fact that the grass consumed is green and contains active microorganisms along with large amounts of water (from the structure of the grass), the fact that the animals leave for pasture in the morning, before the fog rises, the fact that during the day, the grass is dry (in the sense that it has no dew), the fact that the animals also graze during the rain (additional intake of water and microorganisms), the watering of animals (completion of the water requirement for the processes in the stomach) is also valid for grazing and stabling, the floristic diversity of the meadow. Grazing involves the movement of animals over large areas, and the gaseous emissions cannot be captured and treated.

c. The stable

The animals in the barn must be supervised on two levels: on the one hand, gas emissions due to nutrition and digestion; on the other hand, the closed, defective environment, which affects health. In the closed space, the management of greenhouse gases is more strictly controlled, and the solutions that can be applied are easier (ventilation coupled with absorption).

Carbon footprint

The term "carbon footprint" refers to the total amount of greenhouse gas (GHG) emissions, measured in carbon dioxide equivalent (CO_2eq), that are released directly or indirectly by an individual, organization, product, or activity over a specific period. It provides a measure of the impact of human activities on climate change and serves as an indicator of environmental sustainability.

The carbon footprint considers various sources of emissions, including the burning of fossil fuels (coal, oil, and gas) for energy production, transportation, industrial processes, agriculture, deforestation, and waste management. These activities release carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), and other greenhouse gases into the atmosphere, contributing to global warming and climate change.

Carbon Footprint Estimating Tools

In developed countries, significant funding has been allocated to research projects aimed at determining emission factors related to greenhouse gases. These projects focus on identifying the specific factors that contribute to emissions on a farm level, such as feeding practices and manure management. Additionally, researchers investigate various influencing factors, such as the average temperature of the area, that can impact emissions from agricultural activities.

Furthermore, these research efforts have resulted in the development and validation of mathematical models that accurately represent the biological processes involved in greenhouse gas emissions. For instance, complex models have been created to simulate ruminal fermentations, enabling scientists to predict emission levels based on different inputs. These models rely on the formulation and validation of numerous equations that consider various factors influencing greenhouse gas emissions. By inputting specific parameters into these equations, researchers can estimate emissions associated with different agricultural activities. In a later stage of the research process, these equations (simpler/approximate or more complex/exact) were connected within sets of equations (chains of equations) that allow the estimation of greenhouse gas emissions based on input data sets.

A classic reference in this sense are the sets of equations developed in 2006 by the Intergovernmental Panel on Climate Change (IPCC, 2006) and periodically updated (Gómez & Irving, 2019). These sets of equations are accompanied by various documents, periodically developed: technical guides, user manuals, public policy recommendations, descriptions of methodologies, information, research agendas, databases containing emission factors, etc.

The equations allow the estimation of carbon emissions at the emission source level, at the animal level, at the farm section level or at the whole farm level. It is important to note that these equations are publicly available and, to a certain extent, can be adapted to the specifics of countries, regions, etc. by adjusting conversion factors, including updated equations, etc.

Also, to a certain extent, these sets of equations also work when not all input data (a farm's management data) are available, using extrapolations, approximations, etc. Obviously, the lack of data leads to inaccuracies in the calculation/estimation of the carbon footprint and when the proportion of missing data is too high, the models (chains of equations) no longer work.

In the past decade, a multitude of online models and platforms have been developed based on these sets of equations (Popa RA et al. 2021). These tools greatly facilitate and streamline the calculation of carbon footprints, enabling a more efficient and systematic approach. Each of these models and platforms has its own set of advantages and disadvantages. However, it's worth mentioning that at the European level, there is a growing inclination towards standardization. By utilizing these online models and platforms, individuals and organizations can easily input relevant data and parameters to estimate their carbon footprint. These tools leverage the power of computational algorithms to process the inputs and generate carbon footprint calculations in a swift and automated manner.

Regardless of the way of elaboration, the way of constituting the chains of equations used, the interface used (the use or not of an IT interface), in order to be effective, these models (called in current language "tools") have several characteristics common, such as the input data (often the same), the equations and emission / conversion factors used (IPCC 2006, with subsequent updates – adopted faster or slower), the way the results are expressed (greenhouse gases – values individual as well as the integrative value - carbon dioxide equivalent), etc.

There are numerous comparative studies focused on these models/tools, prior to the standardization actions mentioned above, these studies especially highlighting the differences and their effect on the accuracy of estimating the level of greenhouse gas emissions/carbon footprint.

Most importantly, all these models consider the following aspects:

• The environmental impact of animal production can be measured as global warming potential (GWP), acidification potential, eutrophication potential, photochemical ozone-creating potential, ozone-depleting potential, energy use and land use.

• Global warming potential shows how much heat is trapped in the atmosphere and is usually reported as carbon dioxide equivalent (CO_2-eq).

• Measures the accumulated warming over a 100-year period that resulted from a unit mass of gas produced at the beginning of a 100-year reference period.

• Greenhouse gas emissions are calculated for biogenic greenhouse gases: CO_2, methane (CH_4) and nitrous oxide (N_2O).

• The PGI of CO_2 is 1, where CH_4 has the PGI of 28, while the PGI of NO_2 is 265 (IPCC, 2014).

• This means that each kg of CH_4 emitted absorbs the same amount of heat as 28 kg of CO_2 emitted, while one kg of N_2O absorbs the same amount of heat as 265 kg of CO_2 over a period of 100 years.

• Greenhouse gases directly affect global temperature and cause climate change. After net CO_2 emissions have completely ceased, the global warming effect is predicted to last for hundreds if not thousands of years, unless a large amount of net CO_2 is removed over a long period of time.

In this context, one of the roles of these models/tools is to create the framework not only for the determination at a given moment of the carbon footprint at the level of economic operators and agriculture (those who can make management decisions) but also to be able to monitor the effect of the application measures to reduce GHG emissions at the national level, but especially at the level of farms/economic operators. Developing cost-effective mitigation measures for greenhouse gas emissions and ammonia or nitrate leaching requires relational statistics that can only be obtained through farmer survey methods. While on-farm nutrient management tends to vary systematically by farm type (cattle, pigs, etc.) and size, such surveys can be usefully stratified by farm type and size.

Some European countries have already collected farm-level activity data. The surveys were very successful and the national inventories could be improved. Country-specific mitigation options and potentials were identified (Popa D et al. 2026). It was found that the only way forward to a more sustainable and environmentally friendly yet economically viable agriculture was to acquire a better knowledge of farm management practices. Only then can practically feasible, effective, and economical mitigation measures be proposed and implemented.

Agricultural emissions are highly dependent on the livestock housing system and the distribution of the Manure Management System (MMG). These data are a mandatory prerequisite for accurate emission estimates with a low range of uncertainty. The impact of mitigation measures on national emissions reported under the UNFCCC and CLRTP must be documented and this is only possible if representative data on the distribution of GHGs are available. The lack of this data leads to two major disadvantages:

1. Country-specific values can only be integrated to a small extent in the national emission inventory. Major parts of the inventory should be configured with default values that provide a skewed representation of the processes typically found in that country.

2. Due to the lack of breeding and sheltering data, the effect of mitigation measures cannot be included in the national emission inventory.

The methodologies for calculating emissions of greenhouse gases and ammonia are enshrined in international law, so they are not up for discussion. In almost all European states, agriculture is defined as a key source of greenhouse gas and ammonia emissions. As such, Member States are required to use a Tier 2 methodology for stock reporting. Tier 2 methodologies require detailed data that respects the relationships between emission sources. These data can only be collected through large-scale sampling of farms.

Gross nitrogen and phosphorus balances provide holistic indicators of the related environmental pressure exerted by agriculture. For N, there are significant losses to the atmosphere in the form of ammonia, nitrous oxide, nitrous oxide (NO) and molecular nitrogen (N_2). Ammonia, and nitrous oxide are pollutants, while the emission of molecular nitrogen reduces the efficiency of manure and fertilizers and the fertility of soils. Nitrogen is lost to aquatic environments as nitrate, ammonium and dissolved organic nitrogen, all of which can lead to pollution and all of which reduce soil fertility. Unlike greenhouse gas and ammonia emissions, countries are not required to report N and P balances for agriculture as part of any international convention. Consequently, there is no organization equivalent to the IPCC or UNECE that has the responsibility to standardize and improve the methodology for calculating these balances.

Environmental pressure indicators are very important, and some tools (such as CAP'2ER) calculate them as well, in addition to the carbon footprint.

Data requirements for calculating NH_3, CH_4, N_2O emissions and N and P balances are relatively high, especially for large emission sources, because of the accuracy required for these source estimates. Currently, this data is not always available in the Member States. Based on experiences in various countries, it is suggested that farm structure surveys be carried out every five years to collect information on housing systems, manure storage systems and manure application techniques.

Farm activity data, listed under the "minimum requirement" must be collected, as without this data, proper inventory reporting is not possible. The effect of mitigation measures cannot be indicated in the inventory, and the profitability of mitigation measures cannot be assessed. Activity data listed at "optimal requirement" should be collected for more accurate inventory estimation. They offer more possibilities for country-specific and cost-effective mitigation measures and enable the assessment of the environmental impact of farm management practices. For most of this data, the additional effort to collect it is small and the additional effect is large.

Table 3. Inventory of carbon footprint estimation models applicable to ruminants

TOOL	Country	Species covered
CAP'2ER	France	beef cattle, dairy cattle, dairy goats, meat goats, dairy sheep, meat sheep
COOL FARM TOOL	Great Britain	beef cattle, dairy cattle, dairy goats, meat goats, dairy sheep, meat sheep
FARM CARBON CALCULATOR	Great Britain	beef cattle, dairy cattle, meat goats, meat sheep
AGRECALC	Great Britain	beef cattle, dairy cattle, meat goats, meat sheep
CONVIS	Luxembourg	beef cattle, dairy cattle
DECiDE	Belgium	beef cattle, dairy cattle
Kringloopwijzer (ANCA)	Netherland	dairy cattle
AGNAV	Ireland	beef cattle, dairy cattle, meat goats, meat sheep
Agrosfär	Sweden	beef cattle, dairy cattle
ESGreen Tool	Denmark	beef cattle, dairy cattle
Klimrek	Belgium	dairy cattle
KLIR	Swiss	dairy cattle
TEKLa	Germany	beef cattle, dairy cattle, meat goats, meat sheep
BIOCODE	Finland	beef cattle, dairy cattle
ADER 914	Romania	beef cattle, dairy cattle, dairy goats, meat goats, dairy sheep, meat sheep
ArdiCarbon	Spain	dairy sheep, meat sheep
Carbon Sheep	Italy	dairy sheep, meat sheep
Teagasc Sheep LCA	Ireland	meat sheep

Obtaining accurate values for coefficients used in calculating emissions or nutrient balances is essential. The default values provided in the IPCC Guidance 2006 and the EMEP/EEA Guidance 2019 are intended to be reasonable estimates for the specified geographic area. These default values often mask wide geographic variation in actual values, either due to variations in climate or regional variations in agricultural practices. In addition, the default values presented in the various guidance documents generally refer to situations where no mitigation measures have been implemented. Member States are encouraged to use appropriate coefficient values at national or regional level. It is good practice to support the use of these coefficients with empirical measurements. The consequences of relatively small errors in the coefficients can be significant. It is important that the source of the coefficients used is documented. Where default values are used, the source must be indicated. The value of some coefficients varies according to agricultural practice. For example, ammonia emission after land application of animal manure depends on the manure application method used. Coefficients may need to be updated periodically to account for significant changes in farming practices. Most important tools used to estimate carbon footprint in the ruminant farms are presented further.

CAP'2ER

CAP'2ER is a farm-level assessment tool covering mixed cropping and ruminant farming (Milk, Meat, Mixed, Sheep, Goat). Software was developed in 2015, and compute the environmental performances from small ruminant farms, using the variables affecting the carbon footprint of farms. It is widely used in France (about 30,000 assessments and 1,500 users). It is also used in other countries Switzerland, Italy, Germany, Spain, Romania.

The tool (based on life cycle assessment (LCA) assesses the impact on the environment (GHG emissions, nitrogen losses, energy consumption) and the positive contribution (biodiversity, carbon storage, feeding people). It is an advisory tool to build an action plan and provide technical advice. You can do simulation to test the implementation of the practices.

The tool has two assessments level of environmental performances:

Level 1: a simplified web version for large public (farmers, advisers, students), used for a quick evaluation of in farm environmental footprint.

Level 2: a tool for adviser’s decision support (to realize “in depth” assessments, with high level of details).

For comparison, level 1 requests an input of 40 technical data and level 2, an input of 150 technical data. Technical data used are general information about farm, breeds, flock number and structure, crop surfaces, purchased feeds, etc.

A set of equations will estimate production of manure, dry matter intake, emissions allocation, total carbon footprint on unit of production etc. (Cannas et al., 2019)

COOL FARM TOOL

COOL FARM TOOL is widely used in Europe, it is available in several languages (English and German and 15 beta languages). The tool is focused on main areas as: greenhouse gases, biodiversity, water use, food lose and waste.

The tool covers many production systems, have good transparency in the methodology used in the tool (IPCC 2006 methodologies) and good precision for the dairy and crop production system.

The tool is more suited to assess the carbon footprint than to provide technical advice at the farm level, there are few features to build an action plan.

The tool is not well suited to mixed farms, it is more of an aggregation of production (an assessment by production and crop).

Greenhouse gas quantification and soil sequestration are done based on crop and livestock data requirements (yield harvested and marketable products, growing area, fertiliser applied, energy used, transport, herd size, feed, manure management etc.).

Water use is quantified based on farm coordinates, soil moisture, planting time, water general amount, irrigation etc.).

Biodiversity is evaluated using information like: total area and non-productive land of the farm, farm management practices, provision of small and large habitats, etc. (Hillier et al. 2011).

Farm Carbon Calculator

Is a free evaluation tool, used exclusively in Great Britain, and created by an independent company. Is started in 2009 as a help for farmer to reduce greenhouse gases emissions. The tool is very well developed for agricultural use, especially vegetal production. Additionally, a part of toolkit is dedicated to livestock. The tool is also based on IPCC 2006 recommendations.

Provide a practical, scientifically robust, and accurate approach.

Produce guidance and other outputs that are high-quality, accessible and easy-to-understand.

AGRECALC

Is a carbon and economic efficiency estimation tool with the access on the AGRECALC cloud. Widely used tool in the UK (7,000 advisers, 12,000 assessments). It covers a wide type of

production with a farm level approach. Ratings available for multi-production farms. Good methodological precision and sensitivity to practices. Data collection requires data pre-processing when there are multiple production units.
The link between each production unit seems not to be well developed in this tool, and user guide and training are available, but for the methodology, only simplified elements were available on the site.
The tool Conforms to IPCC 2006 calculations for all livestock types & PAS 2050:11 supply chain standards (Smith et al., 2019).

AGNAV
Developed by Teagasc/Bord Bia/ICBF, some features are still under construction. Used in Ireland by Teagasc advisers, it is an advisory tool with a strong link to agricultural practices and environmental outcomes. Attenuation levers are easy to test in the instrument. Is well adapted for use in Ireland, connection to Irish database for data collection. Few chances of use at European level.
(https://www.bordbia.ie/farmers-growers/prices-markets/agri-market-insights/agnav-trusted-solutions-for-everyday-farming/).

ADER 914
It calculates emissions at the animal level, a first for Romania. It is based, like all other systems/models, on the IPCC 2006 equations and allows distinct calculation by category of animals, with an emphasis on the forage module. Significant inputs are needed to be able to compete with much more advanced models/ systems like CAP2ER or CoolFarmTool.

ArdiCarbon
Is a spreadsheet tool, developed in Spain, developed to compute GHG emissions on small ruminant farms. Is relative similar with CAP'2ER tool, but access to tool is very limited.

Teagasc Sheep LCA
Teagasc sheep life cycle assessment (LCA) carries out a life cycle inventory and operates with multiple parameters (Asem-Hiablie S et al. 2018). The method uses the guideline defined by British Standard (BSI, 2008) for potential global warming of sheep farms and caring out LCA guidelines for small ruminants (LEAP, 2025).
The tool is estimating GHG emissions over the one-year production cycles. Are considered all categories of animals from the farm: ewes, lambs, rams, replacement animals etc. The estimation models, sheep-specific emissions are driven by the inputs of the sheep breeding systems: feedstuffs, fertilizers, manure management, gas, and electricity consumption etc. Few chances of being used at European level as it is built on Irish specifics and is only addressed to the sheep farming sector.
It should be mentioned that in addition to the models/platforms developed by public research units, there are numerous private initiatives/companies that offer their services, especially to those interested in trading carbon credits. One such example is Agreena:
https://agreena.com/ro/
It buys carbon credits and assists farmers who intend to reduce their carbon footprint.
According to the company's statements, it already has a portfolio of clients in Romania, mainly those that deal with vegetable crops.

CONCLUSIONS

When faced with the need to reduce emissions, countries are typically faced with a choice between several different mitigation measures. Identifying the most effective cost-reduction measures for agriculture typically requires data beyond what is needed to support a proper approach to calculating emissions. This is because the complex and highly varied nature of agriculture results in large differences between available mitigation measures and their associated costs. In this respect, the availability user-friendly online tools have democratized the process of calculating carbon footprints. It allows a wide range of users, including individuals, businesses, and policymakers, to access and utilize these platforms to make informed decisions regarding their carbon emissions. By providing a more expedient and systematic calculation method, these online models and platforms contribute to raising awareness and facilitating actions to reduce carbon footprints and promote sustainability.

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THE IMPACT OF PRODUCTION SCALE ON PIGLET BIRTHWEIGHT AND SURVIVAL UNTIL WEANING: INSIGHTS FROM A ROMANIAN FIELD TRIAL

Ioan LADOȘI, Alexandru Marius DEAC, Ileana MICLEA, Daniela LADOȘI, Marius ZĂHAN

Faculty of Animal Science and Biotechnology, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, 3-5 Calea Mănăștur Street, 400372, Cluj-Napoca, Romania

Corresponding author email: daniela.ladoși@usamvcluj.ro

Abstract

Piglet birth weight plays a vital role in determining their growth performance and productivity of the farming systems. Moreover, piglet birth weight could be also a predictor for piglet survival and subsequent growth. A negative impact of litter size on piglet birth weight has been acknowledged, as large litters have reduced average piglet birth weights and increased within-litter birth weight variation. Consequently, comprehending the elements that influence piglet birth weight can have substantial financial effects on swine farming. A large scale trial was performed in several Romania commercial farms in order to identify the implications of the size of the production system on the piglet birth weight, survival and growth to weaning. Analysis of the records seems to suggest that there is a high variability in this respect among farms with similar rearing conditions and genetics. However, the survival rate to weaning of the low birth weight piglets (LW <1.3 kg) is low irrespective of the size of the farm. In contrast the high birth weight piglets (HW >1.3 kg) seems to thrive in both large, small or medium size units.

Key words: *birth weight, commercial, farms, piglets, variability*

INTRODUCTION

Low birth weight piglets are associated, by farmers, with increased mortality, reduced weaned pig quality, and slower growth rates thus compromising kilograms marketed (Houben et al., 2017). The subject was investigated in a wide range of farming systems worldwide, of different unit sizes, genetics and environmental conditions. However, defining an ideal or optimum birth weight is still widely debated, mainly as this becomes an issue of animal welfare. It is known that the growth of piglets relies on their weight at birth and access to maternal milk during their initial weeks of existence (Feldpausch et al, 2021; Jankowiak et al., 2020). Evidence suggests that piglets characterized by lower birth weights commonly display diminished vitality, encounter delays in accessing the initial intake of colostrum, and face difficulties in competing against stronger littermates for suckling (Devillers et al., 2011). Moreover, piglet birth weight could be also a significant predictor for piglet survival during lactation and subsequent growth (Houben et al., 2017). A negative impact of litter size on piglet birth weight has been acknowledged, as large litters generally have reduced average piglet birth weights and increased within-litter birth weight variation (Milligan et al., 2002; Kobek-Kjeldager et al., 2020).

A potential approach in order to increase piglet birth weight or decrease variability in piglet birth weights within litters is by adjusting feeding strategies of the sow, both before ovulation and during gestation (Campos et al., 2012; Jin et al., 2018). By optimizing the nutrition of pregnant sows, particularly in terms of protein and energy content, farmers can increase the birth weights of piglets and potentially improve their growth performances. In addition to genetic and nutritional factors, the timing of farrowing can also influence piglet birth weight. Furthermore, low birth weight piglets generally show poor growth performance and have a lower lean percentage of pig carcass at slaughter compared to piglets with higher birth weights (Fix et al., 2010; Zhang et al., 2014; Lujka et al., 2021; Rehfeldt and Kuhn, 2006).

Evidence from various studies supports the assumption that birth weight is a heritable trait and can be selectively bred for. For example, it was found a positive relationship between birth weight and mortality during the first three days after birth in large litters (Muhizi et al., 2022). This implies that selecting sows with higher birth weight piglets could potentially reduce early mortality rates in litters despite that the heritability of this trait is rather low (Damgaard et al., 2003).

Piglet birth weight can also be heavily influenced by the parity of the dam. Sows at parities 3-4 tend to give birth to the most balanced litter weight while parity 1 and 5-6 sows are expected to deliver lighter piglets (Lavery et al., 2019).

Piglets with higher birth weights have been found to exhibit better overall growth performance, nutrient utilization, and muscle development compared to those with lower birth weights (Lanferdini et al., 2018).

Piglets born later in the farrowing process tend to have higher birth weights compared to those born earlier. Furthermore, the variability in birth weight within a litter can also impact piglet growth performances (Zindove et al., 2014). Research has shown that larger litter sizes are associated with lower birth weights, likely due to limited uterine space and competition for nutrients among the developing piglets (Riddersholm et al.,2021).

Studies have consistently shown that birth weight is a reliable indicator of piglet growth and development (Zhang et al., 2014). Thus the economic implications of piglet birth weight should not be overlooked. Consequently, comprehending the elements that influence piglet birth weight and executing suitable tactics to maximize it can have substantial financial effects on swine farming (Opschoor, 2015).

Despite the overwhelming evidence that birthweight is an important trait for commercial pig farming systems, there is limited data investigating the issue concerning the size of the farming operation. It is widely accepted that as the size of the farm routine management practices can become more complex mainly due to the individual skills and professionalism of the caretakers in charge of the activities in the farrowing departments.

The main aim of this study was to assess the various potential implications of the farm size on the piglet birthweight and their subsequent survival and growth until weaning.

MATERIALS AND METHODS

Data collection was performed in 16 Romanian commercial farms divided into two groups based on the herd size. The first group consisted of 7 farms with an average of 2400 sow herd, ranging between 1600 and 4000 heads while the second group consisted of 9 farms with an average sow herd of 600 sows, with limits between 150 and 900 females. All considered farms have a farrow to finish a continuous production system. Farrowing departments have standard commercial housing, ventilation, lighting, feeding, and watering equipment. Farrowing pens in all units have been provided by major EU equipment producers with an average overall surface ranging between 4.6 m^2 and 5.6 m^2 depending on the timing of farm construction or refurbishment. The genetic makeup of the herds was similar in terms of the dam (Large White x Landrace F1 sows) and sire lines (Pietrain). All farrowing was recorded in the same week of June, while the weaning data was recorded in July. No overnight assistance was provided to sows at the time of farrowing. Weaning weight recording was performed individually on the day designated by the farm manager according to each unit's internal protocol. Age at weaning in all units was in line with the legal minimum requirements for pigs as found in the European legislation (Council directive 2008/120/EEC). Concerning nutrition, nursing sows were provided a standard lactation diet based on the genetics and nutrition providers' recommendations, while piglets were provided solid food (commercially available pre-starter) commencing on day 7 after birth. Despite possible and probable differences among farms regarding management, standard internal operating procedures, and health status (respiratory and digestive pathology) at the time of the data gathering no acute symptomatology was signaled by the unit veterinarians, and internal vaccination protocols were strictly followed.

In order to perform the recordings a pack of materials was provided to each unit to designated farm staff containing the followings:

•Weighing plastic bags with the recommenddation to use one bag per litter to help maintain hygiene;

•Digital weigh balance - scaling from 0 to 3 kg;

•Two spray markers - red and green;

•Cards for recording litter details and individual piglet birthweights;

•Button tags - red and green plus pliers to tag pigs at processing (Day 2).

Weighing and data collection protocol. Weighing piglets individually was performed after the sow has finished farrowing and prior to cross-fostering occurring on Day 2 after piglet processing. There were three categories of scaled piglets: a) total born (TB), born alive (BA) and stillborn (SB). Litter cards were completed with individual piglet birth weights and allocated to the following categories: a) piglets with 1.3 kg and above were marked with one spot of green marker (G); b) piglets less than 1.3 kg were marked with one spot of red marker (R) just behind the head. Any pre-weaning death was recorded on the litter cards. Button tags with the same color as the markers were applied the following day, at the time of processing based on the spray markings. Condition score (CS) and parity (P) of the lactating sows were assessed at the time of scaling the birthweight of the piglets and recorded on the litter card. Subsequently, all sow and litter data were transferred from the recording cards onto the spreadsheet provided on a data stick.

Statistical Analysis

Data was statistically analyzed using GraphPad Prism (Version 9.3.1). An unpaired t-test was performed in order to compare the differences in terms of birth weight and weaning weight between the two types of farms. Also, a Pearson's correlation was performed to analyze the linear relationship between birth weight and weaning weight, in both types of units.

RESULTS AND DISCUSSIONS

Farrowing records

A summary of the data recorded at farrowing is represented in Table 1, showing the overall comparison between the Small/Medium (S-M) size and Large (L) size unit groups. When analysing the sow parity score it is evident that there is an important difference between the unit groups of 0.6 more parities in the S-M units meaning that the age of the sows is higher as well (Lavery et al., 2019). A lower average parity score in the L units suggests better management of the parity structure and a consistent policy related to the voluntary replacement rate. Evaluating the average body condition score (BCS) of the sows is leading to similar conclusions as this trait can be closely related to the age of the sows in the herds. Although the differences between the unit groups are minimal, it is worth mentioning that the values are higher in both unit groups than the generally accepted optimal BCS (2.5 to 3.0). However, it must be noted the limited number of sows were assessed in this experiment in comparison to the actual size of the entire herd in these units. Therefore, this sample status might not reflect the overall image of the parity and condition score of all females of the sow herd.

All these might suggest that the nutrition during gestation leads to heavier sows at the time of farrowing and possible effects on the piglet birth weight (BW) as well. The total number of piglets born per litter (TB) is higher in S-M units (+0.57). The same pattern is valid for the piglets born alive (BA) while the difference is slightly lower (+0.52). The average number of stillborn piglets (SB) is similar in both unit groups suggesting that relevant reproductive diseases are controlled efficiently through appropriate vaccination protocols. When compared to averages reported in other industries (Ketchem et al., 2018) the SB in the analyzed units is lower thus confirming at least a sound health control.

Table 1. Summary of farrowing data in the analysed units

Item	Small/Medium Farms (154 litters)		Large Farms (171 litters)	
	n	Mean ± SEM	n	Mean ± SEM
Sow parity score	154	4.4 ± 0.162	171	3.8 ± 0.173
Sow condition score		3.8 ± 0.046		3.4 ± 0.055
Total born/litter	2015	13.08 ± 0.2	2139	12.51 ± 0.161
Born alive/litter	1879	12.23 ± 0.182	2000	11.71 ± 0.141
Still born/litter	119	0.78 ± 0.089	136	0.80 ± 0.095
Litter weigth at birth (kg)		19.51 ± 0.345		18.32 ± 0.258
Born alive piglet birth weight (kg)		1.61 ± 0.021		1.59 ± 0.022
Light piglets/litter (< 1.3 kg)	421	2.73 ± 0.250	458	2.68 ± 0.216
Heavy piglets/litter (> 1.3 kg)	1469	9.54 ± 0.251	1559	9.12 ± 0.221

The situation is highly variable when we are analyze the differences among farms belonging to the same unit size group. In L units (Figure 1) the recorded farrowing parameters are the lowest in Unit 1 while the highest in Unit 4 at least in that specific moment in time when the sampling was performed. The wide difference between Unit 3 and Unit 6 regarding the SB piglets could be attributed either to nutrition practices during gestation or to the specific health status of the herd in the respective farm at the time of the trial. In S-M units, farrowing parameters differences between units are present as well (Figure 2).

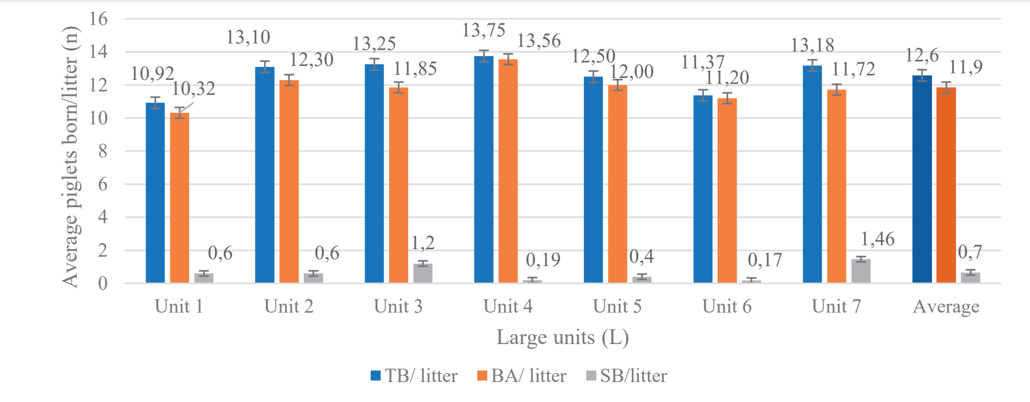

Figure 1. Differences between L units regarding the main recorded farrowing parameters (average and SEM)

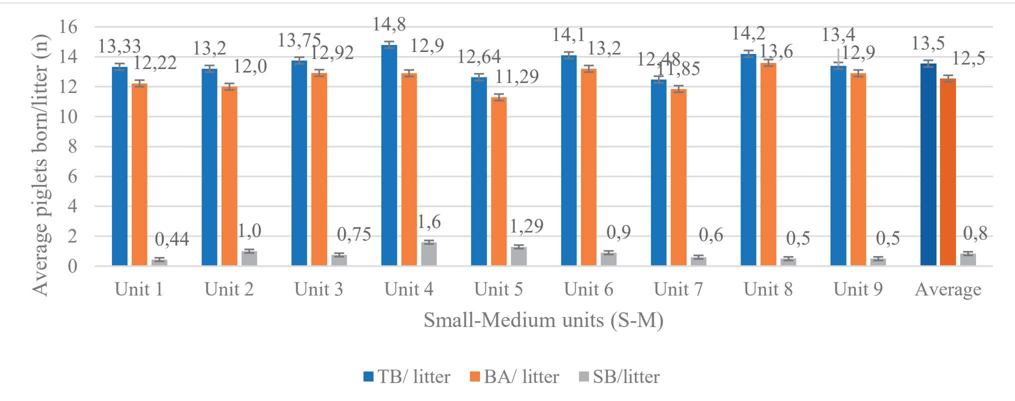

Figure 2. Differences between S-M units regarding the main recorded farrowing parameters (average and SEM)

The wider difference seems to be between Unit 4 and Unit 7 in terms of TB, BA or SB piglets. Reasons for the situation can be multiple and complex as well. However, the main one could be the quality of the sows within the sample at the time of the experiment. As the average parity in Unit 4 (3.8) is much lower than the one in Unit 6 (5.4) this might probably be the main source of variation. This would also mean that Unit 4 sows were younger on average and therefore most probably had a higher genetic index simply due to their age advantage. The same comment would be valid for the differences related to SB piglets. Alternatively, in Unit 4 the recorded condition score (CS) was 3.5 while in Unit 6 the value was 3.8. Therefore it is highly unlikely that this trait had influence over the farrowing traits. All these differences are not unusual even in the situation when the units use the same genetics. This could point out that despite similar operation size there are several other management and/or environmental factors that can influence all the farrowing parameters.

A wider difference between S-M and L unit groups was recorded on the total litter weight at birth with an advantage for the S-M units of 1.19 kg/litter (Table 2).

Table 2. Summary of piglets weights at farrowing in the analysed farms

Item	Small/Medium Farms (154 litters)		Large Farms (171 litters)	
	n	Mean ± SEM	n	Mean ± SEM
Litter weigth at birth (kg)		19.51 ± 0.345		18.32 ± 0.258
Born alive piglet birth weight (kg)		1.61 ± 0.021		1.59 ± 0.022
Light piglets / litter (< 1.3 kg)	421	2.73 ± 0.250	458	2.68 ± 0.216
Heavy piglets / litter (> 1.3 kg)	1469	9.54 ± 0.251	1559	9.12 ± 0.221

This surplus can be attributed to a higher number of BA piglets in these units. These differences favoring the S-M units might be due to better protocols for farrowing assistance and gilt development. Across both group of units the average BW of the piglets was surprisingly similar (1.61 kg/ piglet in S-M farms and 1.59 kg/ piglet in L farms) which could be related rather to the use of same genetics and less to management or the environmental conditions. The same pattern seems to be valid for the number of light (LP under 1.3 kg) versus heavy (HP over 1.3 kg) piglets within litters irrespective of the unit size and it could be attributed probably to genetics. The light and heavy litters average percentages out of the total piglets BA seems to be in the same range in both types of units (22% LP litters vs. 78% HP litters). However, a large variation of this parameter was noted between the units of the same group, ranging from 65.8% to 89.9% (heavy piglet litters) and 11.9% to 29.5% in L units (Figure 3).

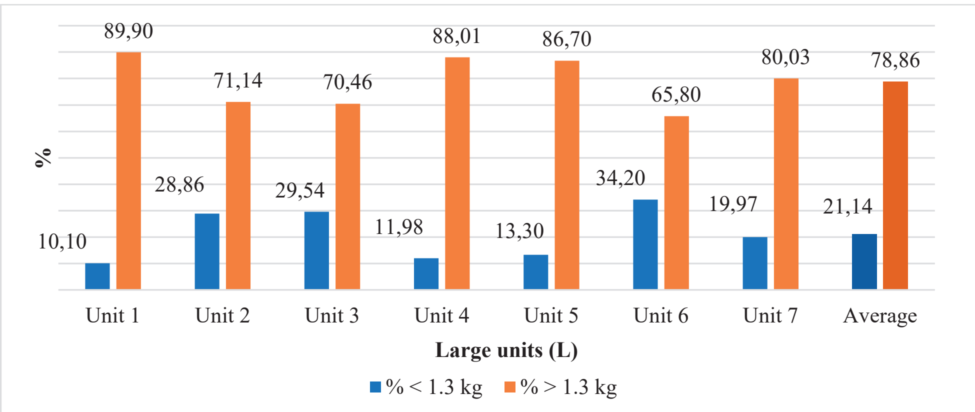

Figure 3. Variation of the ratio of light piglets (LP) and heavy piglets (HP) among the large units (L)

Stating the reasons for the high difference between Unit 1 and Unit 6 might be speculative as it could be related once again to the quality and quantity of the gestation feed or to the actual sow body fat reserves at the time of farrowing. The minimum percentage of light piglets in Unit 1 can be related to the TB and BA, which have the lowest value among examined farms. It is well documented that sows with lower prolificacy tend to have more uniform piglets at birth.

In S-M units the variation of the piglets within litters was even higher ranging between 7.6% and 33.8% (LP litters) and between 66.2% and 92.4% in heavy piglet litters (HP litters) (Figure 4). According to the recorded data, Unit 5 seems to have the most desirable ratio between light and heavy piglets at birth, with the least favorable situation in Unit 2. These differences can be attributed once again to nutrition during gestation and to the quality of the sows in the sample at the time of the experiment mainly from the age perspective (recorded average parity was 4.0 for Unit 5 and 4.7 in Unit 2, respectively). An other explanation of the low number of light piglets in Unit 5 seems to be the correlation with the lowest TB and BA in the entire group of S-M analyzed farms.

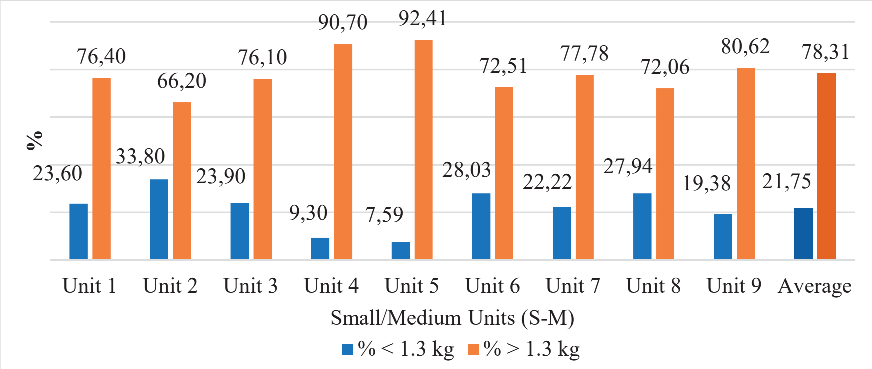

Figure 4. Variation of the ratio of light piglets (LP) and heavy piglets (HP) among (S-M) units

Weaning records

Analysing the records at the time of weaning and comparing the two groups of farms reveals that out of the 3443 piglets weaned of the 3879 BA, there is a clear balance between unit groups not only from the overall piglet number perspective but from the one of the average of piglets weaned/litter as well (Table 3). Thus, this could suggest that the farm size might not have a large impact on this trait after all. There is however a difference related to the piglet age at weaning with one day less in S-M farms suggesting that the litter weaning weight could of been in the favor of the piglets raised in the L farms group. On the contrary, records are showing that weaned litters in L farms are on average 1.53 kg lighter than the ones in S-M group units. This might indicate that S-M units managed to take an overall better care of the piglets in the farrowing house, something possible due to a lower workload and thus a higher attention to details by the caretakers. It is also valid to observe that the average litter weight at birth was 1.19 kg higher in the same S-M units, therefore a clear advantage from the start. This disadvantage for the L units could not be offset however by the extra 1 day spent by the piglets in the farrowing house if we look at the irrelevant difference between unit groups from the perspective of the average piglet weight at weaning. Further analysis of the differences between farm groups regarding the light piglets (LP < 1.3 kg at birth) reveals that the number of weaned litter is lower by 0.26 piglets / litter in S-M units. Similarly, the number of heavy piglets at weaning is higher in S-M units by 0.4 piglets/ litter (Table 3). All these findings might be relevant as the birth weight (BW) was similar in both groups of units (Table 2).

Table 3. Summary of weaning data in the sampled units

Item	Small/Medium Farms (158 litters)*		Large Farms (172 litters)*	
	n	Mean ± SEM	n	Mean ± SEM
Weaned pigs/litter	1667	10.55 ± 0.847	1776	10.33 ± 0.130
Weaning age (days)		27.90 ± 2.241		28.92 ± 0.181
Litter weight at weaning (kg)		75.25 ± 6.045		73.72 ± 1.123
Weigth at weaning (kg/head)		7.18 ± 0.577		7.14 ± 0.079
Light weaners (< 1.3 kg)/litter	298	1.89 ± 0.151	367	2.15 ± 0.206
Heavy weaners (> 1.3 kg)/litter	1339	8.37 ± 0.672	1403	8.16 ± 0.237

*More litters weaned than farrowed due to cross-fostering

Average pre-weaning survival in both groups of units is alike, with a difference of less than 1%.
However, the variation of this trait inside the groups is quite high mainly in one of the large units (66.8 % in Unit 4 to 96.3% in Unit 2). This 30% gap might be attributed primarily to differences in piglet management during lactation or even to the sow nutrition after farrowing (Figure 5).

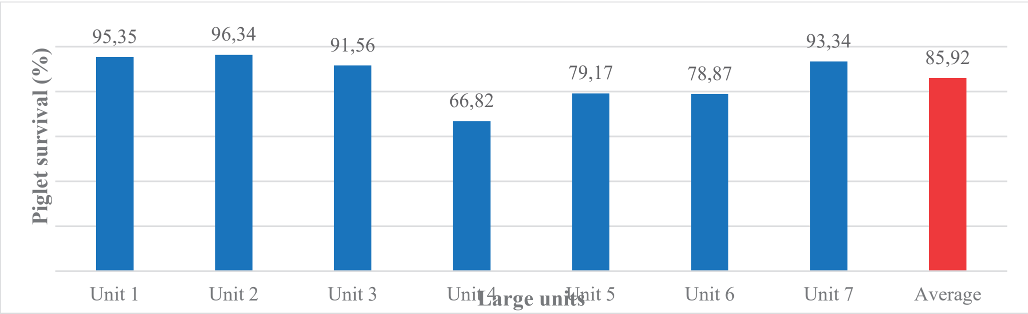

Figure 5. Overall pre-waning piglet survival in the L units group

However, similar pattern can be noticed in the S-M unit group as well with survivability to weaning of only 66.7% in Unit 1 for the same possible reasons (Figure 6).

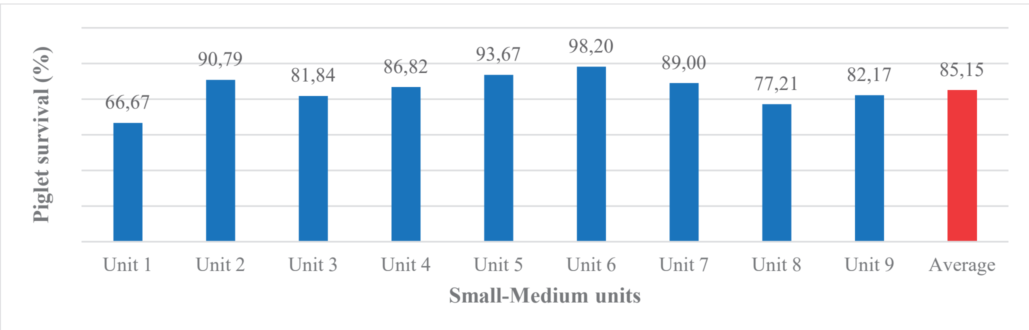

Figure 6. Overall pre-waning piglet survival in the S-M units group

LP survival in the L units is higher than the one in S-M with an average advantage of about 3% (Figures 7 and 8). The explanation could be related to the average sow parity profile and age in these units (3.8) in comparison with 4.4 in S-M units. Having younger sows in the sample would mean an advantage from the quantity of milk available to piglets. It should be also highlighted that the duration of lactation in L units was in average 1 day longer and this can be important for the survival of the lighter piglets.

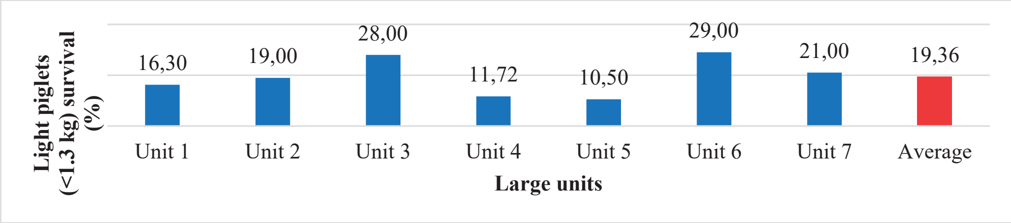

Figure 7. Pre-waning survival of the LP in the L units group

Looking at the variation of the light piglet survival inside the L unit group it can be observed that the highest percentage of weaned animals was in Unit 6 while the lowest in Unit 5 (Figure 7). This might be explained through differences in farrowing house management procedures and /or the effects of sow average parity which is the lowest in Unit 5 (2.2). When it comes to differences in LP survivability in S-M units the variation is more extreme with only 2.7% in Unit 4 up to 30% in Unit 2. This situation can be attributed once again to management and/or to average sow parity which was the highest (5.4) in Unit 4, leading probably to a lower lactating capacity. The same arguments are valid for the best survivability recorded in Unit 2.

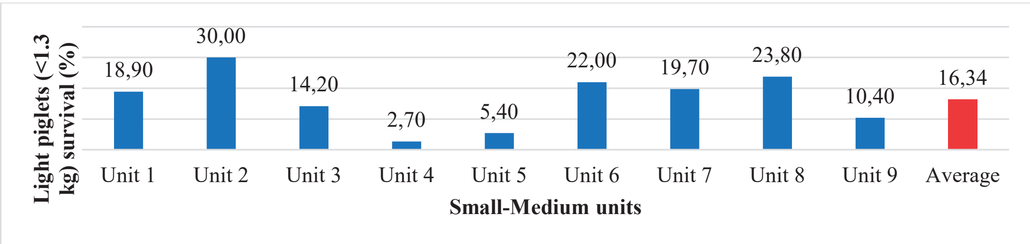

Figure 8. Pre-waning survival of the LP in the S-M units group

Analysing the overall average survival rates of the HP in both L and S-M unit groups shows a difference of only 1.4%. Within the L unit group, the variation is rather limited with the leader being Unit 4, which might be due to the fact that this unit recorded had one of the lowest number of LP at birth (Figure 9).

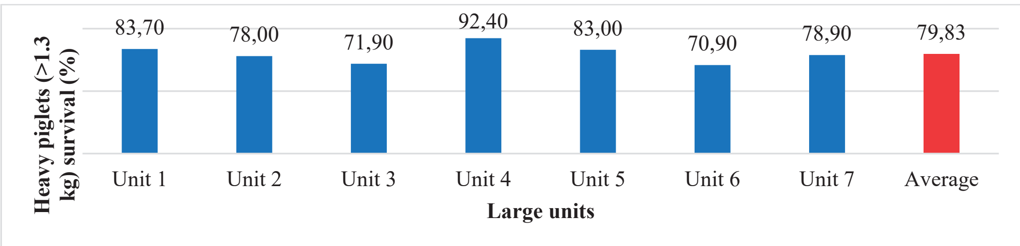

Figure 9. Pre-waning survival of the HP in the L units group

In contrast the variation of survivability of HP in the S-M units is much higher (30%) with a leading unit weaning over 95.5% of the piglets explainable by the low number of light piglets at the time of farrowing. The most unfavorable situation was found in Unit 8, where probably caretakers were not able to apply effective management practices to keep more heavy piglets alive up to weaning (Figure 10).

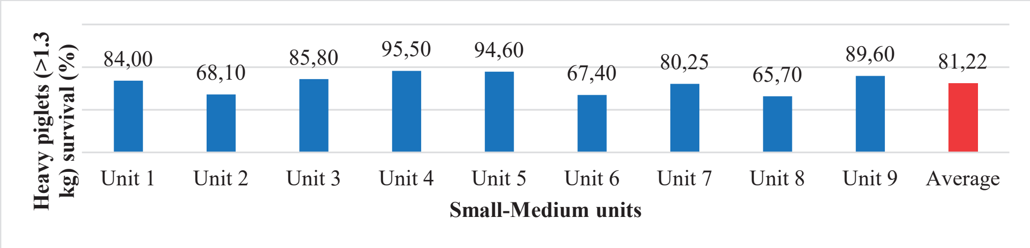

Figure 10. Pre-waning survival of the HP in the S-M units group

In terms of growth performance of the piglets, data seem to suggest that on average results were better in S-M Units than in the L ones. However, the differences are limited (8 g/day). The advantage for the S-M units is 24 g/day in the LP category a value which is more relevant and important for the further ADG in nursery and finishing stages. Also, it might suggest a closer piglet care by the caretakers up to weaning is needed. This difference between unit groups shrinks down to only 6 g/day when HP are considered suggesting that this category of piglets thrive quite the same irrespective of the unit size (Table 4).

Table 4. Summary of growth to weaning data in the sampled units

Item	Small/Medium Farms (158 litters)		Large Farms (172 litters)	
	n	Mean ± SEM	n	Mean ± SEM
ADG to weaning (kg)	1667	0.204 ± 0.016	1776	0.196 ± 0.001
ADG – Light piglets (< 1.3 kg)	298	0.168 ± 0.013	367	0.145 ± 0.005
ADG - Heavy piglets (> 1.3 kg)	1339	0.214 ± 0.017	1403	0.208 ± 0.002

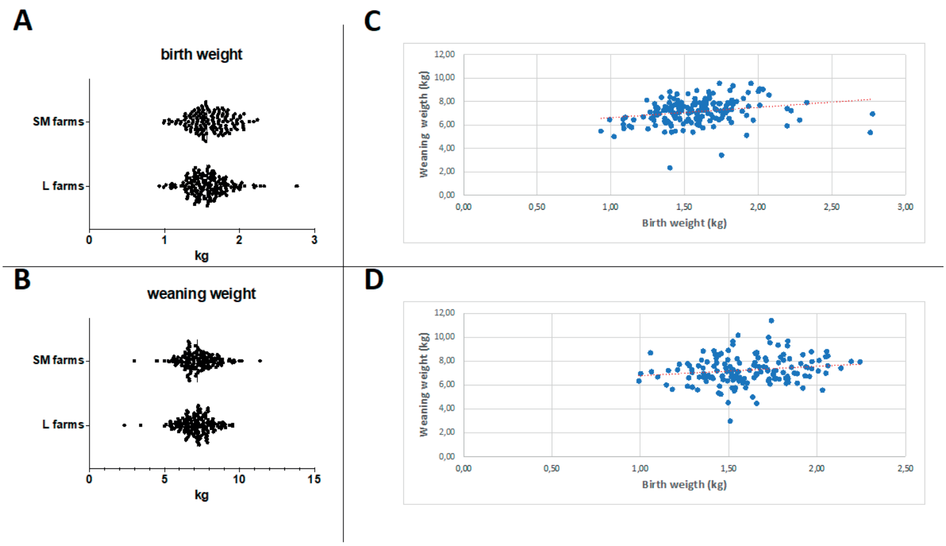

Figure 11. (**A**) Unpaired t-test graph comparing birth weight recorded in the two types of farms; (**B**) unpaired t-test graph comparing weaning weight from the two types of units; (**C**) Pearson's correlation between individual piglet birth weight and weaning weight in Large units; (**D**) Pearson's correlation between individual piglet birth weight and weaning weight in Small-Medium units

The unpaired t-test comparing the results from the two types of farms showed that there were no significant differences neither between the birth weight ($p>0.05$; Figure 11A) nor between the weaning weight ($p>0.05$; Figure 11B). These findings suggest that, despite potential variations in management practices or environmental factors between L farms and SM farms, these factors did not have a substantial impact on the weights of piglets at birth or weaning. Pearson's correlations showed that in L farms birth weight and weaning weight were highly significantly correlated ($p<0.01$; Figure 11C) and in SM farms significantly correlated ($p<0.05$; figure 11D). In L farms, we observed a highly significant positive correlation between birth weight and weaning weight ($p<0.01$). This robust correlation indicates that piglets born heavier tend to have, as well higher weights at weaning in L farms. Conversely, in SM farms, while still significant, the correlation between birth weight and weaning weight was comparatively weaker ($p<0.05$). These findings underscore the importance of considering farm-specific factors when evaluating the relationship between birth weight and weaning weight in livestock. The strong correlation observed in L farms suggests a significant influence of farm-specific factors on piglets growth and development from birth to weaning. In contrast, the weaker correlation observed in SM farms may indicate additional complexities in piglet growth dynamics within this farm type.

CONCLUSIONS

The birth weight of piglets is, undoubtedly influenced by various factors, including genetics, nutrition, litter size, pre-farrowing, management and many others. All these factors might act differently according to the size of the farms for multiple reasons. In the current trial records are suggesting that average litter BW was higher in S-M units mainly due to the extra BA piglets. However, the individual average BW of the piglets was all most the same probably due to the similar genetic makeup both from the sire and dam perspective. Despite this common genetic ground a wide variability among units of the same size was noticed, both in the LW and HW piglets categories suggesting that other influencing factors can play a significant role on the birth weight.

At the time of weaning a clear balance between unit groups was found not only from the overall piglet number perspective but from the one of the average of piglets weaned / litter as well. Therefore, this could suggest that the farm size might not have a large impact on these trait after all. There is however a difference related to the piglet age at weaning suggesting that the litter weaning weight could of been in the favor of the piglets raised in the L farms group. On the contrary records are showing that weaned litters in L farms are on average lighter than the ones in S-M group units. This might indicate that S-M units managed to take a better care of the piglets in the farrowing house, something possible due to lower workload and thus a higher attention to details by the caretakers.

In regard of the growth performance of the piglets from birth to weaning data seem to suggest that results were better in S-M Units than in the L ones. This advantage is more relevant for the LW piglets than in HW ones leading to the conclusion that in S-M units the caretakers allocate more time to the disadvantaged piglets.

Considering the importance of birth weight for piglet survival and growth, it seems essential to implement strategies that optimize birth weight to ensure their further healthy development irrespective of the size unit. Furthermore, our findings highlight that the birth weight is significantly correlated with weaning weigh. While all biological factors influencing the BW of piglets are important, there is a large number of management variables, including as gilt pool control, gestation housing and feeding, prostaglandin induced farrowing and farm hygiene, which all can have their role in boosting piglet weight at birth.

ACKNOWLEDGMENTS

The study was conducted with the full support of the unit owners and managers and with the direct help of the designated caretakers from the farrowing departments. Registered data,

actual unit location, size and personal names were agreed to be confidential.

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GLOBAL QUALITY OF POULTRY MEAT. PECULIARITIES OF POULTRY MEAT

Veronica Denisa LUNGU, Andreea Ionela ZINCA, Andrada Elena MOISE, Dumitru DRĂGOTOIU

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: zinca_andreea20@yahoo.com

Abstract

The quality of poultry meat is largely determined by the evolution of production, breeding practices, and genetic management. Globally, the poultry meat industry has experienced remarkable expansion, continuously adapting to market requirements and sustainability standards. The genotype of the chickens plays an essential role in the quality of the meat, with the advanced selection of breeds aimed at improving the food efficiency and organoleptic qualities of the flesh. Breeding systems range from intensive to extensively organic, each of which has a direct impact on animal health and welfare, as well as on the quality of meat. Chicken feeding, adapted to the specific needs of each genotype, improves the nutritional profile of the meat, optimizing the content of essential fatty acids and micronutrients. Handling chickens, from transport to processing, is crucial for minimizing stress and preventing meat quality defects.

***Key words**: meat quality, poultry meat, quality characteristics, rearing system.*

INTRODUCTION

Chicken is one of the most consumed sources of animal protein worldwide due to its affordable price, nutritional value, and versatility in preparation (Farrell, 2013).

However, the quality of chicken meat is influenced by many factors, such as genotype, breeding system, feeding, and handling of chickens, which can affect the technological and sensory characteristics of the final product. Poultry meat is the fastest-growing agricultural subsector, especially in developing countries. The global poultry sector is expected to continue to grow, as demand for meat and eggs is driven by population growth, rising incomes, and urbanization.

In this context, the poultry sector faces unprecedented challenges (Mottet & Tempio, 2017).

The production and consumption of poultry meat have increased rapidly worldwide and are expected to continue to increase due to its relatively low price compared to other types of meat, the absence of cultural or religious barriers, and its dietary and nutritional properties, as it has a lower level of fat, cholesterol and sodium content, and consumers a greater preference for white meat (Fletcher, 2002).

The US is considered the world's largest producer of poultry meat, it supplies approximately 17% of global poultry meat production, followed by Brazil and China.

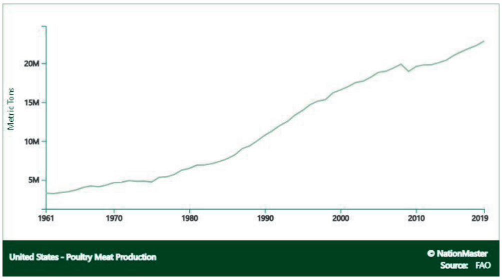

Figure 1. Evolution of poultry meat production internationally (FAO, 1961-2019)

Since 2014, poultry meat production in the United States has increased by 2.3% year-on-year. In 2019, the country was number 1 compared to other countries in poultry meat production at 22.857.595 metric tons. China, Brazil and Russia ranked second, third and fourth in this ranking, respectively. Tajikistan recorded the best average annual growth, with +61.1% per year, while Saint Kitts and Nevis recorded its worst performance at -100% per year.

In 2019, Romania ranked 38th among poultry meat producers in the European Union. Despite the current conditions, our country continues to be a major exporter of poultry meat, by 2020 exporting 42% of the production of fresh and refrigerated unskinned poultry meat.

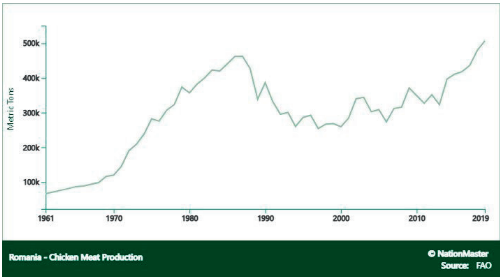

Figure 2. Evolution of chicken meat production in Romania (FAO, 1961-2019)

Since 2014 chicken meat production in Romania has increased by 5% yearly. With 507.106 metric tons in 2019, the country was ranked 38th among other countries in chicken meat production.

Romania is surpassed by Israel, which ranked 37th with 517.106 metric tons, and is followed by Bolivia with 491.098 metric tons. In 2021, around 132.3 million tons of poultry meat were consumed worldwide, making it the most consumed type of meat globally.

Pork was the second most consumed meat in the world, followed by beef and veal (Shahbandeh, 2023). Processed pork also had the highest contamination rates 50% higher than poultry and raw pork.

According to the study by Suler et al., factors contributing to *Salmonella* contamination in meat products included inadequate transport conditions, animal stress during slaughter, poor compliance with hygiene practices, and failures in processing and packaging stages. The predominant types of Salmonella varied with predilection in poultry organs (*S. enteritidis* and *S. enterica*) and raw/processed pork (*S. saintpaul, S. typhimurium, S. tennessee*) (Suler et al., 2019).

MATERIALS AND METHODS

The paper aims to analyze the global quality of poultry meat based on published scientific studies. Articles published in various scientific databases were used for documentation: Elsevier, MDPI, PubMed and Research Gate. This paper, wanted to demonstrate that poultry quality is significantly influenced by advances in production, breeding practices and genetic management.

RESULTS AND DISCUSSIONS

Genotype of chickens

The genotype of chickens is the result of genetic selection, aimed at increasing the productive and economic performance of meat chicks. Thus, hybrid breeds such as Ross 308 or Cobb 500 have been developed, which are characterized by rapid growth, good feed conversion, high body yield, and low-fat content (Hartcher et al., 2020).

However, genetic selection has also had negative effects on the quality of meat, such as reduced water retention, drop loss, and cooking, pale, soft, exudative (PSE) appearance or dark, firm, dry (DFD) (Duclos et al., 2007; Barbut, 2009).

These defects are caused by biochemical and histological changes in muscle tissue, which occur during post-mortem, under the influence of metabolic and oxidative stress to which rapidly growing chickens are subjected (Zhao et al., 2012).

It is therefore necessary to find a balance between productivity and quality, by using breeds adapted to growing conditions and by applying stress reduction measures to chickens (Berri et al., 2001; Bianchi et al., 2006).

A study by Tudorache et al. (2022) highlighted a significant effect of the genotype on growth performance, carcass features, and blood metabolites in slow-growing chickens.

Genotype significantly influences performance parameters such as body weight gain, feed intake, energy intake, feed conversion rate, energy conversion ratio, and production efficiency factor (Tudorache et al., 2022).

Another study by Fanatico et al. (2005) compared the quality of chicken meat from three different genotypes: a rapid commercial hybrid, a slow commercial hybrid, and an inherited breed (Barred Plymouth Rock).

The study found that the genotype had a significant effect on the pH, water retention, color, texture, and lipid content of chicken meat.

The commercial fast-hybrid chicken meat had the lowest pH, the highest cooking loss, the smallest cutting force, and the darkest color, while chicken from the inherited breed had the higher pH, least cooking losses, the largest cutting strength, and the lightest color. Chicken from the slow commercial hybrid had a higher lipid content than the other two genotypes (Fanatico et al., 2005).

Chicken breeding systems

Chicken breeding systems significantly influence the quality of poultry meat, with approaches ranging from intensive systems to extensively ecological ones, each with a direct impact on the final quality of meat. Studies show that non-intensive and organic systems tend to produce meat with superior chemical composition and improved organoleptic characteristics, such as better water retention and better texture, compared to intensive systems, which can promote rapid growth but can adversely affect the quality of meat, through problems such as pale, soft and exudative meat (PSE) and various muscle myopathies (Bošković et al., 2012; Baéza et al. 2021).

The breeding system refers to how chickens are raised and cared for, depending on the space, shelter, light, heat, ventilation, hygiene, and protection they benefit from. The breeding system can be intensive, semi-intensive, or extensive, depending on the degree of freedom and contact with the natural environment of the chickens (Berri et al., 2005).

The intensive system involves breeding chickens in closed, high-density spaces, artificial lighting, controlled temperature, mechanized feeding, and strict prophylaxis.

This system has the advantage of large and fast production, but the disadvantage of low-quality meat due to stress, lack of movement, and exposure to diseases and parasites (Gržinić et al., 2023).

A study conducted by Suler et al. (2021) highlighted species of Listeria that were isolated from various food samples, such as raw pork, raw beef, poultry and bird organs, and processed pork with different contamination rates.

The semi-intensive system involves breeding chickens in open spaces with medium density, natural lighting, variable temperature, mixed feeding, and moderate prophylaxis.

This system has the advantage of balanced production and better quality of meat, due to movement, food diversity, and adaptation to the natural environment (Aksoy et al., 2021).

The extensive system involves breeding chickens in large spaces with low density, natural lighting, variable temperature, natural feeding, and minimal prophylaxis.

This system has the advantage of a higher quality of meat, due to the freedom, food variety, and natural immunity, but the disadvantage of a small and slow production (Bosco et al, 2021).

A study by Castellini et al. (2022) investigated the effect of production technology on the quality of chicken meat.

They compared chicken meat from two different production systems: a conventional system based on intensive growing in narrow spaces and with a high level of stress, and an alternative system, based on extensive growing in open spaces with low levels of stress.

Meat quality parameters such as pH, water retention, color, texture, lipid content, fatty acid composition, and oxidative stress level of chicken were measured.

The results showed that chicken from the alternative system had a higher pH, greater water retention capacity, a lighter color, a softer texture, a lower lipid content, a higher ratio of unsaturated and saturated fatty acids, and a lower level of oxidative stress than chicken coming from the conventional system (Castellini et al., 2002).

Feeding of chickens

The feeding of chickens is an essential factor for their growth and development, but also for the quality of poultry meat (Kralik et al., 2018).

In feeding meat chickens, nutritional strategies aim to optimize their growth and health, taking into account their high requirements for proteins and amino acids. An essential component in the nutrition of chickens is protein, necessary for tissue growth and for the biological functions of the body. In addition, enzymes and hormones, which are essential for the physiology of any living organism, are largely protein compounds (Beski et al., 2015).

The feeding of chickens can be based on concentrated feed, green feed, or natural feed, depending on their source and composition (Alnahhas et al., 2016).

Concentrated feed is the most used in the intensive growing system, as it provides a high energy intake, protein, minerals, and vitamins, which favors rapid growth and meat yield (Bianchi et al., 2005).
Meat chicken feed is often enriched with specialized protein products, both animal and vegetable origin, to support rapid growth and improve growth performance. Also, feeds are formulated to minimize the concentration of anti-nutritional factors and to include immunologically active compounds, to promote the health of the gastrointestinal tract. This is crucial for the early development of the gut and digestive physiology of the chicks, thereby contributing to improving their growth performance and immunity (Beski et al., 2015).
However, concentrated feed can also contain undesirable substances, such as antibiotics, hormones, additives, or pesticides, which can affect the quality and safety of meat.
This paper examines the effect of additives in the feeding of chickens, such as egg yolk, as an alternative to antibiotics. The study indicates that natural additives can improve the quality of meat and reduce the use of drugs that can leave chemical residues (Hussein et al., 2020).
In conclusion, this study provides important data on the use of egg yolk as an additive in poultry feeding, to limit the usage of medicines and improve the quality of meat.
Another study by Khajali & Wideman highlighted that nutritional strategies need to be well balanced to avoid health problems such as pulmonary hypertension, especially at high altitudes where hypoxia can affect the performance of offspring. Low-protein diets are sometimes recommended to reduce oxygen intake and the risk of ascites, but should be managed carefully to avoid compromising the intake of essential amino acids such as arginine.
Another study by Gheorghe et al. (2022) highlighted the importance of dietary manipulation in improving the quality of meat by enriching the diets of animals with bioactive compounds such as PUFA, especially n-3 PUFA which have demonstrated health benefits in alleviating cardiovascular diseases, autoimmune diseases, and certain types of cancer.
Overall, the study recommended the use of ELP30 (a mixture of 30 percent flaxseeds and peas) in broiler diets to improve the nutritional value of meat for consumers, highlighting the positive impact of PUFA-enriched dietary mixtures and probiotics on broiler health and meat quality (Gheorghe et al., 2022).
Green feed is the most used in the semi-intensive breeding system, as it provides a moderate intake of energy, proteins, minerals, and vitamins, which favors balanced growth and meat quality. Green feed can be represented by grass, alfalfa, spinach, and salad which can be served fresh or dried.
Another green feed used in the feeding of meat chickens is represented by sorghum or sorghum-meat, which can be used as partial substitutes for corn and soy flour in the diets of broiler chicks without adversely affecting the chemical composition of the muscle tissue of the broiler or essential amino acids and the concentrations of aromatically related amino acid.
A study conducted by Gheorghe et al. (2021) found that total amino acids, essential amino acids, non-essential amino acids, and the ratio between essential and non-essential amino acids increased in the breast muscle compared to the thigh muscle, indicating differences in the amino-acid deposition between the two types of muscles.
Also, interactions between diets and muscle tissue for specific amino acids were observed, highlighting the influence of the diet on the amino acid composition of the broiler's muscular tissue (Gheorghe et al., 2021).
Natural feed is the most used in the extensive breeding system, as it provides varied energy, protein, minerals, and vitamin intake, which favors slow growth and superior quality of meat (Tufarelli et al., 2018).
Natural feed can be represented by seeds, fruits, vegetables, insects, worms, and slugs that can be found in the natural environment.
The study carried out by Custură et al. (2024) demonstrated that feeding the Barred Plymouth Rock chickens with a low-energy diet, with specific concentrations of nutrients, did not hurt body weight, weight gain, carcass characteristics, or the quality of proteins in the meat. The findings suggest that slow-growing genotypes, Barred Plymouth Rock, can maintain growth performance and meat quality traits even when fed with low-protein and low-energy diets in an organic system.

Another study by Zhang et al. (2010) evaluated the effect of a linoleic acid conjugate feeding treatment (CLA) on the quality of chicken meat. It was given chickens a supplement of CLA (0, 0.5, 1, or 2%) in their diet for 42 days and measured the quality parameters of the meat, such as pH, water retention, color, texture, lipid content, and fatty acid composition.
The results of the addition of CLA in chicken feeding significantly reduced the pH, cooking loss, cutting force, and intense yellow color of the chicken meat, but increased the water retention capacity, the redness index, and lipid content of chicken.
Also, CLA treatment changed the fatty acid composition of chicken meat, reducing the ratio of unsaturated and saturated fatty acids (Zhang et al., 2010).

Manipulation of chickens
Chicken handling refers to how chickens are treated and transported, throughout their breeding period up to the time before slaughter.
The handling of chickens has a direct impact on the quality of the meat, as it can induce stress, injury, bleeding, or even death thereby altering the technological and sensory characteristics of the finished product (Delezie et al., 2007).
The handling of chickens should be as gentle, calm, and quick as possible, to minimize the negative effects on the quality of the meat. Catching chicks should be done manually, with hands, by grabbing the chest and wings, and avoiding shaking, throwing, or hitting them (Jones, 1992).
Chickens must be loaded in suitable cages or containers with sufficient space, adequate ventilation in summer, and a suitable temperature in winter. Chickens must be transported in adapted vehicles with suspension, air conditioning, and monitoring systems. The landing of chickens must be done with caution, avoiding falling, overturning, or hitting cages which usually lead to chicken injury (Nowak & Połtowicz, 2009).
Nishimura et al. analyzed the effect of pre-sacrifice transport and handling on the quality of chicken meat. The chickens were subjected to different transport conditions (duration, temperature, density) and handling conditions (catch method, waiting time, slaughter method), and the meat quality parameters such as pH, water retention, color, texture, lipid content, and stress level of the chicken meat. The results showed that the transportation and pre-sacrifice handling harmed the quality of chicken meat, causing a decrease in pH, an increase in cooking loss, a closure of color, a hardening of texture, a rise in lipid content, and an increased level of stress in the chickens.
Another study by Cockram et al. brought important conclusions about improving the well-being of meat chickens by modifying a mechanized discharge system. The study compared two versions of a swivel discharge system for poultry transport containers, using qualitative and quantitative techniques, including closed-circuit video recording.
The waiting and so-called fasting of the chickens must be done in shaded, cool, and airy spaces, without noise, light, or strong odors together with the withdrawal of food and water to reduce pollution of carcasses and to ensure healthy meat hygiene (Xue et al., 2021).
The sacrifice of the chickens must be done as quickly and humanly as possible, by electrical or mechanical insensitization, followed by neck cutting and the achievement of complete bleeding (Salwani et al., 2015).
Electric shooting in the water bath is still the most commonly used method for chickens regardless of the breeding system used, but controlled atmospheric shooting methods are becoming more and more common, especially at larger slaughterhouses (McNeal et al., 2003).
At the same time, it must be borne in mind that in all methods of astonishment to ensure a reliable and profound process quality the brain mechanisms associated with the induction and maintenance of unconsciousness are carried out appropriately, to avoid unnecessary suffering of the chicken (Berg & Raj, 2015).

CONCLUSIONS

The quality of chicken meat is determined by several factors, which can be controlled or modified by various interventions at the level of genotype, chicken feeding, production technology, transport, and pre-sacrifice handling. These interventions can have positive or negative effects on the sensory, nutritional, technological, and microbiological quality of chicken meat which may also vary depending on the preferences and expectations of consumers. Therefore, it is important to know and monitor these factors, to ensure the optimal quality of chicken meat.

The study highlighted that the alternative extensive breeding system for chicken production resulted in higher meat quality compared to conventional intensive systems, with better pH levels, water retention, color, texture, and lipid content.

Growing conditions also have a significant impact on the quality of chicken meat. The intensive breeding system favors the rapid growth and yield of meat, but can adversely affect the organoleptic and physico-chemical quality of the meat. This is because chickens raised in the intensive system are subjected to metabolic and oxidative stress, which causes biochemical and histological changes in muscle tissue.

The semi-intensive and extensive breeding system favors the balanced growth and superior quality of chicken meat. This is because chickens raised in these systems have more room for movement, more diverse feeding, and better adaptation to the natural environment.

The genotype also plays a significant role in determining growth performance, carcass characteristics, and blood metabolites in slow-growing chickens, highlighting the importance of selecting the right genotypes for desired meat quality results.

Using natural feed in extensive breeding systems can provide a wide range of nutrients, promoting slow growth and superior meat quality in poultry. This approach ensures that chickens maintain growth performance and meat quality even when fed with low-protein and low-energy diets in an organic system.

In conclusion, the research highlights the importance of balancing productivity and quality in poultry production by selecting suitable breeds, implementing stress reduction measures, and optimizing dietary strategies to improve meat quality and overall performance.

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NUTRITIONAL QUALITY OF POULTRY MEAT ACCORDING TO BREEDING SYSTEMS

Veronica Denisa LUNGU, Andreea Ionela ZINCA, Andrada Elena MOISE, Dumitru DRĂGOTOIU

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: zinca_andreea20@yahoo.com

Abstract

The nutritional quality of poultry meat varies depending on the breeding systems used, influencing its composition in proteins, fats, vitamins, and minerals. Poultry meat is recognized as an affordable source of high-quality protein and other essential nutrients. Intensive, semi-intensive and extensive growth systems cause significant differences in nutrient composition. In intensive systems, meat chickens grow fast, having a higher fat content and a lipid profile dominated by saturated fatty acids. Extensive and semi-intensive systems, including outdoor access and diversified feeding, result in a healthier lipid composition with a higher polyunsaturated fatty acids, especially omega-3. In addition, poultry meat from extensive systems has a higher content of vitamins, such as vitamin E and minerals such as selenium, due to the more natural and diversified diet. Attributes such as low fat content and increased concentration of polyunsaturated fatty acids and antioxidants make the resulting products considered healthier and more ethical. In conclusion, the diversity of breeding systems offers opportunities to improve the nutritional quality of poultry meat, aligning it with consumer preferences and trends for healthy and sustainable products.

Key words*: breeding systems, fats, nutritional quality, poultry meat.*

INTRODUCTION

The quality of poultry meat is increasingly recognized as a crucial factor in consumer satisfaction and worldwide marketing. Key attributes that influence the quality of poultry meat include appearance, texture, nutritional value, and safety, each of which plays an essential role in consumer preferences and industry standards.

The appearance of poultry, characterized by the color of the skin and meat, is fundamental for consumer selection and satisfaction, affecting the presentation of meat both raw and cooked. Texture, mainly influenced by factors such as breed and processing techniques, remains a critical sensory attribute that defines the quality of consumption, with an emphasis on trend and succulence. Nutritional quality is another significant aspect, poultry meat provides essential proteins, vitamins, and minerals, beneficial to all types of consumers, regardless of age (Mir et al., 2017).

Safety and ethical production practices have become increasingly important in evaluating the quality of poultry meat, addressing issues such as animal welfare, environmental impact and health risks, such as antimicrobial resistance and disease transmission. The continuing challenges and advances in the field of breeding, nutrition, and processing aim to improve these quality attributes, aligning with global trends towards healthier food choices and ethical products (Iannetti et al., 2021).

Conventional poultry breeding systems are characterized by breeding chickens in enclosed spaces with limited access to natural factors such as sunlight and open space. These systems are more economically efficient but can lead to animal welfare problems and lower meat quality (Küçükyılmaz et al., 2012).

Alternative systems are characterized by the breeding of chickens in open spaces with unlimited access to natural factors. These systems are less economically efficient but give birds more space and a more varied diet, which can lead to better meat quality (Yan, 2017).

The outdoor breeding system is an alternative production strategy that can attract buyers from special markets for high-quality meat demand and is also a low-cost production system in suitable facilities for small-scale individual breeding in developing countries (Li et al., 2017).

MATERIALS AND METHODS

This article aims to determine the variability of the nutritional composition between lots of broiler chickens in different technological breeding systems, comparing the lipid profile of chicken meat and the protein content.
For the phenotypic characterization of lots, classical statistical methods (Snedecor & Cochran, 1989) such as: average, variation, standard deviation, average error and variability coefficient were used. For the study of the homogeneity of the mediums, respectively for testing as statistically significant of the differences observed between the batches, the Student test was used (Fisher, 1992).

RESULTS AND DISCUSSIONS

Protein content of poultry meat

The intensive system has an average higher protein content in the chest (22.95 g vs. 22.78 g) compared with the extensive system (Table 1). The variability is relatively low between the two systems, indicating that the breeding methods slightly influence the protein composition of the meat, so the intensive breeding system has somewhat higher total protein content in the chest compared to the extensive system.

Table 1. The protein content of chest muscle tissue according to the growth systems of the chicks

Specification		U.M.	Intensive Growth Systems		Extensive Growth Systems	
			X	Sx	X	Sx
Breast protein	1	g	21.30	0.3862	21.76	0.1774
	2	g	23.03	0.3005	22.45	0.3031
	3	g	23.51	0.3966	23.59	0.6034
	4	g	23.67	0.6093	24.05	0.6245
	5	g	23.15	0.6631	22.66	0.3245
	6	g	22.45	0.2179	22.04	0.1279
	7	g	22.92	0.7688	22.86	0.7203
	8	g	23.66	0.2614	22.72	0.2597
	9	g	22.84	0.1244	22.88	0.3616
	Total	**g**	**22.95**	**0.4143**	**22.78**	**0.3892**

The variability within each system is low, and the differences between the systems are small and not statistically significant.

Table 2. Protein content of pulp muscle tissue according to chick growth systems

Specification		U.M.	Intensive Growth Systems		Extensive Growth Systems	
			X	Sx	X	Sx
Pulp Protein	1	g	19.10	0.5988	19.79	0.5443
	2	g	20.08	0.4550	18.67	0.2331
	3	g	20.11	0.7420	19.92	0.5029
	4	g	19.42	0.3553	20.63	0.8642
	5	g	18.85	0.2524	18.02	0.2222
	6	g	19.33	0.1429	19.41	0.8716
	7	g	19.54	0.6513	19.98	1.4158
	8	g	19.97	1.6196	19.35	0.6564
	9	g	20.81	0.8030	20.07	0.4605
	Total	**g**	**19.69**	**0.6245**	**19.54**	**0.6412**

The intensive system has an average higher protein content in the pulp (19.69 g vs. 19.54 g) compared with the extensive system (Table 2).
The differences between the intensive and extensive system averages for protein content in pulp are not statistically significant ($p>0.05$).
The protein content of poultry meat is similar in both breeding systems. However, poultry meat grown in alternative systems may have a higher biological value, which means that the human body can use the proteins in this meat more effectively.
The existence of two other specialized studies showed that poultry meat grown in organic farms had a higher content of proteins and essential amino acids, as well as a higher biological value. This means that the human body can more effectively use the proteins in poultry meat grown in organic farms (Ionescu et al., 2022; Yuan et al., 2022).
A study conducted by Jeni et al. (2021) showed that poultry meat grown in alternative systems may have a higher content of protein, essential amino acids and vitamins. However, there were no significant differences between the two growing systems in terms of mineral content.
The study carried out by Custură et al. (2024) demonstrated that feeding the Barred Plymouth Rock chickens with a low-energy diet, with specific concentrations of nutrients, did not have a negative impact on body weight, weight gain, carcass characteristics or the quality of proteins in the meat.
The findings suggest that slow-growing genotypes, Barred Plymouth Rock, can maintain growth performance and meat quality traits even when fed with low-protein and low-energy diets in an organic system (Custură et al., 2024).

Fat content

The extensive system has an average higher content of breast fat (1.41 g vs. 1.24 g) compared with intensive system (Table 3).

The variability is slightly higher in the extensive system, indicating a wider distribution of the fat content.

Table 3. The fat content of chest muscle tissue according to the growth systems of the chicks

Specification		U.M.	Intensive Growth Systems		Extensive Growth Systems	
			X	Sx	X	Sx
Breast Fat	1	g	1.16	0.1926	1.25	0.3585
	2	g	1.71	0.4761	1.33	0.4453
	3	g	1.83	0.4112	1.97	0.5602
	4	g	1.14	0.6142	0.73	0.2552
	5	g	0.49	0.2091	0.66	0.1579
	6	g	0.96	0.5611	1.93	0.4227
	7	g	1.01	0.3398	1.18	0.5152
	8	g	1.75	0.1089	2.29	0.3369
	9	g	1.11	0.4299	1.30	0.4042
	Total	**g**	**1.24**	**0.3714**	**1.41**	**0.3840**

The extensive growth system has a slightly higher total protein content in the chest than the intensive system, but the difference is not statistically significant.

Standard error values show that the estimated averages are relatively accurate, but the variability is higher in the extensive system.

The differences between the intensive and extensive system averages for fat content in pulp are not statistically significant ($p > 0.05$).

The extensive growth system has a slightly higher total fat content in the pulp compared to the intensive system (6.36 g vs. 6.03 g), but the difference is not statistically significant.

The variability is greater in the extensive system (1.4330 g) indicating a wider distribution of the fat content (Table 4).

A study by Socaciu et al. (2023) demonstrate that the fat content of poultry meat is lower in alternative systems than in conventional systems. This is because chickens raised in alternative systems have more physical activity, which leads to a reduction in fat storage.

Fortomaris et al. (2007) compares the impact of different breeding systems on the performance and well-being of chickens. The results showed that broiler chickens raised in alternative systems, such as the free-range system, have betters performance and well-being than conventional systems.

Table 4. The fat content of the muscle mass according to the growth systems of the chicks

Specification		U.M.	Intensive Growth Systems		Extensive Growth Systems	
			X	Sx	X	Sx
Fat pulp	1	g	5.95	1.2246	6.05	1.0396
	2	g	6.01	0.9147	6.37	0.7981
	3	g	6.61	0.6982	7.93	1.2892
	4	g	6.90	1.3987	5.09	1.8624
	5	g	6.77	0.6332	9.22	1.5524
	6	g	5.05	0.9000	5.08	0.4982
	7	g	5.57	0.4919	5.12	2.9781
	8	g	7.76	2.1487	7.18	0.9783
	9	g	3.59	2.2931	5.19	1.9005
	Total	**g**	**6.03**	**1.1892**	**6.36**	**1.4330**

The study results showed that broiler chickens bred in the extensive system performed better than those raised in the conventional system. They had higher body weight at slaughter, better food conversion and lower mortality (Fortomaris et al., 2007).

Aguiar et al. found that broilers raised in a conventional system had the highest lipid content, but lower proportions of polyunsaturated fatty acids and omega-3s compared to free-breeding and alternative chickens.

Free-breeding broilers had lower cholesterol content and a lower pH value compared to chickens raised in a conventional system (Jeni et al., 2021).

Another study by Gheorghe et al. highlighted the importance of dietary manipulation in improving the quality of meat by enriching the diets of animals with bioactive compounds such as PUFA, especially n-3 PUFA which have demonstrated health benefits in alleviating cardiovascular diseases, autoimmune diseases, and certain types of cancer.

Overall, the study recommended the use of ELP30 (a mixture of 30 percent flaxseeds and peas) in broiler diets to improve the nutritional value of meat for consumers, highlighting the positive impact of PUFA-enriched dietary mixtures and probiotics on broiler health and meat quality (Gheorghe et al., 2022).

According to Pandurević et al. (2014) as regards consumer perception, they have shown that consumers consider free-breeding chicken meat to be healthier and tastier than poultry raised in intensive production systems, which makes their overall perception of outdoor production systems positive.

CONCLUSIONS

The study showed that different breeding systems significantly influence the nutritional quality of poultry meat.

Broiler meat grown in alternative systems, such as the free-range and extensive system, had a higher protein content, essential amino acids, and vitamins than conventional systems.

Broiler chickens raised in intensive systems show a higher fat content, with a higher proportion of saturated fatty acids than alternative systems. Instead, chickens raised in alternative systems have a healthier lipid profile with a higher concentration of polyunsaturated fatty acids and omega-3s, thereby reducing the risk of cardiovascular disease.

Studies have shown that alternative breeding systems, such as free-range, offer better chicken welfare and superior performance in terms of body weight, food conversion, and mortality, compared to conventional systems. Consumers also consider chickens bred in alternative systems to be healthier and tastier than those raised in intensive systems, with the overall perception of meat from outdoor production systems being positive.

The diversity of breeding systems offers opportunities to improve the nutritional quality of poultry meat, each system having own advantages and disadvantages.

The choice of the appropriate breeding system depends on the preferences of consumers and the producer's objectives in providing healthy and quality poultry meat.

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THE INFLUENCE OF THE AGE OF SHEEP FOR REPRODUCTION FROM THE GREY KARAKUL BREED (RAMS OF DIFFERENT AGES X 5-YEAR-OLD EWES) ON THE BIRTH WEIGHT AND THE QUALITY OF THE LAMBS SKINS

Iuliu Gabriel MALOȘ, Daniela IANIȚCHI

University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Mărăşti Blvd, District 1, 011464, Bucharest, Romania

Corresponding author mail: dianitchi@yahoo.com

Abstract

This study is focused on the impact of parental age (rams of various ages mated with 5-year-old ewes) on the weight and quality of skins in Karakul lambs - Grey variety. The main objective was to analyze the effects of parental age on the weight and quality of lambs skins. Dates used come from the S.C.D.C.O.C. Popăuţi records, Botoșani County, Romania, and their analysis was conducted at the USAMV of Bucharest. Research methods included weighting of the lambs immediately after their birth and evaluating the quality characteristics of the curl and other relevant traits in the first two days of their life. The results proved a variety of birth weights ranging from 3.18 to 5.05 kg, as well as a performance classified in the Record and Elite zootechnical class. The study's conclusions highlight that parental age plays a significant role in giving the weight and quality of lamb skins at birth.

Key words*: weight, curl, size, sheen, shade.*

INTRODUCTION

Starting from the initial hypothesis regarding the influence of parental age (rams of different ages mated with 5-year-old ewes) on birth weight and lamb skin quality in Karakul lambs - Grey variety, we undertook this study with the aim of contributing to the understanding the phenomenon in the current scientific context.

In the field of zootechnical research, parental age represents a determining factor that influences reproductive performance and phenotypic qualities of offspring in various animal species, including Karakul sheep. Previous studies have revealed a significant correlation between parental age, birth weight of lambs, and skin characteristics, indicating that lambs born to older parents tend to have a higher birth weight, reflecting a more advanced intrauterine development. Regarding skin quality, observations indicate that lambs born from younger parents usually have a finer and more uniform skin texture, while those ones born from older parents may exhibit certain skin imperfections, such as roughness or/and unevenness. It is essential to emphasize that these trends are likely to be influenced by a wide range of factors, including genetics, environmental conditions, and breeding practices. The research in 2023 highlighted that the birth weight of lambs exhibits a phenotypic correlation directly proportional to the age of the ewes at lambing, body dimensions, skin thickness, fiber length, skin surface area, loop size, and constitution, while the relationship with ewe prolificacy is inversely proportional (Buzu, 2023).

The newborn lamb shows the same phenotypic association between body dimensions and body weight, including skin thickness, fiber length, skin surface area; while constitution is directly proportional to body weight, body dimensions, skin thickness, and skin reserve and inversely proportional to skin density.

Some studies conducted on sheep during the year 2022 highlighted that maternal age had a significant influence only on hair length and skin thickness (Rajabov et al., 2022).

The same author argues that loop development increased with maternal age, while loop score and hair quality deteriorated with advancing maternal age, leading him to support the importance of selection processes in improving the Karakul breed by analyzing the characteristics of lamb skin texture, loop quality, and wool fiber thickness, considered essential indicators for this breed. Lambs with different loop types exhibited variations and diversity in loop dimensions, including length and width. Observations revealed that lambs with medium-length loops exhibit significant hereditary inheritance, showing respective percentages of 27.6 ± 5.59% for flat type, 34.2 ± 5.64% for semicircular type, and 24.4 ± 6.84% for ribbed type.

Following statistical analysis regarding the matching of breeding pairs based on age, it was found that the most efficient breeding combination for the Karakul sheep breed, black variety, is obtained by crossing 3-year-old rams with 6-year-old females (Maloș et al., 2005).

This mating configuration is characterized by the following technical indicators: average birth weight of 3.25±3.94 kg, loop type with an average of 90.32±3.94 points, loop size with an average of 18.16±1.38 points, fiber quality with an average of 43.16±3.16 points, fiber luster with an average of 58.00±2.02 points, color hue with an average of 88.16±2.94 points, and own performance with an average of 515.16±19.11 points.

Some authors argue that maternal age appears to be the most significant known environmental effect influencing Karakul skin traits. This effect was more pronounced in certain types than in others, complicating the possibility of adjusting for the maternal age effect. In almost all cases, increasing maternal age led to a deterioration of skin traits (Albertyn & Schoeman, 1993).

Therefore, they recommend not keeping ewes for too long in a flock. These should be eliminated after approximately six years of age.

Other researchers have observed that maternal age and lamb sex significantly influence skin thickness, while birth month does not appear to have a significant impact. Conversely, other scholars contend that no significant differences in skin thickness have been identified among various age groups of ewes (Greeff et al., 1991; Van Niekerk, 1972, as cited in Albertyn & Schoeman, 1993).

MATERIALS AND METHODS

The study was conducted between 2022 and 2023 at the University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Animal Production Engineering and Management, based on data collected from registers completed within the S.C.D.C.O.C. Popauţi unit, regarding the Karakul sheep population of Botoșani, Grey color variety.

The biological material included in the study consisted of 525 sheep, structured as follows: 486 ewes and 39 rams. To ensure the repeatability of the results, the study was conducted under the same conditions over a period of seven years (notated from I to VII), following these steps:

I. All mating pairs were selected based on Grey color and age groups from the mating and lambing register, with rams of different ages paired with 5-year-old ewes, forming the following combinations: 2x5, 3x5, 4x5, 5x5, and 6x5 for the seven years of study.

II. The following determinations were made for the mentioned mating combinations:

Determination of birth weight of offspring – by weighing lambs immediately after birth.

Determination of values (points) for traits related to loop quality – by scoring lambs on the first day after birth, and overall skin traits were evaluated during lamb scoring in the first two days after birth; sex distribution – after lambing, sexes were recorded in registers.

III. The recorded data were statistically processed, calculating: mean, standard deviation, mean error, coefficient of variation, and limits for each trait studied: birth weight, loop type, loop size, fiber quality, fiber luster, color hue, and synthetic index, as well as own performance.

IV. The obtained results were synthesized in tables for a more suggestive interpretation, in comparison with existing bibliographic data.

RESULTS AND DISCUSSIONS

Starting from the initial premise regarding the impact of parental age (rams of different ages mated with 5-year-old ewes) on birth weight and skin quality of Karakul lamb – Grey variety, the following results were obtained:

A. Mating combination between rams of different ages (2, 3, 4, 5, 6, and 7 years) with 5-year-old females - Year I

The offspring resulting from the mating of rams aged 2, 3, 4, 5, and 6 years with 5-year-old ewes (Table 1 presents the following average values for the mating group: 4.48 kg, birth weight - which exceeds the breed standard; loop with a score of 79.82 points - representing the combined type of good glossy value, mirrors; medium loop size with a score below the breed standard, of 18.70 points; fiber quality moderately silky (36.56 points); satisfactory-good luster (56.81 points); good grey color hue, light grey with a score of 101.04 points and own performance of 503.49 points in the "Record" zootechnical class.

Table 1. Influence of the age of sheep for reproduction - rams of different ages x 5-year-old ewes - on birth weight and skin quality of Karakul Grey lambs - Year I

Age	Specification	Characters						
		Birth weight (kg)	Loop type (points)	Loop size (points)	Fiber quality (points)	Fiber gloss (points)	Color shade (points)	Own performance (points)
2x5	$\bar{x} \pm s\bar{x}$	4.47±0.18	82.00±9.61	18.33±6.67	31.6±10.14	56.67±1,67	110.00±15.27	504.33±59.95
	CV%	6.84	20.30	62.98	55.45	5.09	24.05	24.05
3x5	$\bar{x} \pm s\bar{x}$	5.05±0.54	70.00±5.00	12.50±7.50	40.0±10.00	55.00±.20.00	97.50±22.50	447.50±17.50
	CV%	12.60	10,10	84.85	35.36	12.86	32.64	5.53
4x5	$\bar{x} \pm s\bar{x}$	4.36±0.25	87.40±7.05	20.00±3.87	43.00±7.00	59.00±1.00	95.00±8.06	519.40±37.07
	CV%	13.04	18.05	43.30	36.40	3.79	18.98	15.96
5x5	$\bar{x} \pm s\bar{x}$	4.33±0.28	77.00±8.81	21.2±11.76	35.00±9.57	56.25±9.44	96.25±12.48	514.50±29.10
	CV%	13.13	22.88	1.25	54.71	33.55	25.93	11.31
6x5	$\bar{x} \pm s\bar{x}$	4.20±0.17	82.71±7.53	21.43±.2.83	3.14±4.79	57.14±1.84	106.43±9.80	531.71±25.44
	CV%	10.91	24.09	34.91	38.26	8.54	24.37	12.66
Media		4.48	79.82	18.70	36.56	56.81	101.04	503.49

Upon detailed analysis, the following observations can be made:

- Birth weight shows the highest average value among the mating combinations studied at 5.05 kg, with limits of 4.6-5.05 between kg and a coefficient of variation of 12.60% for the 3x5 year mating combination, and the lowest at 4.20 kg, with limits of 3.4-4.8 kg and a coefficient of variation of 10.91%. As observed, birth weight progressively decreases from 5.05 kg in the 3x5 year mating combination to 4.20 kg in the 6x5 year mating combination.
- Loop size presents the best average value of 21.43 points, ranging from medium-small to medium, with limits of 5- 25 points and a coefficient of variation of 34.91% for the 6x5 year mating combination, and the weakest average value of 12.50 points, ranging from small with limits of 5-20 points and a coefficient of variation of 84.85% for the 3x5 year mating combination.
- Loop type appears as a combined type: good glossy value and waves, good glossy value and ribs, etc., with a score of 87.40 points and limits of 60-98 points for the 4x5 year mating combination, while the 3x5 and 5x5 year mating combinations present a combined loop type: long flat tube, medium-long, with a score of 70 points.

Following the mating of 5-year-old ewes with rams aged 2, 3, 4, 5, and 6 years it can be said that the age factor of the ewes led to the attainment of high average values in the 4x5 year mating combination, characterized by: birth weight of 4.36 kg, with limits of 3.7-5.5 kg, superior to the breed standard; loop characterized by a score of 87.40 points, representing a combined type of medium-short tube, medium knob, with limits of 60-98 points; medium-small to medium loop size with an average of 21.43 points and limits of 5-25 points; fiber quality ranging from normal to silky with a score of 43.00 points, and limits of 15-50 points; good fiber luster with a score of 59.00 points, with limits of 55-60 points; color hue of 95.00 points, with limits of 75-120 points, representing a dark grey hue, light grey hue, and other tar-black colors, and own performance classifying the offspring in the "Record" zootechnical class, with limits of 385-605 points and an average value of 519.40 points.

The weakest mating combination resulted from this mating was obtained in the 3x5 year cross, characterized by the following average values: birth weight of 5.05 kg, with limits of 4.6-5.5 kg, exceeding the breed standard; loop of very good glossy wave type, mowing; small loop size, medium-large, size not conforming to the breed standard; normal fiber quality, with a score of 40 points, with limits of 30-50 points; satisfactory-good fiber luster with a score of 55 points and limits of 50-60 points (luster is below the breed standard and undesired by sheep for reproduction); color hue is good, dark grey, with a score of 97.50 points and limits of 75-120 points, and according to the limits, we encounter both dark grey hue and pure grey hue; own performance classifies the offspring in the "Elite" zootechnical class, with a score of 447.50 points and limits of 430-465 points.

B. Mating combination between rams of different ages (2, 3, 4, 5, 6, and 7 years) with 5-year-old females - Year II

The offspring resulted from the mating of rams aged 2, 3, 4, and 5 years with 5-year-old ewes (Table 2) were characterized by the following achieved performances:

- Birth weight: 3.53 kg, a value below the breed standard, with the highest average of 3.93 kg achieved in the 6x5 year mating combination, a value within the breed standard, with limits of 3.5-4.3 kg and a coefficient of variation of 10.27%; the lowest being 3.18 kg in the 4x5 year mating combination, with limits of 2.8-3.9 kg and a coefficient of variation of 14.82%, a value approximately 0.62 kg below the breed standard.
- Loop type: it presents an average value per mating group of 77.05 points, representing a loop type of good glossy wave, small-medium knob, with the best loop in the 3x5 year mating combination, scoring 84.00 points, representing a large knob type, intense glossy wave, with limits of 82-86 points and a coefficient of variation of 3.37%. The weakest loop recorded was in the 6x5 year mating combination, with a loop of short tube, small knob type, with limits of 62-72 points; the limit of 62 points indicates a large knob type loop, undesired on the Karakul lamb skin.
- Loop size: 15.46 points, small to medium-small, with the best loop size being 17.50 points, medium-small to small, with limits of 10-25 points and a coefficient of variation of 60.61%; the weakest loop size recorded was 13.33 points, observed in the 2x5 year mating combination, with limits of 10-20 points and a coefficient of variation of 43.30%.

Table 2. Influence of the age of sheep for reproduction - rams of different ages x 5-year-old ewes - on birth weight and skin quality of Karakul Grey lambs - Year II

Age	Specification	Characters						
		Birth weight (kg)	Loop type (points)	Loop size (points)	Birth weight (kg)	Fiber gloss (points)	Color shade (points)	Birth weight (kg)
2x5	$\bar{x} \pm s\bar{x}$	3.47±0.18	80.00±10.41	13.33±3.33	41.67±8.33	45.67±17.95	95.00±35.00	480.67±88.55
	CV%	8.81	22.53	43.30	34.64	68.07	63.81	31.91
3x5	$\bar{x} \pm s\bar{x}$	3.55±0.75	84.00±2.00	17.50±7.50	40.00±10.00	55.00±5.00	92.50±2.50	506.50±14.50
	CV%	29.88	3.37	60.61	35.36	12.86	3.82	4.05
4x5	$\bar{x} \pm s\bar{x}$	3.18±0.21	76.20±4.95	16.00±3.67	42.00±4.90	59.00±4.58	101.00±12.88	486.80±39.04
	CV%	14.82	14.54	51.35	26.08	17.37	28.52	17.93
6x5	$\bar{x} \pm s\bar{x}$	3.93±0,23	68.00±3,05	15.00±3.05	31.67±10.14	56.67±3.33	88.33±21.28	439.00±27.22
	CV%	10.27	7.78	33.33	55.45	10.19	41.72	10.74

- Fiber quality: Normal-silky, scoring 38.83 points, with the best quality at 42 points observed in the 4x5 year mating combination. All mating combinations scored below 45 points.
- Fiber sheen is a trait sought after by sheep for reproduction due to its economic value to the peltry. The average value across mating combinations is 54.08 points, representing good sheen, with the best sheen observed in the 4x5 year mating combination, scoring 59.00 points, satisfactory-good to good, with limits of 50-75 points and a coefficient of variation of 17.37%. The weakest sheen recorded was 45.67 points, weak-satisfactory to satisfactory, with limits of 10-67 points and a coefficient of variation of 68.07%.
- Color shade: Across mating combinations, it scores an average of 94.21 points, with the highest average of 101.00 points for pure-light grey, pure grey, and the lowest average of 88.33 points for dark grey, with limits of 60-130 points, representing a shade of light grey (60 points) and pure grey (130 points).
- Own performance: "Elite" with the best performance recorded in the 3x5 year mating combination, scoring 506.50 points, with limits of 492-521 points, and a coefficient of variation of 4.05%, while the weakest performance of 439.00 points, "Elite" class, was recorded in the 6x5 year mating combination, with limits of 397-490 points, and a coefficient of variation of 10.74%.
- The best mating combination in this group is between 3-year-old rams and 5-year-old ewes, with a birth weight averaging 3.55 kg; loop score of 84.00 points; loop size medium-small to small with a score of 17.50 points; fiber quality 40 points, normal-silky; fiber sheen satisfactory-good, scoring 55.00; color shade 92.50 points, and "Record" own performance with a score of 506.50 points.
- The weakest mating combination is between 6-year-old rams and 5-year-old ewes, characterized by a birth weight of 3.93 kg, standard for the breed; loop type scored at 68.00 points; loop size small to medium-small, scoring 15 points; fiber quality normal, scoring 31.67 points; fiber sheen satisfactory-good, scoring 56.67 points; color shade scoring 88.33 points, and own performance of 439.00 points, "Elite" class with limits of 397-490 points. Consequently, this combination exhibits individuals with very poor fur quality.

If we make a difference based on fiber quality in the loop, we can say that the best mating combination is between 4-year-old rams and 5-year-old ewes, which means a pure silky fiber with satisfactory-good to good sheen and a very good color shade.

C. Mating combination between Rams of Different Ages (2, 3, 4, 5, 6, and 7 years) with 5-Year-Old Ewes – Year III

The offspring resulted from mating rams aged 2, 3, 4, and 5 years with 5-year-old ewes (Table 3) are characterized by the followings:

- Average birth weight: 3.56 kg, a value below the birth standard, with the highest average recorded in the 3x5 year mating combination, at 3.84 kg, a value within the breed standard, with limits of 2.6-5 kg and a coefficient of variation of 21.87%. The lowest recorded weight was 3.30 kg in the 5x5 year mating combination, with limits of 2.6-4 kg and a coefficient of variation of 30%, a value below the breed standard by approximately 0.24 kg.
- Curl: It relates an average value across mating combinations of 75.75 points, representting a good glossy curl, ribs, or mirrors, with the best curl at 91.00 points, a large bob type, good glossy curl, with limits of 86-96 points and a coefficient of variation of 7.77%. The weakest curl was recorded in the 5x5 year mating combination, with a curl type of glossy-less, ribs, feathers, or only feathers, with limits of 40-71 points. The limit of 40 points indicates a poor curly type, undesirable on Karakul lambskin.
- Curl size across mating combinations is 18.75 points, small to medium-small, with the best curl size at 22.50 points, medium-small to medium, with limits of 20-25 points and a coefficient of variation of 15.71%. The weakest curl size was 15.00 points recorded in the 5x5 year mating combination, with limits of 10-20 points and a coefficient of variation of 30%.
- Fiber quality: It is normal-silky, scoring 38.30 points, with the best quality at 40 points in the 2x5 and 4x5 year mating combinations.
- Fiber luster is a trait closely observed by sheep for reproduction due to its contribution to the economic value of the lambskin. The average value per mating group

is 59.02 points, indicating good luster, with the highest luster recorded in the 4x5-year mating variant, scoring 67.5 points, indicating very good to intense luster, with a range of 60-75 points and a coefficient of variation of 15.71%. Conversely, the lowest luster recorded is 52.50 points, ranging from satisfactory to satisfactory-good, with a range of 30-75 points and a coefficient of variation of 60.61%.

Table 3. Influence of the age of sheep for reproduction - rams of different ages x 5-year-old ewes - on birth weight and skin quality of Karakul Grey lambs - Year III

Age	Specification	Characters						
		Birth weight (kg)	**Loop type (points)**	**Loop size (points)**	**Birth weight (kg)**	**Fiber gloss (points)**	**Color shade (point)**	**Birth weight (kg)**
2x5	$\bar{x} \pm s_{\bar{x}}$	3.65±0.15	78.50±1.50	17.50±7.50	40.00±10.00	57.50±2.50	110.00±20.00	525.50±44.50
	CV%	5.81	2.70	60.61	35.36	4.35	25.71	11.98
3x5	$\bar{x} \pm s_{\bar{x}}$	3.84±0.34	78.00±4.82	20.00±2.89	35.71±3.98	58.57±1.54	102.14±7.44	527.29±22.50
	CV%	21.87	15,15	35.36	27.33	6,45	17,.4	10.45
4x5	$\bar{x} \pm s_{\bar{x}}$	3.45±0.55	91.00±5.00	22.50±2.50	40.00±10.00	67.50±7.50	110.00±20.00	540.00±16.00
	CV%	22.55	7.77	15.71	35.36	15.71	25.71	4.19
5x5	$\bar{x} \pm s_{\bar{x}}$	3.30±0.70	55.50±15.50	15.00±5.00	37.50±12.50	52.5±22.50	77.50±52.50	413.5±112.50
	CV%	30.00	39.50	47.14	47.14	60.61	95.80	38.48

Color shade exhibits an average score per mating group of 99.91 points, with the highest average of 110.00 points observed in the 2x5 and 4x5-year mating variants, representing pure-light grey, pure grey, and the lowest average of 77.50 points, representing dark grey, with ranges from 75-130 points, including a matte and pure grey (130 points).

The average individual performance is classified as "Record," with the highest performance recorded in the 4x5-year mating variant, scoring 540.00 points, ranging from 524-556 points, and a coefficient of variation of 4.19%, while the lowest performance of 413.50 points, classified as "Elite," was recorded in the 5x5-year mating variant, ranging from 301–526 points, with a coefficient of variation of 38.48%.

- The best mating variant in this group is between 4-year-old rams and 5-year-old ewes. Despite having a lower birth weight, this mating exhibits maximum scores in other traits. The average birth weight per mating is 3.45 kg; curl type 91.00 points; curl size medium-small to medium with a score of 22.50 points; fiber quality 40 points, normal-silky; fiber luster very good-intense, scoring 67.5 points; color shade 110.00 points; and individual performance rated as "Record," scoring 540 points.

The weakest mating variant is between 5-year-old rams and 5-year-old ewes. This mating is characterized by a birth weight of 3.30 kg, below the breed standard; curl type scored at 55.50 points; curl size small to medium-large, with a score of 15 points; fiber quality rough-silky, scoring 37.5 points; fiber luster satisfactory to satisfactory-good, scoring 52.5 points; color shade weak, scoring 77.50 points, yet exhibiting a wide range from 25 points (dull) to 130 points (pure grey); and individual performance of 413.50 points, classified as "Elite," ranging from 301-526 points. Consequently, this variant comprises individuals with very poor lambskin quality.

D. The mating variant between rams of different ages (2, 3, 4, 5, 6, and 7 years) with 5-year-old ewes - Year IV

The offspring resulted from mating 4-year-old rams with 5-year-old ewes (Table 4) presented the following average values: birth weight - 3.77 kg, curl type - 75.71 points, curl size - medium to large, with a score of 14.29 points, fiber quality - normal-silky 43.57 points, fiber sheen - good 59.29 points, color hue - 82.86 points, and own performance - 459.57 points, represent the "Record" zootechnical class.

Table 4. Influence of the age of sheep for reproduction - rams of different ages x 5-year-old ewes - on birth weight and skin quality of Karakul Grey lambs - Year IV

Age	Specification	Characters						
		Birth weight (kg)	Loop type (points)	Loop size (points)	Birth weight (kg)	Fiber gloss (points)	Color shade (points)	Birth weight (kg)
4x5	$\bar{x} \pm s\bar{x}$	3.77±0.27	75.71±1.81	14.29±3.35	43.57±7.83	59.29±3.17	82.86±11.94	459.57±22.85
	CV%	18.73	6.32	62.05	25.41	14.14	38.14	13.16
Media		3.77	75.71	14.29	43.57	59.29	82.86	459.57

E. The mating option between rams of different ages (2, 3, 4, 5, 6, and 7 years) and 5-year-old ewes - year V

The offspring resulting from mating rams of different ages (2, 3, 4, 5, 6, and 7 years) with 5-year-old ewes (Table 5) present the following average values for the traits studied:

- Birth weight has an average value of 4.18 kg, within the breed standard, with a maximum average of 4.20 kg in the mating variant 2x5 years, with limits of 4-4.6 kg and a coefficient of variation of 8.25%, and a lower average of 4.15 kg in the mating variant 5x5 years, with limits of 3.7-4.5 kg, and a coefficient of variation of 8.91%.
- Curl type appears as a medium-length tube, short, with medium-sized bobbing, scoring 97.29 points per mating group, with the best curl, good glossy type, scoring 100.33 points, in the mating variant 2x5 years, with limits of 88-115 points, and a coefficient of variation of 13.61%, and the weakest at 94.25 points, a medium tube curl, medium bob, with limits of 70-115 points, and a coefficient of variation of 19.69%.
- Curl size in the mating group is medium-small, scoring 20.21 points, with the best at 21.67 points, medium-small to medium size in the mating variant 2x5 years, and the lowest at 18.75 points in the mating variant 5x5 points, with a coefficient of variation of 33.55%.

Table 5. Influence of the age of the sheep for reproduction - rams of different ages x 5-year-old ewes - on birth weight and the quality of Karakul lambs' fleece - Year V

Age	Specification	Characters						
		Birth weight (kg)	Loop type (points)	Loop size (points)	Birth weight (kg)	Fiber gloss (points)	Color shade (points)	Birth weight (kg)
2x5	$\bar{x} \pm s\bar{x}$	4.20±0.20	100.3±7.88	21.67±1.67	43.33±6.67	58.33±1.67	96.67±3.33	550.33±26.43
	CV%	8.25	13.61	13.32	26.65	4.95	5.97	8.32
5x5	$\bar{x} \pm s\bar{x}$	4.15±0.18	94.25±9.28	18.75±3.15	40.00±5.77	57.50±2.50	96.25±12.48	506.75±33.00
	CV%	8.91	19.69	33.55	28.87	8.70	25.93	13.19
Media		4.18	97.29	20.21	41.67	57.92	96.46	528.54

- Fiber quality is normal-silky with a score of 41.67 points, with the best quality at 43.33 points in the mating variant 5x5 years, with limits of 30-50 points, and the weakest quality recorded in the mating variant 5x5 years, with limits of 30-50 points, and a coefficient of variation of 28.87%.
- Fiber sheen in the mating group is satisfactory-good to good, scoring 57.92 points, with the highest average of 58.33 points, satisfactory-good to good, with limits of 55-60 points and a coefficient of variation of 4.95%, and the lowest average of 57.50 points, satisfactory-good to good, with limits of 50-60 points and a coefficient of variation of 8.70%.
- Color shade presents a good average of 96.46 points, with the highest average on color shade being 96.67 points, with limits of 90-100 points and a coefficient of variability of 5.97%. The lowest is 96.25 points, with limits of 75-130 points and a coefficient of variability of 25.93% at the breeding option of 5x5 years.

Own performance of 528.54 points, that represents the "Record" zootechnical class in the breeding group, with the best performance of 550.333 points at the breeding option of 2x5 years, with limits of 498-583 points and a coefficient of variability of 8.32%. The lowest is 506.75 points, with limits of 444-575 points and a coefficient of variability of 13.19%.

- Out of the two breeding options available in this group, the highest average values were recorded in the breeding option of 2x5 years, while the weakest results were observed in the breeding option of 5x5 years, with both breeding options characterized by relatively close average values between them.

F. Breeding option between rams of different ages (2, 3, 4, 5, 6, and 7 years) with 5-year-old females - Year VI

The average values recorded in the breeding group between rams aged 2 and 3 years (Table 6) place the offspring in the "Record" zootechnical class, mainly due to the following performances: birth weight of 4.17 kg, very good coil with long, medium, short type, medium-small to medium size, normal-silky fiber quality with very good-intense shine, and a color shade rated at 88.33 points.

Table 6. Influence of the age of the sheep for reproduction - rams of different ages x 5-year-old ewes - on birth weight and the quality of Karakul lambs' fleece - Year VI

Age	Specification	Characters						
		Birth weight (kg)	Loop type (points)	Loop size (points	Birth weight (kg)	Fiber gloss (points)	Color shade (points)	Birth weight (kg)
2x5	$\bar{x} \pm s\bar{x}$	4.25±0.25	97.50±4.50	22.50±2.50	40.0±10.00	57.50±2.50	85,.0±5.0	455.0±102.99
	CV%	8.32	6.53	15.71	35.36	6.15	8.32	11.10
3x5	$\bar{x} \pm s\bar{x}$	4.50±0.50	105,. ±7.00	22.50±2.5	48.50±1.50	67.50±7.50	80.00±10.00	550.00±53.00
	CV%	15.71	9.43	15.71	4.37	15.71	17.68	13.62
6x5	$\bar{x} \pm \bar{x}$	3.75±0.05	93.50±2.50	22.50±2.5	37.5±12.50	67.50±7.50	100±10.00	555.50±15.50
	CV%	1.89	3.78	15.71	47.14	15.71	14.14	3.95
Media		4.17	98.67	22.50	42.00	64.17	88.33	520.17

Comparing the average values between the three mating variants of 2x5, 3x5, and 6x5 years, it can be observed that the best mating is between 3-year-old rams and 5-year-old ewes. The resulted offspring exhibit the following performances on the studied traits: a birth weight of 4.50 kg, exceeding the breed standard; a combined type of curl, with long, medium, and short loops and a medium-small to medium size; normal-silky fiber quality with a very good-intense shine and a color shade noted at 80.00 points. The own performance is "Record", with a score of 550.00 points.

The weakest mating variant regarding the studied traits 6x5 years differs from the one presented earlier by lower average values for the curl type and fiber quality, namely 93.50 and 42.00 points, respectively, and by other traits that are superior, however, by very small differences, which explains the good average values on the mating group.

G. Mating between rams of different ages (2 and 3 years) with 5-year-old ewes - year VII

The mating group between 5-year-old ewes and 2- and 3-year-old rams is characterized by the best mating variant between 2-year-old rams and 5-year-old ewes (Table 7), which produced offspring with a very good birth weight of 4.60 kg.

They exhibit a curl type characterized by a good shiny loop, medium-sized, with fiber of normal quality, very good-intense shine, and a color shade with a score of 118.33 points. The own performance, based on the score presented, falls within the "Record" zootechnical class.

The weakest mating variant is the one achieved between 5-year-old females and 3-year-old males, characterized by the following performances: birth weight of 4.00 kg, curls presented in the form of short curls, small bobs, with a good shiny appearance, medium-small curl size, soft-coarse texture, fiber shine ranging from weak to satisfactory, and a dark brumal color shade. The own performance is classified as "Elite" with a score of 434.5 points.

Table 7. Influence of the age of the sheep for reproduction - rams of different ages x 5-year-old ewes - on birth weight and the quality of Karakul lambs' fleece - Year VII

Age	Specification	Characters						
		Birth weight (kg)	**Loop type (ponts)**	**Loop size (points)**	**Birth weight (kg)**	**Fiber gloss (points)**	**Color shade (points)**	**Birth weight (kg)**
2x5	$\bar{x} \pm s_{\bar{x}}$	4.60±0.21	93.33±5.93	23.33±1.67	31.37±10.14	65.00±5.00	118.33±11.67	528.33±46.90
	CV%	7.84	11.00	13.37	55.45	13.32	17.08	15.38
3x5	$\bar{x} \pm s_{\bar{x}}$	4.00±1.40	87.0±15.00	15.00±5.00	20.00±5.00	45.0±15.00	77.50±52.50	434.5±37.50
	CV%	49.50	24.38	47.14	35.35	47.14	95.80	12.20
Media		4.30	90.17	19.17	25.83	55.00	97.91	481.42

CONCLUSIONS

From the analysis of the evolution of birth body weight over the 7 years of study, it is observed that the best combinations were achieved between 5-year-old females and 2-year-old males, except for the first year of study where performances were obtained in all combinations (2x5; 3x5; 4x5; 5x5; and 6x5).

For the assurance of obtaining superior quality, marketable pelts in the process of matching partners in the "major" production, besides other criteria (color, direction of curling of the curl, etc.), the age criterion of the partners must also be taken into account, so that at least one of the partners is an adult: 3, 4, 5, 6 years old (the other partner can be young - 2 years old or old - 7 years old and older).

For the breeding stocks, it is recommended to carry out selection for pelt quality, as much as possible, from the offspring obtained from mating 5-year-old ewes with 6, 2, and 3-year-old rams in Karakul brumăriu.

Analyzing the evolution of own performances, it can be observed that all lambs obtained from mating 5-year-old ewes with rams of different ages of 2, 3, and 4 years are classified into the zootechnical class called Record, while the lambs obtained from mating 5-year-old females with males of 5 and respectively 6 years are classified into the lower zootechnical class compared to the previous Elite.

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According to the studies conducted by researchers, it seems that the age of the mother is one of the environmental factors with the greatest influence on the characteristics of Karakul lamb pelts. In most cases, increasing maternal age has been associated with a degradation of pelt traits, a conclusion that is also confirmed by the results of this study. Therefore, it is strongly recommended to limit the period of time during which ewes are kept in the flock to approximately six years and even younger ages. This approach is essential to minimize the negative effects on lamb pelt quality and to ensure optimal flock management.

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EVALUATION OF SOME CHEMICAL COMPOUNDS IN *Foeniculum vulgare*, *Trigonella foenum-graecum*, AND *Cuminum cyminum* SEEDS AS POTENTIAL FOOD SUPPLEMENTS FOR COWS

Paula POSAN[1], Lucica NISTOR[1], Violeta Alexandra ION[2], Aurora DOBRIN[2], Andra SULER[1], Dana IANITCHI[1], Nicoleta DEFTA[1], Monica MARIN[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Animal Productions Engineering and Management, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Research Center for the Study of Quality Food Products, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: andrasuler@yahoo.com

Abstract

The present study investigates the chemical composition of seeds from Foeniculum vulgare (fennel), Trigonella foenum-graecum (fenugreek), and Cuminum cyminum (cumin) to assess their suitability as food supplements for enhancing milk production in cows. The dry matter, ash content, total nitrogen, total carbon, protein and fiber levels were analyzed to provide comprehensive insights into the nutritional value of these seeds. The experiments in this study were all conducted in triplicate. Results were defined as mean values ± standard deviations. The results are part of a larger project and these findings are integral to the broader project, which aims to develop optimized dietary supplements for dairy cattle. Considering the essential role of nutrition in dairy cattle health and milk production, the identified nutritional components in these seeds hold promise for incorporating them into cattle diets.

***Key words**: chemical composition, cow, galactogenic effect, medicinal plants, milk production.*

INTRODUCTION

Engaging in animal husbandry at the household level grips a significant role in rural economies, offering families a stable income stream and a dependable source of food.

Given the conditions of the world population explosion, an important concern for securing food resources is cattle rearing and exploitation, as this animal species provides raw material for a wide range of food products. Even though the cattle livestock has decreased, milk production has to increase, and farmers need to be interested in exploiting animals with high production potential and ensuring optimal conditions for animal welfare (Defta et al., 2023).

Also, the increasing demand for organic animal products, coupled with restrictions on medicinal substance use and the quest for alternatives to traditional feed additives, underscores the growing interest in natural approaches to enhance milk production. Herbal spices emerge as potential contributors to altering animal diets, with the ultimate aim of positively impacting the secretory tissue of the mammary gland (Mohanty et al., 2014). This influence is anticipated to lead to an enhancement in milk production, aligning with the broader trend towards natural solutions in response to evolving agricultural and health considerations Penagos et al., 2014; Marin et al., 2020.

Researchers have shown a particular interest in galactogenic plants due to their accessibility, affordability, and the absence of toxic residues in milk. Exploring the phyto-pharmacological aspects of medicinal plants holds promise for uncovering innovative methods to incorporate galactogenic plants into the diets of dairy animals. Numerous plants contain a rich array of chemically active compounds with galactogenic properties, presenting viable opportunities for utilizing herbal remedies to augment milk production in animals.

Phytochemical analysis uncovered the presence of active compounds, including phenolic acids such as caffeic acid and chlorogenic acid, α-linolenic acid, curcumin, essential oils like eugenol and limonene, flavonoids such as quercetin and rutin, as well as trigonelline, gentianine, saponins, and galactomannan. These compounds collectively contribute to the galactopoietic effect, as documented by Bharti et al. (2012).

The use of galactogenic plants, as a natural supplement for the growth and support of milk production, is in line with global trends to ensure animal health and welfare for animals and to obtain clean production unaltered by medicinal chemicals, which can be found in milk secretion, thus ensuring the premises for food safety and security. Some of the most used plants in these studies are *Foeniculum vulgare*, *Trigonella foenum-graecum*, and *Cuminum cyminum* seeds, with satisfactory results (Posan et al., 2023).

Several researchers have conducted studies administering these plants to cows at doses ranging from 50 g to 150 g per animal per day. Their findings have demonstrated beneficial effects on both productive qualities (such as improved udder health, oxytocic effects promoting milk ejection by stimulating mammary gland ducts, increased activity of mammary gland alveolar tissue, and enhanced secretory activity of glandular acini) and reproductive aspects (including estrogenic effects, regulation of the sexual cycle, and uterotonic effects) in the respective females (Waghorn et al., 2003; Patel et al., 2016; 2017; Bora et al., 2019; Bhargav et al., 2021).

Recognizing the importance of milk in both human and animal nutrition, but facing pressure to reduce the dairy cattle population due to their implication in methane emissions, contributing to the rise in greenhouse gas concentrations, there is a need to find a sustainable strategy for maintaining high-level milk production. In the year 2020, the European Union maintained a population of around 20 million cows, each yielding an average of 7,300 kg of milk. Projections indicate a continued decline in the number of dairy cows, with an anticipated drop below 20 million by the year 2023. This reflects a reduction of 1.7 million cows since the peak in 2016 and a decrease of 564,000 cows since 2021 (Mihai et al., 2023).

Maintaining the same level of marketable milk quantity, involves increasing production per head of cattle, and the use of galactogenic plants as a dietary supplement can be a viable solution. The current research examines some of the chemical components (dry matter, ash content, total nitrogen, total carbon, protein and fiber levels) of seeds from *Foeniculum vulgare* (fennel - FVE), *Trigonella foenum-graecum* (fenugreek - FGI), and *Cuminum cyminum* (cumin - CI) to evaluate their potential as food supplements for improving milk production in cows.

Fenugreek is widely recognized as one of the most commonly utilized herbal galactogogues. Belonging to the Leguminosae family, it is cultivated extensively in various regions worldwide, with notable cultivation in India, Mediterranean countries, North Africa, and Southern Europe (Abascal & Yarnell, 2008). Fennel, the sole species in the Foeniculum genus, is distributed across temperate zones globally. This perennial and aromatic plant is indigenous to southern Europe, particularly the Mediterranean coast, where it thrives as a wild herb (Manzoor et al, 2016). *Cuminum cyminum*, commonly known as Cumin and belonging to the Apiaceae family, is native to regions spanning from the East Mediterranean to South Asia (Sharif et al., 2018).

While these plants generally originate in the Mediterranean region, they can also be grown in Romania and can be acquired from local producers of medicinal and aromatic plants.

The current study is part of a wider research and besides these elements of chemical composition, determinations of active biological compounds will be made in fennel, fenugreek, and cumin, comparing the seeds grown in other geographical regions (Egypt, India), with those of plants grown in Romania in order to assess the opportunity of their utilization as supplements in the diet of dairy cows.

MATERIALS AND METHODS

The analyses were carried out on seeds of *Foeniculum vulgare*, *Trigonella foenum-graecum*, and *Cuminum cyminum* and the values were reported as percentage of the raw seeds. The seeds were commercially acquired and originated from India (FGI and CI) and Egypt (FVE). From each type of seed, three samples of 200 g each were extracted, labeled as 1, 2, and 3. Prior to analysis, the samples were ground using a grinder for 10 seconds and passed through a 1 mm sieve.

The dry matter, ash content, total nitrogen, total carbon, protein and fiber levels were analyzed to provide comprehensive insights into the nutritional value of these seeds. The experiments in this study were all conducted in triplicate. Results were defined as mean values ± standard deviations.

The determination of dry matter content (DM %) was carried out using the gravimetric method, involving the removal of water through evaporation and weighing, in accordance with the European Pharmacopoeia 7th edition, and the results were expressed in percentages.

The ash determination was conducted following the AOAC Official Method 942.05, "Ash of Animal Feed," using a muffle furnace, and the results were expressed in percentages.

The analysis of total nitrogen content (N%) and total carbon content (C%) was conducted using the Dumas method, employing the elemental analyzer EA 3000, and the protein quantity was calculated by multiplying the total nitrogen content by a factor of 6.25.

The determination and quantification of fibers were performed using the Acid Detergent Fiber (ADF) method according to Van Soest, and, in the end, the calculation formula was applied:

$$ADF\% = \frac{(CW + RW) - CW}{SW} \times 100$$

ADF% = Acid Detergent Fiber
CW - weight of the crucible
RW - weight of the residue
SW - weight of the sample

RESULTS AND DISCUSSIONS

In tables and figures 1, 2, and 3, the values of dry matter and humidity are presented and these parameters exhibit a high degree of similarity among the seeds of the three plant species.

Analysing the dry matter content in galactogenic seeds is crucial for understanding their nutritional composition, formulating effective animal diets, ensuring storage stability, and promoting optimal milk production in livestock.

Table 1. Dry matter and moisture percent for Fennel

Specification	$\bar{x} \pm s_{\bar{x}}$	v %
Dry matter, %	90.97 ± 0.101	0.111
Humidity, %	9.03 ± 0.101	0.115

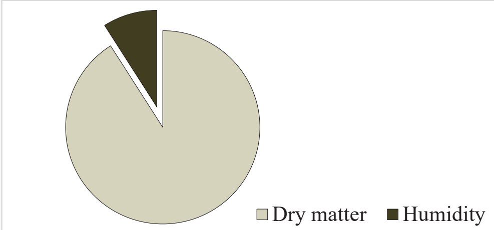

Figure 1. Dry matter and moisture percent for Fennel

In the present study, the dry matter content in fennel seeds measured 90.97%, closely aligning with values reported by other researchers, who found a maximum of 92% (Saber & Eshra, 2019). Consequently, the humidity percentage was 9.03%, differing from the average reported by Hina in 2014 (6.24 ± 0.24). Mehra's 2021 research reveals variations in humidity levels within fennel seeds, ranging from 8.45% to 5.85%, contingent upon their variety or genotype.

Table 2. Dry matter and moisture percent for Fenugreek

Specification	$\bar{x} \pm s_{\bar{x}}$	v %
Dry matter, %	90.51 ± *0.044*	*0.049*
Humidity, %	*9.49* ± *0.044*	*0.464*

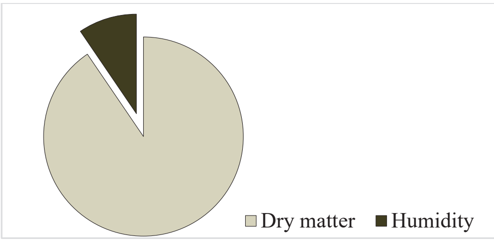

Figure 2. Dry matter and moisture percent for Fenugreek

The dry matter content discovered for Fenugreek in this study, at 90.51%, closely mirrors the findings of Zemzmi et al. (2020), who reported 90.2%. The moisture percentage (9.49%) falls within the range observed by Agrawal et al. in 2015 (11.21%) and Wani & Kumar in 2018 (8.18%).

Table 3. Dry matter and moisture percent for Cumin

Specification	$\bar{x} \pm s_{\bar{x}}$	v %
Dry matter, %	90.49 ± 0.085	0.094
Humidity, %	9.51 ± 0.085	0.464

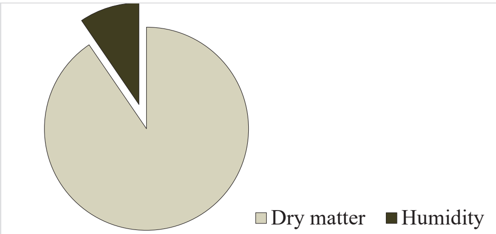

Figure 3. Dry matter and moisture percent for Cumin

The percentage of dry matter for Cumin seeds had an average of 90.49%, a value slightly lower than that found by Kha & Chaudhry in 2010 (96.4%).
In table 4 and figure 4 are presented the percent of ash, from raw seed.

Table 4. Ash percent for Fennel, Fenugreek and Cumin

Specification	$\bar{x} \pm s_{\bar{x}}$	v %
Fennel	8.798 ± 0.241	2.738
Fenugreek	3.317 ± 0.040	1.209
Cumin	6.957 ± 0.195	2.803

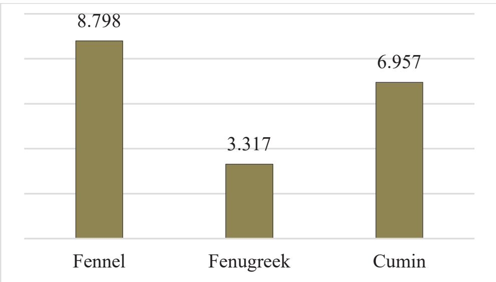

Figure 4. Ash percent from raw seed, for Fennel, Fenugreek and Cumin

In this study, ash content values were determined as 8.79% for fennel, 3.31% for fenugreek, and 6.95% for cumin. These values differ from other researchers, which reported for fennel values ranged between 9.38% and 12.87% (Saber & Eshra, 2019, Mehra et at., 2021, Hina et al., 2014) 3% (Agrawal et al., 2015), and 10.1% for fenugreek (Zemzmi et al., 2020), and 8% for cumin (Kha & Chaudhry, 2010).
In tables 5, 6, 7 and figures 5, 6, and 7 are presented the percentage values of total nitrogen, total carbon and protein from the three types of raw seeds.

Table 5. Total nitrogen percent from raw seed, for Fennel, Fenugreek and Cumin

Specification (N%)	$\bar{x} \pm s_{\bar{x}}$	v %
Fennel	2.494 ± 0.078	3.147
Fenugreek	6.345 ± 0.067	1.059
Cumin	2.369 ± 0.171	7.217

Nitrogen content in galactogenic seeds is pivotal for assessing their potential as feed supplements for enhancing milk production. It provides insights into the protein quality, amino acid composition, and overall nutritional value, influencing the effectiveness of these seeds in promoting lactation in animals.

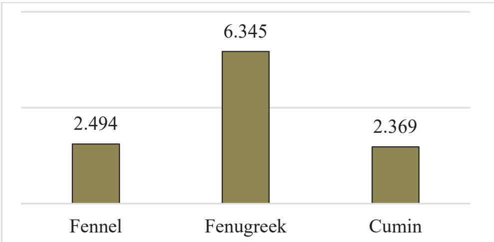

Figure 5. Total nitrogen percent from raw seed, for Fennel, Fenugreek and Cumin

It is observed that the nitrogen values are higher in fennel, compared to those of fennel and cumin. Factors such distinct genetic and metabolic profiles, soil composition, cultivation conditions, and environmental factors can contribute to variations in nutrient levels.

Table 6. Total carbon percent from raw seed, for Fennel, Fenugreek and Cumin

Specification	$\bar{x} \pm s_{\bar{x}}$	v %
Fennel	50.779 ± 1.318	2.596
Fenugreek	47.822 ± 0.137	0.286
Cumin	49.609 ± 2.025	4.082

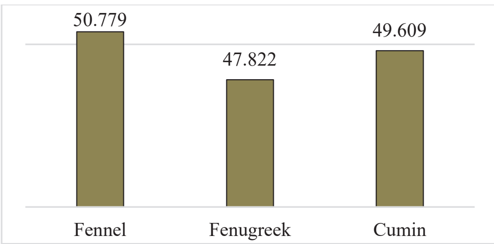

Figure 6. Total carbon percent from raw seed, for Fennel, Fenugreek and Cumin

While nitrogen content is more directly associated with protein synthesis and milk production, carbon content in galactogenic seeds contributes to the energy component of the diet. Balancing both nitrogen and carbon content is crucial for formulating diets that support optimal milk production and overall animal health.

The carbon values recorded were 50.7% for fennel, 47.8% for fenugreek, and 49.6% for cumin. These values are consistent with those reported by other researchers (40.19% for fennel, Saber & Eshra, 2019; 55.49 % Agrawal et al., and 52.3% Wani & Kumar, 2018, for fenugreek).

Table 7. Protein percent from raw seed, for Fennel, Fenugreek and Cumin

Specification	$\bar{x} \pm s_{\bar{x}}$	v %
Fennel	15.590 ± 0.491	3.147
Fenugreek	39.656 ± 0.420	1.059
Cumin	14.808 ± 1.069	7.217

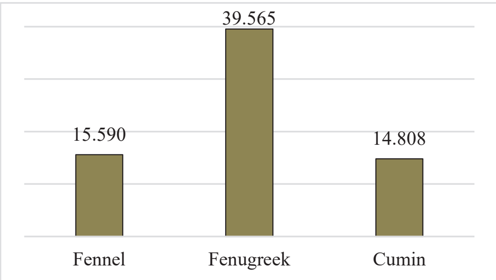

Figure 7. Raw seed total protein from for Fennel, Fenugreek and Cumin (%)

The protein content in seeds is an important factor in determining their nutritional quality. The protein content in these seeds is essential for providing necessary nutrients to animals, supporting growth, and ensuring overall health.

The protein value of the analysed seeds was slightly higher than that found in other authors' studies: 15.59% for fennel, compared to 10.18% (Saber & Eshra (2019) or 12.97% (Hina et al., 2014); 39.56% for fenugreek, compared to 20.9-24.7% (Kha & Chaudhry, 2010), 23.30% (Agrawal et al., 2015), 23-26% (Wani & Kumar in 2018) or 27.57% (Dhull et al., 2021). For cumin seeds the situation is the opposite, the values found in this study (14.8 %) being lower than those found in other authors (22.3% (Kha & Chaudhry, 2010)

In table and figure 8, the percentage values of ADF from raw seed, for the three types of seeds are presented

Table 8. ADF % from raw Fennel, Fenugreek and Cumin seeds

Specification	$\bar{x} \pm s_{\bar{x}}$	v %
Fennel	39.458 ± 0.758	1.921
Fenugreek	14.215 ± 0.739	5.198
Cumin	34.745 ± 1.344	3.868

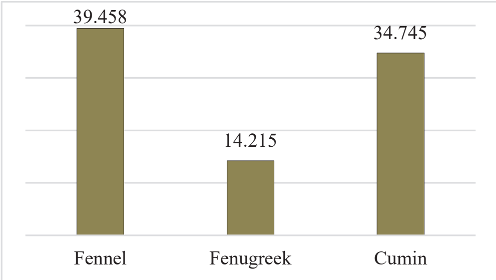

Figure 8. ADF % from raw seed, for Fennel, Fenugreek and Cumin

Acid Detergent Fiber analysis is a valuable tool in assessing the fibrous components of forages and feeds, providing critical information for optimizing animal nutrition, diet formulation, and overall farm management.

In this study, ADF recorded values of 39.45% for Fennel, 14.21% for Fenugreek and 34.74% for Cumin. For Fennel Hina et al. (2014) found values of 43.44%, for Fenugreek Jiang et al. (2007) found values of 26.8%, and Zemzmi et al. (2020), found values of 24.6%. For cumin, the value presented by Kha & Chaudhry (2010) was 24.1%.

The difference in ADF recorded between the different seed samples suggests different values of cellulose, lignin and cutin in the fiber composition of these plants

CONCLUSIONS

Analysing some chemical components provides comprehensive information that is essential for both scientific research and practical applications, especially in the context of using these seeds to enhance milk production in animals. The examination of seed samples indicated that the recorded values align with those reported by previous researchers. Some variations in the values of dry matter/moisture, ash, nitrogen/carbon/protein content, and ADF between the present study and the cited literature arise due to the analysis of plants belonging to different varieties or genotypes, grown under varying soil conditions, temperatures, and cultivation techniques Understanding the chemical composition of these seeds is essential for their proper incorporation into animal feed. This ensures that the seeds can express their maximum potential, ultimately fulfilling their intended purpose, which is to enhance milk production in animals.

ACKNOWLEDGEMENTS

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AMINO ACID AND MINERAL MILK COMPOSITION OF SOWS FED WITH A MIXTURE OF ESSENTIAL OILS

Taras PRUDYUS[1], Anatoly GUTSOL[2], Natalia GUTSOL[2], Roman KAMINSKY[3]

[1]Institute of Animal Biology NAAS, 38 St. Vasyl Stus, 79034, Lviv, Ukraine
[2]Institute of Feeds and Agriculture of Podillia National Academy of Sciences, 16 Yunosti Avenue, 21100, Vinnytsya, Ukraine
[3]Nutrimin Ukraine LLC, Lviv, Ukraine

Corresponding author email: tarasvet126@gmail.com

Abstract

The aim of the work is to find out the influence of essential oils in the diets of sows during farrowing and lactation on the amino acid and mineral composition of milk. The whole milk of sows obtained on the 21st day of lactation was studied, an increase of 9.69% of milk protein in the mass fraction of animals of the experimental group was observed. The amino acid and mineral composition of milk improved. The content of essential amino acids in milk increased: lysine - by 13.18%, alanine - by 13.11%, threonine - by 7.14%, histidine - by 22.22% compared to the control group. In the milk of the research group, an increase in iron content by 13.13%, phosphorus and zinc by 8% and 3.48%, respectively, was noted. The piglets of the research group had a greater increase: on the fifth day of life, the live weight was 5.4% higher; on the 14th day - by 0.4 kg, or by 12.5%, and at weaning (on the 28th day) - by 0.5 kg, or by 6.6% compared to animals of the control group.

Key words*: amino acid composition, essential oils, lactation, milk, sows.*

INTRODUCTION

The efficiency of pig farming in up to date economic environment largely depends on the sows fertility and lactation, as well as on the efficient rearing of suckling piglets and their safety.

The production of multiple litters and well-developed and viable piglets significantly depends on the organisation of complete sow nutrition during the preparation for insemination, farrowing and lactation periods. Gestating sows should be fed enough to meet their physiological need for nutrients for the embryos development and growth, as well as to make appropriate nutrient reserves in the body. The main focus in suckling sows feeding is maintaining good health, increasing lactation, saving all piglets born and achieving an average live weight of 6-9 kg per piglet at one month of age (Povod, 2022; Povoznikov, 2022).

It was proven (Camargo, 2023) that the sow productivity in each subsequent reproductive cycle is closely related to the weight loss during lactation level. When a sow loses less than 5% of her body weight during lactation, her piglets will have a higher weight at weaning, and in addition, she will have a shorter period of barrenness, a productivity increase at the next farrowing, and an increase in the farrowing percentage.

Furthermore, poor productivity negatively affects the parity number per year, piglets born live number, the percentage of replacement stock, which together determine not only the overall productivity of the breeder herd, but also the enterprise profitability (Dang, 2022).

The sow's lactation is one of the important breeding features that largely determines the normal growth and development of suckling piglets, their preservation and further rearing results in the farm. All nutrients contained in colostrum and sow's milk are digested 90-98% and absorbed well by piglets. In this regard, the piglets have a higher growth rate in the first months of life compared to the young of other animal species. At the age of one month, the piglet's live weight increases almost 5 times and 3.6-4.0 kg of mother's milk is needed to consume for 1 kg of gain (Costermans, 2020).

Therefore, it is very important to provide sows with complete feeding, as biologically complete nutrition and proper gestation and suckling sows housing is a key factor in achieving high milk production.

It is impossible to organise biologically complete feeding without the use of biologically active additives that contain the necessary energy and biologically active substances, eliminating their deficiency in feed and acting as catalysts (accelerators) of metabolic processes in the body. Their effective and rational use in pig feeding can significantly increase the digestion and absorption coefficients of feed nutrients, productivity improve and animal welfare (Vuhliar, 2020; Syrovatko & Vuhliar, 2020).

In order to improve the feed products quality and animal diets, it is important to introduce to the feed new non-traditional types of plant material, which contain a balanced complex of proteins, lipids, amino acids, minerals, vitamins and have not only high nutritional feed properties, but also a prophilactic effect (Rooney, 2020; Vuhliar, 2020).

Phytobiotics are phytogenic feed additives made from medicinal plants that perform the following main functions: preventive; therapeutic; tonic and vitamin; immunoprotective, strengthening and normalising; biostimulant; they are resistant to adverse exogenous factors. They have a variety of effects on the body: antimicrobial, antiviral, immunomodulatory, antifungal, anti-inflammatory and are used in animal feeding to increase productivity and improve product quality (Kryzhak, 2020).

The following groups of phytobiotics are distinguished by their biological origin, chemical composition and other characteristics: herbs, spices, essential oils and resins.

Essential oils have the greatest antimicrobial activity. Essential oils have immunostimulating and antimicrobial properties. The use of phytobiotic preparations improves appetite and absorption of nutrients contained in feed, improves digestibility, and stimulates the endogenous enzymes production (Santos, 2023).

However, the widespread use of phytobiotics from non-traditional raw materials in farrowing and suckling sows feeding is constrained by the lack of research on their chemical composition, effect on metabolism and productivity. Therefore, research in this area is relevant and needs to be discussed.

The aim of the study was to determine the effect of feed additive “Activo” in the diets of gestating and suckling sows on the amino acid and mineral composition of milk.

MATERIALS AND METHODS

The effectiveness of the feed additive "Activo" in the complete feed composition was studied in the conditions of the pig complex of LLC "Artsyzka Meat Company", located in the Voskresenka Persha village, Artsyzka district, Odesa region.

For the study, were selected 16 heads of gestating sows of French genetics ADN (meat productivity), divided into two groups of 8 heads each (Table 1). The main methodological technique used in the trial was similar groups method (Vlizlo, 2012). This method involves the selection of animals into groups based on their age, live weight, origin, breed and general condition. The sows were of 2-3 parity and had no overgrazing. The research began when the sows were transferred to the farrowing parlor 5 days before the planned farrowing, or on the 110th day of gestation, and they were kept in individual pens.

Table 1. Trial design

Group	Quantity of animals	Sows		Piglets
		Sows in gestation (110 days)	suckling sows (before weaning 28-days of age)	from 5 days of age and till weaning 28 days
Control	8	BD* (complete compound feed)		BD* (complete compound feed)
Trial	8	BD + 0.1 kg/t «Activo»		BD + 0.2 kg/t «Activo»

*BD - Base Diet

Sows of both groups were fed a complete feed balanced in terms of biologically active substances and nutrients using the Danish premix Nutrimin A/C. The feed additive "Activo" in the amount of 0.1 kg/t of feed was introduced into the sows feed of the trial group from 110 days of gestation to weaning. The animals were fed in doses using feed dispensers. The frequency of feeding was 2 times a day before farrowing, and 3 times a day after farrowing. Access to water was *ad libitum*.

Piglets of both groups were fed pre-starter feed by adding it to the feeders from the fifth day after birth until weaning. The feed for the piglets of the trial group contained the feed additive

"Activo" in the amount of 0.2 kg/t of finished feed. Feed consumption was recorded during the trial.

To determine body weight loss during lactation sows were weighed at the beginning and end of the trial. At farrowing, litter size, litter weight and individual piglets weight at birth were determined. At weaning, litter weight, weight of one piglet, average daily weight gain and piglet mortality during the suckling period were determined.

To study the amino acid and mineral composition of milk, sows were sampled on day 21 of lactation. The amino acid milk composition was determined by analysing amino acids on a Sycam S433 amino acid analyser with post-column derivatisation with ninhydrin in the laboratory of “ES Biolights” LLC.

An inductively coupled plasma atomic emission spectrometer (ICP OES) (Analytik Jena, Germany, model: PlasmaQuant PQ 9000 Elite ICP OES) was used to study the content of macro- and microelements.

The study was carried out according to generally accepted zootechnical and analytical methods (Vlizlo, 2012).

The feed additive "Activo" contains a mixture of essential oils, which is a combination of natural standardised biologically active substances extracted from aromatic herbs and spices, concentrated in one microcapsule. It contains:

- cinnamon essential oil, which is a taste stimulant and antioxidant that enhances the palatability of feed, reduces the stress and disease effects.
- rosemary essential oil, which is an antioxidant and anti-inflammatory agent that reduces oxidative and inflammatory reactions, regulates body temperature, and reduces pain in inflammatory processes.
- chilli extract, which improves digestion by increasing the digestive enzymes activity and gastric juice secretion. Thus, increasing the feed conversion and its taste.
- oregano essential oil, which has bactericidal and antioxidant properties, inhibiting the growth and development of pathogenic fungi and bacteria (Prudyus, 2023).

The statistical processing of the study results was carried out using the standard Statistica package in Microsoft Excel 2013, assessing the reliability of the indicators ($p<0.05$; $p<0.01$; $p<0.001$) according to the Student's criterion (Kramarenko, 2019).

RESULTS AND DISCUSSIONS

The modern genetics achievements in terms of sow productivity allows to produce up to 30 piglets per sow per year. Additionally, the litter weight at birth increases to more than 25 kg. Accordingly, the body's resource expenses increase, and metabolic processes in the body are accelerated. Therefore, it is important to develop a feeding strategy that meets the animal's nutrient requirements at different periods of the reproductive cycle. Compound feed should contain high-quality and easily digestible components and have an optimal amino acid composition (Hall, 2021; Khalak & Hutyi, 2022).

Research has shown that less weight loss and greater muscle retention during lactation improves sow performance, milk composition and nutritional value, and increases piglet weaning weight.

During the lactation period, the sow's organism functions under a significantly greater physiological load. During 28 days of lactation, a sow produces an average of 250-300 kg of milk. Sow’s mammary glands produce milk unevenly in lactation. Most of it is produced in the second and third decades of the lactation period (an average of 22% of the total amount), after which the intensity of lactation gradually decreases. The peak of lactation is considered to be 21 days. The chemical composition of pig milk differs significantly from that of other livestock species. It contains 50-60% more dry matter, proteins, fats and total energy. Compared to milk, colostrum has significantly higher amount of dry matter, protein, and contains up to 40% of γ-globulins, which are part of the immune system, providing piglets with natural immunity against various diseases. Colostrum and sow's milk contain little amounts of iron, copper, calcium and phosphorus, so it is necessary to provide piglets with iron-containing preparations and mineral supplements, otherwise their blood will lack haemoglobin leading to anaemia and rickets.

Addition of the feed additive "Activo" to the sow’s diet in the amount of 0.1 kg/t has a positive effect on their productivity, feed intake and general condition (Table 2).

Table 2. Parameters of live weight of suckling sows during feeding «Activo», n = 8

Parameters	1 group (control)	2 group (trial)
Birth weight, kg	221.5 ± 1.04	220.0 ± 0.23
Weaning weight, kg	199.0 ± 1.34	202.0 ± 0.34
Weight loss during suckling period, kg	22.5 ± 1.14	18.0 ± 1.26
Feed quantity for a sow for suckling period, kg	214.5 ± 0.63	221.0 ± 0.38

Thus, the results of the study show that weight loss during the suckling period of sows in the trial group was 18 kg, or 8.2%, while sows in the control group lost 22.5 kg, or 10.1%. That is, the animals of the trial group that consumed essential oils in the feed lost 4.5 kg or 1.9% less weight during the suckling period. Lower consumption of finished feed also had an impact on the weight loss of sows in the control group. Animals in the control group ate 6.5 kg less feed during the suckling period compared to animals in the trial group. It can be confirmed that the feed additive "Activo" in the feed composition had an impact on the appetite improvement in the animals of the trial group, because sows often refuse the feed after farrowing and during lactation.

The same pattern was observed in the growth rate of suckling piglets. Thus, the piglets of the trial group had a higher live weight of 5.4% ($P<0.01$) on the fifth day of life compared to the control group. Whereas at birth piglets of the control group had a higher live weight by 3.85% compared to piglets of the trial group (Table 3).

Table 3. Sows productivity and piglets growth parameters

Parameters	1 group (control)	2 group (trial)
Sows quantity, heads	8	8
Total born piglets, heads	122	118
Born alive piglets, heads	111	110
Stillborn and mummified piglets, heads	11	8
Piglets live weight at birth, kg	1.3 ± 0.01	1.25 ± 0.03
Piglets live weight at 5 days, kg	2.22 ± 0.04	2.34 ± 0.02**
Piglets live weight at 14 days, kg	3.2 ± 0.03	3.6 ± 0.03***
Piglets live weight at 28 days, kg	7.6 ± 0.08	8.1 ± 0.05***
Mortality, %	-	-
Cases of diarrhea, heads	23	7
Quantity of consumed prestarter, kg (until the weaning moment)	12.15 ± 0.12	12.5 ± 0.07

*$P<0.05$, **$P<0.01$, ***$P<0.001$

The piglets of the trial group at 14 days and at weaning at 28 days had also higher live weight comparing to the control group: by 0.4 kg or 12.5% at two weeks of age and by 0.5 kg or 6.6% at weaning. They had 30.4% fewer cases of diarrhea compared to the control group, which suggests that they had a stronger immune system. Perhaps there was a maternal transfer of biologically active components of plant origin through milk, demonstrating an antimicrobial effect.

Reyes-Camacho et al. (2020) found high concentrations of thymol, anethole and p-cymene in the milk of sows supplemented with plant compounds rich in these components during lactation, suggesting a mother-to-child transmission through milk. There is evidence that the nutrients supply that improve gut health to suckling sows can influence the gut microbiota of the litter within two weeks after weaning (Balasubramanian, 2016). The result was also observed by Tokach (2019), who identified a reduction in the piglets diarrhoea incidence from sows treated with oregano essential oil.

In addition, the piglets of the trial group had a better appetite, they ate 2.88% more pre-starter feed.

One of the important factors in the period of growing suckling piglets is the sows milk yield and its quality. In the study of sows whole milk samples obtained on the 21st day of lactation, an increase of 9.69 % in the protein mass fraction in animals of the trial group was determined compared to the control group (Table 4).

Along with the increase in the protein mass fraction in milk, the amino acid composition has improved. Of particular importance is the increase in the content of essential amino acids in milk.

Thus, lysine, which is essential amino acid responsible for bone formation and growth, calcium absorption, and takes part in the synthesis of antibodies, hormones, enzymes, and collagen formation, was 13.18% higher in the trial group, alanine was 13.11% higher, threonine was 7.14% higher, and histidine was 22.22% higher than in the control group. As for the non-essential amino acids, it was admitted a significant increase in aspartic acid by 11.4%, arginine by 32.25%, and proline by 3.77%.

Table 4. Amino acid composition of milk of lactating sows, n = 3

Parameters	Group	
	1 control	2 trial
Protein mass fraction, %	16.51 ± 1.00	18.11 ± 0.10
Valine, %	0.76 ± 0.06	0.76 ± 0.04
Proline, %	1.59 ± 0.13	1.65 ± 0.05
Phenylalanine, %	0.58 ± 0.08	0.66 ± 0.01
Leucine, %	1.08 ± 0.12	1.17 ± 0.02
Isoleucine, %	0.39 ± 0.05	0.39 ± 0.02
Histidine, %	0.45 ± 0.08	0.55 ± 0.03
Glycine, %	0.50 ± 0.06	0.44 ± 0.18
Glutamic acid, %	2.62 ± 0.31	2.21 ± 1.44
Arginine, %	0.62 ± 0.27	0.82 ± 0.02
Aspartic acid, %	1.14 ± 0.13	1.27 ± 0.04
Alanine, %	0.61 ± 0.06	0.69 ± 0.02
Threonine, %	0.70 ± 0.10	0.75 ± 0.02
Methionine, %	0.20 ± 0.03	0.21 ± 0.01
Lysine, %	0.91 ± 0.11	1.03 ± 0.01
Tyrosine, %	0.47 ± 0.09	0.53 ± 0.02
Serin, %	0.72 ± 0.13	0.83 ± 0.02
Cystine, %	0.38 ± 0.07	0.44 ± 0.05

One of the most important components of the feed is the presence of mineral elements, which are involved in all processes of the body's vital activity. Without them, bone growth, muscle fibre contraction, respiration, bristle growth, etc. cannot take place. Mineral elements intake is ensured by the feed (colostrum, milk, compound feed) consumed by the animal. Another important factor regarding the elements is not only their availability, but also conditions for their assimilation and the process of assimilation and distribution in the body.

All micro- and macronutrients in sow milk are absorbed in the small intestine. The duodenum is responsible for the largest percentage of absorption, which is facilitated by hydrochloric and bile acids. For piglets at an early age, the small intestine is the main organ for nutrient absorption and transport, and a healthy mucosa is important to ensure normal digestive function, as nutrient absorption is largely dependent on small intestinal villi (Prudyus, 2023; Sun, 2022; Zhe, 2022). The level of macro- and micro-nutrients supplied by colostrum and milk has a significant impact on the growth and development of piglets in the first days after birth.

Analysing the data in Table 5, we can see that the calcium level in the control group was 6.1% higher than in the trial group. However, this indicator did not have a significant effect on cell membrane permeability, enzyme synthesis activation and promotion of phosphorus and zinc absorption, and rickets cases, as evidenced by the piglets performance in the trial group before and after weaning.

Table 5. Macro and microelement milk composition of suckling sows, n = 3

Parameters	Group	
	1 control	2 trial
Calcium, g/kg	0.98 ± 0.11	0.92 ± 0.07
Sodium, g/kg	0.76 ± 0.29	0.83 ± 0.09
Ferum, mg/dm^3	1.37 ± 0.50	1.55 ± 0.22
Zink, mg/dm^3	16.07 ± 5.90	16.63 ± 2.40
Magnesium, g/kg	0,09 ± 0.04	0,09 ± 0.01
Phosphorus, g/kg	1.00 ± 0.38	1.08 ± 0.07
Potasium, g/kg	1.08 ± 0.43	1.24 ± 0.02

The first clinical signs of anaemia (iron deficiency) in young piglets appear as early as 5-7 days of life. The full clinical manifestation of this disease is observed in the period of 21-28 days of piglets' life, characterised by pale skin, weakness, diarrhoea, poor feed intake, and mortality. In our study, the iron content was higher by 13.13% in the sows milk of the trial group compared to the control group. In the whole milk sample of sows of the trial group, an increase in Phosphorus and Zinc by 8% and 3.48%, respectively, was noted in relation to the control group. The increase of Phosphorus content has a positive effect as it is a part of the nucleic acids of many enzymes, phospholipids, phosphoproteins, and is involved in metabolism, biological reactions and energy metabolism (Wang, 2022). Zinc prevents the hypotrophic births occurrence, parakeratosis, and reduces the stillbirth rate of piglets (Wei, 2020).

It is scientifically proven (Yang, 2019; Vuhlyar, 2020) that essential oils have antimicrobial, digestive and antioxidant effects. These substances have a significant impact on intestinal health, nutrient absorption through the influence on the stress factors occurrence in these organs (Martyshuk, 2019), and therefore have an impact on milk production.

CONCLUSIONS

The use of the feed additive "Activo" in the amount of 0.1 kg/t of feed for sows, starting from 110 days of gestation and during lactation, helps to improve feed intake and increase productivity. It was found that the sows weight loss during the suckling period of the trial group was 18 kg, or 8.2%, while sows of the control group lost 22.5 kg, or 10.1%.

On the fifth day of life, piglets of the trial group had a higher live weight of 5.4% ($P<0.01$) compared to the control group, at 14 days and at weaning (28 days), the live weight of the trial group also exceeded the control group: by 0.4 kg or 12.5% at two weeks of age and by 0.5 kg or 6.6% at weaning. In addition, they had 30.4% fewer diarrhea cases compared to the control group.

In the study of sows whole milk samples obtained on the 21st day of lactation, an increase of 9.69% in the protein mass fraction and an improvement in the amino acid milk composition was determined.

The use of the feed additive "Activo" in the feed composition for suckling sows has a positive effect on improving the milk quality in relation to its biological value, which has a positive effect on the growth and development of young piglets further.

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EPIDEMIOLOGY, DIAGNOSIS, TREATMENT, CONTROL AND ECONOMIC IMPACT OF TRICHINOSIS

Sabina-Gabriela RĂCĂȘANU (GHIZDAVEȚ)[1], Ionuț RĂCĂȘANU[1], Dănuț-Nicolae ENEA[1], Alexandru MIHAI[1], Stelian BĂRĂITĂREANU[1], Laura Florentina VLĂSCEANU[2], Livia VIDU[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]National Veterinary Sanitary and Food Safety Authority, 1 Presei Libere Square, District 1, Bucharest, Romania

Corresponding author email: sabinna.g@yahoo.com

Abstract

Trichinosis is a disease caused by a nematode parasite of the genus Trichinella spp. This zoonosis has been a major public health problem in many countries. The main source of infection in our country is the domestic pig. It is contaminated by eating food scraps containing raw meat infested with Trichinella spp., eating rats, mice or their droppings or by contact with wild animals. Secondary sources of infection are wild boar and bear. Humans get sick after eating meat infested with larvae enclosed in muscle tissue, insufficiently cooked, curd, roast or smoked. The difficulty in establishing the diagnosis is due to the fact that this disease does not manifest itself clinically and does not present pathognomonic signs and the parasite and its larvae are not visible to the naked eye. The main diagnostic method is represented by the trichinelloscopic examination performed by the veterinarian from a sample of meat collected from certain areas, mainly from the diaphragmatic pillars. In humans, the clinical manifestations are represented by fever, diarrhea, muscle pain, facial edema, eye hemorrhages - retinal and subconjunctival and subungual. All organs, meat and by-products obtained from pigs infected with Trichinella spp. are forbidden to be consumed and are destroyed by burning.

Key words: *disease, pig, trichinosis, Trichinella spp.*

INTRODUCTION

Trichinellosis is a serious parasitozoonosis affecting both wild and domestic animals. While in animals this disease evolves subclinically, only microscopic lesions being evident, in humans the evolution is serious, sometimes deadly. The most common species of trichinella that can cause human disease is *Trichinella spiralis*, although other species of *Trichinella* implicated in human disease are: *T. nativa, T. nelson, T. britovi, T. pseudospiralis, T. murelli, T. papuae*. The most important source of human infection worldwide is the domestic pig. In the last three decades, in Europe it has been proven that horse or wild boar meat is also an important reservoir of infection for humans (Mitrea, 2011; Zarlenga et al., 2016).

The biological cycle is of the autoheterogenous type, it is carried out without passing into the external environment. In the case of *Trichinella* spp. parasites, the definitive host is also the intermediate host. Raw or insufficiently cooked meat, which contains trichinella larva, reaches the stomach and is subject to digestion. The digestive enzymes dissolve the capsule, thus releasing the larva that invades the small intestine, sinking into the columnar tissue. The larvae initially migrate through the lymphatic system, then through the blood system, and reach the general circulation, from here in the organs and muscles. Only the larva that have reached the striated muscles will develop further. Larva can survive months to years, sometimes being viable for the entire life of the host animal. The transition to a new host is made by the ingestion of the parasitized muscles by a receptive animal (Mitrea, 2011; Zarlenga et al., 2016; Şuler et al., 2019).

The evolution of the disease includes several stages (Mitrea, 2011; Furhad & Bokhari, 2023), namely:

- an incubation period lasting between 2 and 28 days;

- an asymptomatic period in which the ingested larva mature into adults, in the small intestine;
- an intestinal stage, manifested by diarrhea with or without fever, abdominal pain, anorexia;
- a stage of muscle invasion that captures the migration of the newly formed larva in the circulatory system, towards the striated muscles; at this stage, the predominant symptomatology is represented by myalgias, swelling of the muscle masses, periorbital or facial edema, urticarial eruptions, fever 38-40°C, neuropsychiatric disorders (headache, up to delirium, coma), respiratory disorders, cardiac disorders (myocarditis with tachycardia, arrhythmia);
- a period of convalescence, corresponding to the confinement of the larva in the muscles.

MATERIALS AND METHODS

Bibliographic sources from the specialized literature, relevant to the topic, were used for the creation of this article. The main aspects followed were related to the epidemiology, diagnosis, treatment, control and economic impact of trichinellosis.

RESULTS AND DISCUSSIONS

Epidemiology. Trichinellosis is a disease present on all continents, in humans it is more frequent in North America and Europe and very rare in Asia, Africa and Australia. Around 10,000 human infestations occur worldwide each year. Cultural factors such as traditional meat-based gastronomy or products derived from raw or insufficiently cooked meat have an important role in the epidemiology of the disease (Franssen et al., 2017).

It has a focal character, with two main outbreaks being known (Mitrea, 2011):

- Synanthropic or domestic outbreak: represented by pig, rat, dog, cat, nutria and lately equine.
- The sylvatic outbreak: represented by wild boar, bear, wolf, fox, etc.

The two outbreaks can be interconnected, mainly through rats, which represent the main core of *Trichinella* that ensure the passage of parasitosis to domestic pigs, the latter being the major source of human infestation (Furhad & Bokhari, 2023).

Contamination is carried out orally, by ingesting the muscles with *Trichinella* larvae. In humans, this can be achieved by consuming meat or meat products from infested animals. In animals, the contamination is similar, in addition, it can be achieved by consuming slaughterhouse by-products or corpses containing cysts or infesting larva. It has been proven that the infestation can also be carried out through the placenta - if it occurs during pregnancy or galactogen pec (Ribicich et al., 2007; Şuler et al., 2019).

The favoring factors are primarily represented by the carnivorous diet. In the case of domestic pigs, the infestation occurs as a result of feeding them with raw or improperly processed slaughterhouse remains, as well as the presence of rats in the breeding area (Ribicich et al., 2007; Mitrea, 2011).

Parasite resistance is reduced in the case of adult helminths; males die after fertilization, but females last 5-6 weeks in animals and approximately 4 months in humans. However, the larva show greater resistance, surviving for many years in the muscles of living animals, and *Trichinella* larvae can remain viable for up to 25 years in humans. In bodies and carcasses, the larva can survive for up to 3 weeks, even in rotten conditions, and when boiled, they are highly sensitive. Cold resistance differs depending on the species of *Trichinella, T. nelsoni* being very sensitive, compared to *T. nativa* which can withstand up to 38 months at -18°C in the meat of white bear or *T. spiralis* whose larva were found live in muskrat corpses, preserved for 35 days at -34°C. Larva eliminated through feces last on the soil for only a few days (Ribicich et al., 2007; Mitrea, 2011).

Diagnostic. In living animals, trichinellosis is impossible to diagnose following a clinical examination because this disease has no pathognomonic signs. Serological tests can be performed, in this case leukocytosis and eosinophilia being correlated with the number of nematodes causing the infection. Serology confirms the suspicion of trichinosis infection when anti-Trichinella IgG antibodies are detected. Creatinekinase, lactate dehydrogenase, aldolase and aminotransferases may be elevated due to parasitic invasion of skeletal muscle causing destruction. At the same time, infested animals can present hypokalemia, hypoalbuminemia and increased serum levels of IgE, but all these tests are non-specific, as they can be observed in other parasitic and autoimmune diseases (Muluken et al., 2020).

The postmortem diagnosis is the one that shows the importance in veterinary medicine, being a mandatory examination for food animals. The trichinelloscopic examination and the artificial digestion method are the two tests used to detect the larvae of *Trichinella* spp. (dspcluj.ro, 2024).

Examination of the fields is done field by field in ascending or descending order noting the integrity of the tissue and the areas of expressed meat juice around the compressed fragments (Savu, 2009; Savu et al., 2012).

In case of trichinellosis, fertile cysts with characteristic structure and shape are found in the interfibrillar spaces and even free larva both interfibrillarly and especially in the peripheral juice. Cysts can be captured in different stages such as spiraling, encapsulation, degeneration or calcification. If the result is positive or uncertain, the diagnosis is carried out by artificial digestion, and the examination is repeated, by collecting other samples; the analysis takes place in an authorized and accredited laboratory (Savu et al., 2012; Mitrea, 2011).

The differential diagnosis in animals is made against cysticercosis, echinococcosis, sarcoccystosis, microascariasis or against tyrosine accumulations in muscles, fat drops, lymphatic filariasis, whipworm (Mitrea, 2011).

In humans, trichinosis is diagnosed as follows (dspcluj.ro, 2024; Muluken et al., 2020):

- On the basis of clinical signs, when at least three of the following six symptoms are present: fever, muscle aches and pains, gastrointestinal symptoms, facial edema, eosinophilia, and subconjunctival, subungual, and retinal hemorrhages.
- Following laboratory tests: demonstration of trichinella larva in tissue obtained by muscle biopsy and demonstration of *Trichinella*-specific antibody response by immunofluorescence, ELISA or Western blot.
- Based on the epidemiological investigation: consumption of infested meat or products and by-products from an animal confirmed positive in the laboratory.

The differential diagnosis in humans is done against (Office International des Epizooties, 2004):

- Salmonellosis, shigellosis and other infections, viral, bacterial or parasitic of the gastrointestinal tract.
- Influenza virus infection
- Glomerulonephritis, serum sickness, toxic-allergic reactions to drugs or allergens, polymyositis, periarteritis nodosa, dermatomyositis.
- Typhoid fever.
- Cerebrospinal meningitis, encephalitis, neuroinfections.
- Leptospirosis, bacterial endocarditis and exanthematic typhus.
- Eosinophilia-myalgia syndromes (for example, eosinophilic fasciitis)
- Fasciolosis, toxocariasis and invasive schistosomiasis.

Treatment. In time, both in humans and in animals, different treatment schemes have been tried, the most effective proving to be benzimidazoles, thiazole derivatives and avermectins. Also, three important aspects were observed, namely (Gómez-Morales et al., 2012; Owen & Reid, 2007):

- It has been proven that the best effectiveness, up to 100%, is on the adult forms of *Trichinella* spp., which are very difficult to diagnose or not at all in live animals.
- On the encapsulated larva, the effectiveness of the drugs is variable, the destruction of the cysts being only partial.
- In the larval migration phase, the effectiveness of the drugs remains high, but it is difficult to diagnose.

Figure 1. The arrangement of the meat samples between the blades for the trichinelloscopic examination

Figure 2. Placement under the microscope of slides with meat samples for the trichinelloscopic examination (Own source)

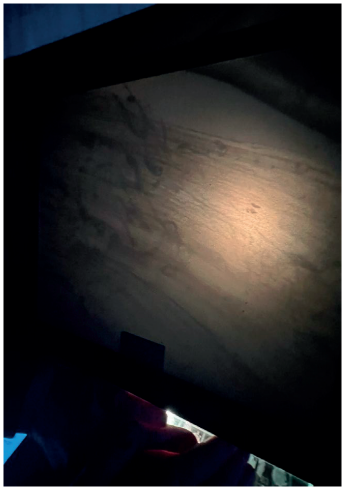

Figure 3. Muscle fiber seen under a microscope (Own source)

The tests carried out showed that increased and repeated doses of anthelmintics are needed, most of the time they are associated with anti-inflammatory and capsulolytic substances. The main anthelmintics used are albendazole and mebendazole, but satisfactory results were also observed following the administration of flubendazole, oxfendazole and avermectin. Albendazole is preferred because in most patients it reaches the required plasma levels, no monitoring is necessary, as opposed to what happens after the administration of mebendazole, whose plasma levels can vary from patient to patient, being continuous monitoring necessary (Gottstein et al., 2009).

It is important to apply an effective and early treatment, especially in the first three days after the infection. A therapy correctly applied and at an optimal time, stops a possible muscle invasion and the development of the disease. Unfortunately, most infected people are diagnosed just a few weeks after infection, when the larva have already settled in the muscles (Gottstein et al., 2009; Owen & Reid, 2007).

The vital prognosis in animals is favorable, the disease being important due to its serious zoonotic aspect, in humans it is a disease with serious symptoms, sometimes even fatal (Gamble, 2022; Office International des Epizooties, 2004).

The control of trichinellosis requires the strict observance of measures such as (Alban & Petersen, 2016; Gamble, 2022):

- The obligation to carry out the trichinelloscopic examination of pork or game meat from animals potentially carrying *Trichinella* spp.
- Confiscation and appropriate processing of carcasses, products and by-products obtained from infested animals.
- Proper sterilization of slaughterhouse products and by-products that are animal feed.
- Disposing of the carcasses of animals that can be the source of *Trichinella* and it is forbidden to feed animals with organs from game animals that have not been thermally treated or treated improperly.
- Encouraging the population to prepare the meat properly, as it is known that smoking, salting or short-term freezing do not destroy the larvae in meat, products and by-products.

In humans, trichinellosis can be controlled in this way (Alban & Petersen, 2016):

- - by exhorting the population to properly heat the meat, as it is known that smoking, salting or short-term freezing do not destroy the larvae in meat, products and by-products;

- carrying out a trichinellosis detection test after slaughtering or hunting;
- raising pigs in hygienic spaces and fighting rodents, which are potentially carriers.

The economic impact of trichinellosis is a significant one considering the costs required for the treatment of both humans and animals, the costs for eviscerating the bodies and for the destruction of the infested meat and products, as well as the potential profit that could be obtained from the sale of meat, products and by-products from meat if they were not affected by *Trichinella* spp. (Cuperlovic et al., 2005).
Between 2016 and 2021, the incidence of human trichinellosis cases was higher in males compared to females (Cuperlovic et al., 2005).

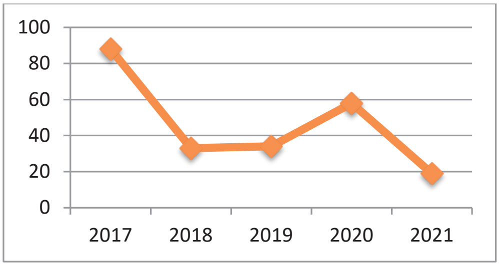

Figure 4. The evolution of human trichinellosis cases in Romania between 2016-2021 (I.N.S.P.)

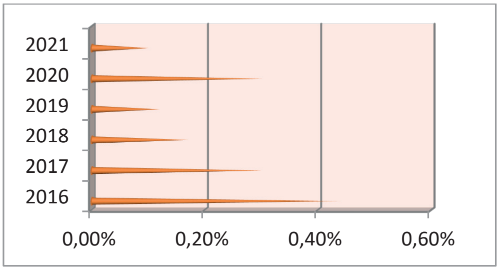

Figure 5. Incidence rate of human trichinellosis cases in Romania during 2016-2021 (I.N.S.P.)

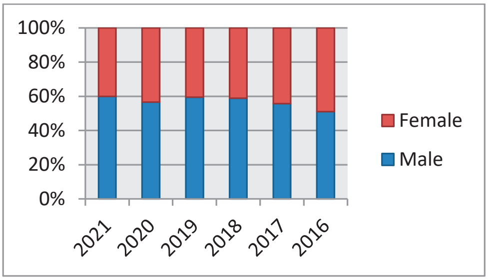

Figure 6. Incidence of human trichinellosis cases according to sex (I.N.S.P.)

CONCLUSIONS

The infection core is represented by domestic animals - pigs, wild or commensal - rats. The larvae of the nematode parasitize the muscles of mammals, and humans can become infected by ingesting raw or insufficiently cooked meat, especially pork, or game - wild boar, bear. When ingested, the muscle larva survive and enter the tissues of the small intestine, where they undergo development to the adult stage.
The disease is not transmitted between humans.
The clinical manifestation of the disease is closely related to the amount of meat ingested, the degree of meat infestation and the body's resistance.
The methods of salting, drying, smoking do not ensure the destruction of the larvae. They can be destroyed if the pork is frozen for almost three weeks at a temperature of at least -15°C. Larvae are also destroyed in the case of thermal processing of meat at a temperature above +70°C.
Romania contributes to European statistics with the most human and animal cases. The cases reported in humans in Romania mainly affect adults
Between 2019 and 2021, in our country no infections with Trichinella spp. were reported in pigs housed in controlled conditions.
In 2021, the proportion of hunted boars that tested positive was 0.07%. The proportion of positive foxes was 1.6% in 2021, higher compared to the rate of 0.9% in 2020.
Trichinellosis in humans can be monitored and controlled through a rigorous reporting and testing system, which requires good interaction between the public health sector and the appropriate veterinary sector.
Trichinellosis has been declared a disease to be monitored, and for European Union member states, this is mandatory under the European Union Zoonoses Directive, 2003/99/EC.

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ANALYSIS OF THE MORPHO-PRODUCTIVE PARAMETERS OF R1 SHEEP RESULTING FROM THE CROSSING OF LOCAL SHEEP FROM THE NORTH-EASTERN AREA OF ROMANIA WITH AWASSI BREED

Ion RĂDUCUȚĂ[1], Costică CRISTIAN[2], Vlăduț Dragoș BULMAGA[2], Andra-Georgiana PĂUN[2], Ion CĂLIN[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]Research and Development Station for Sheep and Goats Breeding Secuieni - Bacău, 54 Holt village, Letea Veche, Bacău, Romania

Corresponding author email: raducion@yahoo.com

Abstract

The aim of the present work was to analyse the morpho-productive parameters of the R1 sheep resulting from the crossing of the local sheep from the north-eastern area of the country with the Awassi breed. The results showed that compared to the milk production of Tsurcana breed during the milking period (120 days), the milked milk production of R1 sheep in the first lactation is higher by 17.30 kg, the differences being very significant (P<0.001). Following the analysis of the external morphological characteristics of the R1 sheep, it emerged that the only character specific to the Awassi breed that was transmitted in a higher percentage is the long and drooping ears (36.7%), the other characters being transmitted in a reduced percentage (3.3-16.7%). The reproductive indices showed the highest values in the case of R1 sheep (compared to the Tsurcana breed, the prolificacy is 28.6% higher, and the numerical productivity index is 41.4% higher). All these results recommend the improvement of local sheep from the north-eastern area of our country through crossbreeding with the Awassi breed up to the second generation of absorption.

Key words*: Awassi breed, crossing, local sheep, milk production, morpho-productive parameters, reproductive indices.*

INTRODUCTION

In the Nord-East region of Romania, most sheep breeders raise sheep from non-improved local breeds, the prevalent system of sheep maintenance is the extensive system and there is no improvement program for production (Popescu, 2020). As a result, the productive and economic performances achieved by these sheep holdings are lower compared to those with intensive and semi-intensive systems of sheep maintenance (Pădeanu & Voia, 2010; Pascal, 2015; NIS, 2024).

Considering those previously mentioned, as well as the interest shown by sheep breeders in the recent period for improving production and implicitly increasing the profitability of these holdings, we proposed to improve milk production by crossing sheep from the north-eastern area of the country with rams from the Awassi breed.

This breed is one of the sheep dairy breeds that was imported to our country in the 80s and proved to have a good adaptability to the extensive exploitation system practiced in the north-eastern area of Romania (Taftă and Răducuță, 1995; Taftă et al., 1997).

The work aims to increase the milk production of native sheep from the north-eastern area of the country, where the reproductive activity was carried out uncontrolled, without a pre-established improvement program, by absorbing crosses of sheep from local breeds with purebred rams Awassi to increase productivity in sheep farms.

MATERIALS AND METHODS

Absorption crosses of local ewes with Awassi rams began in autumn 2018, and during 2023 the R1 crossbred females were in first calving.

In this work, the following determinations were made: evaluation of reproductive parameters and productive performance in the first lactation of R1 females, evaluation of body development of R2 lambs and evaluation of the degree of similarity between R1 products and the Awassi breeding breed.

The working methods used were appropriate for objectives of this kind.

To determine the reproductive parameters of the R1 products, the classic formulas for calculating the reproductive indices existing in the specialized literature (Taftă et al., 1997) were used, the following reproductive indices were calculated: fertility, fecundity, prolificacy, birth rate, lamb mortality, the numerical productivity index, the percentage of abortions and the percentage of lambs weaned.

To determine the total milk production, the control of milk production included the nursing period of the lambs and the milking period of the ewes. The amount of milk during the lactation period was estimated by the method of valorisation of the suckled milk of the lamb, respectively by the weight gain achieved, using the transformation coefficient method.

The amount of milk milked was evaluated by applying successive productive controls (n = 4 controls), at intervals of 30 days. At each control interval, the standard method was used, namely AT4 respecting the technical specifications recommended by the International Committee for Animal Recording (ICAR, 2018).

In order *to evaluate the degree of similarity* between the R1 products and the Awassi breeding breed, a productive and phenotypic analysis of the R1 products was carried out, a comparison was made based on milked milk production, as well as an analysis on a number of 30 adult individuals of female sex of the mode of transmission to R1 crossbred of the main external characteristics specific to the Awassi breed.

Statistical analysis. For the statistical processing of the data, we utilized Microsoft Office Excel 2016. The statistical parameters computed included mean, standard deviation, standard error of the mean, and coefficient of variation. The Student's t-test was employed to assess differences between means, with a significance level set at 0.05.

RESULTS AND DISCUSSIONS

The *evaluation of reproductive indices* in R1 females represented a first objective of our research. The information necessary to calculate the following reproduction indices was extracted from the data entered in the lambing and calving register. Reproduction indices were calculated separately for all existing genotypes within the analysed farm (Tsurcana breed, Awassi breed, F1 crossbred and R1 crossbred) and an average of them for the total farm (Table 1).

Table 1. Reproductive indices at all existing genotypes within the analysed farm (%)

Specification	F1	R1	Tsurcana	Awassi	Farm average
Number of individuals (heads)	45	52	134	170	401
Fertility	95.6	100.0	97.0	98.8	98
Fecundity	97.7	100.0	98.5	99.4	98.9
Prolificacy	134.9	140.4	109.2	110.1	116.5
Birth rate	128.9	140.4	105.9	108.8	114.2
Lamb mortality	0	0	6.3	6.5	4.6
Numerical productivity	128.9	140.4	99.3	101.8	108.9
Percentage of abortions	2.3	0	1.5	0.6	1.0
Percentage of lambs weaned	111.6	140.4	102.3	102.9	111.2

From the analysis of the reproductive indices data, it can be seen that the average percentage of fertility per holding was 98%, and within the genotypes the highest value was recorded by the R1 crossbred, which reached the maximum value of 100%. The same finding is valid for the fertility index. As for the prolificacy index,

it had an average value per holding of 116.5%, the highest value being registered by the R1 crossbred sheep (140.4%), and the lowest value being registered by the breed sheep Tsurcana (109.2%). The same finding, noted for prolificacy, is also true for the birth rate. Regarding the percentage of lamb mortality, it can be noted that at the level of the entire farm it had a value of 4.6%. It should be noted that in the R1 and F1 crossbred no deaths were recorded in the birth-weaning interval, while for the Tsurcana breed and the Awassi breed it had a value of 6.3% and 6.5%, respectively.

The percentage of numerical productivity, which represents the percentage ratio between the number of lambs and the mother herd, is on average per farm 108.9%, the highest values being recorded by the F1 (128.9%) and R1 (140.4 %), and the lowest value being recorded by sheep from the Tsurcana breed (99.3%).

Referring to the percentage of abortions, it can be noted that at the level of the entire holding it had a value of 1.0%. It should be noted that no abortions were recorded in the R1 crossbred ewes, while the F1 crossbred ewes had an abortion percentage of 2.3%. Finally, the highest percentage of weaned lambs is recorded in the R1 crossbred group (140.4%), and the lowest in the Tsurcana breed sheep group (102.3%).

Evaluation of quantitative milk production

The total amount of milk consumed by the lambs during the lactation period was determined after statistical processing of the values obtained from the control weighing of the lambs (25 lambs/batch) at the end of the weaning period and during lactation (Table 2).

From the obtained data it follows that Awassi sheep have a 5.7% higher lactation capacity compared to R1 crossbred sheep (60.60±1.143 kg versus 57.33±0.171 kg), but the difference is insignificant (P>0.05) (Table 3).

Although the R2 lambs had at the time of weaning an average body weight close to that of the Awassi lambs, however, the Awassi ewes have a 5.7% higher lactogenic potential than the ewes in the first lactation R1 crossbred during the nursing period of the lambs (P>0.05).

In conclusion, Awassi lambs benefited from a higher amount of mother's milk and thus recorded a more intense rate in terms of the total gain accumulated over the birth-weaning interval (Pascal et al., 2023).

Table 2. Evolution of body weight in lambs during the suckling period (n = 25 heads/lot) (kg)

Specification	Batch of crossbred lambs R2				Batch of Awassi lambs			
	MEAN±SEM	CV%	Min.	Max.	MEAN±SEM	CV%	Min.	Max.
Weight at birth	4.12±0.081	13.89	2.90	5.00	4.34±0.074	11.99	3.60	5.30
Weight at 28 days	10.11±0.125	8.70	8.47	12.36	10.76±0.150	9.78	8.30	12.80
ADG* 0- 28 days	0.200±0.005	17.17	0.120	0.250	0.214±0.005	16.23	0.137	0.280
Weight at 60 days	15.53±0.228	10.38	12.30	18.90	17.00±0.237	9.84	15.20	20.80
ADG 28- 60 days	0.181±0.009	37.12	0.071	0.315	0.187±0.008	30.26	0.099	0.375

Table 3. Milk production obtained during suckling period (60 days) (n = 50 females and 25 de lambs/batch) (kg)

Genotype	The milk quantity during suckling period					
	0-28 days		28-60 days		Total suckling period	
	MEAN±SEM	CV%	MEAN±SEM	CV%	MEAN±SEM	CV%
Batch of R1 ewes	32.95±0.800	17.17	24.37±1.280	37.12	57.33±1.171	14.45
Batch of Awassi ewes	35.33±0.811	16.23	25.28±1.082	30.27	60.60±1.143	13.33

To determine milk production during the milking period, the AT4 estimation method was used, performing 4 controls, carried out at 30-day intervals. The average daily milk production on the 4 controls for the R1 crossbred group was 649.38±37.03 g with limits between 378.88 and 934.68 g of milk, and for the Awassi breed group of 656.20±23.36 g with limits between 285.00 and 1,197.05 g of milk. The average level of daily milk production for Awassi breed ewes was about 1.05% higher than that of crossbred R1 ewes (Table 4). Differences between controls, including average daily milk production during the milking period, were not significant. The average daily milk production of the ewes from the Awassi group on the 4 controls was 1.05% higher than that of the ewes from the crossbred R1 group, but the differences were insignificant (P>0.05).

The average level of milk production during the milking period for Awassi ewes was 0.57 kg lower than R1 crossbred ewes. It can thus be said that the manifestation of the heterosis effect led to an increase in the milk production of

crossbred R1 sheep, the productive level being similar to that of the Awassi breed.
During the entire lactation period (180 days) R1 crossbred ewes recorded a total milk production of 128.87 kg, which is 2.09% lower than that of Awassi sheep (131.57 kg) (Table 5).

Table 4. Average daily milk production during the milking period (4 checks) (n = 100 ewes/batch) (g)

Specification	Batch of R1 ewes			Batch of Awassi ewes	
	n	MEAN±SEM	CV%	MEAN±SEM	CV%
Check I	50	934.68±37.44	28.32	979.00±27.04	27.04
Check II	50	739.28±37.55	35.92	792.00±31.34	27.98
Check III	50	544.68±37.53	48.72	523.00±24.80	33.53
Check IV	50	378.88±36.40	67.93	330.80±22.53	48.17
Average daily milk production	50	649.38±37.03	40.33	656.20±23.36	25.17

Table 5. Milk production during the milking period (120 days) and during the lactation period (180 days) (n = 50 heads/batch) (kg)

Specification	Batch of R1 ewes			Batch of Awassi ewes	
	n	MEAN±SEM	CV%	MEAN±SEM	CV%
Check I	50	22.43±0.900	28.32	23.50±0.650	19.53
Check II	50	16.26±0.830	35.92	17.42±0.690	27.98
Check III	50	15.80±1.090	48.72	15.17±0.720	33.53
Check IV	50	17.05±1.640	67.93	14.89±1.010	48.17
Total milking milk	50	71.54±4.420	43.73	70.97±2.670	26.56
Total milk (suckling milk + milking milk)	50	128.87		131.57	

In order to evaluate the degree of similarity between the R1 products and the Awassi breeding breed, a productive and morphological analysis of the R1 products was carried out, a comparison was made based on the ratio of milked milk production, as well as an analysis on a number of 30 adult individuals of female sex of the mode of transmission to R1 crossbred of the main external characteristics specific to the Awassi breed. Compared to the milk production of Tsurcana breed during the milking period, the milk production of R1 crossbred females during the first lactation during the milking period (120 days) is higher by 17.30 kg, even in the drought conditions manifested during the period of grazed during the year 2023 (Table 6). The differences are significant (P<0.05), a fact that shows the beneficial influence of the backcrossing of F1 crossbred females with Awassi rams on the milk production of R1 crossbred.

Table 6. The significance of the differences between genotypes in milked milk production

Batches	Batch of Tsurcana 54.24 kg	Batch of Awassi 70.98 kg
Batch of R1 71.54 kg	17.30*	0.56^{NS}
Batch of Awassi 70.98 kg	16.74*	-
Batch of Tsurcana 54.24 kg	-	-

NS - non-significant differences (P>0.05); *significant differences (P<0.05); **distinctly significant differences (P<0.01); ***highly significant differences (P<0.001).

Table 7 presents the analysis of the way of transmission to R1 crossbred of the main external characteristics specific to the Awassi breed. Thus, 8 external characteristics specific to the Awassi breed were considered and 30 female R1 crossbreds were evaluated to see their degree of morphological similarity with the Awassi breed. Following the analysis of the external characteristics of the R1 crossbred, it emerged that 13.3% have a body narrow and long format, 13.3% have a ram-shaped head profile, 36.7% have long and drooping ears, 10% have an ascending spine line towards rump, 6.7% have a bevelled rump, 3.3% have a fat deposit on the tail and 16.7% have a brown colour of hair on face.

Table 7. Morphological similarity analysis of R1 crossbred with the Awassi breed (on-farm analysis)

Specification	R1 crossbred	
	Number of individuals	%
Body format (narrow and long)	4	13.3
Ram-shaped head profile	4	13.3
Long and drooping ears	11	36.7
Ascending spine line towards rump	3	10.0
Bevelled rump	2	6.7
Fat deposit on the tail	1	3.3
Brown colour of hair on face	5	16.7
Total	30	100

CONCLUSIONS

The reproductive indices show the highest values in the case of R1 crossbred sheep (compared to the Tsurcana breed, the prolificacy is 28.6% higher, and the numerical productivity index is 41.4% higher), a fact that recommends improving the sheep by crossing of the Tsurcana breed with Awassi rams up to the second generation of absorption.

Compared to the milk production of Tsurcana sheep during the milking period, the milk production of R1 crossbred females during the first lactation of the milking period (120 days) is higher by 17.30 kg, the differences being significant ($P<0.05$), fact that show the beneficial influence of backcrossing F1 females with Awassi rams. In terms of milk production, the obtained R1 crossbred represents a different population than the Tsurcana breed, being significantly more productive.

Following the analysis of the external characteristics of the R1 crossbred, it emerged that the only character specific to the Awassi breed that was transmitted in a higher percentage is the long and hanging ears (36.7%), the other characters being transmitted in a much lower percentage (3.3-16.7%), from which the conclusion emerges that R1 crossbred have external morphological characteristics closer to the Tsurcana breed.

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THE INFLUENCE OF MANAGEMENT PRACTICES ON MILK QUALITY IN A DAIRY CATTLE FARM

Catalina SANDULEANU[1], Andra-Sabina NECULAI-VĂLEANU[2], Aida ALBU[1], Roxana Nicoleta RAȚU[1], Marius Giorgi USTUROI[1], Vasile VINTILĂ[2], Vasile MACIUC[1]

[1]"Ion Ionescu de la Brad" Iasi University of Life Sciences, 3 Mihail Sadoveanu Alley, 700490, Iasi, Romania
[2]Research and Development Station for Cattle Breeding Dancu, 9 Iasi-Ungheni Street, 707252, Iasi, Romania

Corresponding author email: vmaciuc@yahoo.fr

Abstract

In order to provide an up-to-date perspective on farm management techniques, a survey was conducted in a dairy cattle farm to study the relationships between management practices, milk yield and quality. A number of 310 Holstein Friesian cows reared for milk production in a semintensive farming condition were taken into study. Over the course of a year, individual milk analyses were performed on CombiScope FTIR milk analyzer (Delta Instruments, Netherlands). Milk yield per milking session was recorded daily using the AfiMilk system (Kibbutz, Israel). The analysis and interpretation of the results were correlated with the numerous observations made directly on the farm. The average calculated content, per normal lactation, was 8887.79 kg of milk, 369.05 kg fat, and 306.06 kg protein. A strong, positive relationship between milk yield and fat content, which means that an increase or decrease in milk production results in a corresponding change in the amount of fat contained. The yearly average percent of fat was 3.99 %, protein 3.32 %, casein 27.75 %, lactose 4.9 %, SCC 195,900 cells/ml were obtained in the studied farm.

Key words: *dairy cattle, management analysis, milk quality, welfare.*

INTRODUCTION

The global dairy industry is undergoing rapid transformations, which have created substantial commercial opportunities, as well as several sustainability challenges. The most valued agricultural resource in the world in terms of value is milk along with other dairy products (Wilcox et al., 2017; De Vries et al. 2015).

Milk quality in dairy cattle farms is of utmost importance for both the health of consumers and the economic viability of the farm. By implementing best practices in cattle care, milking hygiene, and milk handling, dairy farmers can ensure the production of high-quality milk that meets consumer demands and industry standards (Galanakis, 2018).

Milk production is essential to enriching diets around the world and provides jobs for billions of people (Garcia-Yuste et al., 2020; Ritter et al., 2023).

Management practices play a critical role in influencing the quality of milk produced on dairy cattle farms. A major role in achieving positive results in the dairy farm is played by the farm manager. The manager plans, controls, and coordinates the activity, ensuring that the farm is managed in compliance with the requirements of the legislation, the established objectives, interests and strategies of the farm (Maltz et al., 2020; Scialabba, 2021).

Dairy farms will have to be in harmony with the environment, and with the community, provide appropriate conditions for the maintenance and exploitation of animals, and at the same time be efficient and economically competitive (Webster, 2020; Amaritii & Maciuc, 2023).

Rearing of dairy cows includes all the technical and organizational measures that take place within the farm, such as cattle body hygiene, housing, technological flow, and cow movement (Berckmans, 2022). All of these measures influence both milk production and the productive lifespan of the animals, reproductive capacity, state of health, behavior of cows, the degree of feed usage, but also the economy of the farm (Bilțiu Dăncuș et al., 2022; Nica & Vidu, 2023).

Depending on the rearing conditions ensured, the qualitative and quantitative production of milk also differs (Goyal, 2023; Rațu et al., 2023). Thus, the farmer must know how the housing conditions can act positively on the production of the cows and use these to his advantage, both for economic purposes and for the dairy cows to express their true genetic potential (Butler et al., 2011; Enea et al., 2023)

The current activity objective is to ensure the stable and sustainable development of the capacity for scientific research, technological development and innovation in the field of rearing and improving dairy cows (Mondal, 2021).

MATERIALS AND METHODS

The research was conducted in the experimental dairy farm of the Research and Development Center for Cattle Breeding Dancu, Iași, on Holstein Friesian dairy cows, primiparous and multiparous.

The experimental dairy farm has as biological material for research a total herd of 791 bovines of the following breeds: 635 Holstein Friesian, of which 315 cows, 72 heifers 248 young cattle, 104 Fleckvieh Simmental of which 47 cows, 8 Heifers, 49 young cattle, and 52 Grey Steppe, 31 cows, 3 heifers, 18 young cattle.

To evaluate associations between management and milk quality with some confidence, we analyzed the technological flow, recorded the individual and total productions, and determined the milk quality during the year 2022.

The individual and total milk production of the Holstein Friesian cows was registered daily in the farm management program Afimilk (Kibbutz, Israel).

The analysis of milk quality was determined on a Combiscope FTIR 600HP milk analyzer, from Delta Instruments (Netherlands), the chemical composition on LactoScope FTIR, and the count of somatic cells on SomaScope LED & flow cytometry. A 50 ml milk sample was heated in a water bath at 37°C and then analyzed for fat, protein, lactose, casein, and SCC count.

The obtained data was analyzed for primary statistical indicators and then proceeded to the statistical significance of the differences, with SPSS and S.A.V.C. programs.

Figure 1. Afimilk Management Software and milk analyzer CombiScope 600HP (original)

RESULTS AND DISCUSSIONS

The activity within the agricultural and livestock farms is under the coordination of the technical director, each farm is conducted by a farm manager, a graduate of higher specialized studies, who is responsible for the achievement of technical and economic indicators.

In the dairy farm, the working schedule is established so that the activities, duration, and sequence are consistent with the biological requirements of the reared cows, thus favoring the maintenance of conditioned reflexes.

Within the livestock farm there are 2 shelters for lactating cows (1,3), between the two shelters is the milking platform (2), shelter for pregnant cows/heifers in advanced stage of pregnancy (4), individual stalls for calves 0-3 months (5), shelter for calves 3-6 months (6), calf shelter 6-12 months (7), heifer shelter (8), area for combined feeds (12), silage cells (11), covered area for straw bales (10), modern manure treatment system (9), summer camp (13), mechanization (14).

To be executed promptly the activities included in the work schedule are carried out on the principle of specialized teams in the intervals 4 – 10 am and 4 – 7 pm (milking, feeding, treatments, artificial insemination, manure removal, cleaning, etc.), reserving the intervals

between 10 am – 4 pm and 7 pm – 4 am for the animals' physiological rest, necessary for rumination, ruminal digestion and milk secretion.
A dairy cattle farm operates on a daily and yearly schedule to ensure the well-being of the cows and the successful production of milk. Compliance with the daily work schedule on the farm results in the creation of conditioned reflexes in the cows, which would otherwise no longer occur. The immediate and long-term effect of not following the program on the farm will be decreased milk production.

Figure 2 The organizational structure of the studied dairy farm (original)

The use of mechanization and automation in the farm means reduced physical effort of the worker and increased labor productivity. Utilizing technological advancements like automated milking systems, data analysis tools, and precision feeding systems can enhance efficiency and improve decision-making (Berckmans, 2022; Wilcox et al., 2017).
The shelters within the farm are closed, equipped with natural and artificial ventilation systems, with automatic brushes for bodily hygiene, and electrically operated. The shelters are provided with a central feeding lane. Access to the feed is achieved through a mechanical grid that individualizes the feed front. During feeding, cows can be restrained using a front feed locking system for administration of hormonal and medicinal treatments, and artificial insemination. A continuous circulation area with a concrete floor is placed between the feeding alley and the rest area. Automatic water bowls are located between the rest areas, on the access aisles, and connected to the public water supply network, with heating to prevent freezing during winter. Providing clean, comfortable, and well-ventilated housing minimizes stress and reduces the risk of disease, impacting milk quality (Maltz et al., 2020).
According to Garcia-Yuste (2020), drinking water on time and in sufficient quantities, at a temperature of 8-12°C, at the smallest possible intervals, ensures 87-88% of the milk content in the water. Efficient water usage for cleaning, irrigation, and animal consumption reduces environmental impact and lowers costs.
Manure evacuation is carried out automatically, with electrically powered scraper plows and sensors for their automatic stop, thus maintaining a high degree of hygiene, reflected in the well-being and health of the animals, but also in the hygienic quality of the milk. The manure ends up in a modern separation system,

which separates the solid part from the liquid part, through decantation and extrusion, thus reducing its volume, and making it easier to store and use in the field. Implementing proper manure storage and handling practices minimizes environmental impact by converting it into fertilizer.

Induction of genetic progress is mainly achieved by using semen from tested Holstein sires, without excluding the other sources of progress - the selection of primiparous cows, respectively the selective reform. Several studies showed that artificial insemination is the most common breeding method on dairy farms and high-quality semen from genetically superior bulls is used to optimize herd performance (Presicce, 2020), also relevant for Romanian dairy farms.

Efficient reproductive management directly impacts the profitability of a dairy cattle farm According to De Vries et al. (2020) reproductive problems are the primary reason for culling cows from the herd. Good reproductive practices help keep culling rates low. Cows that maintain consistent calving intervals contribute to steady milk production levels for the farm. Effective reproduction management allows farmers to selectively breed their best cows, improving the herd's overall genetics over time (Rushen et al., 2017). In the studied dairy farm, the reproduction is organized on the principle of different calving times, which allows for rhythmic milk production throughout the year. Artificial insemination is performed by doctors and veterinary technicians. Correct semen handling and insemination technique by trained personnel are crucial for conception success.

The institution organizes professional qualification courses for employees, both for those with higher and lower education. Educating farm staff on practices like milking hygiene protocols, cow handling, and udder health monitoring ensures consistent implementation and reduces the risk of human error impacting milk quality.

Table 1. Statistics for milk production, by normal lactation, for the Holstein Friesian cattle herd

Parameter	$\overline{X}$	$\pm s_{\bar{x}}$	s	V%	Minimum	Maximum
Milk kg	8887.73	136.734	1304.991	23.684	4263	14527
Fat kg	369.05	5.654	87.041	23.585	175	597
Fat %	4.16	0.01	0.16	3.853	3.52	4.63
Protein kg	306.06	4.7	72.353	23.64	144	477
Protein %	3.44	0.007	0.115	3.344	3.07	3.92

The values of the statistical estimators for milk production calculated for the entire herd are presented in Table 1, where it can be seen that the average production per lactation in the herd is approx. 8888 kg of milk, with 4.16% fat and 3.44% protein, which means very good milk production and quality.

The limits of the variation range are 4263 kg and 14527 kg of milk per normal lactation, with cows that have a percentage of fat in their milk that reaches 4.63%. The amount of fat and protein is dependent on milk production, obtaining an average production of 369 kg of fat and 306 kg of protein. For fat and protein percent, the herd is characterized by low variability, being homogeneous for these characters. (3.85% and 3.34% respectively).

Similar results were obtained by Maciuc (2017), on Holstein Friesian, in analyzing the quality of raw milk for processing.

The analysis of the statistical values calculated monthly for the milk quality indicators in the studied farm indicates that the milk has on average a higher percentage content in the autumn and winter months, the highest average being 4.26% fat in February, this being the highest average value of the year. During the summer months, the fat percentage remains constant, with average values between 3.91% in August and 4.01% in June. The protein percentage is also higher in the winter months, the maximum average value of the protein percentage being that of February of 3.55% and that of casein at 2.85% in November.

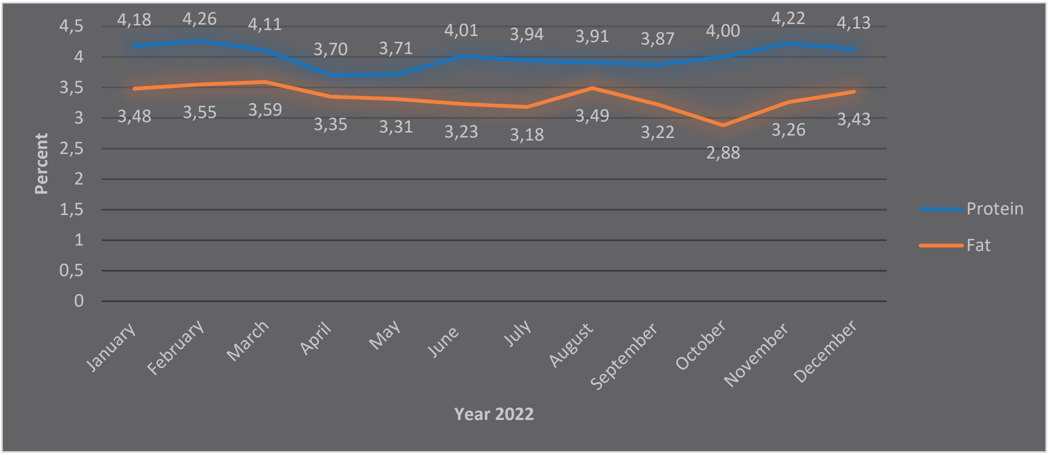

Figure 3. The dynamics of fat and protein average percentage, monthly in year 2022

The herd of dairy cows is characterized by low variability for the characters related to the percentage of fat and protein in milk (values for the coefficient of variation are below 10%), but the same cannot be said when we refer to NCS, in which case the cow population is extremely heterogeneous. The highest SCC averages are recorded during the summer and autumn months, with the maximum average value of 332.000 cells/ml being in August and the lowest in April, its average being 89.670 cells/ml (Figure 4).

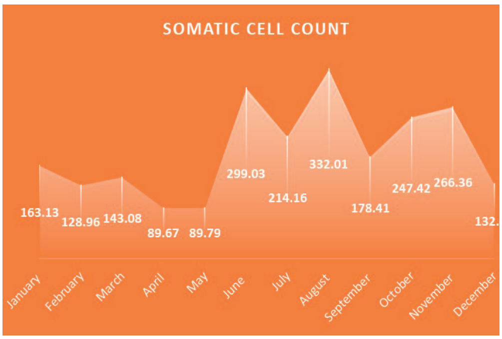

Figure 4. The dynamics of somatic cell count average percentage, monthly in the year 2022

Management practices such as thoroughly cleaning the udder and teats with warm water and a disinfectant before milking reduce bacterial contamination, dipping teats in a disinfectant solution after milking helps prevent the entry of bacteria into the teat canal, and. proper cleaning and maintenance of milking machines and equipment are essential to prevent the spread of bacteria and ensure efficient milking.

Represented in Figure 5 is a Pearson correlation between the amount of fat and milk. The orientation of the regression line and the arrangement of the points on the line denote a positive and strong correlation between milk production and the amount of milk fat, which means that an increase or decrease in milk production results in a change in the same sense of the amount of fat contained.

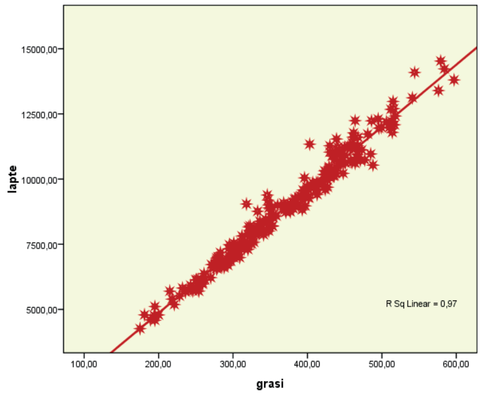

Figure 5. The regression line for the quantity of Milk kg and quantity of Fat kg

In order to statistically characterize the quality indicators of milk for the cattle herd, recorded in 2022, according to the values presented in Figure 6, we can say that milk production in the herd is very qualitative considering the average values of the fat percentage which is approximately 4%, that of protein which on average is 3.32% and that of casein 27.75%.

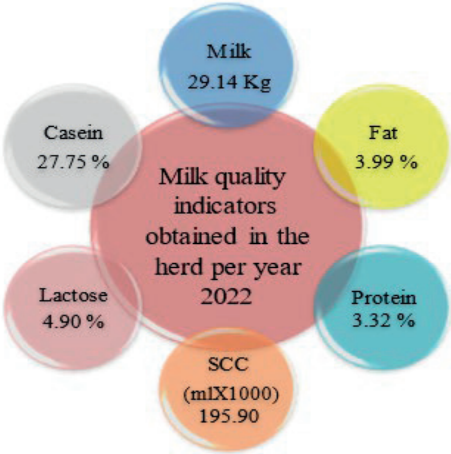

Figure 6. Statistics for the quality indicators of milk, year 2022, in the studied cattle herd

Lactose is 4.9%, a percentage value slightly above the optimal value, and SCC has the average value in the herd of 196.000 cells/ml, below the maximum allowed limit. To characterize the population in terms of the variability of the parameters, we can say that it is homogeneous for % casein, it is medium homogeneous for % fat, % protein, and % lactose being very heterogeneous in for SCC.
Monitoring milk quality parameters and cow health data allows for early detection of potential issues and timely interventions.

CONCLUSIONS

This study investigated the management practices employed at a dairy farm rearing Holstein Friesian cows and analyzed their impact on milk quality. The findings demonstrate that a well-structured management system incorporating best practices contributes significantly to achieving high-quality milk production.
The farm implements a regimented daily schedule for activities such as milking, feeding, and cleaning, ensuring consistency and minimizing stress on the cows. Housing provides a clean, comfortable, and well-ventilated environment with automatic manure removal systems, promoting animal well-being and hygiene. The farm utilizes modern milking equipment and prioritizes staff training on milking hygiene protocols. Effective reproductive management practices, including artificial insemination with high-quality semen, contribute to herd improvement and sustained milk production.
The study revealed an average milk production of 8888 kg per lactation with a fat content of 4.16% and protein content of 3.44%, indicating good overall milk quality.
Milk fat and protein content exhibited low variability, demonstrating herd homogeneity for these traits.
Somatic cell count (SCC) displayed higher values during summer and autumn, suggesting a potential area for improvement in udder health management during these seasons.
This study emphasizes the importance of comprehensive farm management practices for achieving consistent milk quality. By adhering to best practices in areas like cow care, milking hygiene, and data-driven decision-making, dairy farms can ensure the production of high-quality milk that meets consumer demands and industry standards.
Further research could explore targeted strategies for lowering SCC during the summer and autumn seasons to optimize overall milk quality.

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RESEARCH ON CHARACTERS CONSERVATION IN MOUNTAIN ECOTYPE QUEEN BEES

Cristina SURLEA (STOICA-SURLEA)[1], Cristina MATEESCU[2], Georgeta DINITA[1], Iuliana CRINGANU[1], Horia GROSU[1], Carmen Georgeta NICOLAE[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]Apimondia International Federation of Beekeepers` Associations, Corso Vittorio Emanuele 101, Rome, Italy

Corresponding author email: cristina.surlea@apia.org.ro

Abstract

One of the objectives of selection programs in bees is the conservation of the gene pool. The mother-daughter replacement induces a decrease in the rate of loss of sex alleles and the viability of the offspring compared to the population in which the replacement is random. The experiment was carried out in the 2022-2023 bee season. Two queen-breeding colonies and ten drone-breeding colonies were selected. Ten queens were reared from each queen rearing colony. From each drone breeding family, the semen obtained from ten mature drones was collected and homogenized. Each queen was inseminated with the semen obtained by homogenization. Queens were introduced into orphaned colonies of equal strength. In the bees resulting from these colonies, morphological and behavioural characters were measured and compared with those of the parental population. According to the statistical results, the difference between the average of the studied characters of the parental population and the descendant population was very significant.

Key words*: breeding, cubital index, homogenized semen, morphometry.*

INTRODUCTION

Within the bee breeding program, emphasis is placed on outlining taxonomic characters valid for bee appreciation at different stages, quantifying the main preselection criterion and the other primary and secondary criteria (Cebotari, 2006; Cebotari & Buzu, 2011; https://www.anarz.eu).

The correct application of the method of breed improvement, formation of hybrids depends on the and economic conditions of the territory (Alpatov, 1929; 1948).

The taxonomic characters preferred by Linnaean systematics are the morphological ones because they are easily visible, are less influenced by the environment, have a higher heritability.

Modern taxonomy also accepts characters according to the degree to which certain populations can be differentiated (Ruttner, 1975; Ruttner et al., 1978).

Ruttner (1986) has analysed and categorized honeybees from all over the world based on morphometric characteristics in: African races of south of the Sahara; West Mediterranean races, and East rases.

Morphological traits of honey bees can be quantified to describe honey bee populations (Louveaux, 1969a; 1969b). To profile honey bee populations, a common approach involves gathering random samples of honey bee workers from various hives and locations (Abou-Shaara et al., 2012).

The study of bee breeds based on morphometry has remained extremely important until today, reflecting also the presence and absence of isolation because in some areas the local bee is hybridized due both to frequent import phenomena of other breeds, but especially to periodic unguided migrations.

This morphometric study is motivated by the need to know and recognize the mountain bee according to measurable criteria, a starting point for its conservation.

MATERIALS AND METHODS

The experiment was conducted during the 2022-2023 beekeeping season. Two queen bee breeding colonies and ten drone breeding colonies were selected from an apiary considered representative of the mountain population. From each queen bee breeding colony, ten queen bees were bred. From each drones breeding colony, semen was collected from a number of ten mature drones. The semen collected was homogenized. Each queen bee was artificially inseminated (Cebotari & Buzu, 2021), with semen obtained by homogenization. The queens were introduced into orphaned colonies of equal strength. In bees resulting from these colonies, morphological and behavioural characters/traits were measured and compared with those in the parental population, to check the degree of variability between parents and offspring (Cornuet et al., 1975). The morphological characters: proboscis (trunk) length, upper wing length, tergite-3 length, and cubital index were individually measured.

The observed behavioural characteristics were: gentleness, quiet behaviour on honeycombs, anecbalia (lack of swarming), honey capping (relative appreciation), degree of nest blockage (% area) (Oztokmak et al., 2023).

The measurements were carried out on ten bees from each colony that received an artificially mated queen (were an artificially mated queen was introduced).

Bee sampling

Bee samples for morphometric examination were taken from the hive and not from bee entrance. Adult bees were generally taken from the last frames with honey. After harvesting, they were sacrificed, and then put in boiling water to soften the chitinous plates (Chauvin, 1968). The bees were dissected using tweezers, scalpels and very fine scissors. After detachment, the chitinous pieces on which the measurements were made were degreased, cleaned and stretched on microscopic slides. Each slide contained 20 pieces. Measurements were made using a microscope and stereoscopic magnifying glass provided with an ocular micrometre.

Morphological measurements and indicators

The *cubital index* (%) - is the ratio of two segments of the wing ribs in bees. This index is used in the analysis of anatomical shape and structure, being a method of distinguishing species and subspecies from living organisms. The pattern of the ribs of the forewing is peculiar to each breed of bees. The measurements were made on the two ribs that form an obtuse angle with the base of the 3rd cubital cell. 40x magnification is used. The cubital index is the ratio of A/B (Figure 1) (Ruttner, 1986).

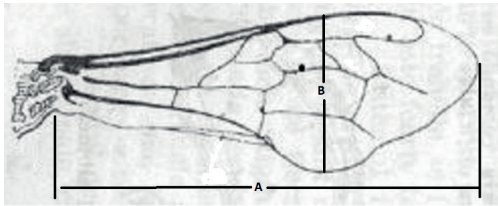

Figure 1. Upper wing width length in bees (after Ruttner, 1986)

Proboscis length (trunk) (mm) - measurement was made from the extremity of the prementum to the extremity of the gloss and the spoon (la bellum). The measurements were made with a magnification of 16x (Ruttner, 1986).

Upper wing length (mm) - the length of the forewing was measured with a magnification of 16x (Ruttner, 1986).

Tergit-3 length (mm) - was measured to establish the degree of hybridization of a bee population. It is transmitted hereditarily to the lineage, being a character with high heritability (0.7-0.8) (Ruttner, 1986).

The measurement of behavioural indices was achieved by awarding points for the characters as gentleness, quiet behavior on the honeycombs and anecbalia, indicating the type of ticking (wet dry or mixed), and percentage for the degree of blocking of the nest, the bee colonies from which the samples originated (Ruttner, 1975; Ruttner et al., 1978) (Table 1).

Table 1. Score for establishing Behavioural indices

Crt. No.	Behavioural aspect	Unit of measurement
1.	Gentleness	Max. 4 points
2.	Quiet behavior on the honeycombs	Max. 4 points
3.	Anecbalia (lack of swarming)	2-4 points (minimum 3)
4.	Honey caulking (relative appreciation)	Type of wet, wet or mixed cap (%)
5.	The degree of blockage of the nest	% area with brood (minimum 40%)

The results of the measurements were statistically processed to establish differences between the parental population and the bee population obtained from artificial insemination with homogenized semen. Data analysis was performed using Fisher Test for check the homogeneity of the variances and the modified Student Test, Behrens-Fisher variant, for the case of heterogeneous variances.

RESULTS AND DISCUSSIONS

A number of 200 bees were taken both from the parental population - queen breeding colonies and drone breeders - and from bee colonies obtained by introducing artificially inseminated queens with homogenized semen.
The samples were collected from bee colonies with high honey yields. The yields obtained were generally over 20-25 kg annually.
The sampled colonies had uniform bees, even though it included yellow corners on the abdominal tergites. All samples were collected during the active season (summer) so that there were no differences between samples.

Cubital Index

The values measured at the cubital index in the parental and offspring of the mountain bee population have values lose to those of the specific values found in the specialized literature (Table 2).

The measurements performed on bees from the Republic of Moldova and Ukraine, revealed higher values of the cubital index (40.00% and 52.80%) (Eremia & Cataraga, 2021), compared to the mean values determined in the present study for the bee mountain ecotype (37.20%, and 39.04%).

Table 2. Values of statistical parameters of the cubital index

Specification	Parental population (PP)	Descending population (DP)
Mean	0.390	0.372
Variance	0.00315	0.00301
Standard deviation	0.0560	0.0547
Maximum	0.5333	0.5133
Minimum	0.25	0.25

After applying the Fisher test, it was found that the variances of the two populations are heterogeneous.
Thus, the Fisher test, obtained by reporting the larger variant to the smaller one, led to the value of 1.0465.
The critical value of the Fisher test obtained at 398 DF and the significance level of 0.1% has the value 1.

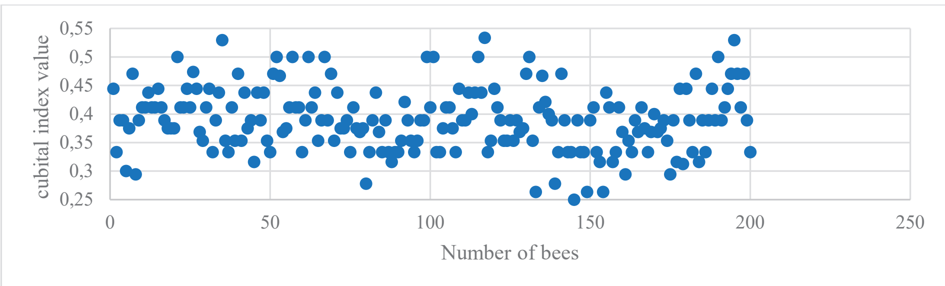

Figure 2. Cubital index values for the parental population (PP)

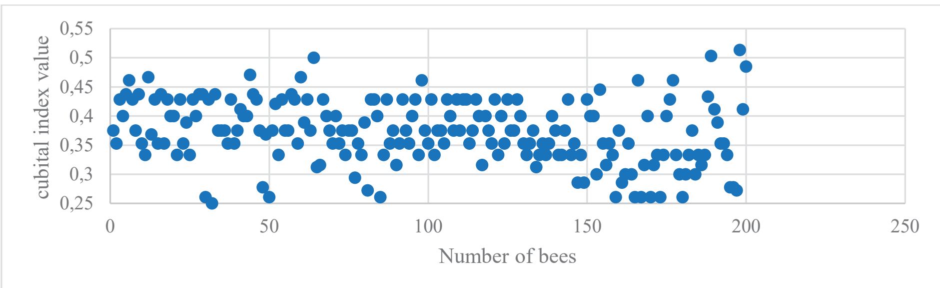

Figure 3. Cubital index values for descending population (DP)

Figures 2 and 3 indicate the distribution of determined values in the parental population compared to the descending population.
Since the variances are heterogeneous, the Behrens-Fisher Student's Test was used to verify the equality of the means of the two populations (Table 3).

Table 3. Student Test values of the two populations for the cubital index

Groups	Sample size	t calculated	t critical, $\alpha = 0.001$
PP	200	3.315***	3.290
DP	200		

As a result, the difference of cubital index values in the two populations studied was very significant.

Proboscis Length

Regarding the proboscis length, differences were noted between the parental and descendant populations. A heterogeneous distribution of the measured values, with a lower standard deviation was noted (Table 4).

Table 4. Values of the statistical parameters of proboscis lengths

Specification	Parental population (PP)	Descending population (DP)
Mean	6.355	6.447
Variance	0.0441	0.0289
Standard deviation	0.2101	0.1701
Maximum	6.8	6.8
Minimum	5.5	5.7

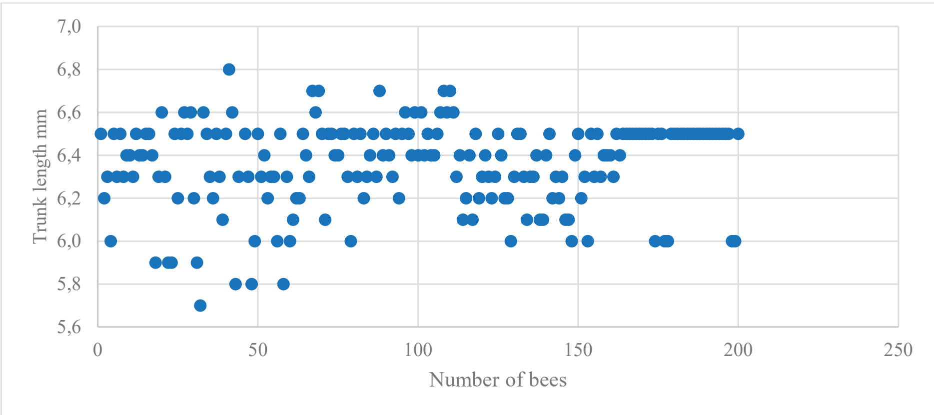

Figure 4. Proboscis length values for the parental population (PP)

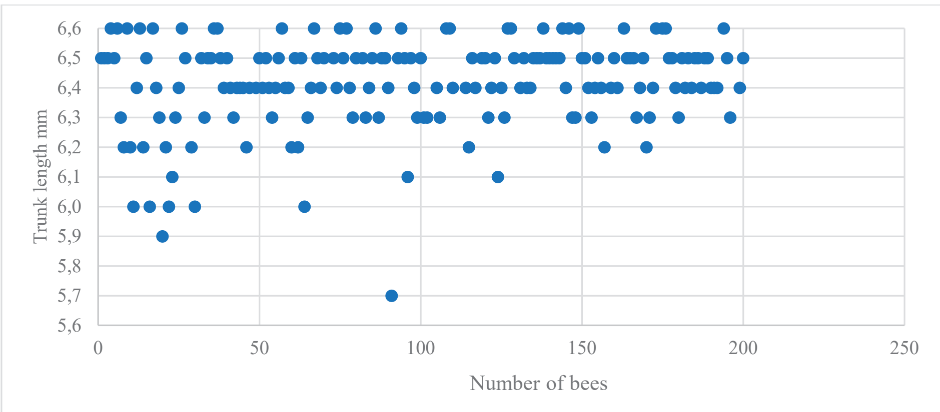

Figure 5. Proboscis length values for descending population (DP)

The average values obtained for the proboscis length (6.355 mm, respectively 6.447 mm) are slightly higher than those in the specialized literature, where the values are in the range of 5.950-6.340 mm (El-Aw et al., 2012; Rahimi & Mirmoayedi, 2013; Mohammed et al., 2017; Eremia & Cataraga, 2021).
The proboscis length character also shows a more uneven distribution of measured values in the parental population (Figure 4) compared to the descending population (Figure 5).
The Behrens-Fisher Student's Test was used to test the significance of the differences between the means of the two populations (Tables 5).

Table 5. Student Test values of the two populations for the proboscis length character

Groups	Sample size	t calculated	t critical, $\alpha = 0.001$
PP	200	4.799***	3.290
DP	200		

As a consequence, the difference between the two populations regrading proboscis length character was very significant.

Upper Wing Length
The distribution of upper wing length character values has the same characteristic as previous characters.
The average values obtained of the upper wing length (9.563 mm, respectively 9.478 mm) are higher than those in the specialized literature, where the values are in the range of 8.70-9.25 mm (El-Aw et al., 2012; Rahimi & Mirmoayedi, 2013; Mohammed et al., 2017; Eremia & Cataraga, 2021).
The variances of the two populations, the descending population and the parental population, are heterogeneous (Table 6).

Table 6. Values of upper wing length statistical parameters

Specification	Parental population (PP)	Descending population (DP)
Mean	9.563	9.478
Variance	0.0628	0.0560
Standard deviation	0.2506	0.2368
Maximum	10.0	10.0
Minimum	9.0	9.0

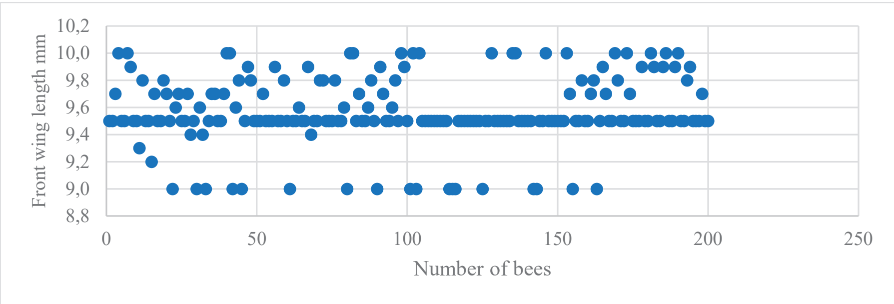

Figure 6. Upper wing length values for the parent population (PP)

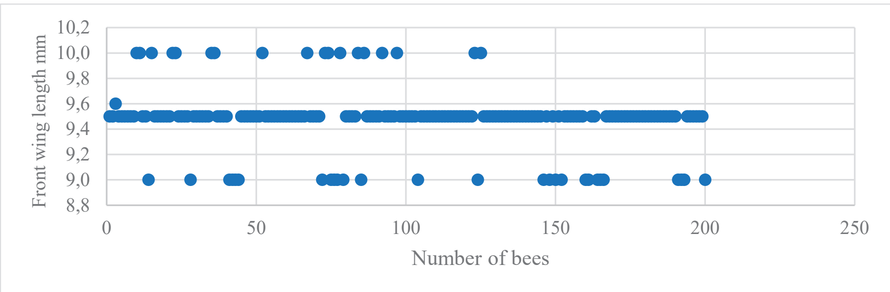

Figure 7. Upper wing length values for descending population (DP)

For the upper wing length character, a more uneven distribution of measured values is observed in the parent population (Figure 6) compared to the descending population, where measured values are distributed over three levels (Figure 7).
The measured character values are comparable in the two populations. Applying the Fisher test revealed that the variances of the two populations are heterogeneous (Table 7).

Table 7. Student Test values of the two populations for the for the upper wing length character

Groups	Sample size	t calculated	t critical, α = 0.001
PP	200	3.485***	3.290
DP	200		

To test the significance of differences in the means of the two populations, Behrens-Fisher Student's Test was applied. The difference of the values of the upper wing length character in the two populations was very significant.

Tergit-3 length

For the tergit-3 length character, a heterogeneity of measured traits mean values it was observed in both populations (Table 8).
The average values obtained for tergit-3 length (2.936 mm, respectively 2.862 mm) fall within the range found in the specialized literature, where the values are in the range 1.98-4.37 mm (Rahimi & Mirmoayedi, 2013; Eremia & Cataraga, 2021).

Table 8. Statistical parameter values for tergit-3 length

Specify	Parental population (PP)	Descending population (DP)
Mean	2.936	2.862
Variance	0.0166	0.0068
Standard deviation	0.1288	0.0830
Maximum	3.3	3.1
Minimum	2.6	2.6

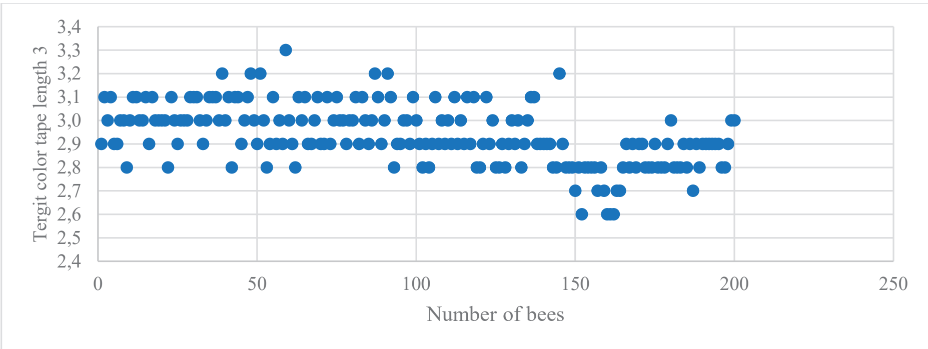

Figure 8. Tergit-3 length values for the parent population (PP)

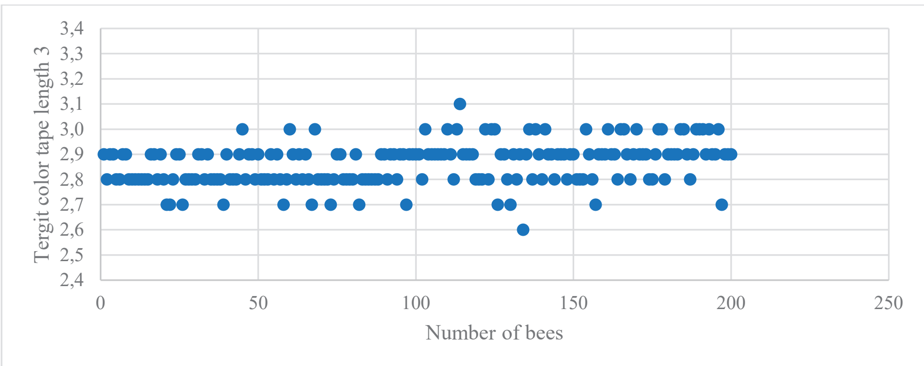

Figure 9. Tergit-3 length values for descendant population (PD)

For the tergit-3 length character, it is observed that the distribution of measured values in the parental population is more homogeneous (Figure 8) compared to the descending population (Figure 9).

Table 9. Student Test values of the two populations for the tergit-3 length character

Groups	Sample size	t calculated	t critical, $\alpha = 0.001$
PP	200	6.874***	3.290
DP	200		

The difference of values of the tergit-3 length character in the two populations is very significant ($P < 0.001$) (Table 9).

Behaviour Characters

Behavioural character measurements show small differences between the parental and descendant populations (Table 10).

This highlights the fact that the descendant population has productive behaviour quite similar to that of the parental population.

Table 10. Behaviour analysis

Specification	Parental population	Descendant population
Gentleness	3.4	3.5
Quiet behavior on the honeycombs	3.7	3.4
Anecbalia (lack of swarming)	2.8	3.0
Honey caulking (relative appreciation)	64.4% dry caulking	60.2% dry caulking
The degree of blockage of the nest	54.3%	55.2%

CONCLUSIONS

Morphological character values measured in *Apis mellifera carpatica* bees, mountain ecotype, are within or close to the average morphological character values identified by other studies.

For all the characters studied, the statistical analysis highlighted the fact that there are very significant differences between the means of the two populations. These results are valid for the samples taken in the study. In other conditions, for other samples, the results could be different.

Conservation studies of the mountain ecotype of the *Apis mellifera carpatica* breed have not been carried out until now in our country.

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HARMONIZING GLOBAL HEALTH - EXPLORING THE ROLES OF WAHIS, EMPRES-i AND ADIS IN ANIMAL HEALTH SURVEILLANCE AND RESEARCH

Dimitar TANCHEV, Gergana BALIEVA

Trakia University, Students campus, 6015, Stara Zagora, Bulgaria

Corresponding author email: dimitar.tanchev@trakia-uni.bg

Abstract

In an era where timely and reliable animal disease reporting is more critical than ever before, this research paper meticulously examines the pivotal global animal disease reporting systems-World Animal Health Information System (WAHIS), Global Animal Disease Information System (EMPRES-i), and European Union Animal Disease Information System (ADIS). The study provides a systematic analysis of their distinct features, data management strategies, and visualization approaches, exploring their interconnectedness with national and international legislative frameworks. Amidst the evolving landscape of emerging zoonotic diseases with pandemic potential, this research sheds light on the roles of WAHIS, EMPRES-i, and ADIS in global animal health surveillance and research. The findings helps to understand transboundary disease monitoring mechanisms within regulatory landscapes, fostering a harmonized approach to advancing global animal health.

Key words*: animal health, disease surveillance, information database, transboundary diseases.*

INTRODUCTION

In order to successfully and effectively fight contagious animal diseases and reduce economic losses from morbidity and mortality, it is extremely important each country to have in place measures for disease prevention and control, including early detection of outbreaks (Kshirsagar et al., 2013). Many regional local and worldwide systems are established with the overarching goal of bolstering global capacity for animal disease surveillance, monitoring, and reporting. These initiatives emerged from a recognition of the critical need for centralized platforms capable of efficiently collecting, analyzing, and disseminating comprehensive information on animal diseases on a worldwide scale. By facilitating early detection and swift response mechanisms, these systems aim to not only safeguard the health of animals but also protect public health and preserve the integrity of international trade. Furthermore, they play a vital role in supporting informed decision-making processes, enabling risk assessment, and fostering the development of effective policies pertaining to animal health and veterinary public health. In addressing transboundary disease outbreaks, these initiatives seek to mitigate the socio-economic repercussions, ensuring the resilience and sustainability of agricultural practices while safeguarding livelihoods and food security.

In this article, we examine three systems established to address specific needs in global animal health surveillance and management.

WAHIS

Being recognized as the referral organisation for animal health by WTO (World Trade Organisation) and a standard-setting intergovernmental worldwide presented structure, WOAH (World Organisation for Animal Health) ensures freedom of trade in live animals and animal products internationally thorough maintaining rigorous and transparent information system on animal health status. For this purpose, WOAH publishes annually a list of notifiable animal diseases and zoonoses, with a great health impact and economic importance, for which all member states have to provide data on timely manner regarding occurrence, reoccurrence and emergence of new diseases. All data provided by the WOAH members are entered, recorded, processed, analyzed and used through WAHIS (World Animal Health Information System) for disease prevention and control. Due to the constantly changing environment, climate change, human activities and many others, the challenges for WOAH related to animal health are more complex and there is a need of a continuous improvement and update within the organization, including to WAHIS. At present, the system operates based on three main pillars:

1. A tool for early warning and immediate notification of disease alerts after confirmation of an outbreak of a WOAH-listed disease.
2. A tool for monitoring the development of the WOAH-listed animal disease through six-monthly updates.
3. Mandatory annual reports from the Member states` competent authorities on the status of the notifiable animal diseases and zoonoses, population of susceptible animal species, capacities and resources for disease control and prevention.

Furthermore, as an additional module WOAH had developed WAHIS-Wild which operates with data on wildlife diseases that are not on the notifiable list but still require surveillance (WOAH, 2023b).

EMPRES-i

EMPES-i is also an internet-based system, which operates under the United Nations (FAO) in order to ensure the access and timely management on transboundary and contagious animal diseases with high heath and economic impact on international level, including zoonoses. This Global Animal Disease Information System is aimed at methodologically active assistance to veterinary services in their official control activities, in line with the need of progress and improvement (FAO, 2021).

ADIS

At European level, a secure tool to provide relevant and timely information on animal health situation was established by the European Commission as Animal Disease Information System - ADIS. The system operates as a centralized hub for receiving, processing and circulating precise data on animal disease status among the EU member states and associated countries through notifications. The digital platform of ADIS allows competent authorities to improve data accuracy upon entry, to simplify reporting procedures and reduce administrative burden. As ADIS is functionally in synchronization with the WAHIS, it is a valuable tool for the decision makers when it comes to the need of accurate information on animal health status and contagious diseases outbreaks (European Commission, 2024a).

MATERIALS AND METHODS

Data collection

For the purposes of this study, a comprehensive approach was adopted to collect data from official sources corresponding to each of the three systems under examination. Information pertinent to the World Animal Health Information System (WAHIS) was meticulously sourced from the official website of the World Organization for Animal Health (WOAH). This entailed accessing a plethora of reports, publications, and databases housed within the WOAH platform, all of which were deemed relevant to the realm of disease surveillance within the global animal health landscape (WOAH, 2023).

Similarly, data pertaining to the European Union Animal disease information system (ADIS) were diligently gathered from official publications, reports, and databases sanctioned by the European Union. This encompassed a comprehensive review of EU publications pertaining to animal health and disease surveillance, as well as personal access to specific databases and repositories housing relevant data within the EU.

In the case of the Emergency EMPRES-i a thorough examination of data was conducted through access to the official website of the Food and Agriculture Organization of the United Nations (FAO). This involved targeted retrieval of global information on transboundary animal and plant diseases via EMPRES-i reports, maps, and datasets, ensuring a comprehensive understanding of disease dynamics and surveillance efforts on a global scale.

Data analysis

Comparative analysis

The collected data underwent rigorous comparative analysis aimed at identifying not only similarities but also differences in the characteristics, data management approaches, and visualization methods employed by each of the three systems under investigation - WAHIS, ADIS, and EMPRES-i. This involved a meticulous examination of various parameters, including but not limited to, the scope of disease surveillance, reporting structures, response mechanisms, and data dissemination protocols. By conducting a detailed comparative assessment, insights were gleaned into the unique strengths and potential limitations of each system, thereby facilitating a nuanced understanding of their respective contributions to global animal health surveillance and management.

Thematic Analysis

In addition to comparative analysis, a thematic analysis approach was adopted to delve into the alignment of WAHIS, ADIS, and EMPRES-i with national and international legislative frameworks governing animal health surveillance and management. This multifaceted examination involved a systematic review and synthesis of relevant policies, regulations, and guidelines enacted at both the national and international levels. These legislative frameworks were meticulously mapped to the functionalities and operational conditions of each system, allowing for an in-depth exploration of their compliance with regulatory requirements and their capacity to effectively address emerging challenges in animal health surveillance and management. By juxtaposing the operational modalities of WAHIS, ADIS, and EMPRES-i with established legal frameworks, this thematic analysis shed light on the extent to which these systems are poised to meet the evolving needs of global animal health governance.

RESULTS AND DISCUSSIONS

Legal framework

WAHIS

The legal basis for operation of WAHIS is laid down by Art. 1.1.2. of the WOAH Terrestrial Animal Health Code which stipulates that all member states have to report to the WOAH Headquarters the available information of notifiable animal diseases in order to improve globally their management and control by minimizing the spread of outbreaks and pathogens.

EMPRES-i

The legal framework of EMPRES-i is multifaceted and encompasses a combination of international agreements, national legislation, FAO governance structures, and collaborative partnerships, all aimed at promoting global cooperation and coordination in addressing transboundary animal and plant health threats.

ADIS

Within the European legislation, Regulation (EU) 2016/429 Animal Health Law defines the categories of contagious animal diseases with economic and health importance to the EU, for which occurrence, reoccurrence and development of outbreaks have to be registered by the member states through the ADIS. Regulation (EU) 2020/2002 additionally lays down the uniform rules for procedures and notifications of listed animal diseases to the EU which are applied with the computerized information system.

Data collection

WAHIS

In compliance to the rules set in the WOAH Terrestrial Animal Health Code by Article 1.1.3., all member states shall provide the WOAH Headquarters through their competent veterinary authorities with on-time notifications within 24 hours of the laboratory confirmed diagnose accurate information through WAHIS platform on first occurrence on a WOAH listed disease or pathogen on the territory of the country or part of it (zone, compartment). The same information shall be provided for recurrence of an eradicated listed disease or a pathogen in one of the member states or a part of it, as well as a change in increase of virulence/morbidity/mortality of any of the diseases from the WOAH list, including the pathogens causing animal diseases and zoonoses in an unusual animal host species.

To ensure the quality and relevance of the information, training and support services are provided. Since 2006, annual training sessions have been conducted focusing on the practical utilization of the reporting system (WAHIS) and key aspects of disease notification. These sessions primarily target WOAH Focal Points for Animal Disease Notification, who serve as the WOAH's national contact points for disease reporting.

In terms of support services, the WOAH directly assists the Veterinary Services of its Member States. This assistance encompasses clarifying notification requirements and enhancing Members' awareness of their obligations for disease notification to the WOAH. Additionally, the WOAH's regional and sub-regional Representations are available to provide support in utilizing the reporting system (Caceres et al., 2020).

The quality and relevance of the information are significantly influenced by the quality of veterinary services, emphasizing their importance in ensuring accurate and pertinent data. Like unique intergovernmental organisation WOAH has established a number of international standards, some of which regulate and enforce a framework for ensuring the quality of Veterinary Services worldwide (WOAH, 2023a).

As developed and improved constantly, the OIE PVS Tool for the evaluation of performance of Veterinary Services - encompasses four fundamental components (WOAH, 2019). The aim of the PVS Tool is to ensure all necessary factors for the effective performance of Veterinary services are in place, up-to date and efficient in order to maintain high level of protection of animal health and public health and food safety:

1) adequate human, physical and financial resources under proper coordination and allocation, with staff excelled in technical and leadership competences;

2) technical authority and capability which include veterinary laboratory capacities and aim at control, surveillance, emergency preparedness and response to animal diseases, food and feed safety, antimicrobial resistance and animal welfare as well;

3) interaction with stakeholders expressed through regulation, communication and consultation with interested parties at various scales with the goal to enhance joint collaboration and up-to date performance;

4) sustainable access to markets ensuring safe trade in animals, animal products, food and feed through introduction of international veterinary legislation, certification, sanitary agreements and harmonization of standards and defining of zones and compartments with disease free-status (WOAH, 2019).

It's evident that the ability of veterinary services to substantiate claims regarding the health status of animals through disease surveillance data, monitoring program results, and detailed disease history is highly relevant (Vallat & Wilson, 2003).

In order to enhance the effectiveness of its animal health information system and acquire a comprehensive understanding of the global animal health situation, WOAH initiated an active search for non-official information related to animal health and public health starting from 2002. The acquired information is subjected to assessment with regard to the predominant animal health status within the respective zone, region or country. If the information is confirmed, it is verified by the member state with official confirmation and potential publication (Caceres et al., 2017).

For this purpose, Member states have granted WOAH Headquarters the authority to directly contact their national Delegate for all cases of reported through media or other non-official channels news on animal health incidents and outbreaks which may require legally action of immediate disease notification to WOAH system. However, before being officially confirmed by the competent authority of the member state this information is not to be announced and published.

As certain procedures have been set regarding reporting events with importance to animal health, the prerequisite of receiving official confirmation on notifiable animal disease by the member states is invaluable and mandatory to have the reported information disseminated afterwards through official forms (WOAH, 2023c). Percentage of this notification vary by the years (Figure 1).

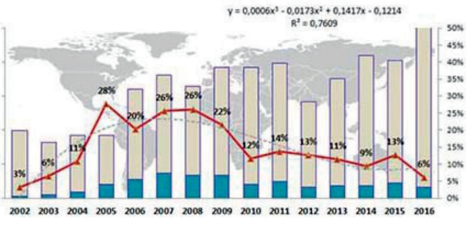

Figure 1. Percentage of alert messages obtained due the tracking activities (Source: WOAH website)

EMPRES-i

This platform is designed to provide joint information on notifiable animal diseases at global level through consolidation of data from various sources shared on a regular basis between collaborative partners and FAO. Besides the official information fed to EMPRES-i from the global networks, the platform also uses non-official channels.

Informal information on animal health situation enters EMPRES-i through media reports from the countries or networks like ProMED (Program for Monitoring Emerging Diseases, managed by the International Society for Infectious Diseases) (ISID, 2023) and GPHIN - Global Public Health Intelligence Network (developed by Canada`s Public Health Agency, also part of WHO alert system) (Government of Canada, 2023).

Sources of formal information to EPMRES-i are provided by WOAH, WHO, FAO, European Commission and partnering Non-Governmental Organizations.

However, the unconfirmed information on animal diseases events has to be verified through a coordinated process with the relevant organisations - WOAH and WHO. The process includes disease-tracking in order to confirm or deny the information. When necessary veterinary officers from national competent authorities are contacted to verify data provided within official reports on the location and time of animal disease outbreaks, and is further detailed through mapping tools like Google Earth. The same verification process and contacts are used for confirmation of data coming from informal channels (Farnsworth et al., 2010).

ADIS

The process of reporting data to ADIS (Animal Disease Information System) is governed and standardized according to the legal framework established by the European Union. This regulatory framework is detailed in Article 3 of the Commission Implementing Regulation (EU) 2020/2002 with regard to reporting listed disease to the EU with the List of diseases of terrestrial animals provided in Annex I of the Regulation.

The legislation implies that in case a primary outbreak of a notifiable animal disease is registered within the territory of a member state, it is mandatory to alert the European Commission (EC) within 24 hours after the laboratory confirmation of the diagnosis. Regarding secondary outbreaks, a rigorous reporting procedure requires Member states to notify the EC on the events on the first working day of the week at the latest with information covering the previous week.

Upon receipt of a report, WOAH verifies, processes, translates and subsequently displays the information in the WAHIS, publicly available. Information is sent promptly to all WOAH members by email (Lin et al., 2023).

The alert procedure on listed diseases is defined by Article 1.1.5 of the OIE Terrestrial code which assigns the Headquarters the duty to communicate all relevant information on animal disease outbreaks to the member states` competent authorities through electronic means - email or WAHIS interface (WOAH, 2023).

EMPRES-i

EMPRES-i disseminates scientific data by presenting it on its publicly accessible profile. Users have the ability to explore “Disease Events”, which are visually represented on a map interface. Further analysis is facilitated through the selection of various optional layers available for examination. This dissemination strategy ensures that stakeholders have prompt access to pertinent information concerning animal health situation and emerging threats worldwide.

ADIS

Based on the mode of operation, ADIS requires the information on a primary animal disease outbreak to be entered into the platform. After receiving the initial report, the system generates timely notifications on automated mode which are emailed to all member states-users of the application.

Additionally, to ensure comprehensive communication, a detailed weekly email bulletin is circulated to all ADIS members every Friday. This bulletin serves to summarize and provide insights into both primary and secondary outbreaks that have been documented throughout the week. For the purpose of effective and efficient management on information regarding listed animal diseases, ADIS works in a close joint collaboration with WAHIS. Figure 2 explain ADIS dissemination of data and connection with WAHIS.

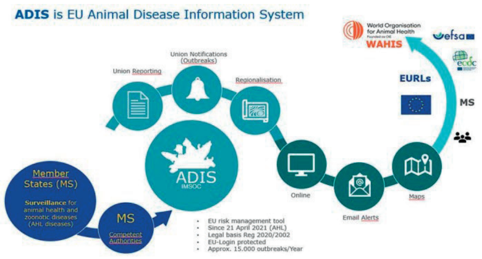

Figure 2. ADIS dissemination of data
(Source: An official website of the European Union https://food.ec.europa.eu/animals/animal-diseases/animal-disease-information-system-adis/unveiling-adis_en)

Both databases function at international level and manage the crucial task to provide its members with precise and timely information on current animal health events, thus allowing the interested parties to implement measures to prevent and stop contagious animal diseases to spread across state boundaries (Lin et al., 2023). Despite that they contribute to global animal health surveillance, they serve distinct purposes and user bases.

WAHIS functions as an extensive official global repository for data on animal health events, facilitating standardized reporting and analysis to support disease monitoring and control efforts on a global scale. It focuses on promoting transparency, collaboration, and data exchange among member countries and international organizations to enhance global animal health governance. According to the OIE Manual 6: Animal health information system: "An animal health information system is only as good as the data it contains" (WOAH, 2018). With the aim to provide information with integrity and reliability, the WOAH not only furnishes a comprehensive database but also disseminates standardized laboratory manuals tailored for disease diagnosis. Additionally, WOAH undertakes the evaluation of veterinary services, aiming to synchronize their capacities and bolster their preparedness in effectively managing outbreaks of emerging diseases.

The data within WAHIS are verified and official, featuring sensitive and specific information on more than 120 animal diseases. They characterize the endemic situations for over 200 territories, including all current 183 members and other interested parties who willingly provide information to WOAH. Because of these features, there exists a significant diversity in the utilization of data from WAHIS (Caceres et al., 2023).

WAHIS offers a suite of tools and features for data submission, validation and visualization, including standardized reporting formats, interactive maps, and data analysis tools. It emphasizes the importance of official reporting channels and data quality assurance mechanisms to ensure the reliability and comparability of reported information. Because of these features, there exists a significant diversity in the utilization of data from WAHIS (Mur et al., 2019).

The valuable resource of WAHIS could be interpreted in two ways: first, to discover an occurrence/reoccurrence of listed animal diseases through alert messages and follow-up reports, and second, to observe an absence of a disease within the mandatory reports each six months. Combined at regional, national and global level, the information from both datasets is further analyzed and used for risk assessment which in combination with data on control measures could enhance disease management in terms of the noted absent listed diseases (Caceres et al., 2023).

Furthermore, data from animal health platforms could be used to improve members` preparedness based on the analysis of disease patterns. By tracing the changes in disease development, the animal health experts could use the platform to identify the evolution and spread of diseases (Bianchini et al., 2022).

And not least, WAHIS is a part of WOAH role in international trade regulation. The information system is used to ensure safe trade in live animals and animal products with the help of the notifications disseminated to the member states that allow them to impose timely measures to limit the spread of pathogens from the infected country to other territories (Cardoen et al., 2017).

In the other hand EMPRES-i is specifically designed as an early warning system for animal diseases, emphasizing real-time monitoring, rapid detection, and response to disease outbreaks and emerging threats. It prioritizes the timely dissemination of actionable information to stakeholders to mitigate the impact of diseases on animal health, livelihoods, and food security. Data on animal disease events are

entered in EMPRES-i based on formal and informal reports. The information is provided through various sources among which reports from FAO and some partnering organizations and national authorities on field missions and regional projects, media and computerized health monitoring systems (Perez et al., 2011).
By harnessing data from numerous and varied sources, EMPRES-i can capture a comprehensive snapshot of the global animal health landscape in real-time. This multifaceted approach to data aggregation ensures that the system remains responsive to emerging disease threats, rapidly detecting and disseminating information on new outbreaks or disease patterns. Furthermore, the diversity of data sources allows for cross-validation and triangulation of information, enhancing the reliability and accuracy of the insights provided by EMPRES-i.
In essence, the wealth of sources available to EMPRES-i empowers the system to maintain a state of heightened situational awareness regarding animal health events worldwide. This dynamic and expansive information ecosystem positions EMPRES-i as an indispensable means for early warning, decision support, and strategic planning in the realm of animal disease prevention and control. It accelerates the dissemination of disease information at regional and international scales. At the meantime, it is also used for risk assessment of the current animal health situation with present and emerging diseases. The platform is a valuable tool for planning and implementation of surveillance measures and detailed epidemiological analysis on a particular listed disease (FAO, 2015).
The utilization of EMPRES-i not only facilitates the examination of the geographic dispersion of diseases but also enables the visualization of human or animal populations within a given area (Figure 3). This capability extends beyond mere mapping to include the activation of topographic features, thereby providing a comprehensive understanding of natural barriers that may influence disease spread dynamics. Moreover, EMPRES-i offers the flexibility to identify the source of information, regardless of its official or unofficial nature, thereby contributing to a nuanced assessment of disease events. The integration of a vast repository of disease data and the capacity to generate diverse graphical models further augment EMPRES-i effectiveness as a tool for rapidly comprehending infectious disease processes in animal populations.

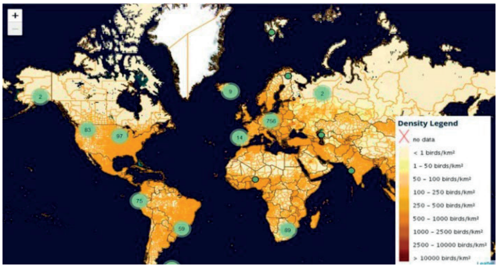

Figure 3. Map with visualized livestock density (personal access)

Another unique opportunity offered by EMPRES-i is the genetic module within the platform which encompasses data from real health events on registered influenza outbreaks and data on the characteristics of the virus itself. By integrating the specific viral features within the information system it could be possible to trace the distribution of the viruses, track their strains with molecular markers, analyze the effects on viral distribution after vaccination for control and prevention purposes. In the case of influenza outbreaks an algorithm is developed that automatically computes the probability of related disease outbreaks and viral sequences, using information provided by EMPRES-i on animal health events and virus characteristics available in OpenfluDB (OpenFluDB, 2024) . The suggested links between the outbreak event and particular viral strain as a cause has to be mandatory validated by experts (Claes et al., 2014).
Based on the current legislative framework and the categorization of contagious animal diseases with significance to the EU, as defined by the Animal Health Law (Regulation (EU) 2016/429), ADIS operates as a tool for disease management and control of epidemiological situation among the member states connected to the application. The system provides immediate access to the EU countries and the partnering organisations on the current status of animal health (occurrence, reoccurrence of outbreaks, emerging threats) and through the early warning mechanism enables the countries to undertake

urgent measures and response to ensure the safety and health of their animal populations and the public as well (European Commission, 2024). Based on its automated mode that requires only one-time reporting on a disease event by the member states, ADIS operation leads to less administrative workload. By the dissemination of information via email to a large number of veterinary specialists at various levels ADIS works as a valuable tool for decision-makers to allow them use the latest and most consistent data on animal health situation and thus develop and implement reasonable and efficient decisions and policies on animal health management (WOAH, 2023).

On one hand, the system enables more specific data retrieval not only by country but also by region within the EU. This allows for obtaining information regarding disease zoning. On the other hand, the automatic linkage with WAHIS facilitates reporting of diseases by member states.

CONCLUSIONS

Despite sharing certain superficial resemblances, such as geographical data visualization and data exchange functionalities, the examined systems occupy distinct niches within the realm of global animal health surveillance and research. This necessitates a comprehensive understanding of the nuanced disparities between them, encompassing aspects such as legislative frameworks, methods of data acquisition, export functionalities, and approaches to data visualization. This nuanced understanding facilitates the judicious selection of the most suitable system for conducting specific analyses, thereby ensuring the acquisition of precise and reliable data. Conversely, in the context of global analysis and varied dissemination needs, it is common practice to leverage multiple or all of these systems, as they often complement one another, synergistically enhancing the comprehensiveness and depth of insights garnered, and contributing to a more holistic understanding of the prevailing circumstances.

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STUDY ON THE MANAGEMENT OF TECHNOLOGICAL FLOW IN BEEF CATTLE FARMS IN THE NORTH-EAST REGION OF MOLDOVA

Felicia ȚENU (DASCĂLU)[1], Raluca-Elena DONOSĂ[1], Andreea ȘERBAN[1, 2], Vasile MACIUC[1]

[1]"Ion Ionescu de la Brad" University of Life Sciences of Iași, Mihail Sadoveanu Alley no. 3, Iasi, 700490, Romania
[2] Research and Development Station for Aquaculture and Aquatic Ecology, "Alexandru Ioan Cuza" University, Carol I, 20A, 700505, Iaşi, Romania

Corresponding author email: feliciatenu@yahoo.com

Abstract

This study investigates the management practices and technological processes within beef cattle farms in the North-East region of Moldova, focusing on six distinct farms. Analyzing data from these farms, encompassing a total of 1838 cattle, revealed notable disparities in animal stock, labor force, and forage cultivation areas. Additionally, diverse housing systems, waste disposal methods, and breeding practices were identified. Noteworthy results include Farm F5's effective waste management strategies and Farm F6's adoption of advanced breeding techniques. These findings underscore the necessity of tailoring management approaches to individual farm contexts to optimize operational efficiency and productivity. By providing actionable insights, this research contributes to ongoing efforts aimed at improving beef cattle farming practices in the region and fostering sustainable agricultural development.

Key words*: Aberdeen Angus, bovine husbandry, livestock management.*

INTRODUCTION

Livestock farming, a vital component of the agricultural sector, plays a significant role in boosting rural incomes at both macroeconomic and microeconomic levels (Upton, 2004).
The downturn in this sector has been influenced by several issues, including a reduction in animal numbers, climate variations affecting both breeds and fodder production, and European legislation concerning animal health (Grigoras, 2016; Popescu, 2017; Popescu, 2014; Sterie & Chetroiu, 2021, Nica & Vidu, 2023).
The practice of cattle husbandry serves as a cornerstone in upholding nutritional equilibrium and propelling agricultural and food sectors on a global scale (Velea, 2012). The Aberdeen Angus breed, distinguished for its exceptional marbling and tenderness attributes, stands as an exemplar of high-quality beef production (Gociman et al., 2020; Manea, 2022), representing a substantial 70% share of the global beef cattle population (Maciuc et al., 2015; Țenu, 2023). In the Northeast region of Moldova, characterized by a convergence of historical, cultural, and natural richness, cattle rearing flourishes, especially with breeds well-suited to local conditions such as the Aberdeen Angus.
This study focuses on beef cattle management in Moldova's North-East region, encompassing Botoșani, Iași, and Vaslui counties. Despite the region's agricultural dominance, there's room for improving cattle productivity. The study aims to enhance performance and economic efficiency in beef cattle farming, considering rearing and utilization systems.
Various cattle breeds, including those specialized in milk and meat production, coexist in the region (Vidu et al., 2014) While many breeds have adapted well, there's scope for enhancing productivity. Effective farm management is essential for optimizing outcomes, necessitating managers to stay abreast of industry advancements from diverse sources.
This study seeks to unlock the full potential of beef cattle farming in Moldova's Northeast, leveraging its rich agricultural heritage and fostering sustainable practices for future prosperity.

MATERIALS AND METHODS

This study aimed to investigate various aspects of cattle farming across the N-E region of Romania. Specifically, we focused on six farms located in the counties of Botoșani, Iași, and Vaslui.

The farms were selected based on specific criteria including geographical diversity, establishment year, rearing system, etc.

The farms included in this study (Table 1) were chosen to represent a diverse range of locations and operational characteristics. Farms were identified through a combination of online databases, agricultural directories, and local government records. The selection criteria prioritized farms with a significant presence in their respective communities, ensuring a representative sample of the region's cattle farming industry.

Table 1. Studied Farms Overview

Farm	Township	County	Year of Establishment	Area (ha)	Number of Taurines	Rearing System
F1	Prăjeni	Botosani	2015	25	28	Semi-intensive
F2	Ibănești	Botosani	2008	90	139	Extensive
F3	Cozmești	Iasi	2009	360	280	Semi-intensive
F4	Ţigănaşi	Iasi	1996	8500	433	Intensive
F5	Banca,	Vaslui	2016	80	103	Semi-intensive
F6	Târzii	Vaslui	2018	5000	850	Intensive

Data collection was conducted through a combination of online research, direct communication with farm owners or managers, and on-site visits.

Rearing systems were categorized into three main types: semi-intensive, extensive, and intensive. The semi-intensive system involved moderate levels of input, including partial confinement and supplemental feeding, whereas the extensive system relied primarily on grazing with minimal intervention. In contrast, the intensive system utilized high levels of input, including full confinement and controlled feeding regimes.

To ensure the accuracy and reliability of the collected data, multiple verification measures were employed. This included cross-referencing information obtained from farm records with official agricultural statistics and conducting on-site visits to confirm reported data. Any discrepancies or inconsistencies were addressed through further investigation and consultation with farm personnel.

RESULTS AND DISCUSSIONS

Geographical Characteristics of the Studied Farm

The investigation into the six distinct cattle farms situated across the N-E region of Romania sheds light on the geographical diversity and agricultural landscapes characterizing each area (Table 2).

Table 2 Regional Characteristics of Studied Farms

Farm	Township	County	Description
F1	Prăjeni	Botoșani	Favorable climatic conditions conducive to semi-intensive rearing.
F2	Ibănești	Botoșani	Optimal conditions for extensive cattle grazing.
F3	Cozmești	Iași	Rich agricultural heritage and diverse farming activities.
F4	Țigănași	Iași	Expansive farmlands and intensive farming practices.
F5	Banca	Vaslui	Fertile valleys ideal for semi intensive cattle grazing.
F6	Târzii	Vaslui	Expansive agricultural lands and diverse farming practices.

Situated in the north-eastern part of Romania, F1 is nestled within the picturesque township of Prăjeni, located in Botoșani County. This region is renowned for its lush green pastures and favorable climatic conditions, making it conducive to cattle farming. The fertile soils and ample rainfall contribute to the abundant growth of nutritious forage, essential for the sustenance of the semi-intensive rearing system practiced at F1.

Farm F2 is situated in Ibănești, Botoșani County, which shares similar geographical characteristics with Prăjeni. The region boasts expansive grasslands and vast open spaces ideal for extensive cattle grazing. Ibănești benefits from a temperate climate and ample sunshine, providing optimal conditions for the flourishing of extensive farming practices.

Cozmești, Iași County is home for F3. The region is renowned for its rolling hills and fertile plains, providing an idyllic setting for agricultural pursuits. The broader region of Cozmești is characterized by its rich agricultural heritage and diverse farming activities.

Iași County is the location of F4 to, within the township of Țigănași. This region is distinguished by its expansive farmlands and well-developed infrastructure, reflecting its long-standing history of agricultural prominence. The fertile soils and favorable climate support intensive farming practices, facilitating the high-density cattle production observed at F4.

The region where F5 is located is characterized by its undulating terrain and fertile valleys, providing an ideal environment for semi-intensive cattle grazing. Banca benefits from abundant rainfall and moderate temperatures, creating optimal conditions for the thriving of semi-intensive farming systems.

Situated in Vaslui County, F6 is nestled within the scenic township of Târzii. The region is known for its expansive agricultural lands and diverse farming practices. The broader region of Târzii is celebrated for its rich agricultural traditions and commitment to sustainable farming practices.

Feeding Methods, Waste Management, and Cattle Care in Studied Farms

The feeding methods, waste management practices, and cattle care strategies employed in the studied farms exhibit a diverse range of approaches tailored to the specific needs and resources available in each county (Table 3).

Table 3 Feeding Methods and Care Practices Across Studied Farms

Farm	Feeding Methods	Waste Management	Cattle Care
F1	Semi-intensive grazing	Regular removal and proper disposal	Regular monitoring of health and wellbeing
F2	Extensive grazing	Rotational grazing practices	Regular health assessments and preventive measures
F3	Semi-intensive grazing	Regular removal to maintain cleanliness and hygiene	Adequate care inferred based on general practices
F4	Intensive feeding	Systematic removal and disposal	Comprehensive care, including health monitoring and vaccination
F5	Semi-intensive grazing	Rotational grazing to prevent overgrazing	Prioritization of cattle welfare, including access to water and shelter
F6	Intensive feeding	Systematic removal and disposal	Meticulous care, including regular health assessments and disease prevention

In **F1**, a semi-intensive feeding system is implemented. Cattle are provided with a balanced diet consisting of a combination of grazing and supplementary feed. This approach ensures adequate nutrition while leveraging natural forage resources.

Dejections are managed through regular removal and proper disposal. Efforts are made to minimize environmental impact by utilizing waste management practices that prevent contamination of soil and water sources (Evans et al., 2019; Pykälä, 2019).

Cattle receive attentive care, with regular monitoring of health and wellbeing. Veterinarian services are utilized as needed to address any health issues promptly.

F2 employs an extensive feeding system, emphasizing grazing on open pastures. Cattle have access to abundant forage resources, supplemented with minimal concentrated feed as necessary (Fernando et al., 2010; Khafipour et al., 2011; Russell & Rychlik, 2001).

Dejection management involves natural decomposition processes facilitated by rotational grazing practices (David et al., 2023). This approach enhances soil fertility and minimizes the need for external waste removal.

Cattle health and welfare are prioritized, with regular health assessments and preventive measures in place to mitigate disease risks (da Silva et al., 2006). Attention is given to ensuring adequate nutrition (NASEM, 2016) and access to clean water (NASEM, 2000).

Considering the geographical location and climatic conditions of **F3**, a combination of grazing and supplementary feeding is employed to sustain cattle throughout the year (Figueiredo et al., 2007; Cazzuli et al., 2023).

The approach to waste management involves regular removal of dejections to maintain cleanliness and hygiene within the premises. Proper disposal methods are implemented to prevent environmental pollution (Chaudary et al., 2018).

Cattle receive adequate care, with attention to their nutritional needs (Olson et al., 2021), and overall wellbeing.

F4 implements an intensive feeding system to meet the nutritional requirements of a larger cattle population. This involves a combination of grazing, silage, and concentrated feed to optimize growth and productivity (Lahart et al., 2020).

Given the scale of operations, waste management practices include systematic removal and disposal of dejections to prevent accumulation and maintain hygienic conditions (Chaudary et al, 2018).

Cattle receive comprehensive care, with regular health monitoring, vaccination protocols, and nutritional management strategies in place to support optimal growth and performance.

Similar to F1, **F5** employs an semi-intensive feeding system, allowing cattle access to open pastures for grazing. Supplementary feeding is provided during periods of limited forage availability.

Waste management practices involve rotational grazing to prevent overgrazing and soil degradation. Dejections decompose naturally, contributing to soil fertility (Aliyev, 2022).

Cattle welfare is prioritized, with measures taken to ensure access to clean water, adequate shelter, and veterinary care as needed (Nalon et al., 2021).

In **F6**, the intensive feeding system is implemented to support a substantial cattle population. This involves the provision of high-quality concentrated feed to meet nutritional demands, without relying on pasture-based grazing (Doyle et al., 2023).

Age Distribution of Cattle: Implications for Farm Management and Productivity

Each farm implements distinct rearing systems and management practices tailored to their specific operational objectives and environmental conditions, consequently influencing the age distribution of their cattle population (Table 4). This variability in rearing approaches reflects the diverse strategies employed by farmers to optimize productivity, animal welfare, and sustainability within their respective contexts.

Table 4. Distribution of Cattle by Age Across Studied Farms

Farm	Adult Cattle (over 18 months)	Yearlings (12-18 months)	Calves (0-12 months)	Total Cattle
F1	14	9	5	**28**
F2	95	40	36	**171**
F3	160	80	40	**280**
F4	120	105	209	**434**
F5	50	29	24	**103**
F6	752	18	80	**850**

F1 maintains a **semi-intensive** rearing system. The majority of its cattle population comprises animals aged over 18 months, representing 50% of the total herd. Additionally, 32.14% of the herd consists of yearlings (12-18 months), while the remaining 17.86% comprises calves (0-12 months). With a moderate number of taurines (28), F1 may focus on selective breeding to enhance genetic traits conducive to semi-intensive rearing systems (Otoikhian et al., 2022). Breeding decisions likely prioritize traits that improve adaptability to local conditions and optimize meat production efficiency. Given the small herd size, feeding regimens on F1 is more easily tailored to individual animal requirements, ensuring adequate nutrition for optimal growth and development. Close monitoring of animal health is feasible on this farm, allowing for timely intervention and disease control measures. Collaboration with local veterinarians may be integral to maintaining herd health.

Considering the specific characteristics of F1, including its semi-intensive rearing system, distribution of cattle by age, and management practices, There was conducted a SWOT analysis to assess its strengths, weaknesses, opportunities, and threats (Table 5).

Table 5. The SWOT Analysis of F1

STRENGTHS	WEAKNESSES
1. **Focus on selective breeding** for genetic enhancement, contributing to the improvement of desirable traits in the cattle population. 2. **Ability to tailor feeding regimens** to individual animal needs due to a moderate herd size, ensuring optimal nutrition and growth. 3. **Close monitoring of animal health** facilitated by the manageable scale of operations, allowing for timely intervention and disease control measures.	1. **Limited economies** of scale due to the relatively small herd size, potentially hindering efficiency and profitability compared to larger-scale operations. 2. **Dependence on local resources and infrastructure**, which may limit access to specialized services and technologies available in larger farming operations. 3. **Vulnerability to market fluctuations and external factors** due to a smaller market presence and limited bargaining power.
OPPORTUNITIES	**THREATS**
1. **Expansion of the cattle herd** through strategic breeding programs and targeted investment in herd management practices, leveraging the farm's existing strengths in selective breeding and individualized feeding regimens. 2. **Diversification of revenue streams** by exploring alternative markets or value-added products derived from cattle production, such as organic or specialty beef products. 3. **Collaboration with local agricultural institutions, veterinarians, and industry stakeholders to access resources, expertise, and market networks, enhancing the farm's competitive position and resilience.**	1. **Intensifying competition** from larger-scale operations or industrialized farming practices, posing challenges to market differentiation and profitability. 2. **Regulatory changes or policy developments** impacting agricultural practices, environmental standards, or animal welfare regulations, requiring adaptation and compliance measures. 3. **Environmental and climatic risks**, such as extreme weather events or natural disasters, affecting pasture quality, animal health, and overall farm productivity.

F2 adopts an **extensive** rearing system. The distribution of cattle by age is relatively evenly spread, with approximately 22.22% of the herd aged 0-12 months, 23.39% aged 12-18 months, and 54.39% aged over 18 months. With a larger herd size (171 cattle), F2 has the potential to implement more sophisticated breeding programs aimed at improving genetic traits relevant to extensive rearing systems (Brito et al., 2021; Berry, 2022). Emphasis may be placed on traits such as maternal instincts, foraging abilities, and environmental adaptability. The extensive nature of this farm requires strategic grazing management and supplementary feeding practices to ensure adequate nutrition across a larger herd size and varying pasture conditions. Vigilant disease surveillance and vaccination protocols are essential for maintaining herd health and preventing disease outbreaks in extensive grazing systems.

Analyzing the SWOT of F2's extensive rearing system reveals key insights into its operational dynamics and strategic considerations (Table 6).

Table 6. The SWOT Analysis of F2

STRENGTHS	WEAKNESSES
1. **Focus on extensive rearing system**, leveraging larger herd size for potential economies of scale. 2. Relatively **even distribution of cattle by age,** facilitating diverse breeding programs and genetic enhancement. 3. Emphasis on genetic traits **relevant to extensive grazing systems, such as maternal instincts and environmental adaptability.**	1. **Potential challenges in managing a larger herd size**, including grazing management and supplementary feeding practices. 2. **Dependency on pasture conditions and environmental factors**, which may affect nutrition and overall herd health. 3. **Limited capacity for individualized care** and monitoring due to larger scale of operations.
OPPORTUNITIES	**THREATS**
1. **Exploration of alternative markets** or value-added products derived from extensive rearing practices. 2. **Collaboration with industry stakeholders and experts** to optimize grazing management and herd health protocols. 3. Adoption of advanced technologies and management practices **to enhance efficiency and productivity.**	1. **Intensifying competition** from larger-scale operations, posing challenges to market differentiation and profitability. 2. **Environmental and climatic risks**, such as droughts or extreme weather events, impacting pasture quality and herd health. 3. **Regulatory changes or policy developments** affecting agricultural practices and environmental standards.

Farm F3 employs a semi-intensive grazing system, managing a total of 280 cattle. The distribution by age reflects strategic management practices. Approximately 57% of the herd consists of adult cattle over 18 months old, while 29% are yearlings aged 12-18 months. Calves aged 0-12 months make up the remaining 14%. F3's focus on rearing taurines suggests a commitment to breeding programs geared towards meat production. Genetic selection may prioritize traits such as growth rate, feed efficiency, and carcass quality (Brito et al., 2021; Berry, 2022). With a significant land area (280 ha), Farm F3 likely benefits from ample pasture resources, supporting extensive grazing and minimizing the need for supplementary feeding during the grazing season. Proximity to local veterinary services may facilitate regular health checks and disease management protocols, ensuring the well-being of the herd.

The SWOT analysis (Table 7) provides a comprehensive overview of F3's, shedding light on key factors influencing its operational dynamics and strategic decision-making.

Table 7. The SWOT Analysis of F3

STRENGTHS	WEAKNESSES
1. **Utilization of semi-intensive grazing system**, optimizing land resources and supporting extensive grazing practices. 2. **Majority of the herd comprised of adult cattle**, reflecting strategic breeding programs and emphasis on meat production. 3. Proximity to local veterinary services**, facilitating regular health checks and disease management protocols.**	1. **Limited data availability**, potentially hindering comprehensive analysis and decision-making. 2. **Relatively high proportion of adult cattle**, which may increase vulnerability to market fluctuations and production risks. 3. **Dependence on natural pasture resources**, subjecting the herd to environmental variability and seasonal fluctuations.
OPPORTUNITIES	**THREATS**
1. **Exploration of alternative breeding strategies and genetic selection criteria** to enhance meat production efficiency and carcass quality. 2. **Implementation of innovative grazing management practices** to optimize pasture utilization and minimize supplementary feeding requirements. 3. Collaboration with industry experts and research institutions **to access specialized knowledge and resources for herd management.**	1. **Market volatility and unpredictable economic conditions**, impacting demand for meat products and farm profitability. 2. **Environmental risks**, such as droughts or disease outbreaks, affecting pasture quality and overall herd health. 3. **Regulatory changes or policy developments** affecting agricultural practices and land management policies.

F4, operates an intensive rearing system. The majority of its cattle population is concentrated in the age group of 12-18 months, accounting for 60% of the total herd. Cattle aged over 18 months represent 30% of the herd, while the remaining 10% consists of younger animals aged 6-12 months. As one of the largest farms with an extensive herd (433 taurines), F4 may invest in advanced breeding technologies and genetic selection methods to enhance productivity and profitability in an intensive rearing system (Brito et al., 2021; Berry, 2022). Intensive production on F4 necessitates precision feeding programs to meet the nutritional requirements of a large herd and optimize feed efficiency. The scale of operations on F4 requires robust health management protocols, including routine vaccinations, biosecurity measures, and disease surveillance to minimize health risks and maintain herd productivity (Doyle et al., 2023).

Following the detailed analysis of each farm's operations, the SWOT analysis (Table 8) was applied to elucidate the keys inherent in their respective management strategies and environmental contexts.

Table 8 The SWOT Analysis of F4

STRENGTHS	WEAKNESSES
1. Extensive herd size**, providing economies of scale and potential for increased production efficiency.** 2. Concentration of cattle in the 12-18 months age group**, facilitating standardized management practices and targeted breeding programs.** 3. Potential for investment in advanced breeding technologies and genetic selection methods **to optimize productivity and profitability.**	1. **Limited diversity in age distribution**, with a relatively small percentage of younger animals aged 6-12 months, potentially limiting flexibility in breeding and production strategies. 2. **High reliance on intensive rearing practices**, which may entail increased resource inputs and management complexity. 3. **Vulnerability to market fluctuations and external factors** due to the large-scale nature of operations and dependence on specialized inputs.
OPPORTUNITIES	**THREATS**
1. Adoption of innovative technologies and management practices **to enhance productivity, efficiency, and sustainability.** 2. Diversification of product offerings or market channels **to mitigate risks associated with market volatility.** 3. Collaboration with industry stakeholders and research institutions **to access resources, expertise, and market networks, fostering innovation and competitiveness**	1. **Regulatory changes or policy developments** impacting intensive farming practices, environmental standards, or animal welfare regulations, necessitating adaptation and compliance measures. 2. **Exposure to health risks and disease outbreaks**, requiring robust biosecurity measures and proactive disease management strategies. 3. **Intensifying competition** from other large-scale producers or industrialized farming operations, potentially affecting market share and profitability.

Farm F5, follows an extensive rearing system similar to F1. Here, approximately 35% of the herd is aged 6-12 months, 45% is aged 12-18 months, and 20% is aged over 18 months. F5's focus on semi-intensive rearing and a moderate-sized herd (103 taurines) may prioritize breeding for traits such as adaptability to outdoor environments, grazing efficiency, and reproductive performance. Farm F5 operates under a semi-intensive grazing system, managing a total of 103 cattle. The distribution across different age groups is as follows: adult cattle over 18 months old comprise 48.5% of the total, yearlings aged 12-18 months account for 28.2%, and calves aged 0-12 months make up the remaining 23.3%. Despite the semi-intensive approach, the farm relies primarily on natural forage resources for grazing, supplemented as needed to ensure adequate nutrition and herd health. Regular health monitoring and access to veterinary services remain critical for preventing disease and maintaining optimal herd conditions within the semi-intensive production system.

The SWOT analysis (Table 9) offers a clear evaluation of F5 farm, shedding light on key factors influencing its operational dynamics and strategic positioning.

Table 9. The SWOT Analysis of F5

STRENGTHS	WEAKNESSES
1. Strategic focus on semi-intensive rearing system**, capitalizing on natural forage resources and outdoor grazing opportunities.**	1. **Limited economies** of scale compared to larger operations, potentially impacting efficiency and profitability.
2. Diversified age distribution of cattle**, reflecting flexibility in management practices and breeding strategies.**	2. **Reliance on natural forage resources** may pose challenges during periods of scarcity or adverse weather conditions.
3. Moderate-sized herd **allows for more personalized care and attention to individual animal needs.**	3. **Moderate-sized herd** may limit bargaining power and market presence, affecting competitiveness.
4. Semi-intensive grazing **approach promotes animal welfare and environmental sustainability.**	**4. Dependence on external veterinary services and infrastructure** for health management and disease control.
OPPORTUNITIES	**THREATS**
1. Exploration of value-added products or niche markets **to diversify revenue streams and enhance profitability.**	1. **Fluctuations in feed costs or market prices** may impact production costs and overall profitability.
2. Collaboration with local agricultural institutions and industry stakeholders **to access resources, expertise, and market networks.**	2. **Regulatory changes or policy developments** affecting agricultural practices, environmental standards, or animal welfare regulations.
3. Adoption of innovative technologies or management practices **to improve efficiency, productivity, and sustainability.**	3. **Environmental and climatic risks**, such as droughts or extreme weather events, affecting pasture quality and animal health.
4. Expansion of the semi-intensive grazing system **to optimize land use and production potential.**	4. **Intensified competition from larger-scale operations or industrialized farming practices**, potentially squeezing profit margins and market share.

Lastly, F6 operates an intensive rearing system, with a majority of its cattle population aged 12-18 months, comprising 88.5% of the total herd. Cattle aged over 18 months represent 8.5% of the herd, while those aged 6-12 months constitute the remaining 3%. With a significant herd size of 850 taurines, F6 has the capacity to implement comprehensive breeding programs aimed at maximizing meat production efficiency and genetic potential in an intensive rearing system. Intensive production on Farm F6 requires precise nutritional management, with formulated diets tailored to meet the specific needs of different age groups and production stages. Rigorous health monitoring protocols, disease prevention measures, and access to veterinary expertise are essential for ensuring the well-being and productivity of the large herd on F6 (Doyle, 2023).

An analysis of Farm F6's SWOT (Table 10) provides valuable insights into its current position and future prospects within the intensive cattle rearing sector.

Table 10. The SWOT Analysis of F6

STRENGTHS	WEAKNESSES
1. Extensive experience in intensive cattle rearing**, leveraging knowledge and expertise accumulated over time.**	**1. Reliance on intensive rearing methods** may increase operational costs and resource requirements.
2. Large herd size **provides economies of scale and potential for increased production efficiency.**	**2. Limited flexibility in grazing management** due to the scale of operations and infrastructure constraints.
3. Capacity to implement sophisticated breeding programs **aimed at enhancing meat production efficiency and genetic potential.**	**3. Potential challenges in managing a large herd**, including disease control, nutrition, and environmental impact.
4. Robust health monitoring protocols and access to veterinary expertise **ensure optimal herd health and productivity.**	**4. High initial investment and ongoing maintenance costs** associated with intensive production systems.
OPPORTUNITIES	**THREATS**
1. Expansion into value-added products or niche markets to capitalize on consumer preferences for premium beef products.	**1. Market volatility and fluctuations in input costs** may impact profitability and financial stability.
2. Strategic partnerships with local agricultural institutions or industry stakeholders to access resources, expertise, and market networks.	**2. Regulatory changes or environmental policies** affecting intensive farming practices or land use.
3. Adoption of innovative technologies and practices to improve operational efficiency and sustainability.	**3. Competition from other livestock producers or alternative protein sources** may erode market share and pricing power.
4. Diversification of revenue streams through complementary activities such as agro-tourism or renewable energy production.	**4. Environmental risks** such as climate change, natural disasters, or disease outbreaks could disrupt operations and supply chains.

The distribution of cattle by age varies significantly across the studied farms, influenced by factors such as rearing system, management practices, and regional characteristics.

CONCLUSIONS

The study provides valuable insights into the complexities of cattle farming practices in northeastern Romania. By elucidating key aspects of farm management, breeding programs, feeding and nutrition strategies, and health management, our study contributes to the broader body of knowledge on livestock production systems. These insights can inform decision-making, policy development, and future research aimed at advancing the sustainability and productivity of cattle farming in Romania and beyond.

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RESEARCH ON THE EFFECT OF CROSSBREEDING PROLIFIC PALAS EWES WITH PALAS MEAT RAMS COMPARED TO PALAS MERINO BREED ON QUANTITATIVE AND QUALITATIVE MEAT PRODUCTION IN MALE LAMB FATTENING

Alexandru Gabriel VARTIC[1, 2], Corneliu Ion NEACSU[1], Petru Gabriel VICOVAN[1], Raducu RADU[1], Constantin PASCAL[2]

[1]Research and Development Institute for Sheep and Goat Breeding Palas, 248 I. C. Bratianu Blvd, Constanta, Romania
[2]"Ion Ionescu de la Brad" Iasi University of Life Sciences, Faculty of Food and Animal Sciences, 8 Mihail Sadoveanu Alley, Iași, Romania

Corresponding author email: gabriel.alexandru88@gmail.com

Abstract

The research was carried out within the Research and Development Institute for Sheep and Goat Breeding (R.D.I.S.G.B) Palas - Constanta and aimed at testing the fattening performances of meat crossbreeds (Prolific Palas breed x Meat Palas breed) compared to contemporaries from the Palas Merino breed. The two batches were fattened intensively for a period of 73 days, using granulated fodder with a content of 88% dry matter, 2570 Kcal and 160g digestible protein/kg during fattening. The final body weight was higher by 5.89%, and the average daily gain achieved during the entire fattening period was 14.62% higher in meat crossbreeds compared to contemporaries from the Merino Palas breed. The specific consumption of nutrients was lower in the group of meat crossbreeds that consumed 12.75% less Kcal metabolized energy and respectively 11.03% less protein compared to Merino de Palas lambs. The slaughter yield was higher by 4.7 percentage points in the meat hybrid group. The tissue composition of the carcass was characterized by a higher content in muscle by 1.9 percentage points and lower in bones by 1.22 percentage points in the meat hybrid group compared to contemporaries from the Palas Merino breed. The carcasses of the crossbreeds fell entirely into the U_2 class (very good, low-fat carcasses) compared to those of the Palas Merino lambs which were classified into the R_2 class (good, low-fat carcasses). Researches have demonstrated the superiority of crossbred meat lambs compared to contemporaries from the Palas Merino breed).

Key words: *crossbreeds, Palas Merino, sheep, yield.*

INTRODUCTION

The diversity of productions obtained through the exploitation of sheep constituted and constitutes a particularly important advantage of this species. This led to the increase of sheep herds, in certain areas of the world, pursuing specialization for certain productions, increasing the volume but also the value of the productions made per head of exploited sheep, the level of productions being determined by the breed of sheep and the system of growth used. Romanian research in the field of animal husbandry, over time, has been concerned with increasing meat production in local sheep breeds, because the adaptation and breeding of specialized sheep for meat from other countries have not yielded results in our country, causing many losses through mortality, and decreasing significantly fertility and birth rate. The solution to improve meat production in local Romanian sheep breeds is the creation of F1 and R1 crossbreeds in the direction of this production, using local and improved breeds, created in other countries, and also benefiting from the effects of the heterosis phenomenon, which represents the increase in the productivity of hybrid organisms in the first F1 generation compared to the parental forms. Sheep breeding is one of the important activities of animal husbandry, which ensures the need for high-quality proteins used in human nutrition. In order to improve the carcasses obtained from the sheep species, the best solution is the crossbreeding of local breeds with specialized breeds, this determines the quantitative and qualitative improvement of meat production and implicitly, an increase in the economic

efficiency of sheep farms. In the current economic situation, the raising and exploitation of sheep must be oriented towards the realization of productions based on market requirements, aiming at the same time, the creation of lines and breeds of sheep with pronounced skills for these productions, as well as the obtaining of superior quality carcasses, in accordance with EUROP grid (Fahmy, 1995).
In developed countries (England, France, Australia, New Zealand, etc.) for the profitability of sheep farms, the most used method is that of industrial crossings to obtain first-generation crossbreeds with superior quality carcasses. (Casas et al., 2004; Fathala et al., 2014; Jakubec et al., 1977; Ciobanu et al., 2023; Nechifor et al., 2022; Pascal et al., 2023).

MATERIALS AND METHODS

The research was carried out within R.D.I.S.G.B. Palas - Constanța, on two batches of 20 heads each, as follows: Batch 1: mixed meat males (Prolific breed Palas x Meat breed Palas) Batch 2: Palas Merino males. Within the two batches, their growth performances were tested in the fattening process, determining the increase in weight, the consumption of nutrients as well as the quality of the obtained carcasses. For fattening the lambs, granulated mixed feed with an energy content of 2570 Kcal/kg, 16% crude protein, 3.5% crude fat and 8.5% crude cellulose was used. The length of the growing and fattening period was 73 days; the administered food was weighed daily and the leftovers after a period of 2-3 days, after which the average consumption of feed and nutrients per head and day as well as the specific consumption of nutrients obtained during the present experiment was calculated. The animals were kept in collective boxes, previously subjected to the disinfection process and ensuring a surface of 0.85 m^2 per head in both batches. Drinking water was provided at discretion, in watering troughs and before the start of the experience the lambs were subjected to the deworming process. After the end of fattening, 3 animals were chosen from each batch with body weights similar to the average of the batch, later being weighed and sacrificed after a 24-hour diet. Weighed the warm carcasses, which were refrigerated at temperatures of 2-4°Celsius, for 24 hours. The following day, the carcasses were weighed individually, sectioned on the median line, proceeding to be cut into regions (the jig, the front limb and the rest of the carcass). Each region was carefully dissected, separating muscle from fat (intermuscular and covering and bone). Slaughter yields were determined:

$$\text{Yield 1} = \frac{\text{Cooled carcass weight (kg)}}{\text{Live weight (kg/head)}} \, x \, 100$$

$$Yield\ 2 = \frac{\text{Chilled Carcass Weight (kg)}}{\text{Empty Live Weight} * \text{ (kg/head)}} \, x \, 100$$

*Empty live weight from which the gastrointestinal mass has been subtracted. (Pădeanu, 2000; Vicovan, 1980).
The tissue composition (%) of the carcasses consisting of muscle, fat and bone was determined.
The carcasses were classified according to the European classification grid (Czeslawa et al., 2001). All data were processed statistically, and the Fischer test (analysis of variance) was used to interpret the differences between the groups (Laville et al., 2002; Snedecor, 1968).
The solution to improve the quality of meat production in Romanian sheep breeds is the creation of F1 and R1 crossbreeds for this production, using local breeds and improved breeds, the latter being created in other countries, this causing the phenomenon of heterosis, which increases the vigor of hybrid organisms in the first F1 generation, compared to the parental forms (Stanford et al., 1998; Pascal et al., 2022).
Body measurements performed on live animals were performed with zoometer, compass and tape as follows:
- Width at the coxofemoral joints, measured with the compass between the coxofemoral joints;
- The perimeter of the jig, measured with a tape, passing over the knee joint and the buttock point;
- The length of the jig, measured with the ribbon well stretched on the inner side of the right hind limb, between the ischiopubic symphysis and the middle of the tibio-metatarsal joint.
Based on the body dimensions, the following body and constitution indices were calculated:
- Gigot compactness index according to the formula (Laville et al., 2002):

$$C.I.G = \frac{\text{Width of coxofemural joints}}{\text{Length of gigot}} x100$$

Muscularity index of the gigot (Vicovan et al., 2010)

$$M.I.G = \frac{\text{Perimeter of the gigot}}{\text{Lenght of gigot}} x100$$

RESULTS AND DISCUSSIONS

The body weight of the two batches at the beginning of fattening showed close values, respectively 28.09-28.65 kg (Table 1).

Table 1. Evolution of body weight and average daily gain in lambs subjected to intensive fattening

Spec	Nr.	Initial Body Weight (kg)		Final Body Weight (kg)		Average Daily Gain (g)	
		$\bar{x} \pm s_{\bar{x}}$	V%	$\bar{x} \pm s_{\bar{x}}$	V%	$\bar{x} \pm s_{\bar{x}}$	V%
Meat crossbreeds lambs	20	28.65 ± 0.79	12.33	43.15 ± 1.26	13.06	198.70 ± 5.21	11.73
Palas Merino lambs	20	28.09 ± 0.65	10.35	40.75 ± 1.20	13.17	173.35 ± 4.92	12.69

SUMMARY			
Groups	*Count*	*Sum*	*Average*
2	20	3467	173.35
1	20	3974	198.7
ANOVA			
Source of Variation	*F*	*P-value*	*F crit*
Between Groups	4053.377696	3.17	4.098171731
Within Groups			
Total	6486.4702	39	

At the beginning of the experiment, the body weights were between 28.09 kg for lambs from the Palas Merino breed and 28.65 kg for the group of mixed meat lambs, the groups being homogeneous and similar in terms of age, sex and body weight. At the end of the intensive fattening period (73) days, the body weights were between 40.75 kg and 43.15 kg, being higher in meat hybrids by 5.89% compared to the batch of contemporary Palas Merino lambs. The average daily gain achieved in the two batches was 173.35- 198.70 g, being 14.62% higher in the batch with mixed meats, the differences being significant ($p < 0.05$). The statistical analysis was calculated with the help of the ANOVA program, which ensures the statistical processing of the data from the experiments carried out, while at the same time, with the help of the aforementioned program, a correct interpretation of the results obtained within the experiments is followed. The p value is extremely small (3.17) which indicates that there is a significant difference between the groups. So, the null hypothesis that there are no differences between the groups can be rejected. This interpretation is reinforced by the fact that the F value (4053.377696) is much higher than the F crit (4.098171731). The data obtained in the present experiment agree with those obtained by crossing sheep from the Transylvanian Merino breed with rams from the Suffolk, Merinofleisch and Berrichon du Cher breeds (Pascal, 1997). Thus, from their study, it is found that the groups subjected to fattening recorded relatively similar increases in growth (Table 2).

Table 2. Results obtained during the fattening of crossbreeds and purebred lambs (Maier, cited by Pascal, 2007)

Variant	Initial Body Weight (kg)	Final Body Weight (kg)	Average Daily Gain (g)
	$\bar{x} \pm s_{\bar{x}}$	$\bar{x} \pm s_{\bar{x}}$	$\bar{x} \pm s_{\bar{x}}$
Suffolk x Transylvanian Merino	18.011 ± 0.97	43.117 ± 1.80	209 ± 8.92
Merinofleisch x Lowland Transylvanian Merino	15.864 ± 0.53	41.382 ± 1.04	212 ± 7.81
Berrichon du Cher x Lowland Transylvanian Merino	15.864 ± 0.53	41.342 ± 1.04	212 ± 7.81
Lowland Transylvanian Merino	18.305 ± 0.35	42.147 ± 0.76	198 ± 6.08

It can be seen that the four groups achieved close weight gains. It should be noted that the crossbreed lambs resulting from crossing the Transylvanian Merino with the Merinofleisch and Berrichon du Cher breeds achieved the highest daily average gain, being 1.43% higher than the Suffolk x Transylvanian Merino variant and 7.07% higher compared to the daily average obtained by fattening the Transylvanian Merino breed.

The daily average feed consumption per head and day was identical (1.16 kg/head) in the two groups studied, while the daily intake of dry matter was identical in the two groups studied, respectively 1020 g D.M./head.

Table 3. Feed consumption and nutrients in crossbred meat lambs (Prolific Palas breed x Palas Meat breed) compared to Merino de Palas lambs

Specification	Combined Feed Consumption (kg/head)		Daily Dry Matter Intake (g/head)	Dry Matter Intake (% of live weight)	Feed Conversion Efficiency (growth increment g/kg DM)
	Per lot daily	Per head per day feeding			
Crossbred meat Lambs	23.13	1.16	1020	2.36	195
Palas Merino Lambs	23.13	1.16	1020	2.50	170

If we report the intake of D.M. in terms of body weight, this is 2.36% in the meat crossbred batch and 2.50% in the batch of Palas Merino lambs, being higher in the latter by 5.93%. Regarding the efficiency of feed conversion, this was 195 g increase in growth in meat crossbreeds and 170 g in lambs from the Palas Merino breed, being superior to the first by 14.71% (Table 3).
The specific consumption of nutrients in the two batches is presented in Table 4.

Table 4. Feed consumption and nutrients in crossbred meat lambs (Prolific Palas breed x Palas Meat breed) compared to Merino de Palas lambs

Specification	Specific Consumption					
	Metabolized Energy (Kcal)			Digestible Crude Protein (g)		
	Total period per head	Per head per day feeding	Per 1 kg weight gain	Total period per head	Per head per day feeding	Per 1 kg weight gain
Crossbred Meat Lambs	217613	2981.2	15009	13548.80	185.60	952
Palas Merino Lambs	217613	2981.2	17193	13537.12	185.44	1070

In order to achieve 1 kg increase in live weight, 15,009 Kcal of metabolizable energy was consumed in the group of meat crossbreds and 17,193 Kcal of metabolizable energy in the group of Palas Merino lambs, being 12.70% lower in the crossbred meat group. The specific consumption of digestible crude protein per 1 kg of live body weight was between 952 g in the group of mixed meat lambs and 1070 g in the group of Palas Merino lambs, being higher in the latter by 12.39%.
After finishing the fattening period, the main indices of body conformation and constitution of the animals from the two batches under study were determined (Table 5).

Table 5. Body measurements and body indices on the live animal related to the development of the rear train, depending on the genotype

Nr. crt	Specification	Genotype	
		Crossbred lamb batch (Prolific breed x Palas Meat breed)	Merino Palas lamb batch
		$\bar{x} \pm s_{\bar{x}}$	$\bar{x} \pm s_{\bar{x}}$
1	Coxofemoral joint width (cm)	22.55 ± 2.05	21.98 ± 1.14
2	Perimeter of the gigot (cm)	56.33 ± 3.92	56.03 ± 3.56
3	Length of gigot (cm)	26.85 ± 2.07	26.75 ± 2.21
4	Compactness index of the gigot (C.I.G.)	83.99 ± 6.73	82.17 ± 6.23
5	Muscularity index of the gigot (M.I.G.)	209.80 ± 8.98	209.00 ± 8.62

From the data obtained in Table 5, it follows that the body dimensions are different, depending on the group studied. Thus, in the two batches the width of the carcasses showed dimensions between 21.98 ± 1.14 cm and 22.55 ± 2.05 cm at the coxo-femoral joints, the perimeter of the jig was between 56.03 ± 3.56 cm and 56.33 ± 3.92 cm, while the jig had a length between 26.75 ± 2.21 cm and 26.85 ± 2.07 cm, being higher in the meat crossbred group by 0.37-2.59%.
The compactness index of the jig was between 82.17 ± 6.23% and 83.99 ± 6.73%, and the muscularity index of the jig showed values between 209.00 ± 8.62% and 209.80 ± 8.89%, the values obtained being superior in both indices to the lot of meat crossbreeds (Prolific Palas breed x Palas Meat breed) compared to contemporaries from the Palas Merino breed.
The live weight at the experimental control slaughters was between 42.03 kg (the group of meat crossbreeds) and 43.93 kg (the group of Palas Merino lambs) as shown in Table 6. The empty weight was 37.19 kg for the group of meat mestizos and 38.77 kg for the batch of lambs from the Palas Merino breed. The weight of the cooled carcass presented values between 18.64 kg (lambs from the Palas Merino breed) and 19.43 kg (crossbred lambs), the values obtained being superior to the latter by 4.24%. Yield 1 at slaughter was between 42.40 and 47.10%, while the yield 2 at slaughter was between 48.10% and 53.24%.

Table 6. Slaughter yield in crossbred meat lambs (Palas Prolific breed x Palas Meat breed) compared to Palas Merino lambs

Specification	Units	Crossbred Lambs			Palas Merino Lambs		
		n	$\bar{x} \pm s_{\bar{x}}$	V%	n	$\bar{x} \pm s_{\bar{x}}$	V%
Live Weight	kg	3	42.03 ± 2.75	11.34	3	43.93 ± 1.64	6.46
Empty Body Weight	kg	3	37.19 ± 2.78	12.94	3	38.77 ± 1.65	7.37
Chilled Carcass Weight	kg	3	19.43 ± 1.67	14.58	3	18.64 ± 0.79	7.34
Yield 1	%	3	47.10 ± 1.28	4.71	3	42.40 ± 1.25	5.11
Yield 2	%	3	53.24 ± 1.32	4.29	3	48.10 ± 1.12	4.03

There is a superiority Yield 1 at slaughter in meat hybrids by 4.7 percentage points compared to Palas Merino lambs, the differences being significant ($p < 0.05$).
The slaughter yield 2 was between 48.10% and 53.24%, higher by 5.14 percentage points in the meat crossbreeds, the differences being significant ($p < 0.05$).
The tissue composition of the carcasses reveals that the meat crossbreeds had more muscle, by 1.9 percentage points, compared to the Palas Merino lambs (Table 7).

Table 7. The tissue composition of carcasses in meat crossbreeds (Palas Prolific breed x Palas Meat breed), compared to Palas Merino breed

Specification	Tissue Composition (%)		Differences between Crossbred Lambs and Merino Palas ± percentage points
	Crossbred Meat Lambs	Palas Merino Lambs	
Total, of which:	100	100	
Muscle	59.96	58.06	+ 1.9
Fat	16.14	16.82	- 0.68
Bone	23.90	25.12	- 1.22

Also, compared to lambs from the Palas Merino breed, the meat crossbreds had less fat and bones respectively in the carcass, with 0.68 and 1.22 percentage points, respectively.
The tissue composition of the carcass in the two groups was characterized by the following values: muscle 58.06-59.96%, fat 16.14-16.82% and bones 23.90-25.12%.
Table 8 presents the classification of the carcasses resulting from the intensive fattening of the two batches studied.

Table 8. The tissue composition of carcasses in meat crossbreeds (Palas Prolific breed x Palas Meat breed), compared to Palas Merino

Specification		Breed			
		Crossbred Meat lambs		Palas Merino lambs	
		Nr. of carcasses	%	Nr. of carcasses	%
Conformation Class	E	-	-	-	-
	U	3	100	-	-
	R	-	-	3	100
	O	-	-	-	-
	P	-	-	-	-
Fatness class	1	-	-	-	-
	2	3	100	3	100
	3	-	-	-	-
	4	-	-	-	-
	5	-	-	-	-
Total		3	100	3	100

It is observed that all the carcasses of the meat crossbreeds (Prolific Palas breed x Palas Meat breed), according to conformation, are of class U (very good carcasses), and according to the stage of fattening, they fall into class 2 (low-fat carcasses). The carcasses of contemporaries from the Palas Merino breed fall into class R (good carcasses), and according to the stage of fattening they are of class 2 (low-fat carcasses).

CONCLUSIONS

Following the intensive fattening of crossbred meat lambs (the Prolific Palas breed x Palas Meat breed) and those from the Palas Merino breed, the following conclusions were drawn:
The body weight at the beginning of the fattening period was between 40.75 kg and 43.15 kg, being higher by 5.89% in the batch of crossbred meat lambs (Prolific Palas breed x Palas Meat breed).
The daily average gain recorded was 173.35-198.70 g, the batch of crossbred meat lambs achieving a 14.62% higher gain compared to the Palas Merino lambs.
The combined average feed consumption per head and per day presented identical values (1.16 kg) in the two batches.
For 1 kg of D.M. ingested, crossbred meat lambs achieved a 195 g increase in growth compared to 170 g in Palas Merino lambs, the increase being higher in crossbred lambs by 14.71%. The specific consumption of nutrients was lower in the group of crossbreeds who consumed 12.70% more Kcal of metabolizable energy and

respectively 11.03% less protein compared to contemporaries from the Merino Palas breed.

The slaughter yield 1 was between 42.4-47.10%, being higher for meat hybrids by 4.7 percentage points.

The slaughter yield 2 was 53.24% in the meat crossbreeds and 48.10% in the batch of Palas Merino lambs, the difference of 5.14 percentage points being a significant one.

The tissue composition of the carcass was characterized by the following values: muscle: 58.06-59.96%; fat: 16.14-16.82%; bones: 23.90-25.15%.

The meat hybrids had more muscle in the carcass by 1.9 percentage points, and fewer bones, by 1.22 percentage points compared to the batch from the Palas Merino breed.

The carcasses of the crossbreeds fell into the U2 class (very good, low-fat carcasses) and the carcasses of the contemporaries from the Palas Merino breed fell into the R2 class (good, low-fat carcasses).

By determining the main indices of conformation and constitution of the males belonging to the two groups under study, higher values are obtained in the crossbred meat group compared to the lambs from the Palas Merino group.

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THE EFFECTIVENESS OF THE INFLUENCE ON THE EMISSION OF HARMFUL GASES FROM PIG MANURE DURING STORAGE IN LAGOONS THE ADDITION OF MINERAL FERTILIZERS

Mariia VOROBEL[1], Oleh KLYM[1], Vasyl KAPLINSKYI[2], Myron PETRYSHYN[1], Taras PRUDYUS[2], Oksana SMOLIANINOVA[2], Olha STEFANYSHYN[2], Mariia TSAP[2], Andrii PYLYPETS[2], Ivan LUCHKA[2]

[1]Institute of Agriculture of the Carpathian Region NAAS, 5 Mykhaila Hrushevskoho Str., v. Obroshyne, Lviv Region, Ukraine
[2]Institute of Animal Biology NAAS, 38 Vasylya Stusa Str., Lviv, Ukraine

Corresponding author email: vorobelmariia@gmail.com

Abstract

In the structure of the animal husbandry production, a prominent place belongs to the pig industry thanks to the valuable products and quick payback. However, when providing the population with food, a large amount of waste accumulates, which is not only a valuable organic fertilizer, but also a producer of environmental pollution, since gaseous air pollutants are emitted into the atmosphere during their decomposition. As a result of the conducted research, the effectiveness of the investigated mineral fertilizers – phosphorite flour and slaked lime in reducing the level of ammonia (NH_3), carbon dioxide (CO_2), methane (CH_4), nitrogen oxide (NO) and hydrogen sulfide (H_2S) emissions from pig manure at storing it in lagoons was established. In particular, it was established that the addition of phosphorite flour and slaked lime to the pig manure in lagoons contributes to lower emissions of gases (NH_3, CO_2, CH_4, NO, H_2S), respectively by 18.4-33.6% and 8.8-30.8%. At the same time as the emission of the studied gases decreases, mineral fertilizers provide a lower level of the pH, i.e. it shifts to the acidic side. Thus, the establishment of the effectiveness of the investigated mineral fertilizers on the reduction of emissions of harmful gases from pig manure during its storage in lagoons indicate the perspective of their use to prevent environmental pollution in the pig industry.

Key words: *harmful gases, mineral fertilizers, pig farming, pollution, waste.*

INTRODUCTION

Animal husbandry is a strategic branch of the national economy, as it provides not only the domestic market of our country with food, but is also a significant reserve for the country's export potential. It also provides the crop industry, in particular agriculture, with organic fertilizers, that is, manure (Demchuk et al., 2010; Palapa et al., 2016; Zakharchenko, 2017). The largest amount of manure is produced in cattle breeding – 44%, pig farming – 39%, poultry farming is responsible for the accumulation of 17% of waste (Pinchuk & Borodai, 2019). In particular, 4 kg of excrements is produced for each liter of milk (with a productivity of 5 thousand liters per year), 11 kg for 1 kg of pork (for live weight gains of 600 g per day), 30 kg for 1 kg of beef (for gains of 1 kg per day), and 4.6 kg of waste is generated for 1 kg of poultry meat. And this is without taking into account water and litter that enters the manure (Buzovskyi et al., 2008).

Agricultural production is closely related to environmental conditions, the availability and possibility of exploitation of natural resources – land, water, forests, plants and animals (Smith et al., 2008; Demchuk et al., 2010; Mykhailova, 2016). Agricultural enterprises are powerful polluters of air, water sources and soil, which leads to a decrease in their profitability and competitiveness. Only one pig farm of 100,000 pigs pollutes the natural environment as much as a large industrial center with a population of 400-500,000 people (Maksishko & Malik, 2012; Zakharchenko, 2017). The specific smell from a piggery complex with 108,000 pigs spreads to a distance of up to 5 km (Dubin & Vasylenko, 2014). In modern conditions, environmental protection is one of the most important issues. Effective management of the livestock industry is impossible without a purposeful, scientifically based approach in this area (Smith et al., 2008; Tubiello et al., 2014; Caro, 2019). Therefore, the priorities in the livestock industry are not only to meet food needs, but also the minimization of the negative impact of by-products on the environment, ensuring the welfare of animals and at the same time increasing the profitability of the industry.

In agriculture currently the second place in the production of livestock products, after poultry, is occupied by the pig industry (Nykyforuk & Zhukorskyi, 2014). The anthropogenic impact of the functioning of agricultural enterprises on the atmosphere occurs as a result of the entry into it of the decomposition products of organic waste – harmful gases that cause the deterioration of the ecological situation and, therefore, climate change (Khodorchuk et al., 2014; Pinchuk & Borodai, 2019; Caro, 2019). The fight against climate change is a global challenge, the ways to solve this issue are reflected in a number of successively concluded international agreements: the United Nations Framework Convention on Climate Change (1992), the Kyoto Protocol (1997) and the Paris Agreement (2016). (Kholod, 2009; Udova et al., 2014; Pinchuk, 2015). Taking into account that with the help of the tools provided by the first two agreements, it was not possible to achieve a significant reduction of greenhouse gas emissions, the Paris Agreement was concluded. The latter is aimed at reducing greenhouse gas emissions to a level that by 2030 will not exceed 60% of the 1990 level and limiting the increase in air temperature to 1.5ºC from the pre-industrial level (Mykhaylova, 2016; Tymoshchuk et al., 2022).

According to estimates by the World Food and Agriculture Organization, the livestock sector is responsible for 18% of all greenhouse gas emissions, which is more than emissions from transport (14%) (Tubiello et al., 2014; Palapa et al., 2016; Korbych, 2021). The livestock sector accounts for the formation of about 9% of global carbon dioxide emissions (the duration of its retention in the atmosphere is 50-200 years), 37% of anthropogenic methane (the global warming potential is 21-34 times higher than CO_2), 64% of ammonium emissions and 65% – nitrous oxide (potential of influence is 265-310 times higher than CO_2) (Gerber et al., 2013; Binkovska and Shanina, 2016). An indirect source of potential greenhouse gas – N_2O is ammonia, which causes eutrophication of water bodies, leads to acid rain, soil acidification and is associated with the formation of aerosols (Blunden & Aneja, 2008; Korbych, 2021). Among the by-products of manure decomposition, the most toxic gas with an unpleasant smell is considered to be hydrogen sulfide, which is one of the factors in the occurrence of acid rain and, therefore, climate change (Blunden & Aneja, 2008). It is predicted that emissions of the main greenhouse gases will increase by 25-90% by 2030 relative to the indicators of 2000, if a number of measures to improve the situation are not adopted (Smith et al., 2008; Udova et al., 2014).

According to the National Register of Anthropogenic Emissions of Greenhouse Gases in Ukraine, the second place belongs to animal excrement, and pig farming occupies 46%. Methane emissions from the decomposition of pig manure are known to be higher (3.19 kg/head/year) compared to emissions from intestinal fermentation (1.5 kg/head/year) (Herman, 2009).

In the sources of scientific and and patent literature there are known methods of reducing emissions into the atmosphere of some particular gases from pig farming waste, mainly ammonia, hydrogen sulfide, somewhat less methane, with the use of peat (Portejoie et al, 2003), an additive "ManureMax" (Shah & Kolar, 2012), basalt tuff (Broschak et al., 2017), the preparation "Bioprogress" (Broschak et al., 2020). At the same time, it is urgent to reduce the negative impact of emissions of a complex of harmful gases on the environment – NH_3, CO_2, CH_4, NO, H_2S from organic waste.

The purpose of the study was to establish the effectiveness of the influence of mineral fertilizers on the emission of harmful gases (NH_3, CO_2, CH_4, NO, H_2S) from pig manure when stored in lagoons.

MATERIALS AND METHODS

A study on the effectiveness of using mineral fertilizers to reduce the emission of harmful gases from pig manure when it is stored in lagoons was conducted at Ugryniv Eco Ferm LLC, Chervonohrad District, Lviv Region. The experiment was performed using: electrochemical, mathematical-statistical and analytical methods.

To reduce the level of emissions of harmful gases (NH_3, CO_2, CH_4, NO, H_2S) from pig manure in the lagoons, mineral fertilizers were applied in the optimal pre-installed (*in vitro)* dose – 2.5%: Option I – control (without adding substances); Option II – phosphorite flour;

Option III – slaked lime. The emission level of the studied gases was determined with the help of a portable gas analyzer – Dozor C-M-5 (device inspection certificate type UA.TR.001 212-18 and certificate of conformity No. UA.TR.002.CB.1234-19). Determination was carried out once a week for a month after the application of the studied mineral fertilizers.In the course of the experiment, acidity was monitored using the pH-Meter Typ N5170.

The statistical analysis of the obtained research results was carried out by the methods of variational statistics using the standard package of Microsoft Excel and AtteStat application programs using the Student's t-test. The differences between the average arithmetic values were considered probable for: * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

RESULTS AND DISCUSSIONS

On the basis of the conducted experimental studies, it was established that the most noticeable effect in reducing the emission level of the studied gases – NH_3, CO_2, CH_4, NO, H_2S when using mineral fertilizers – phosphorite flour and slaked lime from pig manure during its storage in lagoons was observed on the seventh day. The duration of the positive effect on the reduction of emission of harmful gases from pig manure in the lagoons with the use of the studied fertilizers gradually decreased up to 31st day of research.

In the process of conducting experimental studies, it was established that the addition of phosphorite flour to pig manure in lagoons contributes to a lower level of ammonia emission (Figure 1), depending on the day of the experiment (1st, 7th and 31st), respectively by 21.3%, 27.0 ($P<0.05$) and 9.8% ($P<0.05$). When using slaked lime, a decrease in the NH_3 release from pig waste when stored in lagoons was noted: by 11.4% on the 1st day, by 19.7% ($P<0.05$) on the 7th day and by 6.6% on the 31st day of the experiment.

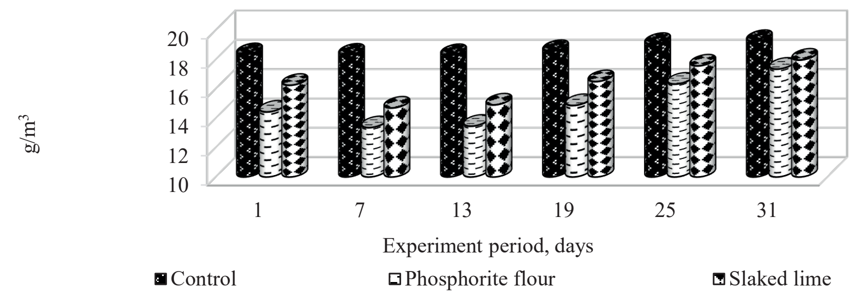

Figure 1. Effect of mineral fertilizers on the level of NH_3 emission from pig manure during its storage in lagoons

The analysis of the results shows the effectiveness of phosphorite flour on the level of carbon dioxide emission (Figure 2) from pig manure in the lagoons, which decreases compared to the control, respectively: by 27.0% ($P<0.05$), 28.4 ($P<0.05$) and 8.7% depending on the day of the experiment – 1st, 7th and 31st day.

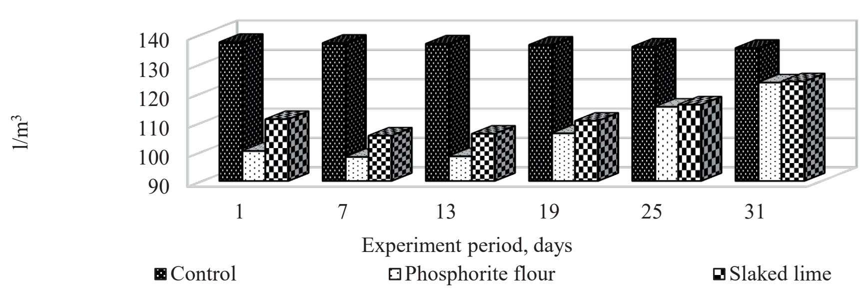

Figure 2. The influence of mineral fertilizers on the level of CO_2 emissions from pig manure during its storage in lagoons

When applying slaked lime, there is a decrease in CO_2 release from pig waste when stored in lagoons – by 19.1% (P<0.01) on 1st day, 23.1% (P<0.01) on 7th day and by 8.5% – on the 31st day of research.

According to the research results, it was established that the addition of phosphorite flour causes a lower level of methane emission (Figure 3) from pig manure in the lagoons, respectively: on the 1st day – by 15.0% (P<0.05), on the 7th day – by 18.4% (P<0.05) and on the 31st day – by 5.7% (P<0.05) relative to the control. Somewhat worse results regarding the reduction of CH_4 emissions from pig waste when stored in lagoons were obtained with the use of slaked lime. The difference, compared to the control, was 7.1%, 8.8 and 3.2%, depending on the day of research – 1st, 7th and 31st day.

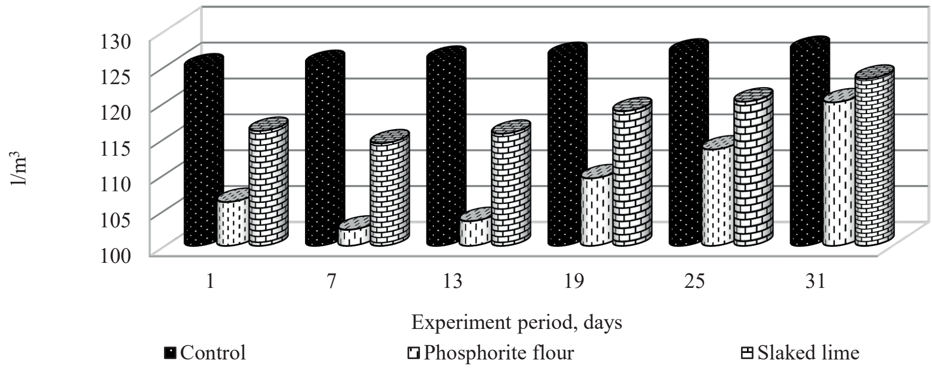

Figure 3. The influence of mineral fertilizers on the level of CH_4 emission from pig manure during its storage in lagoons

The introduction of phosphorite flour helps to reduce the level of nitrogen oxide emissions (Figure 4) from pig manure in the lagoons, depending on the day of the experiment, respectively: by 23.7% (P<0.05) – on 1st day, 33.6% (P <0.05) – on the 7th day and by 7.7% – on the 31st day. When slaked lime is added to pig waste in lagoons, NO release decreases by 19.7% (P<0.05), 30.8 (P<0.05) and 6.9%, depending on the period of research – 1st, 7th and 31st day.

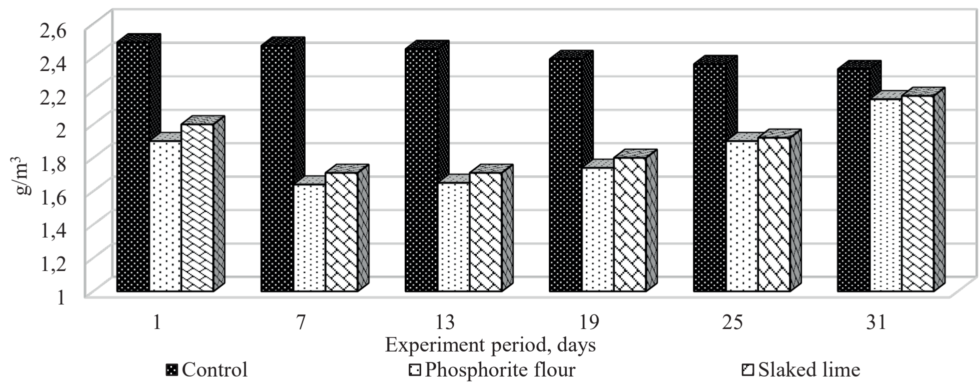

Figure 4. Effect of mineral fertilizers on the level of NO emission from pig manure during its storage in lagoons

Experimental data confirm that the use of phosphorite flour contributes to a lower level of hydrogen sulfide emission (Figure 5) from pig manure when it is stored in lagoons, compared to the control, depending on the day of the experiment: on the 1st day – by 15.8%, on the 7th day – 27.9% (P<0.05) and on the 31st day – by 7.4%. The addition of slaked lime causes a decrease in H_2S release from pig waste in lagoons, respectively – by 10.5% (P<0.01), 24.6% (P<0.01) and 6.1% (P<0.05), depending on the period of research (1st, 7th and 31st day).

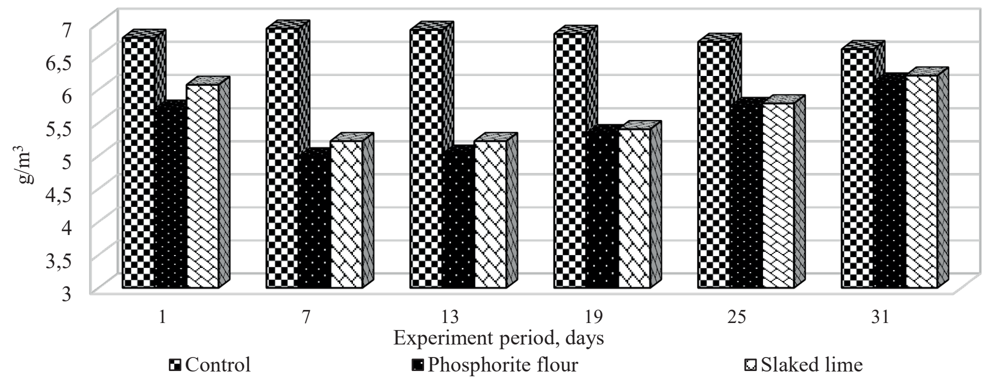

Figure 5. Effect of mineral fertilizers on the level of H_2S emission from pig manure during its storage in lagoons

On the basis of the obtained results, it was established that the pH value of pig manure when stored in lagoons (Figure 6) in control was 6.44-6.58, and when mineral fertilizers were applied – phosphorite flour and slaked lime – it decreased to 5.65 and 5.81, respectively.

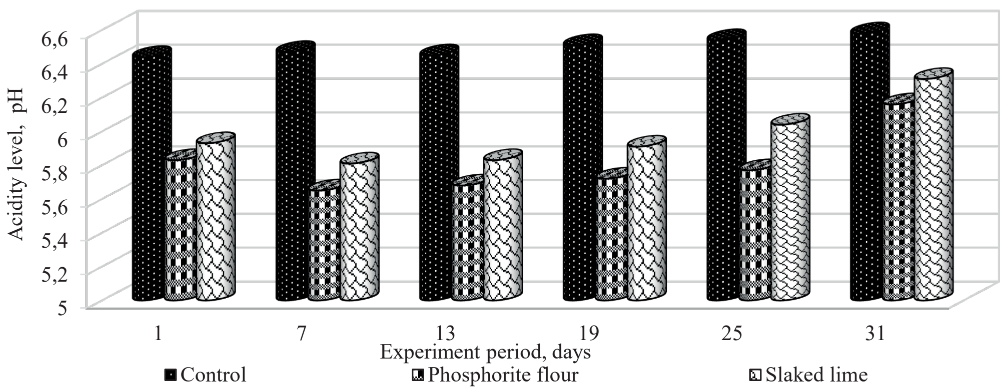

Figure 6. Changes in the level of acidity of pig manure when it is stored in lagoons, options with mineral fertilizers

Summarizing the above, it should be noted that those investigated substances, which provided the lowest pH value, made it possible to reduce the emission level of harmful gases, NH_3, CO_2, CH_4, NO, H_2S, from pig manure when stored in lagoons to the greatest extent.

Therefore, the analysis of research results shows the effectiveness of mineral fertilizers – phosphorite flour and slaked lime on reducing emission of the studied gases from pig manure when it is stored in lagoons.

It was experimentally established that the best results of the tested mineral fertilizers (phosphoric flour and slaked lime) in reducing by 18.4-33.6% the level of gas emissions, NH_3, CO_2, CH_4, NO, H_2S, from pig manure when it is stored in lagoons were obtained using phosphorite flour. Slaked lime contributed to lower gas emissions relative to the control on 8.8-30.8%. Thus, phosphorite flour has a more effective effect on reducing the emission of the studied gases from pig manure in lagoons – by 2.8-9.6%, relative to slaked lime.

Therefore, the research results indicate the feasibility of adding the studied mineral fertilizers to pig manure in lagoons to reduce emissions of harmful gases when storing by-products of animal origin, which will make it possible to prevent environmental pollution and preserve nature purity for future generations, and thus increase the profitability and competitiveness of the pig farming industry.

CONCLUSIONS

The expediency of using the studied mineral fertilizers – phosphorite flour and slaked lime to reduce the emission of harmful gases – NH_3, CO_2, CH_4, NO, H_2S from pig manure during its storage in lagoons due to changes in the pH of the substrate has been theoretically substantiated and proven. It was experimentally confirmed that in the comparative evaluation of the action of mineral fertilizers – phosphorite flour and slaked lime, the most effective reduction of emissions of the studied gases from pig manure in lagoons occurs by the use of phosphorite flour – 18.4-33.6%.

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ANALYSIS OF CASES OF DOG BITES IN STARGARD, POLAND

Daniel ZABORSKI[1], Katarzyna M. KAVETSKA[2], Katarzyna SABATOWICZ[2]

[1]Department of Ruminants Science, West Pomeranian University of Technology, Klemensa Janickiego 29, 71-270 Szczecin, Poland
[2]Department of Animal Anatomy and Zoology, West Pomeranian University of Technology, Klemensa Janickiego 33, 71-270 Szczecin, Poland

Corresponding author email: daniel.zaborski@zut.edu.pl

Abstract

The aim of this study was to analyse dog bites (n = 213) in the area supervised by the County Veterinary Inspectorate in Stargard between 2018 and 2020. The proportion of dog bites was significantly higher in 2018 (44%; $p < 0.05$) and in the summer (36%; $p < 0.05$). Adults (84%) were bitten significantly more often ($p < 0.05$) than children (16%). Most frequently (56%; $p < 0.05$) the bite took place in the home or on the owner's property. The highest percentage of bites (39%) was characteristic of 0 to 4-year-old dogs ($p < 0.05$). The proportion of biting males (70%) was significantly higher ($p < 0.05$) than that of females (30%). Mixed-breed dogs (77%) predominated ($p < 0.05$). The highest percentage of bites (16%) was caused by the smallest dogs (below 10 kg of body weight; $p < 0.05$). The proportion of bites caused by vaccinated dogs (81%) was significantly higher than that for unvaccinated animals ($p < 0.05$). The problem of dog bites requires further investigation, since the large proportion of cases is not reported.

Key words: *cynology, bites, dog breeding.*

INTRODUCTION

Millions of dog bites take place around the world every year (Desai, 2020). Most of these incidents are not serious, but the police have noted fatalities in Poland as well (Fiszdon and Boruta, 2012). In the vast majority of cases the victims are children (Abuabara, 2006; Chen et al., 2018), who very often experience trauma as a result (Fiszdon and Boruta, 2013; Reinholz, 2017). Failure to adequately supervise dogs leads to serious legal conflicts, injuries, and emotional disorders (following trauma) in people (Fiszdon and Boruta, 2012). Depending on the extent of the injuries, they may result in disability, deformation and mutilation of the body (Abuabara, 2006; Chen et al., 2018).

The subject of dog bites is linked to every aspect of raising a dog (Bernardo et al., 2000). Its later life will depend on the owner's level of awareness, commitment, and inclination (Edo and Estrada, 2016). Dog bites do not only result from aggression; they are more often due to a lack of awareness about dog breeds, breeding, diseases, and most importantly, a dog's individual needs (Abuabara, 2006; De Keuster et al., 2006). Very often it is the owners who bear responsibility for dog bites, when they purposely or unintentionally trigger aggression in the dog (Matthias et al., 2015). A lack of adequate supervision is the most common cause of dog bites, as dogs are territorial animals that defend their territory and may behave aggressively towards strangers (Abuabara, 2006).

Taking into account the above, the aim of this study was to analyse cases of dog bites in the area under the supervision of the County Veterinary Inspectorate in Stargard from 2018 to 2020, taking into account the sex and age of the victims, the circumstances of the incident, the time of year when it took place, and the type of dog.

MATERIALS AND METHODS

The research material consisted of documenttation provided by the County Veterinary Inspectorate in Stargard. These were documents from epizootic investigations containing statements from dog owners regarding cases of dog bites, taken down by a veterinarian. The data covered the period from 17 January 2018 to 31 December 2020.

The area under the supervision of the County Veterinary Inspectorate in Stargard includes the city of Stargard and the Stargard rural commune (Figure 1).

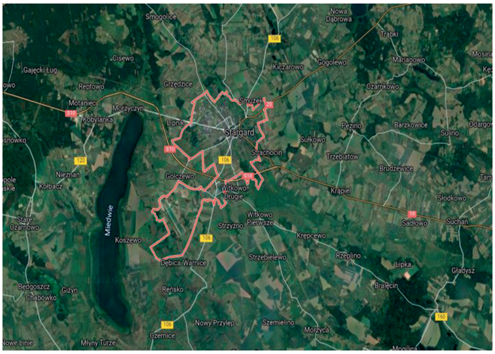

Figure 1. Map of the commune of Stargard, Poland
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A total of 213 cases of dog bites were described during the study period. The study took into account factors pertaining to the dog, the circumstances of the incident, and characteristics of the victims. For the analysis of dog age and body weight, four groups were distinguished (0 to 4, 5 to 8, 9 to 12 and 13 to 15 years, as well as 0 to 10, 11 to 24, 25 to 39 and 40 to 70 kg, respectively). Detailed analysis of dog bite cases was analysed using Microsoft Excel (Microsoft Inc., Redmont, WA, USA) and Statistica (v13.3, Tibco Inc., Tulsa, OK, USA). The Pearson's Chi-square test was used for determining the significance of differences in the number of dog bites. Statistical significance was declared at $p < 0.05$.

RESULTS AND DISCUSSIONS

The analysis of the reports of the County Veterinary Inspectorate in Stargard (2018-2020) showed that the number of dogs subject to epizootic control following a bite decreased from year to year (Figure 2).

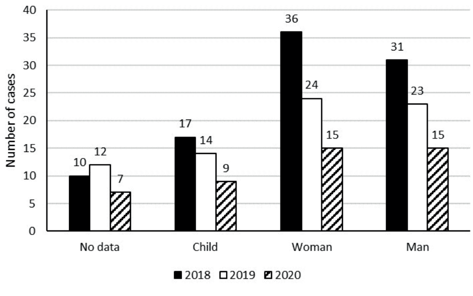

Figure 2. Characteristics of dog bite victims
from 2018 to 2020

The number of dog bites was significantly higher in 2018 ($p < 0.05$; about 8 cases per month, 94 in total). In 2019 the number of dog bites fell slightly, by two cases a month (73 in total). In 2020 the number was only half of that recorded in 2018 (46 cases in total).

This may have been linked to lockdowns during the COVID-19 pandemic, when people's movements were severely restricted. Shoesmith et al. (2021) confirmed changes in the behaviour of dogs associated with the pandemic. More than 65% of those surveyed reported that during the first lockdown in 2020 they observed changes in their dogs' behaviour, e.g. apathy, depression and passivity.

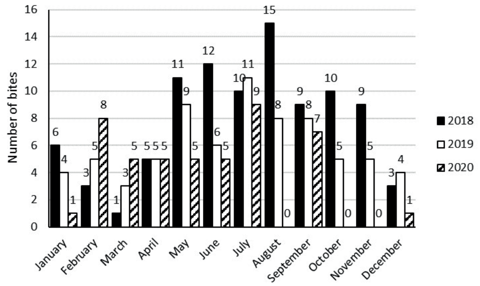

Figure 3. Number of dog bites
in different months each year

Analysis of the circumstances of the incidents revealed that dog bites significantly more often took place in the summer ($p < 0.05$; Figure 3). About one-third of all bites occurred in the three-month summer period (from July to September). The increased number of dog bites in summer is probably explained by the fact that people were more active, and dogs spent more time outside of the home (Borud & Friedman, 2000; Fiszdon & Boruta, 2013). A similar pattern was observed by Fiszdon & Boruta (2013); in the period from June to October, twice as many dog bites were noted as in the remaining months of the year. Maksymowicz et al. (2016) also observed a large number of dog bites during the summer period (29.04% of all cases), similarly to Schalamon et al. (2006) and Rosado et al. (2009), who found the peak incidence of dog bites during August.

Among the victims, adult women were bitten more frequently (n = 94; 53%) than adult men (n = 84; 47%), but the difference was not statistically significant. The sex of minor victims (under 18 years of age) was not reported in the bite records. Adults (n = 178; 84%) were bitten significantly more often ($p < 0.05$) than children (n = 35; 16%). A detailed information of child age was not provided in the records. Contrary to our findings, a study carried out in Spain (Rosado et al., 2009) showed a much higher percentage of children among victims of dog bites (30%), while research in the Netherlands revealed that dog bites were three times as common in children as in adults (Cornelissen and Hopster, 2010). According to the results of a Canadian study on the age of people bitten by dogs, 64% were adults and 36% were children (Guy et al., 2001a). A report on dog bite cases in various parts of the United States stated that the most common victims were children, and in 60-75% of cases the victims were under 20 years old (Overall & Love, 2001). The data pertaining to the site of the incidents (provided in 175 cases) are noteworthy (Figure 4).

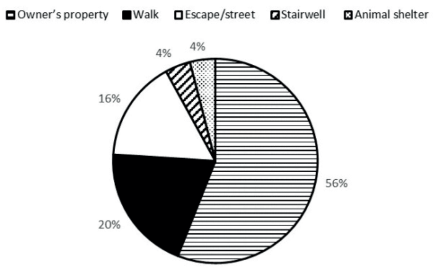

Figure 4. Sites of occurrence of dog bites from 2018 to 2020

Most often (56% of cases; $p < 0.05$) the bite took place in the home or on the owner's property. This was probably because the dog was defending its own territory or owner. Certain breeds are more likely to exhibit territorial behaviour and do so instinctively, without training. Most cases of this form of aggression, however, are taught and reinforced, more or less intentionally, by the dog's owner, who rewards this type of behaviour (Love & Overall, 2001; Jacobs et al., 2003; Taylor & Mills, 2006). In 20% of cases the dog bite took place away from the home (on a walk) (Figure 4). The cause of this type of bite may be 'lead reactivity', i.e. a dog's dynamic, unpleasant behaviour when a certain element appears in its surroundings (such as vehicles, objects, people or animals) (Shih et al., 2020; van Haaften et al., 2020). Not all behaviours that resulted in a bite were necessarily a specific form of aggression. The documentation did not contain information on any illnesses the dogs may have suffered from, their psychological state, or external factors.

Research conducted in the United States over 20 years (1979-1998) showed that about 75% of dog bites took place on the owner's property, of which 29% were associated with the dog being kept tethered (Sacks et al., 2000). Among 1,078 cases of dog bites in the Netherlands, 556, and thus slightly more than half, took place on the dog's territory, and in most cases (74%) the victim was the owner or another member of the household. These cases were usually less serious than those taking place in public spaces, and the victims did not usually require medical assistance (67% of cases) (Cornelissen & Hopster, 2010). Similar results were obtained in a study of dog bites in children. Among 100 such cases, 65 took place in the home, and 61 of the children bitten belonged to the family of the dog's owner. The bite usually took place during interactions between the child and the dog (Kahn et al., 2003; Horisberger et al., 2004; De Keuster et al., 2006). The reports analysed in that study did not contain information as to whether the property was inadequately secured or marked, or whether a stranger had intruded on the premises.

The animals observed under the supervision of the County Veterinary Inspectorate in Stargard also included very young dogs that had caused minor injuries (with their claws or teeth) to their owner/handler or a child belonging to the family. Many bites reported by the County Sanitary and Epidemiological Station in Stargard to the County Veterinary Inspectorate in Stargard were actually scratches.

In the present study, the age of the dogs ranged from 11 weeks to 15 years (mean = 5.8 years, SD = 3.5 years, n = 186; Figure 5). The highest number of bites (n = 74; 39%) was characteristic of 0 to 4-year-old dogs ($p < 0.05$). Further division of age groups according to breed was not possible due to the single representatives of individual breeds (most animals were mixed-breed dogs). Research conducted in Poland showed that the average age of a biting dog was about 5 years, but the study population also included two- and three-month-old puppies as well as 15-year-old dogs (Fiszdon and Boruta, 2012).

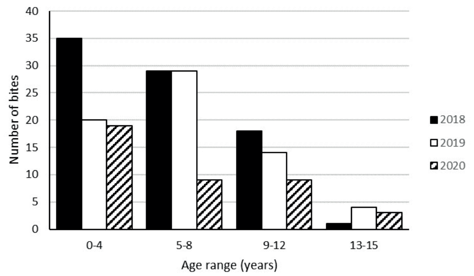

Figure 5. The number of dog bites according to dogs' age from 2018 to 2020

Beaver (1983) also reported a wide age range (from 9 weeks to 11 years among 120 analysed dogs), whereas Rosado et al. (2009) recorded values from 0 to more than 12 years among 657 examined dogs. In a Canadian study including 227 biting dogs, the average age was five years. All dogs were at least 6 months of age, and the maximum age was 15 years (Guy et al., 2001c). Another study carried out in Canada on 3,027 dogs showed the average age of four years. Most of the dogs were more than one year old, and the maximum age was 18 years (Guy et al., 2001b). The sex of the dogs was recorded in 196 cases (Figure 6).

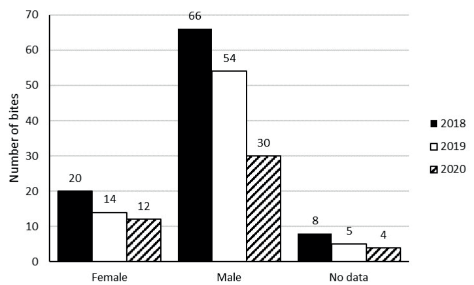

Figure 6. The number of dog bites according to dogs' sex from 2018 to 2020

The number of males (n = 150; 70%) was significantly higher ($p < 0.05$) than that of females (n = 46; 22%), although the number of dogs (both males and females) decreased from year to year. Ethological research suggests that male dogs display aggressive behaviour more often than females (Rosado et al., 2009). Offensive or territorial aggression may be positively correlated with testosterone levels (Nelson & Chiavegatto, 2001; Haug, 2008), but it is difficult to explain aggression caused by fear in this manner (Jacobs et al., 2003). Such a pronounced over-representation of males in the study material may also have been due to the preferences of dog owners who choose to keep male dogs rather than females (Fiszdon & Boruta, 2012).

From 2018 to 2020 dog bites were most often attributed to dogs of mixed breeds ($p < 0.05$; Table 1).

Table 1. Number of bites according to breed from 2018 to 2020

Breed of dog	2018	2019	2020
Mixed breed	66	52	29
French Bulldog	0	0	1
Polish Lowland Sheepdog	0	0	1
Shiba Inu	0	1	0
Saint Bernard	0	1	0
Polish Hunting Dog	0	1	0
West Highland White Terrier	1	0	0
Rottweiler	1	0	1
Australian Silky Terrier	1	0	0
Boxer	1	0	0
Beagle	1	1	0
German Shepherd Dog	6	5	3
Shih Tzu	2	1	1
Cocker Spaniel	1	1	1
Siberian Husky	1	1	0
American Staffordshire Terrier	2	2	2
Bull Terrier	1	1	0
Poodle	1	0	0
Golden Retriever	1	0	0
No data	8	6	7

According to a Canadian study, mixed breeds were predominant among canine veterinary patients which had bitten a member of the household (41.9%), followed by the Labrador Retriever (6.6%), Springer Spaniel (4.0%), Golden Retriever (3.5%), Lhasa Apso (3.1%), and Shih Tzu (3.1%) (Guy et al. 2001a). Analysis of more than 30 breeds showed that those most inclined to display aggressive behaviour in various situations were the Dachshund, Chihuahua and Jack Russell Terrier breeds (Duffy et al., 2008). Different percentages have been obtained for Poland: mixed breeds more than 65%, German Shepherd about 14%, and Dachshund 4.1%, with only single cases in other breeds (Fiszdon & Boruta, 2012). A similar distribution of breeds of biting dogs was obtained by Maksymowicz et al. (2016), with mixed breeds accounting for 41% and German Shepherds for 21%. This distribution is evidence of the popularity of these breeds rather than their behavioural predispositions. In Poland, dogs of mixed breeds are the most popular, while among pure breeds, German Shepherds account for 8.6% of pet dogs and

Dachshunds for nearly 3% (ZKwP, 2022). A Belgian study on dog bites in children showed that German Shepherds, which make up 29.3% of the population, accounted for 51.9% of cases. Second place was occupied by Rottweilers (20.4% of bites), with a 27.7% share of the overall population, followed by Labradors (16.7% of bites), for which the percentage of bites was significantly lower than their proportion in the overall population (38.1%) (De Keuster et al., 2006).

In the Netherlands, an analysis of cases of dog bites in various breeds in relation to their representation in the local population showed a significantly higher risk of bites in the case of Belgian Shepherds, Bouvier des Flandres, Dobermans, German Shepherds, Jack Russell Terriers, and Rottweilers, while the risk from mixed breeds was significantly lower (Cornelissen & Hopster, 2010).

The size of the dogs under observation was determined by veterinarians in 92 cases, who classified them as follows: small dogs up to 10 kg, medium dogs 11 to 25 kg, and large dogs over 25 kg (Table 2). The highest number of bites was caused by the smallest dogs ($n = 34$; $p < 0.05$). In 2019 and 2020 there was an increase in the number of bites from dogs weighing 25 to 39 kg. An interesting finding of the research is that dogs with body weight above 40 kg accounted for the lowest percentage of bites during the three-year period (max. 17% in 2019; $p < 0.05$). This may confirm the widespread opinion that giant breed dogs are house dogs that are not interested in physical activity and thus less likely to bite people (Shulan, 2010). It is difficult to state conclusively whether it was the predominance of large dogs in the population that explained their large share in reports of dangerous behaviour. In the research material there were no cases of dog bites resulting in death, and the dogs under observation often caused only minor injuries. A large dog, irrespective of its behaviour, often appears more dangerous, although dogs of any breed can be dangerous and very serious injuries to children were inflicted even by toy breeds (Collier, 2006; Fiszdon and Boruta, 2012). It may be presumed that small dogs that have bitten a member of the household were not reported and not placed under observation. However, it is worth noting a Canadian study, in which increasing body size was associated with a reduced odds of biting (Guy et al., 2001b). The average body weight of biting dogs was lower than that of non-biting dogs (Guy et al., 2001c).

Table 2. Percentage of bites by dogs' body weight from 2018 to 2020

Body weight range [kg]	Percentage of bites		
	2018	2019	2020
≤10	35	34	27
11–24	37	26	37
25–39	17	23	36
≥40	11	17	0

Information about vaccinations was given in 194 cases. The number of dog bites caused by vaccinated dogs was significantly higher than that for unvaccinated animals (158 vs. 36; $p < 0.05$; Figure 7).

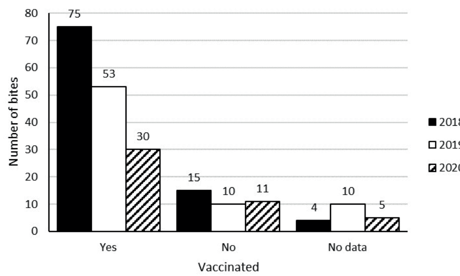

Figure 7. The number of dog bites according to vaccination status from 2018 to 2020

In 2018 the owners of 80% of dogs were able to present certification of rabies vaccination. The percentage of dogs confirmed to have been vaccinated against rabies was 73% and 65% in 2019 and 2020, respectively. This means that about 16 – 24% of owners did not have their dogs vaccinated, despite the fact that annual vaccination against rabies is the obligation of every dog owner. Veterinarians have no way to enforce this obligation, but can only recommend it during examinations. However, it cannot be directly determined whether unvaccinated status increases the frequency of biting (Fiszdon & Boruta, 2013).

CONCLUSIONS

The study indicates that the problem of dog bites has not been well investigated, and a large proportion of cases are not reported. Cases are most often reported when dogs are placed under observation and the consequences of the bites are more serious. The victims of most dog bites in the study area were adults, while children were a clear minority. Bites most often occurred in the summer, usually on the owner's property. In most cases the dogs were 0 to 4 years old, and males were found to be more aggressive. Mixed-breed dogs predominated and the vast majority of animals were vaccinated.

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TECHNOLOGIES OF THE AGRO FOOD PRODUCTS PROCESSING

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FROM WASTE TO VALUABLE FOOD: DEVELOPMENT AND QUALITATIVE DIFFERENTIAL CHARACTERIZATION OF BONE BROTHS FROM JUVENILE AND ADULT CATTLE

Bianca Georgiana ANCHIDIN, Ioana GUCIANU, Florin-Daniel LIPȘA, Marius Mihai CIOBANU, Elena-Iuliana FLOCEA, Paul Corneliu BOIȘTEANU

"Ion Ionescu de la Brad" Iasi University of Life Sciences, 3 Mihail Sadoveanu Alley, 700490, Iasi, Romania

Corresponding author email: paul.boisteanu@iuls.ro

Abstract

Following the slaughtering and processing of animals for meat, only one-third of them is meat, while the remainder consists of byproducts and waste, which need to be processed and utilized appropriately. Industrial byproducts constitute costly losses for these industries and pose challenges in their eco-friendly disposal. These costs can be offset through innovation to generate value-added products that increase profitability. Efficient utilization of byproducts has a direct impact on the economy and the environment. This study explores the potential to create collagen-rich bone broths and to this end, four batches were developed, two made from bones sourced from adult cattle and two from bones sourced from juvenile cattle. These were analyzed physicochemically and microbiologically to characterize them qualitatively. The findings suggest that both types of bone broths offer significant nutritional value, with variations influenced by the age of the cattle and the vegetable additions, as evidenced by highly significant differences ($p < 0.001$) among batches obtained through statistical processing. These findings underscore the importance of efficiently exploiting resources and the potential to develop valuable food products from seemingly residual sources.

Key words: *animal by-products, bone broth, new valuable products, quality characterization.*

INTRODUCTION

Meat and meat products constitute an important segment of the human diet as they provide essential nutrients that cannot be easily obtained through vegetables and their derivatives (Byers et al., 2002). They offer a means to reduce malnutrition and increase food security in households (Chikwanha et al., 2018). Over the past 20 years, the demand for meat and meat products has increased in many parts of the world (including Africa, Asia, Europe, and the United States of America), leading to a rapid growth in animal production for sustainable food security (Sans & Combris, 2015).
The slaughter of animals not only provides meat but also valuable by-products. The yield of these by-products has been reported to represent approximately 10 to 15% of the value of the live animal in developed countries, but following the slaughter and processing of animals, only one-third is meat, while the rest are by-products and waste (two-thirds), which need to be processed and utilized appropriately. The global meat industry economy requires the utilization of animal by-products for the livestock industry to remain economically competitive against plant protein sources (Irshad & Sharma, 2015). Processing by-products can turn a low-value product or one requiring costly disposal into a product capable of covering all processing and disposal costs, with higher added value and reduced environmental impact (Toldrá et al., 2012). Furthermore, any industry that transforms residual products into valuable products should be commended (Irshad & Sharma, 2015).
Bone broth is defined as a liquid obtained by boiling bones, and optionally adding vegetables, herbs, and spices to the boiling process (Gimbar, 2017). Bone broth, a food with a centuries-old history, has experienced a significant increase in popularity in the food and health industry in recent years. This trend can be partially attributed to the rise of the Paleo diet, which emphasizes foods consumed in the pre-agricultural era, including meat from sustainable sources, fish, nuts, vegetables, and fruits while

avoiding legumes, dairy products, refined sugar, grains, and processed foods. Bone broth recipes are often found in popular cookbooks that follow the principles of the "Paleolithic" diet and are promoted as an effective means of alleviating various conditions, from arthritis to wound healing (Gimbar, 2017). The success of a product involves the combined efforts of various professional branches, especially nutritionists, epidemiologists, food technologists, chemists specializing in natural products, and others (Anchidin et al., 2023).

Bones are essentially connective tissue similar to cartilage, composed of cells located in lacunae and collagen fibers (Uddin et al., 2021). Bone consists of a cell present in each lacuna and is connected to others through a series of bones traversing a matrix. This matrix contains collagen fibers, albuminoid substances, and calcium salts (Mushtaq et al., 2022). Bone is a hard tissue in the body, composed of two types of tissue, namely compact tissue and spongy tissue, which contain almost the same amount of collagen (Alipal et al., 2021). The color of fresh bone is yellowish-white, and when boiled, it becomes completely white. Bone contains organic and inorganic materials, the majority of which are inorganic materials such as calcium phosphate and calcium carbonate. At the same time, the rest are ions such as magnesium, potassium, fluoride, and chlorine (Rigueto et al., 2022). The inorganic materials in bone function to provide hardness to the bone structure.

The bone tissue is composed of bone cells that remain alive throughout the animal's life (Aykın-Dinçer et al., 2021). Bone cells are responsible for the bone matrix containing salts of several minerals such as sodium, potassium, phosphorus, calcium, and magnesium, type I collagen, and other proteins (proteoglycans, glycoproteins, and sialoproteins), in addition to acid mucopolysaccharides (hyaluronic acid, chondroitin sulfate, and heparin) (Aljumaily, 2011; Hay & Dane, 2016; Haluk et al., 2018). Bovine bones represent a highly promising material as a new material, especially for applications in the food, medical, textile, and medical textile fields (El-Aassar et al., 2021). This is because the content of compounds in bovine bones is highly compatible to be accepted by the body, such as collagen and gelatin (Asadi et al., 2021). In general, inedible by-products, such as bones, are used in the manufacture of fertilizers, animal feed, and fuels, but there is also a growing market for their use in obtaining protein hydrolysates and collagen. The main sources for obtaining collagen are the by-products resulting from the slaughter of pigs and cattle (Jia et al., 2010; Silva & Penna, 2012). Obtaining these products, which have high added value, represents a better alternative for the use of these by-products, which would otherwise be disposed of.

The word "collagen" comes from ancient Greek, where "kola" means glue and "gen" means producer. Collagen is a fibrous structural protein present in the extracellular matrix and connective tissue of animals (Ramshaw et al., 2009). It is the most abundant protein in the animal kingdom. Collagen is not present in plants and unicellular organisms, where its role is taken over by polysaccharides and cellulose. In the case of invertebrates, it is present in the body walls and cuticles. Collagen accounts for 25-30% of the protein content of the entire body, especially in mammals. It is found in the cornea, bones, blood vessels, cartilage, etc. (Müller, 2003), and its abundance in the animal kingdom is attributed to its unique thermal and chemical stability characteristics, which are conferred by intermolecular and intramolecular forces (Shoulders & Raines, 2009).

According to research conducted by Grand View Research (2021), the easy availability of collagen sources will lead to an increase in collagen-based product production until 2027, at an estimated annual growth rate of 5.9%. The growing demand for collagen in food products, beverages, cosmetics, and medical applications is expected to drive the demand for collagen. A significant portion of the collagen market is dominated by Europe and North America, with an increasing application of collagen in food, nutraceuticals, and cosmetics. In the Middle East and Africa, bovine collagen applications are on the rise due to easy availability. Additionally, the demand for non-genetically modified products is increasing in these regions due to the demand for 100% natural products.

Many of the collagen-rich products originate from by-products of the beef industry due to its high production (Silva & Penna, 2012). Besides bovine collagen, various sources such as porcine, poultry, fish, and marine algae can be utilized. In 2020, the global collagen market yielded 34.9% from bovine-derived products, which can be attributed to the large number of available bovine by-products (Grand View Research, 2023). Utilizing these by-products (tendons, skins, bones, hides) for collagen production represents an alternative for improving the circular economy of this sector.

Collagen is an economically renewable source (Yorgancioglu et al., 2020) because its extraction adds value to the by-products resulting from animal slaughter, and this reuse is considered of great importance in the quest for a clean, sustainable, and circular economy (Masilamani et al., 2016; Schmidt et al., 2016). In this context, skin processing by-products are valuable materials due to their composition, which contains substances ideal for producing gelatin and collagen peptides (Ali et al., 2020).

The necessity of optimizing the utilization of abattoir by-products represents a significant challenge for processing units globally. Bones, a by-product obtained from meat deboning, containing compounds of high value, can be processed into valuable products. This not only minimizes the environmental waste impact but also enhances the sustainability and profitability of the meat industry. The present study investigates the main characteristics of the physico-chemical and microbiological quality of a valuable product obtained from a by-product considered "waste" in the meat industry. This product is represented by bone broth, elaborated by utilizing bones from juvenile and adult cattle. The study aims to identify the qualitative differences between these two categories of raw materials and the influence brought by vegetable additives.

MATERIALS AND METHODS

The products subject to this study were obtained within the Meat Microproduction Workshop at the "Ion Ionescu de la Brad" University of Life Sciences in Iași.

In this study, cattle bones containing high amounts of marrow (femur and tibia), pelvic bones (coxal bone - consisting of ilium, ischium, and pubis), and the patella were used to obtain bone broth as raw material. These bones were purchased from a local abattoir, S.C. IASICARN S.R.L. (Tomești commune, Iași county). Root and bulb vegetables added to the broths included: parsley (root), carrot, and onion.

The process of making bone broth involved several steps, as follows: baking the bones at 120ºC for 60 minutes, slow boiling the baked bones for 12 hours at 110ºC, followed by two filtration stages to remove larger and smaller debris, portioning into jars and sterilizing them, thermostating, drying the jars, labeling, and storing at refrigeration temperatures (0-4ºC). In addition to these steps, vegetable-added bone broth involved several additional stages that occurred after filtering the bone broth and consisted of boiling the vegetables at 95-100ºC for 30 minutes and filtration, followed by the steps described above for bone broth without vegetable additives.

For the chemical determinations, samples of approximately 100-150 grams were collected from each batch. These samples were analyzed using the Food-Check apparatus (Bruins Instruments - a KPM Analytics brand, Puchheim, Germany) via an infrared light source (Boișteanu et al., 2023). This is a spectroscopic technique that utilizes the electromagnetic spectrum. The NIR (Near-Infrared) region is the spectrum defined by wavelengths ranging from 700 nm to 2500 nm.

The physical determinations of the bone broth batches consisted of pH value determinations, instrumental colorimetric determinations, and the calculation of process yield and losses. The pH values of the bone broth samples were determined using the Testo 206-pH2 pH meter for semi-solid food products (Testo Rom S.R.L., Cluj-Napoca, Romania). Instrumental color was determined in the CIELAB system using the luminosity scales (L*), red-green complementary color coordinates (a*), and yellow-blue complementary color coordinates (b*) using the Konica Minolta Chroma Meter CR-410 color analyzer, as in the study by Gucianu et al. (2023). The light source of the device was D65, and the observation angle was set at 10°C, in accordance with the work of Manoliu et al. (2023). Based on the results of the parameters concerning the CIE colorimetric coordinates a* and b*, Hue (H*) and Chroma (C*) were calculated using equations (1) and (2), according to Long et al. (2024). The C* value represents color saturation, indicating the distance covered by the gray achromatic central axis of the color space, and the hue angle (H*) reflects the chromaticity or color tone, ranging from 0° (red) - 90° (yellow) - 180° (green) - 270° (blue) - 360° (red) (Long et al., 2024).

$$H(*) = tan^{-1} \times b^*/a^* \quad (1)$$

$$Chroma\ (C^*) = \sqrt{(a*)^2 + (b*)^2} \quad (2)$$

For the measurement of microorganism count (Plate Count Agar - PCA) and certain bacterial species - *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* - in the bone broth samples, the method of successive dilutions was used. The samples were collected in accordance with the specifications of ISO 6887-2:2017 standard. The procedure involved preparing dilutions (10^{-1}, 10^{-2}, and 10^{-3}) from the collected samples using peptone water. Then, for each dilution, 1 ml was taken and transferred onto individual Petri dishes. These plates were subsequently used for bacterial cultivation on three distinct culture media: Rapid *Staph.* Agar, Plate Count Agar, Rapid *E. coli*, and Rapid *Salmonella*. The selection of culture media was determined by the specific type of target microorganism identified in each dilution. Plates with specific culture media were incubated for 24-36 hours depending on the target microorganism. After completing this step, the plates were removed and visual examination was performed using a colony counter with a counting grid and digital counting pencil (BOECO Colony Counter CC-1, Germany).

For each batch, a total of five trials were conducted for each of the analyses described above.

The results obtained after conducting the physico-chemical analyses were subjected to a one-way analysis of variance (ANOVA), followed by Tukey's post hoc test at a significance level of 5% ($p < 0.05$), to compare the mean values among the four batches of bone broth, as in the study by Ciobanu et al. (2023). IBM SPSS Statistics V21 software was used for statistical analysis.

RESULTS AND DISCUSSIONS

The results of the raw chemical composition of the bone broth samples studied are presented in Table 1. These consist of the mean values and standard deviation.

The moisture content of all bone broth samples ranged from 68.10% to 76.56%. As observed in Table 1, the highest moisture content values were recorded in the batches where the raw material was obtained from juvenile cattle (YBS1 and YBVS2). This may be due to a higher moisture content in the carcasses of young cattle, as observed by Coleman et al. (1993) for two different cattle breeds. Additionally, studies by Arthaud et al. (1977) show that the total moisture content of cattle carcasses decreases with age. The highest moisture content value was obtained in the sample of bone broth from juvenile cattle without vegetable additives (YBS1), which was 76.56 ± 0.089%, while the lowest was identified in the batch of broth obtained from bones of adult cattle with vegetable additives (ABVS4), which was 68.10 ± 0.600%. By consulting Table 1, it can be observed that the moisture content is higher in batches of bone broth without vegetable additives, regardless of the raw material used, even though they have high water content (Knez et al., 2022). These results may be due to the water absorption properties of root vegetables during heat treatment (Bradbury & Holloway, 1988). As these are eliminated at the end of the heat treatment, they led to a reduction in the total moisture content of the broths in which they were added.

The differences in moisture content between the four batches of bone broth were highly significant ($p < 0.001$). However, the mean values obtained were relatively similar within the batches where the same raw material was used, especially in the YBS1 and YBVS2 batches, where the difference was only 0.24%.

The lipid content varied considerably among the batches (Table 1), with highly significant differences ($p < 0.001$) observed upon statistical testing. The primary influence on this parameter was the origin of the raw material, with high values recorded in the batches where bones from adult animals were used, at 11.52 ± 0.760% (ABVS4) and 8.36 ± 1.677% (ABS3), compared to those where the bone tissue originated from young cattle, which had lower values of 1.26 ± 0.089% in the batch without vegetable additives and 1.62 ± 0.084% in the batch with vegetable additives. By examining these data, a slight increase in this parameter value can be observed in both batches where bones from adult cattle and those from juvenile cattle with added root vegetables were used. The differences were much more significant in the batches where the raw material consisted of bones from adult cattle, with differences of 3.16% between the two batches. Even though the amounts of lipids contained in the added vegetable products are extremely low, it can be assumed that they solubilized during the heat treatment in the liquid where it took place (the future bone broth), as observed in the studies by Bradbury & Holloway (1988), where the fat content of root vegetables decreases after the heat treatment stage, resulting in values very close to or even equal to 0.00. However, the fat contribution provided by the vegetable addition is not sufficient to account for such a large difference (3.16%) between the batches where bones from adult cattle were used. For this reason, it can be assumed that the reason for the much higher fat content in batch ABVS4 compared to batch ABS3 may be due to a better extraction of this qualitative parameter. This superior extraction can be attributed to a more rigorous adherence to the heat treatment, exceeding the established values for it, or more frequent opening of the boiling kettle in the batch with a lower fat content (ABS3), which led to a decrease in temperature and slower return to the temperature set in the product technical sheet, resulting in a more deficient fat extraction. Additionally, another cause may be attributed to the non-uniformity in sample collection. The bone broth from adult animals exhibited a significant fat layer, constituting approximately 20 - 30% of the jar's volume, compared to the broths where the raw material originated from young animals, where the fat layer was <5% of the jar's volume. This latter cause is presumed to be correct, considering that the other parameters of the chemical quality studied were not affected.

Table 1. The proximate chemical composition of the bone broth batches

Parameters (%)	Batches				p-value
	YBS1	YBVS2	ABS3	ABVS4	
Moisture	76.56 ± 0.089^{c}	76.32 ± 0.045^{c}	70,70 ± 1,389^{b}	68.10 ± 0.600^{a}	0.000 (***)
Lipid	1.26 ± 0.089^{a}	1.62 ± 0.084^{a}	8.36 ± 1.677^{b}	11.52 ± 0.760^{c}	0.000 (***)
Protein	22.06 ± 0.054^{c}	21.96 ± 0.055^{c}	20.42 ± 0.396^{b}	19.68 ± 0.164^{a}	0.000 (***)
Collagen	20.44 ± 0.055^{c}	20.34 ± 0.054^{c}	18.64 ± 0.428^{a}	17.90 ± 0.200^{a}	0.000 (***)
pH	8.80 ± 0.060^{c}	6.44 ± 0.047^{a}	8.69 ± 0.087^{c}	7.49 ± 0.059^{b}	0.000 (***)

$^{a, b, c}$ – Superscripts on different means within the same row differ significantly, $p > 0.05$; *** $p < 0,001$.
YBS1 – juvenile bovine bone soup; YBVS2 – juvenile bovine bone soup with vegetable addition; ABS3 – adult bovine bone soup; ABVS4 – adult bovine bone soup with vegetable addition.

The protein content of the studied samples, as presented in Table 1, is higher for batches made from bones of juvenile cattle, where values of 22.06 ± 0.054% (YBS1) and 21.96 ± 0.055% (YBVS2) were obtained, compared to batches made from bones of adult cattle, which had values of 20.42 ± 0.396% (ABS3) and 19.68 ± 0.164% (ABVS4). These values are much higher than those obtained by Ozturk & Kerimoğlu (2022), where the highest protein content value in one of the bone broth batches studied was 16.58 ± 0.44%, under the condition that sheep meat was also added, and for the batch obtained exclusively from bones (tibia), the protein content was only 3.27 ± 0.18%.

Collagen also shows highly significant differences ($p < 0.001$) among the four studied batches (Table 1). Batches made with bones from adult animals (> 24 months) have a lower collagen content of 18.64 ± 0.428% (ABS3) and 17.90 ± 0.200% (ABVS4), compared to the values of the same parameter obtained in batches where bones from cattle still in the growth and development phase (< 24 months) were used as raw material. Although at first glance, these results seem to contradict those in the literature, which indicate an increase in collagen content in animal tissues directly proportional to age, Williamson et al. (2001) observed a slight decrease in collagen content in adult cattle compared to that recorded in calves, but higher than the collagen content in animals in the fetal stage. Their study concluded that this decrease from younger to adult cattle could be due to the normal evolution of the biochemical properties of bones and joint cartilage during development. These results imply changes in the collagen component of articular cartilage as having important functional consequences during normal development and growth (Williamson et al., 2021). Furthermore, we can observe that batches of bone broth with vegetable additives have lower collagen content values, a fact motivated by the absence of collagen in plant-based products, which is specific only to animal tissues.

The average values obtained for the pH parameter are high (alkaline) in the case of three of the studied batches - YBS1 (8.80 ± 0.060%), ABS3 (8.69 ± 0.087%), and ABVS4 (7.49 ± 0.059%), as can be observed in Table 1. The results of the statistical test of variation of the samples (ANOVA) show that there are highly significant differences between all the studied samples in terms of the pH parameter, just like in the case of all the other parameters of the chemical quality of the bone broths. The relatively high average values of this parameter may be due to the relatively alkaline water in the municipality of Iași, as shown by the results obtained by Cohl et al. (2014). Another reason for the alkalinity of the studied batches could be the high microbiological load of the bones, which is not entirely destroyed during heat treatment, or the dissolution of minerals present in the bone tissue, especially calcium (Field et al., 1974), which can lead to an overall increase in the pH value of the analyzed products.

Table 2 presents the Pearson correlations between the studied chemical parameters. As can be seen in this table, the moisture content of the samples shows distinct significant correlations ($p < 0.01$) with all the other chemical parameters, with the exception of pH, where the interaction is nonsignificant ($p > 0.05$). Strong positive correlations of the moisture content of the samples are observed for the protein content and for the collagen content. In addition to these, there is also a negative correlation (-1.000**) between the moisture content of the samples and the lipid content, which is in line with the specialized literature (Cobos & Díaz, 2015).

Table 2. Pearson correlations between qualitative parameters of bone broth samples

Parameters	Moisture	Lipid	Protein	Collagen	pH
Moisture	1	**-1.000****	**.999****	**.999****	-.098
Lipid		1	**- .999****	**.999****	,093
Protein			1	**.998****	-.086
Collagen				1	-.096
pH					1

**Correlation is significant at the 0.01 level.

The fat content shows a significant negative correlation (- .999**) with the protein content (Table 2), as an increase in the latter implies a decrease in the former. Consequently, batches with higher lipid content (ABS3 and ABVS4) obtained lower scores for protein content (Table 1). The relatively significant differences in the average fat content between batches made with bones from juvenile cattle compared to those produced from bones from adult cattle are considered one of the causes of the highly significant differences ($p < 0.001$) in the average values for the protein parameter within the studied batches.

The proteins and collagen of the samples exhibit a significant correlation (.998**) between the studied samples, which represents a completely natural result, considering that collagen is a component of animal proteins. It is entirely normal for the collagen content to increase alongside the increase in crude protein content (Table 2).

The pH value does not significantly influence the other chemical parameters (Table 2), but its elevated values are most likely influenced by the presence of mineral elements (Field et al., 1974).

Color is considered the most important visual characteristic that can affect consumer preferences and satisfaction. Since it has been found that color parameters are influenced by recipe compositions in numerous meat systems (Ozturk & Kerimoğlu, 2022), we evaluated the variations in color parameters in the case of bone broths (Table 3).

The average values of the L* parameter can be observed to be higher in the case of broths made from bones from adult cattle (ABS3 and ABVS4, Table 3). The same trend can be observed within the broths made with bones from the same age category, where the broths without vegetable additions recorded higher values for the colorimetric parameter L* (YBS1 and ABS3). The broth made from bones from adult cattle without vegetable additions (ABS3) obtained the highest value for the L* parameter (71.24 ± 0.903), followed by the broth made with the same raw material but with vegetable addition (ABVS4), which obtained a value of 67.65 ± 0.882. From this, we can deduce that the most significant influence on the brightness of the sample was the raw material used and, subsequently, the addition of root vegetables, the latter leading to a slight "darkening" of the samples. The higher values for the colorimetric parameter L* in the case of samples using bones from adult cattle can also be attributed to the much higher fat content in these batches (Table 1), which is opaque white in color. All values for L* are statistically significantly different ($p < 0.001$).

The parameter a* obtained higher and closer values within the YBVS2 and ABVS4 batches, which are 2.81 ± 0.387 and 2.91 ± 0.084, respectively (Table 3). By analyzing the values in Table 3, significant differences ($p < 0.05$) can be observed between the L* and a* parameters, which are negatively correlated (-.467). Therefore, batches YBS1 and ABS3, which have higher values of the L* parameter, obtained lower values for the a* parameter, which are 0.15 ± 0.048 and 0.47 ± 0.063, respectively (Table 3). The colorimetric coordinate b* recorded higher values in the batches where the value of the coordinate a* was higher and where the brightness (L*) was lower (Table 3). To reinforce this finding, we can analyze Table 4, where positive correlations (.995**) can be observed between the a* and b* parameters, which are significant at a level of 0.01. As with the colorimetric parameter a*, a negative correlation (-.493*) was recorded between the b* parameter and brightness, which is significant ($p < 0.05$). The correlations obtained for the CIE L*, CIE a*, and CIE b* parameters are in line with those obtained by Ozturk & Kerimoğlu (2022).

Table 3. Colour parameters (L* - lightness, a* - redness and b* - yellowness), chroma and hue values (mean ± st dev) of the batches

Parameters	Batches				p-value
	YBS1	YBVS2	ABS3	ABVS4	
L*	67.04 ± 1.417^{b}	62.13 ± 3.699^{a}	71.24 ± 0.903^{c}	67.65 ± 0.882^{bc}	0.000 (***)
a*	0.15 ± 0.048^{a}	2.81 ± 0.387^{b}	0.47 ± 0.063^{a}	2.91 ± 0.084^{b}	0.000 (***)
b*	14.66 ± 0.591^{a}	37.03 ± 2.367^{c}	18.06 ± 0.385^{b}	35.554 ± 0.374^{c}	0.000 (***)
Chroma	14.67 ± 0.591^{a}	37.14 ± 2.388^{c}	$18.07 \pm 0,376^{b}$	35.67 ± 0.376^{c}	0.000 (***)
Hue	1.56 ± 0.000^{c}	1.49 ± 0.009^{a}	1.55 ± 0.005^{b}	1.49 ± 0.000^{a}	0.000 (***)

a, b, c – Superscripts on different means within the same row differ significantly, $p > 0.05$; *** $p < 0,001$.
YBS1 – juvenile bovine bone soup; YBVS2 – juvenile bovine bone soup with vegetable addition; ABS3 – adult bovine bone soup; ABVS4 – adult bovine bone soup with vegetable addition.

Table 4. Correlations between color parameters (L* - lightness, a* - redness and b* - yellowness), chroma and hue

Parameters	L*	a*	b*	Chroma	Hue
L*	1	**-.467***	**-.493***	**-.493***	,420
a*		1	**.995****	**.995****	**-.992****
b*			1	**1.000****	**-.986****
Chroma				1	**-.986****
Hue					1

*Correlation is significant at the 0.05 level.
**Correlation is significant at the 0.01 level.

The degree of color saturation (Chroma) shows a direct proportional increase with the a* and b* coordinates of the CIELAB system, as seen in the table. Consulting Table 4, which presents the Pearson correlations of the studied color parameters, it can be observed that there are strongly positive and significant correlations ($p < 0.01$), of .995** (the correlation between Chroma and a*) and 1.000** (Chroma and the b* parameter), the latter being a perfect correlation. The highest values of Chroma, 37.14 ± 2.388 (YBVS2) and 35.67 ± 0.376 (ABVS4), are observed in the broths with vegetable additions, indicating that this addition has the highest influence on this parameter.

The color tone (Hue) correlates positively only with the CIE L* coordinate, where we observe the value of .420 (Table 4), but this correlation is not significant. Negative and significant correlations ($p < 0.01$) are observed between hue and the other colorimetric parameters (a* -.992** and b* -.986**), as well as between hue and chroma (-.986**) (Table 4). Higher average values are observed in the broths without vegetable additions, which are 1.56 ±0.000 (YBS1) and 1.55 ± 0.005 (ABS3) (Table 3). Lots with higher values of color coordinates a* and b* have slightly lower values of the hue parameter (Table), which are 1.49 ± 0.009 and 1.49 ± 0.000 in the YBVS2 and ABVS4 lots, respectively.

Following the application of statistical tests, highly significant differences ($p < 0.001$) were obtained among all color parameters, chroma, and hue of the studied lots (Table 3).

Table 5 includes the microbial species for which microbiological analyses were conducted and the results obtained for them. All microbial species studied, except *Salmonella*, were identified in the samples examined.

Staphylococcus aureus exhibited the highest value (3.07 ± 0.045 log CFU/cm^2) in the ABVS4 batch, followed by the YBVS2 batch, with a value of 2.75 ± 0.010 log CFU/cm^2. Batches YBS1 and ABS3 obtained average values of 1.93 ± 0.025 log CFU/cm^2 and 2.34 ± 0.046 log CFU/cm^2, respectively (Table 5). From these results, it can be observed that batches without vegetable additives obtained lower values for this microorganism. Additionally, it can be noted that the values are slightly lower in batches where the raw material originated from juvenile cattle.

Escherichia coli, unlike *Staphylococcus aureus*, obtained lower values in batches where root vegetables were added (YBVS2 and ABVS4), at 1.83 ± 0.031 log CFU/cm^2 and 2.09 ± 0.036 log CFU/cm^2, respectively, compared to batches made exclusively from bones, where the recorded values were 2.14 ± 0.017 log CFU/cm^2 for YBS1 and 2.32 ± 0.021 log CFU/cm^2 for ABS3 (Table 5).

The PCA (Plate Count Agar) analysis was used to determine the number of aerobic bacteria in the samples studied. By analyzing the values in Table 5, it can be observed that the values resulting from this analysis are higher in batches where bones from young cattle were used for soup production and in batches where root vegetables were added. The highest value of the PCA analysis was 4.92 ± 0.020 log CFU/cm^2, obtained by batch YBVS2, which was made from both bones from young cattle and vegetable additions. The lowest value of this microbiological test was identified in batch ABS3, at 3.74 ± 0.032 log CFU/cm^2 (Table 5).

Salmonella was absent in all batches studied, consistent with the provisions of Regulation (EC) No. 2073/2005, as are the other results of the microbiological analyses conducted in this study.

All batches of bone broth (YBS1, YBVS2, ABS3, and ABVS4) showed significantly different results ($p < 0.001$) for the three microbiological analyses where colony-forming units were identified (*Staphylococcus aureus, Escherichia coli*, and Plate Count Agar) (Table 5).

Given that pH has a significant influence on the microbial contamination of meat and meat products (Sofos, 2014), we decided to perform Pearson correlations (Table 6) to observe if it might have had an influence on the microbiological quality of our products, considering that some of them recorded quite high pH values (Table 1) Moreover, its value also increases due to the high pressure during heat treatment, which promotes intensive and rapid multiplication of microorganisms that cause food spoilage (Hygreeva & Pandey, 2014). Certain protein-degrading bacteria can lead to the increase of pH in meat products, such as *Enterobacteriaceae* bacteria. The growth of microorganisms and their ability to cause spoilage in meat products depends on a variety of properties. These include intrinsic factors such as the type of organism causing spoilage and the initial load of bacteria present, pH, and water activity as well as the availability of substrates used for energy (Gribble, 2014).

Table 5. The average values of the microorganisms analyzed in the batches of bone broth studied

Batch	*Staphylococcus aureus* (log CFU/g)	*Escherichia coli* (log CFU/g)	Plate count agar (log CFU/g)	*Salmonella* (log CFU/25g)
YBS1	1.93 ± 0.025[a]	2.14 ± 0.017[c]	4.36 ± 0.026[c]	Abs.
YBVS2	2.75 ± 0.010[c]	1.83 ± 0.031[a]	4.92 ± 0.020[d]	Abs.
ABS3	2.34 ± 0.046[b]	2.32 ± 0.021[d]	3.74 ± 0.032[a]	Abs.
ABVS4	3.07 ± 0.045[d]	2.09 ± 0.036[b]	4.11 ± 0.010[b]	Abs.
p-value				
Sig.	0.000 (***)	0.000 (***)	0.000 (***)	-

a, b, c, d – Superscripts on different means within the same column differ significantly, p > 0.05; ns – non significant; *** p < 0,001.
YBS1 – juvenile bovine bone soup; YBVS2 – juvenile bovine bone soup with vegetable addition; ABS3 – adult bovine bone soup; ABVS4 – adult bovine bone soup with vegetable addition.

Table 6. Pearson correlations between the studied microorganism species and pH

Comparative indicators	*Staphylococcus aureus*	*Escherichia coli*	PCA	pH
Staphylococcus aureus	1	0.381	-0.180	0.266
Escherichia coli		1	**0.918****	**0.908****
PCA			1	**-0.735****
pH				1

** Correlation is significant at the 0.01 level.

The results obtained from the application of Pearson correlations were positively significant between *Escherichia coli* and PCA (0.918**) and between *Escherichia coli* and pH (0.908**). A highly significant negative correlation was identified between pH and PCA results (-.735**) (Table 6). The values for *Staphylococcus aureu*s did not show significant correlations either with the values of other microbiological analyses or with the pH value.
These results indicate that pH may have an influence on the presence of microorganisms in the samples studied, but not for all bacterial species.

CONCLUSIONS

The research conducted in this investigation highlights notable variations among bone broths based on the composition of the ingredients used, whether of animal or vegetable origin, in terms of physicochemical, colorimetric, and microbiological characteristics. Although derived from the same species, the choice of raw materials for bone broth production, whether it be from juvenile or adult bovine bones, has been associated with significant differences in the final product's quality. The influence of vegetable additives has also been crucial in determining the qualitative differences among batches of bone broth.
Specifically, broths prepared with bones from young bovines (under 24 months of age) exhibited higher protein and collagen contents compared to those obtained from adult bovine bones, and the addition of bones was associated with higher average lipid content in the samples studied. Additionally, vegetable additives had a significant impact on the pH of the broths, resulting in significant differences between those with and without vegetable additives.
Regarding the instrumental color of the samples studied, it was greatly influenced by the presence or absence of vegetable additives in the bone broth samples. The use of root vegetables in the production of two of the bone broth batches led to increased brightness (L*) and higher values of the colorimetric parameters a* (redness) and b* (yellowness) of the samples.
The results of the microbiological analyses conducted on the bone broth samples indicate significant variation between batches, except for *Salmonella*, which was absent in all samples. *Staphylococcus aureus*, PCA, and *Escherichia coli* showed different values depending on the composition and origin of the ingredients used. pH values were also variable, with some samples recording high values.
Ultimately, both bone broths derived from juvenile and adult cattle, with or without vegetable additives, exhibited a high level of quality, particularly in terms of protein and collagen content. However, the significant variations between these types of broth were evident and deserve special attention.

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ENHANCING ANTIOXIDANT CAPACITY IN FUNCTIONAL MEAT PRODUCTS THROUGH INFUSION WITH SEA BUCKTHORN OIL TO COMBAT INHERENT ANTIOXIDANT DEFICIENCY

Bianca Georgiana ANCHIDIN, Florin-Daniel LIPȘA, Diana-Remina MANOLIU, Marius Mihai CIOBANU, Mihai Cătălin CIOBOTARU, Ioana GUCIANU, Paul Corneliu BOIȘTEANU

"Ion Ionescu de la Brad" Iasi University of Life Sciences, 3 Mihail Sadoveanu Alley, 700490, Iasi, Romania

Corresponding author email: paul.boișteanu@iuls.ro

Abstract

Given the growing concern in recent years for a healthier diet, attention must also be directed towards improving the quality profile of meat products and transforming them, as much as possible, into functional foods that combine the benefits of plant-based products with those of animal-origin products. With this in mind, we aimed to develop a functional meat product, given the recent scrutiny these products have faced, by using an oil with antioxidant effects to enhance the antioxidant profile of products with insignificant endogenous antioxidant levels. To achieve this, three batches of pork tenderloin were injected with 1%, 3%, and 5% sea buckthorn oil, and were analyzed in terms of antioxidant capacity, physicochemical and microbiological quality, and sensory perception. Most results showed highly significant differences ($p < 0.001$) between batches, with superior quality observed in the batch injected with 5% sea buckthorn oil and subjected to heat treatment. However, consumers preferred the batch injected with 3% sea buckthorn oil due to its more balanced taste. This research underscores the potential to develop meat-based functional foods with enhanced nutritional benefits.

***Key words**: antioxidants, functional meat products, meat products, sea buckthorn oil.*

INTRODUCTION

Meat and meat products are important foods with essential nutritional components such as essential amino acids, fatty acids, vitamins, and minerals, which form a significant component for normal physiological and biochemical processes (Kausar et al., 2019).

There is an increasing demand for healthier meat and meat products that contain lower levels of fat, reduced cholesterol, reduced sodium chloride and nitrite content, an updated fatty acid profile, and the addition of health-enhancing ingredients among consumers worldwide (Kausar et al., 2019).

The term of functional foods was first introduced in Japan in the mid-1980s and refers to processed foods that contain ingredients which help specific functions of the body in addition to being nutritious (Kumar et al., 2012). The concept of functional foods stems from the traditional paradigm of providing methods for preventing nutritional deficiencies; this paradigm includes foods that offer health benefits through micronutrient fortification (Katan & De Roos, 2004). To date, Japan is the only country that has formulated a specific regulatory approval process for functional foods. Known as Foods for Specified Health Use (FOSHU), these foods are eligible to bear an approval seal from the Japanese Ministry of Health and Welfare (Kumar et al., 2012).

The European Commission's Concerted Action on Functional Food Science in Europe (FuFoSE), coordinated by the International Life Sciences Institute (ILSI) Europe, defined functional foods as: 'food products that, in addition to their basic nutritional impact, have beneficial effects on one or more functions of the human body, thereby improving general and physical conditions and/or reducing the risk of disease progression. The amount of consumption and the form of functional foods should be as typically expected, consumed in the daily diet. Therefore, they should not be in the form of pills or capsules, but only in the form of conventional foods' (Hoffmann et al., 2010). The International Life Sciences Institute of North America (ILSI) states that functional foods are 'foods that, due to physiologically active food components, provide health benefits beyond basic nutrition.' This definition is vague, but encompasses the scope of functional foods (Vattem & Maitin, 2016).

One of the most interesting functional ingredients that can be added to meat products is sea buckthorn oil, which contains high concentrations of vitamin C, carotenoids, tocopherols, and other bioactive compounds with a strong antioxidant role (Anchidin et al., 2023).

Common sea buckthorn (*Hippophae rhamnoides*), also known as Siberian pineapple, is a spiny, dioecious shrub (or tree) from the family Elaeagnaceae that can grow up to 7 meters in height (Fu et al., 2014; Wang et al., 2014). Sea buckthorn has a unique nutritional composition, containing vitamins (A, C, D, E, F, K, P, and the B complex), 18 free amino acids, and a unique profile of unsaturated fatty acids, making it the only plant source of omega-7. Its bioactive phytochemical compounds possess various biological activities such as antioxidant, immunomodulatory, anticarcinogenic, hepatoprotective, cardioprotective, anti-atherogenic, and radioprotective properties (Shah et al., 2021).

Sea buckthorn fruit oil contains an average of 35% palmitoleic acid (16:1n-7) (Suryakumar & Gupta, 2011). Sea buckthorn oil is rich in antioxidant compounds such as vitamins A and E, sterols, and flavonoids (Ursache et al., 2017). It has been used in traditional medicine in Eastern Europe and Asia for the treatment of asthma, circulatory disorders, and other conditions (Suryakumar & Gupta, 2011; Zadernowski et al., 1997). Seed oil contains high concentrations of tocopherols (140 mg/100 ml), 1% phytosterols, and small amounts of tocotrienols (Yang & Kallio, 2001).

The antioxidant effects in fruits and vegetables may arise from phenolic compounds such as flavonoids and phenolic acids, or from nitrogenous compounds such as alkaloids, chlorophyll derivatives, amino acids, and amines. These flavonoids and other phenolic compounds of plant origin have been reported as scavengers and inhibitors of lipid peroxidation (Song et al., 2013). Sea buckthorn oil has been reported as a potent antioxidant in all in vitro model systems it has been added to (Chauhan et al., 2007).

This study aimed to assess the effect of sea buckthorn oil injection on relevant quality parameters of pork tenderloin (psoas major) and derived products with compact texture subjected to heat treatment. The central objective was to investigate the potential of this oil as a natural antioxidant, with the potential to replace synthetic antioxidants. Thus, the influence of sea buckthorn oil on essential physicochemical aspects of meat, as well as its antioxidant and antimicrobial capacity, was analyzed, with a focus on products exposed to heat treatment.

MATERIALS AND METHODS

In order to achieve the aim of the study, three functional products with pork tenderloin were developed, by injection with 1%, 3%, and 5% sea buckthorn oil. The pork tenderloin, as the raw material, was purchased from Metro Cash & Carry România S.R.L. in Iași. The organic sea buckthorn fruit oil (*Hippophae rhamnoides* L.) used for injecting the experimental batches was obtained from Zorian Export S.R.L. in Iași County, produced through cold pressing and without any chemical treatment.

The total 6 experimental batches are presented in Table 1, along with the ingredients used in their manufacture and the heat treatment applied to them. For the laboratory analyses necessary for characterizing the studied products, samples from these batches were accompanied by samples of sea buckthorn oil (*Hippophae rhamnoides* L.) and of the meat used as raw material in the batches. The laboratory analyses conducted included physicochemical antioxidant capacity, and microbiological analyses. The experimental batches and the qualitative analyses were carried out at the "Ion Ionescu de la Brad" Iași University of Life Sciences (IULS) and at the Institute of Research for Agriculture and Environment (ICAM).

The experimental protocol for the pork tenderloin samples (M1S, M2S, and M3S) involved salting the meat pieces with 2% salt (dry brining) and injecting them with sea buckthorn oil, in the concentrations presented in Table 1. The prepared pieces were vacuum-sealed to facilitate better absorption of the oil into the meat and refrigerated under vacuum for 3 days until physicochemical analyses were conducted. The pastrami batches (P1S, P2S, and P3S) followed the same initial experimental protocol as the M1S, M2S, and M3S batches, but in addition to these, they underwent a heat treatment (Table 1) consisting of 4 stages, each lasting between 20 and 30 minutes.

Table 1. The composition of the experimental batches and the applied heat treatments

Experimental batches	Ingredients				Heat treatment (°C)			
	Pork tenderloin (%)	Sea buckthorn oil (%)	Salt (%)	Pepper crust (present or absent)	Drying I	Smoking	Boiling	Drying II
U	-	100	-	-	-			
C	100	-	-	-	-			
M1S	97	1	2	-				
M2S	95	3	2	-	-			
M3S	93	5	2	-	-			
P1S	97	1	2	X	65	65	72	80
P2S	95	3	2	X	65	65	72	80
P3S	93	5	2	X	65	65	72	80

The crude chemical determinations included quantitative analysis of moisture content, protein quantity, collagen, fat content, and salt concentration using a versatile method of near-infrared spectroscopic determination (NIR) (Gucianu et al., 2023) employing the Food Check meat analyzer (Bruins Instruments, Germany).

The assessment of the antioxidant activity of the experimental batches involved determining the content of polyphenols, flavonoids, and the scavenging activity of DPPH and ABTS radicals.

The extraction of compounds exerting antioxidant capacity is a crucial step in determining the antioxidant capacity of any food (Echegaray et al., 2022; López-Fernández et al., 2020). Meat is no exception, and extraction processes are particularly important for subsequent correct analysis. In this regard, solid-liquid extraction is the most commonly used in meat matrices. However, this process is performed in various ways, as different conditions can be used for this purpose (e.g., different solvents, different extraction times, and different extraction temperatures) (Wu et al., 2008). For this reason, we decided to use multiple methods for determining antioxidant compounds, as can be seen above. The solvents we used were represented by hexane-acetone (ABTS), ethanol (DPPH, polyphenols, and flavonoids).

For the total polyphenol content (TPC), the Folin-Ciocâlteu colorimetric method was used, as described by Blainski et al. (2013). The flavonoid content of the samples was determined using the method described by Zhishen et al. (1999) and Dewanto et al. (2002).

The determination of antioxidant capacity by the DPPH method was carried out following the procedure described by Pires et al. (2017), while that by the ABTS assay was conducted according to the method described by Dumitrașcu et al. (2022).

To detect the absorption of the samples, a UV-vis spectrophotometer Specord Plus 210 (Analytik Jena, Germany) was used. Sampling was done after vortex mixing to ensure reproducibility. The results were expressed as mg/g DW (for flavonoids), mg GAE/g DW (for polyphenols), µMol Trolox/g DW (for the DPPH assay), and µMol Trolox/g DW (for the ABTS assay). The solutions used in the antioxidant value determination analyses were purchased from Sigma Aldrich Steinheim (Darmstadt, Germany).

For the microbiological analyses, the following solutions/culture media bases were used: peptone water, Rapid *Staph* Agar base, Rapid *E. coli* 2 Agar base, Rapid *Salmonella* Agar base, and Plate Count Agar (PCA). All of these were purchased from Bio-Rad (Marnes-la-Coquette, France), except for the last culture medium, which was purchased from Scharlau (Barcelona, Spain).

The steps of evaluating the microbiological contamination of the meat and oil samples studied were as follows: (a) homogenization and weighing of the samples, (b) dilution, (c) inoculation on appropriate media, (d) incubation at specific temperatures and times, and (e) counting specific colonies indicating the presence of microorganisms (Otero et al., 1998). All analytical procedures performed to determine the microbial load were carried out in a sterile environment, with five replicates for each sample. One gram of meat/oil was homogenized with 9 milliliters of peptone water. This represented a 10-1 dilution, followed by vortexing and serial dilution of each analyzed sample to a concentration of 10-3, by taking one milliliter from the previous dilution and diluting it in 9 mL of peptone water. One milliliter of the prepared samples was inoculated onto Petri dishes, onto which the specific culture medium previously prepared according to the instructions on the technical sheet was added. After the incubation period, colonies of microorganisms from the meat and oil samples were manually counted, and the results were expressed as logarithmic colony forming units per gram (log CFU/g).

The color of the samples was determined using the portable colorimeter Konica Minolta CR-410, in the CIE tridimensional color system, measuring the color parameters L*, a*, and b* with illuminant D65 at a 10-degree observation angle (Boișteanu et al., 2023; Manoliu et al., 2023). The instrument was calibrated on a white calibration plate for standard values before starting the measurements.

The colorimetric determinations for Chroma and Hue angle were determined using formulas (1) and (2), following the model of Turgut et al., 2017.

$$H(*) = tan^{-1} \times b^*/a^* \qquad (1)$$

$$Chroma\ (C^*) = \sqrt{(a*)^2 + (b*)^2} \qquad (2)$$

pH measurements were conducted using a HANNA HI 99163 Meat pH-meter. The electrode was inserted into the meat following prior calibration in buffer solutions with known pH values (an acidic solution with pH 4.01 and a neutral solution with pH 7.01). The probe of the meter was cleaned with distilled water after calibration and between readings to ensure that the results obtained were not influenced (Ciobanu et al., 2022).

A sensory evaluation of the pork tenderloin samples that were thermally treated (pastrami – P1S, P2S, and P3S) was conducted 24 hours after completing all technological stages by 12 experienced panellists (6 males and 6 females). The samples were labeled with random three-digit numbers. Generalized Procrustes Analysis (GPA) was used for processing the sensory data to evaluate the results of the sensory tests conducted by the tasting panel. All these analyses were performed using XLSTAT, an add-in software for Microsoft Office Excel (Trial Version 2024, Addinsoft, Paris, France).

All experiments were performed in five replicates. The results are expressed as mean ± standard deviation. Statistical comparisons were conducted using one-way analysis of variance (ANOVA) with IBM SPSS Statistics V21 software. Differences were considered significant when p-values were less than 0.05.

RESULTS AND DISCUSSIONS

Table 2 presents the main physico-chemical characteristics analyzed within the studied batches (moisture, proteins, collagen, fat, salt, and pH).

The batches of pork tenderloin injected with sea buckthorn oil and not thermally treated (M1S, M2S, and M3S) exhibited lower average moisture values of 75.74 ± 0.114% (M1S), 75.46 ± 0.114% (M2S), and 74.68 ± 0.109% (M3S) compared to the control batch (C), where this parameter value was 76.02 ± 0.130%. However, these values were higher than those of the batches of pork tenderloin thermally treated for the same oil injection amount (P1S, P2S, and P3S). The P1S batch obtained the highest moisture value among the thermally treated batches (75.54 ± 0.114%), with this value increasing directly proportional to the increase in the amount of oil contained in the pork tenderloin. The batch treated with 3% sea buckthorn oil (P2S) showed a decrease in moisture to 74.74 ± 0.251%, while the batch injected with 5% sea buckthorn oil and thermally treated (P3S) recorded the lowest moisture among all studied batches, at 74.30 ± 0.071% (Table 2). The decrease in moisture following thermal treatment is a consequence of the increased temperature in the center of the product. The amount of moisture lost depends on the method of thermal treatment and the amount of connective tissue in the meat (Vinnikova et al., 2019). Pork tenderloin is known to be one of the most tender anatomical regions with a low content of connective tissue, as shown by the study of Nishimura et al. (2009). The progressive decrease in moisture in both thermally treated and untreated samples is also due to the increased addition of sea buckthorn oil. As the amount of lipids in meat or meat products increases, whether endogenous or exogenous, it leads to a decrease in moisture, as observed by Petrov et al. (2008) for meat with different amounts of fat.

The amount of fat (Table 2) exhibited progressive increases among batches, concurrent with the percentage of sea buckthorn oil added to them. The lowest fat content was observed in the control batch (C), at 1.82 ± 0.148%, which is expected considering that the psoas major is one of the anatomical portions with the lowest fat quantities. The batches not thermally treated had lower fat values, at 2.38 ± 0.179% (M1S), 3.06 ± 0.167% (M2S), and 3.80 ± 0.187% (M3S), compared to the batches of pork tenderloin that were thermally treated, where the mean values obtained were 2.54 ± 0.114% (P1S), 3.22 ± 0.192% (P2S), and 4.00 ± 0.158% (P3S). These changes in the fat percentage are attributed to the exogenous addition of vegetable fat (sea buckthorn oil) and the loss of moisture during the thermal process, but may also correlate with an inhibition of lipid oxidation induced by sea buckthorn oil. Both within batches subjected to the same thermal treatment and across all batches analyzed together statistically, very significant statistical differences are observed ($p < 0.001$) (Table 2). These results largely correlate with those obtained by us in previous research on this type of thermally treated product. In the current study, the mean value of the fat parameter increases directly proportional to the increase in the quantity of sea buckthorn oil added, which contrasts with our previous study where in the batch injected with 3% sea buckthorn oil, the value is lower than in the batches injected with 1% and 5% sea buckthorn oil (Anchidin et al., 2023). This discrepancy could be due to faulty injection of the sea buckthorn oil into the meat pieces, uneven distribution within the product mass, and sampling from a portion where an inadequate amount of sea buckthorn oil reached in line with the injected value. The issue we consider to have led to this "error" might be the type of injection used, namely manual injection.

Regarding the protein content in the studied batches, a slight gradual decrease is observed in the batches that were not thermally treated compared to the control batch (C) (Table 2). The mean protein content in this latter batch is 22.00 ± 0.100%, which is the highest value in our study for this qualitative parameter. From this value, it decreases successively to 21.80 ± 0.070% (batch M1S), 21.74 ± 0.134% (batch M2S), and 21.34 ± 0.114% (M3S) in the case of thermally untreated pork tenderloin. Regarding the batches of pork tenderloin that were thermally treated, since heat can modify/decompose proteins, it can significantly influence the nutritional properties of meat products (Yu et al., 2017). For this reason, in some of the thermally treated batches with the same quantity of injected sea buckthorn oil, a slightly lower mean value of this qualitative parameter is observed, as is the case with batches M1S and P1S, where the recorded values are 21.80 ± 0.070% and 21.42 ± 0.070%, respectively. The same situation was observed in batches M2S and P2S, where the obtained values were 21.74 ± 0.134% and 21.58 ± 0.084%, respectively. The only batches that did not follow this trend were the ones injected with 5% sea buckthorn oil (M3S and P3S), where the protein value of the thermally untreated sample was 21.34 ± 0.114%, lower than the 21.36 ± 0.134% obtained by the batch injected with 5% sea buckthorn oil and thermally treated. Thermal treatment had a lesser influence on the fluctuation of the mean protein value compared to the addition of sea buckthorn oil, but the differences between the studied batches were still distinctly significant (0.010**) (Table 2).

The collagen content of the pork tenderloin samples injected with sea buckthorn oil shows, similar to moisture and protein content, a decreasing trend as the percentage of injected oil increases. The only analyzed sample that does not follow this trend is the pork tenderloin injected with 1% sea buckthorn oil and thermally treated (P1S), where the highest collagen value was recorded, 20.60 ± 0.100%, which is even higher than the control batch (C), where its value was 20.38 ± 0.084. This represents the only instance where a parameter of chemical quality, which typically decreases with increasing sea buckthorn oil quantity injected, shows an increase in its value surpassing the control batch (Table 2). The rest of the mean collagen values for pork tenderloin samples injected with sea buckthorn oil, like moisture, showed successive decreases from the control batch value (20.38 ± 0.084) as follows: 20.24 ± 0.089 in batch M1S, 20.06 ± 0.114 in batch M2S, 19.78 ± 0.148 in batch M3S. Similarly, the values of batches subjected to thermal treatment also showed a decrease in the mean collagen value compared to the control batch (C), starting from batch P2S, with a value of 19.83 ± 0.130%, and continuing with P3S - 19.78 ± 0.084%. The differences recorded following the application of the analysis of variance (ANOVA) for collagen value variation were distinctly significant (0.007**) for the thermally treated pork tenderloin batches (P1S, P2S, and P3S) and highly significant (0.000***) for all analyzed batches (Table 2).

Table 2. Means ± standard deviation of the physicochemical results obtained by the studied batches

Experimental groups	Physicochemical parameters (%)					
	Moisture	Protein	Collagen	Fat	Salt	pH
M1S	75.74 ± 0.114[c]	21.80 ± 0.070[b]	20.24 ± 0.089[b]	2.38 ± 0.179[a]	1.92 ± 0.109[a]	5.91 ± 0.035[a]
M2S	75.46 ± 0.114[b]	21.74 ± 0.134[a]	20.06 ± 0.114[b]	3.06 ± 0.167[b]	2.22 ± 0.084[b]	6.09 ± 0.047[b]
M3S	74.68 ± 0.109[a]	21.34 ± 0.114[a]	19.78 ± 0.148[a]	3.80 ± 0.187[c]	2.22 ± 0.084[b]	6.09 ± 0.046[b]
p-value	**0.000 (***)**	**0.000 (***)**	**0.000 (***)**	**0.000 (***)**	**0.000 (***)**	**0.000 (***)**
P1S	75.54 ± 0.114[c]	21.42 ± 0.070[a]	20.60 ± 0.100[b]	2.54 ± 0.114[a]	1.98 ± 0.164[a]	6.184 ± 0.029[a]
P2S	74.74 ± 0.251[b]	21.58 ± 0.084[b]	19.83 ± 0.130[a]	3.22 ± 0.192[b]	2.14 ± 0.134[a]	6.22 ± 0.029[a]
P3S	74.30 ± 0.071[a]	21.36 ± 0.134[a]	19.78 ± 0.084[a]	4.00 ± 0.158[c]	2.12 ± 0.084[a]	6.24 ± 0.036[a]
p-value	**0.000 (***)**	**0.010 (**)**	**0.007 (**)**	**0.000 (***)**	0.154 (ns)	0.064 (ns)
C	76.02 ± 0.130[d]	22.00 ± 0.100[d]	20.38 ± 0.084[b]	1.82 ± 0.148	–	5.79 ± 0.079
Total p-value	**0.000 (***)**	**0.000 (***)**	**0.000 (***)**	**0.000 (***)**	**0.001 (***)**	**0.000 (***)**

Different letters on the same column indicate significant difference (estimated by ANOVA analysis and Tukey's test, $p \leq 0.05$).
M1S - pork tenderloin injected with 1% sea buckthorn oil, untreated thermally; M2S - pork tenderloin injected with 3% sea buckthorn oil, untreated thermally; M3S - tenderloin injected with 5% sea buckthorn oil, untreated thermally; P1S - tenderloin (pastrami) injected with 1% sea buckthorn oil, thermally treated; P2S - tenderloin (pastrami) injected with 3% sea buckthorn oil, thermally treated; P3S - tenderloin (pastrami) injected with 5% sea buckthorn oil, thermally treated.

The values for the salt content of the studied batches do not include the control sample (C) because it was not subjected to the salting process like the other studied batches. In the batches where sea buckthorn oil was injected, a prior salting process also took place. The values referring to the salt content of the studied samples do not show major fluctuations; however, the batches that were not subjected to thermal treatment recorded highly significant differences ($p < 0.001$) among the studied batches during statistical analysis. These results are in contrast to those obtained for the thermally treated samples, where the differences were not significant ($p > 0.05$). The differences observed between the batches subjected to thermal treatment and those that were not treated thermally were extremely significant, presenting a statistical significance level of up to 0.001*** (Table 2).

The highest average value for the salt content was obtained in batches M2S and M3S, both registering 2.22 ± 0.084%. The lowest value for the same parameter was observed in one of the batches that did not undergo thermal treatment, namely M1S, with a value of 1.92 ± 0.109% (Table 2). Intermediate values were recorded in batches that underwent thermal treatment – P1S, P2S, and P3S, with values of 1.98 ± 0.164%, 2.14 ± 0.134%, and 2.12 ± 0.084%, respectively.

Statistical differences for the pH of the analyzed samples were, as with the salt content, non-significant (0.064) for the batches subjected to thermal treatment and highly significant (0.000***) for the batches only injected with sea buckthorn oil and not subjected to other technological operations. The statistical analysis of all studied batches, as shown in Table 2, revealed highly significant differences (0.000***) among them. pH values showed a continuous increase, concurrent with the increase in the percentage of injected oil, starting from the value of the control batch (5.79 ± 0.079%). Thus, batches M1S, M2S, and M3S recorded mean pH values of 5.91 ± 0.035%, 6.09 ± 0.047%, and 6.09 ± 0.046%, respectively. An increase in pH is also observed after the thermal treatment stage, with the following results obtained: 6.184 ± 0.029% (P1S), 6.22 ± 0.029% (P2S), and 6.24 ± 0.036% (P3S).

In continuation of this study, we evaluated the antioxidant capacity of the batches injected with sea buckthorn oil (M1S, M2S, M3S, P1S, P2S, P3S). The spectrophotometric analyses used to measure the antioxidant activity in the aforementioned batches consisted of determinations for: total flavonoid content (TFC), total polyphenol content (TPC), DPPH assay, and ABTS assay.

Sea buckthorn oil is rich in antioxidant compounds such as vitamins A and E, sterols, and flavonoids (Ursache et al., 2017). In the non-heat-treated batches, the lowest value of total flavonoid content was observed in batch M1S, at 0.10 ± 0.004 mg CE/g DW, gradually increasing in batches M2S and M3S (where average values of 0.14 ± 0.014 and 0.20 ± 0.014 were obtained), while in the heat-treated batches, these values increased significantly ($p < 0.05$) up to 0.43 ± 0.005 (P3S). This significant increase in phenolic content suggests that temperature has an effect on the stability of flavonoids and their biological activity (Chaaban et al., 2017). Additionally, we observe a consistent increase in antioxidant activity parallel to the increase in the percentage of sea buckthorn oil in the analyzed products.

The analysis of total polyphenolic compounds in the batches of injected and non-heat-treated pork tenderloin revealed significantly different values ($p < 0.05$) from one sample to another, with values of 0.23 ± 0.036 mg GAE/g DW (M1S), 0.33 ± 0.016 mg GAE/g DW (M2S), and 0.47 ± 0.012 mg GAE/g DW (M3S). This increase could reflect the percentage variations in the injection of batches with sea buckthorn oil. Furthermore, the batches of injected pork tenderloin subjected to heat treatment showed a significant increase ($p < 0.05$) in polyphenolic content compared to the non-heat-treated batches, with values ranging from 0.87 ± 0.069 to 1.08 ± 0.017 mg GAE/g DW (Table 3). This increase could be attributed to the synergistic effects between heat treatment and the compounds in the injected oil, which could promote the release or formation of new polyphenolic compounds. The antioxidant activity of food matrices containing phenolic compounds, after heat treatment and exposure to light, may remain constant, increase, or decrease. The evolution depends on the interactions between molecules and food matrices, as well as operating conditions (Ioannou et al., 2020).

The DPPH assay, like the other antioxidant capacity analyses in the present study, highlights positive effects on antioxidant activity directly proportional to the increase in the percentage of sea buckthorn oil added to the products, as well as to the heat treatment. Batches M1S and M2S show relatively similar levels of μMol Trolox/g DW, at 4.62 ± 0.150 μMol Trolox/g DW and 4.99 ± 0.030 μMol Trolox/g DW, respectively, with no significant differences between them ($p > 0.05$). From the injection of 5% sea buckthorn oil into the pork tenderloin, a more significant increase in antioxidant activity is observed, reaching 7.10 ± 0.112 μMol Trolox/g DW (Table 3). According to statistical analysis, the differences between these three described batches are highly significant ($p < 0.001$). Significant differences ($p < 0.05$) are observed between batches M1S, M2S, M3S, and between the batches of pork tenderloin injected with sea buckthorn oil and subjected to heat treatment (P1S, P2S, and P3S). Batch P1S, injected with 1% oil and subjected to heat treatment, obtained a significantly different value ($p < 0.05$) of 9.60 ± 0.152 µMol Trolox/g DW compared to batch M3S, injected with 5% sea buckthorn oil but not subjected to heat treatment, which measured 7.10 ± 0.112 µMol Trolox/g DW. Batches P2S and P3S, injected with 3% and 5% sea buckthorn oil, respectively, showed values with nonsignificant differences ($p<0.05$) between them (10.28 ± 0.152 µMol Trolox/g DW and 10.42 ± 0.188 µMol Trolox/g DW, respectively). Statistical differences show distinct significant differences ($p<0.01$) between the batches subjected to heat treatment (P1S, P2S, and P3S) for the DPPH test (Table 3).

The last test used in our study to determine antioxidant activity was the ABTS assay, as shown in Table 3. The highest values of antioxidant activity were obtained in this assay. In the untreated batches, batch M3S (injected with 5% sea buckthorn oil) obtained the highest antioxidant value in this category, at 1617.17 ± 14.753 µMol Trolox/g DW, with the lowest being obtained by the batch injected with 1% sea buckthorn oil (M1S). In the case of batches subjected to the ABTS assay that underwent heat treatment, a different situation was observed compared to the results of the other antioxidant analyses conducted (Table 3). This translates to a lack of successive increase in antioxidant capacity values, as seen in the other tests. Instead, a decrease in antioxidant capacity was observed from 1688.84 ± 15.238 µMol Trolox/g DW (P1S – pastrami injected with 1% sea buckthorn oil) to 1522.24 ± 4.681 µMol Trolox/g DW (P3S - pastrami injected with 3% sea buckthorn oil). This latter value is the lowest antioxidant activity value obtained using the ABTS assay among the samples injected with sea buckthorn oil, regardless of the presence or absence of heat treatment. This result could be due to uneven distribution of sea buckthorn oil in the product mass or defective extraction, considering that batch P3S obtained the highest antioxidant value in this study, at 1802.28 ± 3.770 µMol Trolox/g DW (Table 3). In all phytochemical activity characterization tests, highly significant differences ($p<0.001$) were observed within the same test for all analyzed samples (Table 3).

Table 3. Characterization of the phytochemicals in the studied batches

Experimental groups	Phytochemical content			
	TFC, mg/g DW	TPC, mg GAE/g DW	DPPH µMol Trolox/g DW	ABTS µMol Trolox/g D.W.
M1S	0.10 ± 0.004^{b}	0.23 ± 0.036^{b}	4.62 ± 0.150^{b}	1566.81 ± 9.002^{c}
M2S	0.14 ± 0.014^{c}	0.33 ± 0.016^{c}	4.99 ± 0.030^{b}	1584.35 ± 6.179^{c}
M3S	0.20 ± 0.014^{d}	0.47 ± 0.012^{d}	7.10 ± 0.112^{c}	1617.17 ± 14.753^{d}
p-value	**0.000 (***)**	**0.000 (***)**	**0.000 (***)**	**0.003 (**)**
P1S	0.31 ± 0.016^{e}	0.87 ± 0.069^{e}	9.60 ± 0.152^{d}	1688.84 ± 15.238^{e}
P2S	0.38 ± 0.008^{f}	0.97 ± 0.025^{f}	10.28 ± 0.152^{e}	1522.24 ± 4.681^{b}
P3S	0.43 ± 0.005^{g}	1.08 ± 0.017^{g}	10.42 ± 0.188^{e}	1802.28 ± 3.770^{f}
p-value	**0.000 (***)**	**0.003 (**)**	**0.002 (**)**	**0.000 (***)**
C	0.05 ± 0.015^{a}	0.09 ± 0.013^{a}	3.06 ± 0.176^{a}	651.25 ± 16.74^{a}
p-value	**0.000 (***)**	**0.000 (***)**	**0.000 (***)**	**0.000 (***)**

Different letters on the same column indicate significant difference (estimated by ANOVA analysis and Tukey's test, $p \leq 0.05$).
M1S - pork tenderloin injected with 1% sea buckthorn oil, untreated thermally; M2S - pork tenderloin injected with 3% sea buckthorn oil, untreated thermally; M3S - tenderloin injected with 5% sea buckthorn oil, untreated thermally; P1S - tenderloin (pastrami) injected with 1% sea buckthorn oil, thermally treated; P2S - tenderloin (pastrami) injected with 3% sea buckthorn oil, thermally treated; P3S - tenderloin (pastrami) injected with 5% sea buckthorn oil, thermally treated.

The results regarding antioxidant activity show that the ABTS assay recorded the highest values of antioxidant activity compared to the DPPH assay, with significant differences (Table 3). These highly different results can be explained by the type of antioxidants each of these two tests can measure. The ABTS assay measures both hydrophilic and lipophilic antioxidants, while the DPPH assay applies only to hydrophobic systems (Munteanu & Apetrei, 2021).

Following the Pearson correlations between phytochemical and physicochemical parameters, strong and significant relationships (p<0.01) were identified among all studied parameters (Table 4).

The strongest positive correlations of TFC were observed with TPC (0.992**) and DPPH (0.977**). Very strong correlations were also observed between TPC and DPPH (0.987**), indicating a close association between flavonoid and phenolic content and antioxidant activity. Very strong positive correlations between the results of these antioxidant capacity determination analyses and pH were observed, indicating that an increase in pH value leads to an increase in antioxidant activity. Strong negative correlations of TFC, TPC, and DPPH are observed with the chemical parameters moisture, protein, and collagen (Table 4). The chemical parameter fat strongly correlates with the results of antioxidant capacity determination tests, as seen in Table 4. These results are compatible with those in Tables 2 and 3, where an increase in the amount of fat in the batch is observed as the percentage of sea buckthorn oil injected increases, but there is also an increase in antioxidant activity in batches with a higher addition of sea buckthorn oil. Moisture, protein, and collagen show significantly negative correlations with TFC, TPC, DPPH, and ABTS (Table 4). These results suggest that batches with lower moisture, protein, and collagen content had a higher concentration of antioxidant compounds.

Table 4. Pearson correlations between the results of the phytochemical compound analysis and those of the physicochemical analysis

Parameters	TFC	TPC	DPPH	ABTS	Moisture	Protein	Collagen	Fat	Salt	pH
TFC	1	**0.992****	**0.977****	**0.637****	**-0.765****	**-0.694****	**-0.630****	**0.673****	**0.562****	**0.875****
TPC		1	**0.987****	**0.635****	**-0.711****	**-0.697****	**-0.634****	**0.624****	**0.550****	**0.874****
DPPH			1	**0.672****	**-0.721****	**-0.758****	**-0.672****	**0.642****	**0.605****	**0.906****
ABTS				1	**-0.581****	**-0.717****	**-0.731****	**0.682****	**0.941****	**0.785****
Moisture					1	**0.709****	**0.663****	**-0.941****	**-0.577****	**-0.775****
Protein						1	**0.929****	**-0.731****	**-0.611****	**-0.788****
Collagen							1	**-0.741****	**-0.658****	**-0.770****
Fat								1	**0.685****	**0.777****
Salt									1	**0.782****
pH										1

** Correlation is significant at the 0.01 level.

For the determination of the color of all analyzed samples, the CIELab system was used, and its results are presented in Table 5, along with the chromatic parameters Hue Angle and Chroma, which were calculated based on the values obtained using the colorimeter. The CIELab model consists of the color brightness parameters L*, a*, and b*, where L* = 0 and L* = 100 are considered to be black and white, respectively. The parameter a* is used to represent negative values for green and positive values for red, while negative values for blue and positive values for yellow are displayed by b*.

The ranges of the L*, a*, b*, H*, and C* values were 38.36 ± 1.21 - 52.90 ± 1.486, 13.49 ± 0.358 - 21.82 ± 0.912, 4.87 ± 0.240 - 20.68 ± 0.738, 0.30 ± 0.023 - 0.84 ± 0.030, and 16.70 ± 0.951 - 28.69 ± 1.081, respectively (Table 5). All values of the CIE a* and CIE b* colorimetric parameters were positive, indicating that the studied samples fall within the red and yellow color range. By analyzing the mean values of the control samples, it can be observed that the luminosity value (L*) decreases with the addition of sea buckthorn oil, with lot M3S (injected with 5% sea buckthorn oil) showing the lowest value (38.36 ± 1.210) for this parameter among all the studied lots, while the control lot that was not injected with sea buckthorn oil (52.90 ± 1.486) obtained the highest value (Table 5). Following heat treatment, an increase in the luminosity of the samples is observed up to a value of 52.00 ± 0.626 (P3S) within the lot injected with 5% sea buckthorn oil.

The CIE a* value initially decreases in the lot injected with 1% sea buckthorn oil and not subjected to heat treatment (13.49 ± 0.358), compared to the control lot (C), which obtained a value of 15.97 ± 1.000. Subsequent values of the parameter a* for the lots that were not heat-treated reached a maximum value of 21.82 ± 0.912 for the lot injected with 5% sea buckthorn oil (M3S). Regarding the values for the heat-treated samples, there is also an increase in the a* parameter up to a maximum of 18.70 ± 0.668 in the lot injected with 5% sea buckthorn oil and subjected to heat treatment (P3S), which is lower than that obtained in the lot injected with the same percentage of sea buckthorn oil but not subjected to heat treatment (M3S).

As with the CIE a* parameter, the CIE b* parameter also shows a continuous increase, up to the maximum value of 20.68 ± 0.738 in the pastrami lot injected with 5% sea buckthorn oil and subjected to heat treatment (P3S, Table 5). These continuous increases, directly proportional to the amount of sea buckthorn oil injected, indicate an intensification of the yellow color, which is characteristic of sea buckthorn oil. The intense color of this oil is due to its concentration of carotenoids (Koskovac et al., 2017). In the case of lots M1S and M2S (Table 5), a decrease in the b* parameter value is observed from 10.07 ± 0.516 to 9.75 ± 0.331, even though the latter lot was injected with 3% sea buckthorn oil, and the former with only 1%. However, in the lot injected with 5% sea buckthorn oil belonging to the same category (without heat treatment), a substantial increase in the average value of the b* parameter is observed (18.60 ± 1.316), a value lower than that for the heat-treated lots injected with 3% and 5% sea buckthorn oil, where values of 19.70 ± 0.552 and 20.68 ± 0.738 were obtained, respectively. From this, we can deduce a combined effect of heat treatment and the addition of sea buckthorn oil on the value of the CIE b* parameter. The results are similar to those obtained by Bobko et al. (2019) on minced pork meat to which sea buckthorn juice and oil, both organic, were added.

The Hue values for the untreated muscle vary slightly but fall within a relatively narrow range (0.53 ± 0.009 - 0.71 ± 0.043). These values are lower than those for the heat-treated muscle, which range from 0.83 ± 0.025 to 0.84 ± 0.030, indicating a different color hue induced by heat treatment. Although meat is a dead skeletal muscle tissue, it is not inert. The complex interactions between myoglobin (Mb) and biomolecules in the muscle's food matrix critically influence the internal color of heat-treated meat (Suman et al., 2016). The control sample has the lowest Hue value (0.30 ± 0.023), indicating a distinct hue compared to the other samples (Table 5).

Table 5. Chromatic parameters (Luminance L*, color coordinates a* and b*, Hue angle, and Chroma)

The tested samples	L*	a*	b*	Hue angle*	Chroma*
M1S	41.06 ± 0.775^{b}	13.49 ± 0.358^{a}	10.07 ± 0.516^{b}	0.64 ± 0.013^{c}	16.83 ± 0.582^{a}
M2S	39.88 ± 0.521^{ab}	16.88 ± 0.616^{bc}	9.75 ± 0.331^{b}	0.53 ± 0.009^{b}	19.50 ± 0.674^{b}
M3S	38.36 ± 1.210^{a}	21.82 ± 0.912^{e}	18.60 ± 1.316^{d}	0.71 ± 0.043^{d}	28.69 ± 1.081^{e}
p-value	**0.001 (***)**	**0.000 (***)**	**0.000 (***)**	**0.000 (***)**	**0.000 (***)**
P1S	46.68 ± 0.742^{c}	14.26 ± 0.374^{a}	15.52 ± 0.548^{c}	0.83 ± 0.014^{e}	21.08 ± 0.582^{c}
P2S	49.61 ± 0.996^{d}	17.84 ± 0.777^{cd}	19.70 ± 0.552^{de}	0.84 ± 0.030^{e}	26.59 ± 0.563^{d}
P3S	52.00 ± 0.626^{e}	18.70 ± 0.668^{d}	20.68 ± 0.738^{e}	0.83 ± 0.025^{e}	27.89 ± 0.712^{de}
p-value	**0.000 (***)**	**0.000 (***)**	**0.000 (***)**	0.870 (ns)	**0.000 (***)**
C	52.90 ± 1.486^{e}	15.97 ± 1.000^{b}	4.87 ± 0.240^{a}	0.30 ± 0.023^{a}	16.70 ± 0.951^{a}
Total p-value	**0.000 (***)**	**0.000 (***)**	**0.000 (***)**	**0.000 (***)**	**0.000 (***)**

Different letters on the same column indicate significant difference (estimated by ANOVA analysis and Tukey's test, $p \leq 0.05$).
M1S - pork tenderloin injected with 1% sea buckthorn oil, untreated thermally; M2S - pork tenderloin injected with 3% sea buckthorn oil, untreated thermally; M3S - tenderloin injected with 5% sea buckthorn oil, untreated thermally; P1S - tenderloin (pastrami) injected with 1% sea buckthorn oil, thermally treated; P2S - tenderloin (pastrami) injected with 3% sea buckthorn oil, thermally treated; P3S - tenderloin (pastrami) injected with 5% sea buckthorn oil, thermally treated.

The untreated muscle batches exhibit highly significant differences (p = 0.000***) in Chroma among the studied lots, with values of 16.83 ± 0.582 (lot M1S), 19.50 ± 0.674 (lot M2S), and 28.69 ± 1.081 (lot M3S) (Table 5). Similarly, highly significant differences (p = 0.000***) are observed among the studied lots that underwent heat treatment (Table 5), where the values were higher than those in untreated lots for batches P1S (1% sea buckthorn oil injection) and P2S (3% sea buckthorn oil injection), with values of 21.08 ± 0.582 and 26.59 ± 0.563, respectively. Batch P3S, with a mean Chroma value of 27.89 ± 0.712, injected with 5% sea buckthorn oil and subjected to heat treatment, is the only batch that did not exceed the value obtained by this parameter for the untreated batch with the same injection

percentage (M3S), which had a value of 28.69 ± 1.081 (Table 3).
The differences were highly significant ($p<0.001$) for all studied batches regarding all colorimetric parameters, except for the heat-treated batches (P1S, P2S, and P3S) for the Hue angle.
In the present study, the in vitro antimicrobial activity of sea buckthorn (Hippophae rhamnoides) oil against microbial activity was evaluated qualitatively and quantitatively by the presence or absence of colony-forming units on Petri plates. For this purpose, bacterial colonies were analyzed for the following species: *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella*. Additionally, the total number of microorganisms in the samples was measured (Plate Count Agar). The statistical results show a significant diversity among the values for the same microbiological analysis, as evidenced by the highly significant differences (0.000***) identified between the analyzed batches (Table 6).

Table 6. The mean values ± SE of the batches of pork tenderloin injected with sea buckthorn oil regarding microbiological contamination

Batch	*Staphylococcus aureus* (log CFU/g)	*Escherichia coli* (log CFU/g)	Plate count agar (log CFU/g)	*Salmonella* (log CFU/g)
O	0.00 ± 0.000^{a}	0.00 ± 0.000^{a}	0.00 ± 0.000^{a}	*Absent*
C	4.17 ± 0.032^{g}	3.99 ± 0.031^{f}	4.12 ± 0.026^{f}	
M1S	3.55 ± 0.040^{de}	2.91 ± 0.055^{c}	3.58 ± 0.026^{b}	
M2S	3.63 ± 0.010^{ef}	3.10 ± 0.012^{e}	3.70 ± 0.015^{c}	
M3S	2.59 ± 0.032^{b}	2.59 ± 0.021^{b}	4.02 ± 0.015^{e}	
P1S	3.70 ± 0.040^{f}	3.47 ± 0.021^{f}	4.03 ± 0.025^{e}	
P2S	3.20 ± 0.030^{c}	2.97 ± 0.020^{cd}	3.94 ± 0.015^{d}	
P3S	3.48 ± 0.035^{d}	3.04 ± 0.032^{de}	4.02 ± 0.015^{e}	
p-value	**0.000 (***)**	**0.000 (***)**	**0.000 (***)**	-

Different letters on the same column indicate significant difference (estimated by ANOVA analysis and Tukey's test, $p \leq 0.05$).
M1S - pork tenderloin injected with 1% sea buckthorn oil, untreated thermally; M2S - pork tenderloin injected with 3% sea buckthorn oil, untreated thermally; M3S - tenderloin injected with 5% sea buckthorn oil, untreated thermally; P1S - tenderloin (pastrami) injected with 1% sea buckthorn oil, thermally treated; P2S - tenderloin (pastrami) injected with 3% sea buckthorn oil, thermally treated; P3S - tenderloin (pastrami) injected with 5% sea buckthorn oil, thermally treated.

By analyzing Table 6, we can observe that the sea buckthorn oil samples (O) evaluated for all four microbiological analyses showed no microbial contamination. These results can be explained by the fact that plants produce a variety of antimicrobial compounds to protect themselves from biotic attacks (Gupta et al., 2011). Furthermore, experiments conducted by other authors have demonstrated that sea buckthorn oil exhibits pronounced antimicrobial activity (Yue et al., 2017). However, even though sea buckthorn oil influenced the antimicrobial activity of the samples in this study, the test results did not show consistently lower or higher values but rather appeared chaotic, with both decreases and increases observed (Table 6). Even in this scenario, the batches injected with sea buckthorn oil still exhibited lower mean values of log CFU/g compared to those obtained for the control batch C, where 4.17 ± 0.032 log CFU/g was identified for *Staphylococcus aureus*, 3.99 ± 0.031 log CFU/g for *Escherichia coli*, and 4.12 ± 0.026 log CFU/g for the total germ count (Table 6).
The analysis of the presence of *Staphylococcus aureus* showed that the mean values ranged from 2.59 ± 0.032 to 3.70 ± 0.040 log CFU/g. The highest value (3.70 ± 0.040 log CFU/g) was observed in batch P1S, which represents one of the thermally treated batches, followed by the value of batch M2S, at 3.63 ± 0.010 log CFU/g, which is relatively close to the former. In the batches injected with 3% sea buckthorn oil, a slight decrease in microbial load was observed after thermal treatment, with a value of 3.20 ± 0.030 log CFU/g obtained, while the untreated batch showed significant differences ($p < 0.05$), with a value of 3.63 ± 0.010. Batches injected with 1% (M1S and P1S) and 5% (M3S and P3S) sea buckthorn oil showed higher values for *Staphylococcus aureus* after thermal treatment.
The microbiological tests for *Escherichia coli* showed the same trend as in the case of tests for *Staphylococcus aureus*, with reductions

observed before and after thermal treatment in the microbial load only in the batch injected with 3% sea buckthorn oil (M2S and P3S). The results for this bacterial species ranged from 2.59 ± 0.021 log CFU/g, identified in batch M3S, to 3.47 ± 0.021 log CFU/g (Table 6).
Regarding the results for Plate Count Agar, we observe that the highest values obtained in this analysis, aside from the control sample, were obtained by batches P3S (4.02 ± 0.015 log CFU/g), P1S (4.03 ± 0.025 log CFU/g), and M3S (4.02 ± 0.015 log CFU/g), as shown in Table 6. The results for two of these batches, P1S and P3S, positively correlate with the results of the tests for the identification of the bacterial species *Staphylococcus aureus* and *Escherichia coli*, as these batches exhibited among the highest values for these bacteria among the studied product batches injected with sea buckthorn oil.
Salmonella was absent in all the batches studied, a result that complies with the microbiological requirements set by Commission Regulation (EC) No 2073/2005, similar to the values of all microbiological tests conducted in this study.

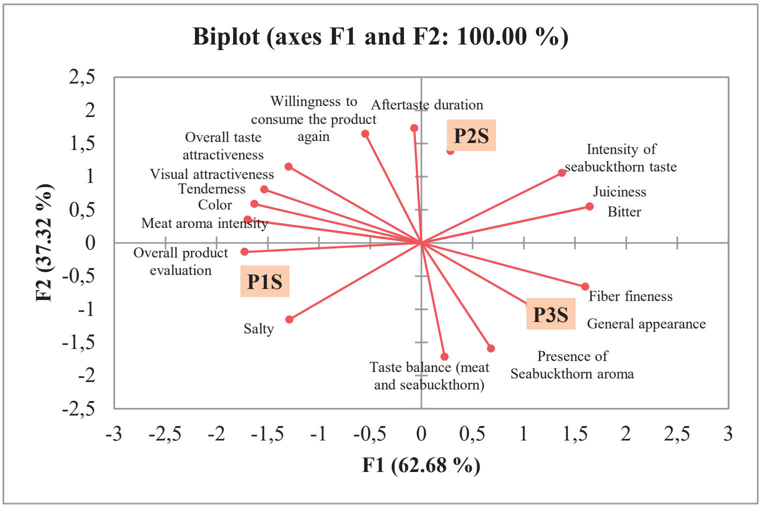

Figure 1. Representation of correlations between sensory traits using Principal Component Analysis (PCA)

For a comprehensive sensory characterization, we decided to perform a PCA graphic, considering the following characteristics: General appearance, Color, Visual attractiveness, Presence of Seabuckthorn aroma, Meat aroma intensity, Fiber fineness, Juiciness, Tenderness, Salty, Bitter, Intensity of seabuckthorn taste, Overall taste attractiveness, Taste balance (meat and seabuckthorn), Overall product evaluation, Willingness to consume the product again, Aftertaste duration (Figure 1). Figure 1 displays the products and attributes in a single plane, providing an overview of the relationships between products and attributes. PCA graph – principal component analysis involves evaluating the perception differences among the heat-treated batches in this study (batches P1S, P2S, and P3S). The biplot obtained through PCA analysis offers a clear view of the variations and relationships between the evaluated sensory characteristics and the batches of pork tenderloin injected with sea buckthorn oil that were heat-treated (pastrami).
By analyzing Figure 1, we can observe that the characteristics located in the upper-left quadrant are represented by Meat aroma intensity, Color, Tenderness, Visual attractiveness, Overall taste attractiveness, Willingness to consume the product again, and Aftertaste duration, which have negative contributions on axis F2 but positive contributions on axis F1. The positioning of these sensory characteristics suggests that product P2S (closest to these characteristics and positioned on the positive side of the Biplot) is perceived favorably by consumers in terms of aroma and appearance. However, it

also exhibits a higher intensity of seabuckthorn taste, greater juiciness, and a slightly bitter taste, as seen in batch P3S (the batch injected with 5% seabuckthorn oil). These latter characteristics, located in the upper-right quadrant, were perceived by consumers as positive.

Batch P1S, located in the bottom-left quadrant, is perceived as having certain negative characteristics. It is perceived as salty, even though its salt content, according to chemical analysis, was lower than in the other batches (Table 3), and it received a generally poor evaluation, meaning it obtained low scores for most of the evaluated characteristics. The perception of saltiness in the product could be due to its extremely low fat content, which fails to counterbalance this aspect.

Batch P3S, characterized primarily by a high degree of muscle fiber fineness, a pleasing overall appearance, the presence of seabuckthorn aroma, and, last but not least, a balanced taste between meat and seabuckthorn oil. However, it is less favored than batch P2S, which is preferred by consumers.

By analyzing the PCA results, we can observe that batch P2S (the batch injected with 3% seabuckthorn oil) emerged as the frontrunner in terms of consumer preferences. It was characterized by a strong seabuckthorn taste, juiciness, and an attractive appearance. Visual cues are often the first to be perceived and capable of influencing perception (Ciobanu et al., 2023). Batch P3S also received relatively high scores in the sensory analysis, similar to P2S. Panelists appreciated the balance of flavors and appearance in the muscle injected with 5% seabuckthorn oil (P2S). The only batch characterized negatively among all three was batch P1S (injected with 1% seabuckthorn oil), which received low scores in the sensory analysis and, consequently, an overall negative evaluation.

CONCLUSIONS

The analysis conducted on various batches of pork tenderloin reveals significant variations in physicochemical characteristics among them. Although some batches recorded higher values for certain parameters, there is no clearly superior batch in all aspects.

The analyses of the antioxidant capacity of pork tenderloin (psoas major) injected with sea buckthorn oil have shown positive effects of adding it to the analyzed samples. The most suitable method for determining antioxidant compounds, according to the results obtained, is ABTS. This method yielded values much higher than those obtained in the DPPH test, suggesting that the ABTS test is highly suitable for this type of food matrix.

By analyzing the parameters of the CIE*a*b* system, Hue angle, and Chroma, it can be observed that the addition of sea buckthorn oil positively influenced all colorimetric parameters, leading to their increase, especially in the case of samples subjected to heat treatment.

The microbiological analyses conducted indicate that the sea buckthorn oil injected into the pork tenderloin (psoas major) batches does not exert a strong antimicrobial effect, but it still has a slight positive influence, resulting in a minor reduction in microbial contamination.

Overall, our results indicate the valuable potential of *H. rhamnoides* in developing natural antimicrobial and antioxidant agents. Sensory-wise, the products were well received by consumers, especially the batches injected with 3% and 5% sea buckthorn oil. The batch injected with 1% sea buckthorn oil was negatively perceived by panelists due to low scores obtained for positive characteristics, resulting in an overall unfavorable evaluation. Panelists appreciated the presence of sea buckthorn flavor in the products and penalized the weak or imperceptible presence of it.

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INVESTIGATING THE EFFECTS OF STORAGE TEMPERATURE ON THE QUALITY OF FROZEN CEPHALOPODA: A MULTIMODAL APPROACH

Cătălina Nicoleta BOIȚEANU[1], Nicoleta CIOCÎRLIE[1], Florin NEACSU[2], Magda GONCIAROV[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]Baylor University, Department of Chemistry and Biochemistry, Waco, TX 76798, USA

Corresponding author email: catalina-nicoleta.boiteanu@fmvb.usamv.ro

Abstract

Cephalopoda classes are highly valued for their culinary appeal and nutritional value. However, their perishability necessitates careful handling and optimal storage conditions to maintain their quality. This study aimed to investigate the influence of storage temperature on the quality attributes and sensory properties of five commonly consumed cephalopod types: whole squid, whole squid tubes, octopus, Loligo spp. (squid tubes), and Sepia spp. (cuttlefish tubes) classified in five different species (Loligo vulgaris (European squid), Loligo gahi (Japanese squid), Octopus vulgaris (Octopus), Sepia officinalis (Cuttlefish) and Illex argentinus (Argentine shortfin squid)). Lower temperatures (-23°C) significantly prolonged shelf life, delaying spoilage onset and preserving taste. The product named calamar had the fastest spoilage rate, followed by whole squid and tube squid, while Octopus had the slowest spoilage rate. Appropriate storage practices are vital for ensuring the freshness and palatability of frozen cephalopods.

Key words*: frozen cephalopods, quality attributes, sensory properties, storage temperature.*

INTRODUCTION

Cephalopoda, members of the phylum Mollusca, are a diverse group of marine animals recognized for their set of tentacles, making them distinct. They are highly favoured as a seafood delicacy. This category encompasses a variety of species, including squid, octopus, cuttlefish, and nautilus (Ahne et al., 2000).

Molluscs play a significant role in meeting global seafood demand, providing consumers with high-quality protein, essential amino acids, and valuable nutrients. Their economic importance in aquaculture, coupled with their health benefits and nutritional value, underscores the importance of sustainable molluscs farming practices to support food security and healthy diets worldwide (Petcu, 2013).

Octopus, a versatile marine cephalopod, has garnered immense popularity worldwide, captivating culinary enthusiasts with its delicate flavour, satisfying texture, and nutritional richness. Its growing demand has fuelled a surge in global fish production, with the volume reaching 186.6 million metric tons in 2023, up from 184.6 million metric tons in 2022 (Statista, 2024). This unprecedented demand has placed octopus at the forefront of the global seafood market, with China and the Republic of Korea emerging as dominant players in the live, fresh, or chilled octopus trade over the past two decades.

While China and Korea have long held the reins of the octopus market, recent years have witnessed a shift in the trade dynamics. European and Mediterranean countries, including Spain, Portugal, Italy, and Morocco, have ascended to prominence, establishing themselves as key octopus importers and exporters. This transformation reflects the growing global appreciation for octopus and the adaptability of international markets.

The escalating demand for octopus necessitates a comprehensive understanding of its culinary properties, preservation techniques, and the factors influencing its quality. Previous research articles on the freshness and sensory attributes of octopus highlight the critical importance of understanding and evaluating these factors to ensure optimal quality and consumer satisfaction (Atrea et al., 2009). This article delves into the scientific realm of octopus, exploring its nutritional value, sensory

attributes, and the impact of storage conditions on its shelf life. By unravelling the science behind octopus, we gain valuable insights into optimizing its preservation and ensuring its continued popularity as a culinary delicacy (Ospina-Alvarez et al., 2022).
The global seafood industry is projected to experience significant growth in the coming years, with a projected value of $155.32 billion by 2023. China, Indonesia, and India currently hold the top positions for fish production, while Asia is the world's leading aquaculture producer, with a production volume of approximately 70.5 million tons in 2020 (Figure 1). Notably, finfish was the most produced species in aquaculture, accounting for over 57.46 million metric tons. China, with its extensive fish and seafood processing industry, generated revenue of $31.6 billion in 2018. These figures underscore the immense global demand for seafood and the crucial role of aquaculture in meeting this demand (Statista, 2024).

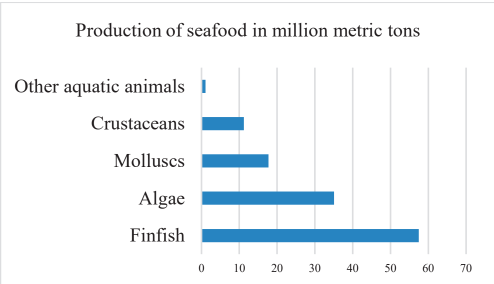

Figure 1. Global aquaculture production worldwide and major species traded in 2020 (after Statista, 2023)

Seafood consumption in Europe has exhibited a downward trend in recent times, with a projected decline of 7% in 2022, reaching 9.42 million metric tons (live weight equivalent). This decrease is attributed to various factors, including the ongoing conflict between Russia and Ukraine, which has disrupted supply chains and impacted seafood imports.
Prior to the 2022 decline, global seafood consumption had been steadily increasing. In Europe, the consumption of seafood products has predominantly been in frozen form, primarily due to the lower per capita consumption compared to other fish and mollusc species. This trend suggests that frozen cephalopod products are primarily consumed for their extended shelf life and convenience.

Over time, efforts have been made to optimize the preservation and utilization of these products, potentially affecting their quality and consumer satisfaction, resulting in millions of research articles worldwide. However, solutions are still sought to enhance their commercial and organoleptic quality, and to find the best storage and freezing conditions for edible cephalopods.

MATERIALS AND METHODS

The research was conducted at the Max Rubner Institute, Hamburg branch, Germany, using the appropriate equipment, and methodology.

Sample collection

In this study, we examined five different species of cephalopods: *Loligo vulgaris* (European squid), *Loligo gahi* (Japanese squid), *Octopus vulgaris* (Octopus), *Sepia officinalis* (Cuttlefish) and *Illex argentinus* (Argentine shortfin squid) (Figures 2 and 3).

Figure 2. *Illex* spp. (own source)

Five cephalopod marketed assortments – whole squid, whole squid tubes, octopus, *Loligo* spp. (squid tubes), *Illex* spp. and *Sepia* spp. (cuttlefish tubes) – were selected for the study. The products were purchased from local seafood markets and stored at three distinct temperatures: -15°C, -18°C, and -23°C for three months. At regular intervals, samples were collected for chemical, physical, and sensory analyses.
Thawing test: The products were stored at +4°C for three weeks, then thawed at room temperature for one hour. The pH and TVB-N content of the products were measured before and after thawing, at each storage temperature.

Chemical analysis

The samples were prepared for analysis following the guidelines outlined in BVL L

06.00-1. The pH and volatile basic nitrogen (TVB-N) content were analysed to assess microbial spoilage. pH serves as an indicator of the acidity or alkalinity of the product, according to BVL L 06.00-2 guidelines.

Figure 3. *Octopus* spp. (own source)

On the other hand, TVB-N levels are indicative of the accumulation of biogenic amines, compounds generated during protein degradation, further contributing to the assessment of spoilage.

Physical analysis

Color and texture were assessed as physical parameters of product quality. Color was measured using a Hunter Lab colorimeter, while texture was evaluated based on firmness, elasticity, and cohesiveness.

Sensory evaluation

The sensory analysis process requires a comprehensive understanding of sensory evaluation methodologies, a profound knowledge of food composition and processing, and substantial practical experience to ensure accurate and reliable results (Derndorfer, 2023).

The sensory analysis panel consisted of 10 experienced researchers who received additional training before each sensory analysis session. The trained sensory panel evaluated the overall palatability of the products using a 9-point hedonic scale ranging from 1 (dislike extremely) to 9 (like extremely). The panel assessed flavour, texture, appearance, and overall acceptability (Busch-Stockfisch, 2005).

Preliminary tests

Prior to the main tests, a series of preliminary tests were conducted to assess the freshness of the cephalopod products. The tests included:

pH measurement: the pH value was measured using a glass single-rod electrode introduced into product homogenate diluted 1:1 with distilled water. The electrode was always calibrated with pH 4.0 and pH 7.0 before the samples were measured. In order to prevent the values from changing due to long storage times, the measurement was carried out immediately after the samples had been homogenized. The homogenate, being relatively dry, was diluted with distilled water in a 1:1 ratio before measurement. Despite this dilution, the pH of the homogenate was not affected by the addition of distilled water, as the water used was within a neutral pH range. For the measurement, 20 g sample homogenate was used and a double determination was carried out in each case (Oehlenschläger et al., 2002). Fresh cephalopod products should have a pH between 6 and 7.5.

Before the TVB-N determinations were carried out, the homogenate was prepared. For this purpose, 180 ml perchloric acid solution of 6% concentration was added to a 20 g sample and then extracted for one to two minutes using an Ultraturrax. The subsequent filtration over filter paper was facilitated using Witt's pot to prevent clogging of the filter by the homogenate, which could otherwise halt the filtration process. For each investigation, two samples of the same homogenate were consistently analysed.

Volatile basic nitrogen (TVB-N) content:

The TVB-N content of the products was measured to assess the degree of microbial spoilage and protein degradation, which are indicated by the presence of various nitrogen compounds including amines and ammonia. TVB-N level, in crustaceans, shells and molluscs was determined according to the German reference method, § 64 LMBG (German Food & Feed Code, 2008) and EU recommendations (EU, 2005). However, the amount of homogenate specified in the European regulation was doubled in order to obtain a more representative sample. The volatile nitrogenous bases were extracted from the samples with perchloric acid as described above. The resulting extract (Figure 4) was subjected to steam distillation after alkalinization. The volatile basic components were absorbed in an acid reservoir. The TVB-N content was determined by titrimetric determination of the absorbed bases. The analysis was automatically carried out using a Vapodest 50 distillation device. The TVB-N content in mg/100g was calculated (Figure 4) using the following formula (EU, 2019):

$$TVB-N = \frac{(V1 - V0) \times 2 \times 100 \times 0.14}{M}$$

where:

V1 = volume of 0.01 mol standard hydrochloric acid solution in milliliters for the sample,
V0 = volume of 0.01 mol standard hydrochloric acid solution in milliliters for the blank,
M = mass of the sample in grams.

Figure 4. Samples preparation for TVB-N extraction from perchloric acid solution (own source)

To verify the results (the initial ones from day I are included in Figures 5-7), duplicate determinations of the individual extracts were performed. Given the absence of precise limit values for this type of product, values between 25 and 35 mg/100 g were considered critical. These values are derived from Commission Regulation (EC) No. 2074/2005 for certain fishery products. From this perspective, fresh cephalopod products should ideally have a TVB-N content of less than 30 mg/kg.

Sensory evaluation: The panel of 10 experienced tasters, as previously mentioned, evaluated the appearance, smell, taste, and texture of the products. Each taster was instructed to rate the products on a scale ranging from 1 to 9, with 1 representing the lowest score indicating poor quality, and 9 representing the highest score indicating excellent quality. To assess the effects of different storage temperatures on the quality of cephalopod products, two main tests were conducted. The first test investigated the effects of storage temperature on the pH, TVB-N content and sensory properties of the products. The products were stored at -15°C, -18°C and -23°C for one, two and three months.

The second test investigated the effects of thawing on the quality of the products. The pH, TVB-N content and sensory properties of the products were then measured.

RESULTS AND DISCUSSIONS

Various studies have examined the chemical and physical properties of cephalopods during storage (Sykes et al., 2009). These studies were mainly conducted on non-frozen products. The results from these studies suggest that the content of biogenic amines and volatile basic nitrogen compounds correlate with the sensory properties of cephalopods (Özogul, & Serhat, 2011; Sun et al*.*, 2003; Vieira et al., 2008).

The present study investigated the changes in chemical and physical parameters of three cephalopod sample types (squid, cuttlefish and octopus) during storage at different temperatures. The results showed that the pH and TVB-N content of all five species: *Loligo vulgaris* (European squid), *Loligo gahi* (Japanese squid), *Octopus vulgaris* (Octopus), *Sepia officinalis* (Cuttlefish) and *Illex argentinus* (Argentine shortfin squid) increased over time, while the sensory properties of the products deteriorated. The sensory results of the profile tests did not show any significant differences, while the scalar rating test revealed that the product named calamar had the fastest spoilage rate, followed by whole squid and tube squid. Octopus had the slowest spoilage rate.

Previous research made through omission and addition tests and sensory evaluations of synthetic extracts, identified the taste-active components in the mantle muscle of oval squid (*Sepioteuthis lessoniana*) to include various amino acids, nucleotides, and ions. A simplified synthetic extract containing these key components was able to closely replicate the taste of the complete extract of *S. lessoniana* muscle. This replicated taste was consistent across other squid species tested, including *Loligo bleekeri, Loligo edulis*, and *Todarodes pacificus*, by adding specific components tailored to each species. The findings suggest

that specific combinations of taste-active components play a crucial role in determining the overall taste profile of squid species, with the synthetic extract approach offering insights into taste preferences and potential applications in food product development (Kani et al., 2008).
Innovatively, our research uncovered a compelling negative linear relationship among key biochemical indicators - pH, total volatile basic nitrogen (TVB-N) content and sensory scores - in cephalopod products. This intriguing correlation suggests a novel approach for assessing the overall freshness of cephalopods. By leveraging the levels of these biochemical compounds as indicators, we propose a groundbreaking method to precisely evaluate cephalopod freshness, providing valuable insights for both industry practitioners and consumers alike.
The storage time for cephalopods at a temperature of +4°C (39°F) without significant deterioration of sensory properties can vary depending on factors such as the species of cephalopod, initial freshness, handling practices, and storage conditions. However, it is generally true that cephalopods can be stored for a limited period at this temperature without significant adverse effects on their sensory properties.
In some cases, cephalopods can be stored for up to 14 days at +4°C without experiencing noticeable changes in sensory properties such as appearance, odor, taste, and texture. However, it's important to note that the quality of cephalopods may gradually decline over time, even at refrigeration temperatures. Therefore, while storage at +4°C may extend the shelf life compared to higher temperatures, it's still essential to monitor the quality of the product regularly and adhere to recommended storage times to ensure optimal freshness and safety.

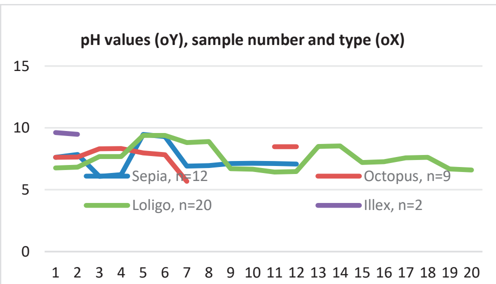

Figure 5. Results regarding initial pH values in all analyzed samples (on day 1 of storage at 4°C)

The results of the preliminary tests (Figure 5) showed that *Octopus vulgaris* and *Illex argentinus* had the highest pH values (7.2-9.6), while *Sepia officinalis* and *Loligo vulgaris, Loligo gahi,* had the lowest pH values (6.6-6.8). The results of the main tests showed that the storage temperature had a significant effect on the pH, TVB-N content (Figures 6 and 7) and sensory properties of the products.
The products stored at -23°C had the lowest pH values, TVB-N content, and the best sensory properties. The products stored at -18°C had intermediate values, while the products that were stored at -15°C had the highest pH values.
Various techniques are employed in the seafood industry, including the use of ice, chilling, super-chilling, freezing with different types of freezers and freezing mediums, freeze drying, ultra-low temperature freezing, low-temperature packaging, and additives. Each preservation method is associated with specific preservation temperatures, technologies, additives used, packaging materials, storage conditions and preferred shelf life (Siddiqui et al., 2024).

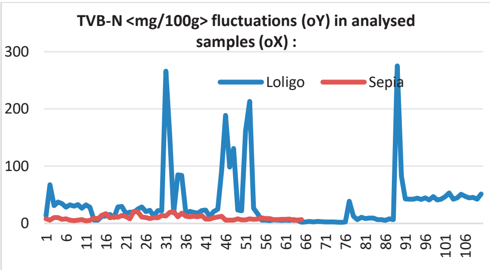

Figure 6. Results regarding the initial value of TVB-N in frozen, thawed calamar *(Loligo* spp.) and thawed squid *(Sepia* spp.) samples (on day 1 of storage at 4°C)

Chilling is a common preservation method that involves maintaining seafood at temperatures between 0-5°C. This technique utilizes vacuum packaging and modified atmosphere packaging to preserve seafood freshness by avoiding exposure to air and moisture. The preferred shelf life for chilled seafood ranges from 2-3 days for fresh products, emphasizing the importance of minimizing air and moisture contact to extend product quality.
Super-chilling extends preservation temperatures below 0°C to enhance the shelf life of seafood. With the absence of additives, super-chilling utilizes vacuum packaging and

modified atmosphere packaging to maintain product quality. Fresh seafood can be preserved for 2-3 weeks, while processed seafood can last up to 1 month, highlighting the efficacy of super-chilling in prolonging seafood freshness.
Freezing seafood at temperatures below -18°C stops bacterial growth and enzymatic activity, thereby extending the shelf life of seafood products. This preservation method commonly employs vacuum packaging, modified atmosphere packaging, or freezer-safe contain-ners to maintain optimal storage conditions. Most seafood items can be stored for 6-12 months, with certain types lasting up to 18 months, showcasing the versatility of freezing in preserving a wide range of seafood products.
Ultra-low temperature freezing takes presser-vation to the next level by storing seafood below -50°C, significantly extending the shelf life of products. Similar to freezing, this method utilizes vacuum packaging, modified atmosphere packaging, or freezer-safe containers to ensure product quality. The extended shelf life of 2-3 years for most seafood items demonstrates the effectiveness of ultra-low temperature freezing in preserving seafood freshness over an extended period.
Freeze-drying is a unique preservation tech-nique that involves removing moisture from seafood while maintaining its nutritional value and flavour. This method requires airtight packaging, often in the form of pouches or jars, to prevent exposure to air and moisture. Seafood preserved through freeze-drying can be stored at room temperature or below for 1-2 years, showcasing the longevity of this preservation method in maintaining seafood quality (Siddiqui et al., 2024).
The utilization of low-temperature preservation technologies is essential in the seafood industry to ensure the quality, safety, and shelf life of seafood products. By understanding the specific preservation temperatures, technologies, packaging materials, and storage conditions associated with each method outlined in Table 3, seafood producers and consumers can make informed decisions to enhance freshness and prolong the shelf life of seafood items. These preservation techniques not only preserve seafood quality but also contribute to reducing food waste and meeting consumer demands for high-quality seafood products.

As of the latest scientific knowledge and technological advancements, storing cephalon-pods at -23°C is technically feasible using commercial freezer units and cryopreservation techniques. While storing cephalopods at -23°C is technically possible with current technology, practical considerations such as equipment availability, storage logistics, quality concerns, and cost-effectiveness need to be carefully evaluated before implementing this storage method on a large scale.
The results of the thawing test showed that the products did not regain their original freshness after thawing.

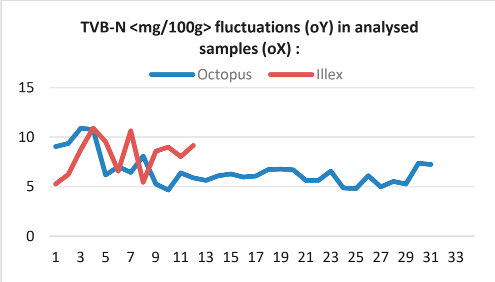

Figure 7. Results regarding final value of TVB-N in frozen, thawed Illex *(Illex argentinus)* and *Octopus (Octopus* spp.) samples

The pH values of the products were slightly higher after thawing (Figure 5); the TVB-N content and sensory properties of the products were still lower than the values of the fresh products.

CONCLUSIONS

The results of this study show that the storage temperature and thawing time have a significant effect on the quality of cephalopod products. The products that are stored at the lowest temperature (-23°C) and thawed for the shortest time have the best quality. However, even these products do not regain their original freshness after thawing. Retailers with access to commercial-grade freezers or blast freezers may indeed be able to store cephalopod products at temperatures as low as -23°C or even lower. These ultra-low temperatures are commonly used in commercial food storage facilities to extend the shelf life of perishable products, including seafood.

As for consumers, while it may be challenging to replicate such low temperatures in standard household freezers, they can still maintain the quality of cephalopod products by storing them at the lowest temperature settings available. Rapid thawing methods, such as placing the product under cold running water or in a bowl of cold water, can help preserve quality by minimizing the time spent in the thawing process. Additionally, using a microwave with a defrost function or a refrigerator for gradual thawing can also be effective methods, although they may take longer.

The study provides compelling evidence that storage temperature plays a pivotal role in preserving the quality of frozen cephalopods. Lower storage temperatures, such as -23°C, significantly delay the onset of spoilage and maintain palatability for a longer period due to several mechanisms:

1. Slowing down of biological processes: at lower temperatures, the metabolic activities of microorganisms, enzymes, and other biological agents responsible for spoilage slow down. This reduction in metabolic rate decreases the rate of food degradation and spoilage, extending the shelf life of the product.
2. Inhibition of microbial growth: many microorganisms responsible for food spoilage have optimal growth temperatures within a certain range. By storing food at temperatures well below their optimal growth range, microbial growth is inhibited or significantly slowed down, reducing the risk of contamination and spoilage.
3. Enzyme inactivation: enzymatic reactions that contribute to food deterioration are also slowed down or halted at low temperatures. This includes enzymatic browning, lipid oxidation, and other enzymatic reactions that degrade the quality of food over time.
4. Water activity reduction: lower temperatures reduce the water activity (amount of available water) in the food matrix. This decrease in water activity inhibits the growth of microorganisms and slows down chemical reactions, further extending the shelf life of the product.
5. Preservation of texture and flavour: lower temperatures help preserve the texture, flavour, and overall quality of the food by minimizing changes in the food's physical and chemical properties over time.

These findings have significant implications for consumers and retailers, emphasizing the importance of proper storage practices to ensure the freshness and enjoyment of these seafood delicacies.

Based on the results of our experiment, an innovative extension of the hypothesis could be that the exposure of cephalopods to extremely low temperatures like -23ºC not only extends their storage time but also enhances the retention of key nutritional compounds and flavour profiles. This could lead to the development of novel storage techniques that preserve the quality and sensory attributes of cephalopods for longer periods, potentially opening up new opportunities in the seafood industry and culinary applications.

An innovative approach to cephalopod conservation involves utilizing rapid freezing techniques combined with cryoprotectants at -23ºC to enhance both the quality and sustainability of cephalopod food products. This method aims to preserve the delicate texture, flavour, and nutritional profile of cephalopods while minimizing the formation of ice crystals that can degrade quality during freezing and thawing processes.

By employing this advanced freezing technology, cephalopod producers can effectively extend the shelf life of these valuable seafood items without compromising their sensory attributes. Additionally, the use of cryoprotectants can help maintain the structural integrity of cephalopods, ensuring that they retain their natural taste and appearance upon thawing. This innovative freezing method not only enhances food quality but also contributes to reducing food waste and promoting sustainable seafood practices in the industry.

Further research and development in seafood preservation technologies could lead to more efficient and sustainable methods for extending the shelf life of cephalopods in the future.

Investigations on the effects of packaging and thawing methods on the quality of frozen cephalopods are also needed. By understanding the intricate relationship between storage conditions, chemical changes, and sensory perceptions, we can optimize the preservation and consumption of these delectable marine invertebrates.

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IMPACT OF DISODIUM DIPHOSPHATE ON THE COLORIMETRIC PROFILE IN A MEAT PRODUCT WITH HETEROGENEOUS STRUCTURE: AN ANALYSIS IN ACTUAL TECHNOLOGY

Marius Mihai CIOBANU, Bianca-Maria MĂDESCU, Elena-Iuliana FLOCEA, Mihai Cătălin CIOBOTARU, Diana-Remina MANOLIU, Bianca Georgiana ANCHIDIN, Ioana GUCIANU, Paul Corneliu BOIȘTEANU

"Ion Ionescu de la Brad" Iasi University of Life Sciences, 3 Mihail Sadoveanu Alley, 700490, Iasi, Romania

Corresponding author email: paul.boisteanu@iuls.ro

Abstract

The aim of this work focused on the analysis and evaluation of the emulsion stabilizer, $Na_2H_2P_2O_7$ on the colorimetric profile in a meat product with a heterogeneous structure of Sus scrofa domesticus. Water (%), fat (%), protein (%), collagen (%), and salt (%) contents were also monitored. Depending on the fat and water content, the agent absorption may vary, which may affect the uniformity or intensity of the color of the finished product. The experimental samples consisted of a control batch and 0.09% $Na_2H_2P_2O_7$ per 3000 g of meat fed to the experimental batch. Data distribution was evaluated using SPSS Statistics 26.0 software and Graph Pad Prism 9 software. A T-test was applied to evaluate the influence of the $Na_2H_2P_2O_7$ on quality biomarkers (%). Linear regression was applied to determine if there was a linear relationship between $Na_2H_2P_2O_7$ and the CIE(Lab) system parameters: L(D65), a*(D65), and b*(D65). The results showed significant effects of $Na_2H_2P_2O_7$ in fat content (%) and water (%), thus influencing the colorimetric profile.*

Key words: *emulsion stabilizer, meat product, quality.*

INTRODUCTION

There is now a shift in dietary patterns in modern society, with consumers increasingly aware of the relationship between food and health. In this context, some meat products are often perceived as unhealthy due to their composition (Câmara et al., 2020; Boișteanu et al., 2023; Ciobanu et al., 2023).

The many negative connotations of processed products can be alleviated by decreasing the amounts of harmful elements such as saturated fats, salt, nitrites, and phosphates (Alirezalu et al., 2019; Anchidin et al., 2023). Studies have indicated that reducing the level of these components can lead to more technological limitations in meat products (Ciobanu et al., 2023; Câmara & Pollonio, 2015).

From a technological point of view, lipid reformulation using vegetable oils is a great challenge because it influences the physical and chemical stability of the emulsified matrix (Câmara & Pollonio, 2015).

Phosphates offer a wide range of possibilities when used in meat production. They are used in meat products for several reasons, such as modifying and/or stabilizing the pH value, and increasing water holding capacity to lead to higher yields from a technological point of view. Phosphates in meat products are also sources of phosphorus supply to consumers through feed (Long et al., 2011).

Phosphates are important for human health because they are responsible for the growth, maintenance, and repair of tissues and cells of living organisms. However, an avoidable health risk results from the increased use of phosphates as food additives and preservatives (Ritz et al., 2012).

Diphosphates can immediately dissociate the actomyosin complex of meat, and tri- and polyphosphates help activate meat proteins by partially chelating Mg^{2+} and Ca^{2+} proteins, both of which lead to increased solubilization of myosin and actin and depolymerization of thick and thin filaments (Glorieux et al., 2017). This work aimed to analyze and evaluate the effect of the emulsion stabilizer, $Na_2H_2P_2O_7$, on the colorimetric profile of a meat product from domestic pigs with a heterogeneous structure. In addition, the content of water, fat, protein, collagen, and salt was monitored.

MATERIALS AND METHODS

The experimental samples were designed in accordance with (Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008). The experimental samples consisted of a control batch and 0.09 % $Na_2H_2P_2O_7$ per 3000 g of meat fed to the experimental batch. The samples used in this study, together with the reference material, were obtained in the Meat Microproduction Department (IULS Iași), following ISO quality standards. The raw materials, from a local producer, were stored in cold storage for 24 hours before the start of the study. For the research, muscle samples from *Musculus gluteus maximus* were taken for the study after the removal of excess fat and connective tissue. Only one type of salt was used: NaCl (table/natural salt) for both the control and experimental groups. These were mixed manually with the muscle samples and then subjected to a 12-hour maturation period at a controlled temperature of 2-4°C. After maturation, the samples were coarsely grinded by Grinder WP - 105 using a sieve with a diameter of 3 mm, then the emulsion was obtained using the Cutter Titane V 45L. The samples were mixed with the control emulsion and the emulsion containing the emulsion stabilizer 0.09 % $Na_2H_2P_2O_7$ per 3000 g of meat fed to the experimental batch. The resulting paste was placed in collagen membranes with a diameter of 45 mm, which were previously hydrated to form elasticity. The samples were subjected to a specific heat treatment according to Table 1.

Table 1. Heat treatment steps for the control sample (meat product with heterogeneous structure without $Na_2H_2P_2O_7$) and experimental samples (meat product with heterogeneous structure with $Na_2H_2P_2O_7$)

Heat treatment stage	Time	Temperature inside the cell	Temperature in the thermal center
	minutes	°C	°C
Drying	30	60	50
Smoking	25	60	58
Boiling	-	76	72
Drying	10	80	76

These samples were vacuum-sealed in two-layer bags, the inner layer being 60 μm polyethylene, suitable for food contact, and the outer layer being 15 μm polyamide, UV-filtered, using ATM Machinery equipment with a 630W chamber cooled to 2°C and separated from light for 12 hours. After this period, samples were transported to the control laboratory for further analysis.

The final products were subjected to physicochemical evaluations to test the influence of Na2H2P2O7 on the heterogeneous structure product. Instrumental color characteristics of the product were determined using a Chroma Meter CR-410 colorimeter from Konica Minolta Inc., Japan, on the CIELAB scale. These characteristics were expressed as L*, a*, and b* values. The primary color coordinates L* (brightness), a* (complementary red-green color coordinate), b* (complementary yellow-blue color coordinate) were evaluated according to CIE (Commission Internationale de l'Eclairage) space LAB on the sample surface (presented as the average value of measurements at five equally distributed locations on the sample) and section using a Hunter Minolta CM-2600d colorimeter, with an observation angle of 2° and a measurement area with a diameter of 8 mm, illuminating an area of 50 mm in diameter, according to (Manoliu et al., 2023), (Tapp et al., 2011). Fat (%), protein (%), collagen (%), and salt (%) contents were also monitored using the Omega Bruins Food-Check Near Infrared (NIR) spectrophotometer (Bruins Instruments GmbH, Puchheim, Germany).

Data distribution was evaluated using SPSS Statistics 26.0 software and Graph Pad Prism 9 software. A T-test was applied to assess the influence of $Na_2H_2P_2O_7$ on quality biomarkers (%). Linear regression was applied to determine if there was a linear relationship between $Na_2H_2P_2O_7$ and the CIE(Lab) system parameters L*(D65), a*(D65), and b*(D65).

RESULTS AND DISCUSSIONS

The results of the analysis of the parameters fat (%), protein (%), collagen (%), and salt (%) in the control and experimental samples with the addition of $Na_2H_2P_2O_7$ are shown in Table 2. The current technology may have a significant impact on the raw chemical composition.

Protein is an essential element in determining the quality of the finished product and the current technology may play a significant role in influencing the concentration. Protein is often considered a measure of the nutritional value of a food product and is essential in determining the texture, taste, and nutritional value of the final product. Samples from the control batch show an average protein of 20.02 ± 0.0447%, while the experimental batch records an average of 21.52 ± 0.1924%. The quantity of protein increased significantly. Visible differences can be seen in water and fat content. The addition of $Na_2H_2P_2O_7$ can help to reduce water loss during product processing. The control samples show an average of the water parameter of 69.02 ± 0.1095%, while in the experimental batch, the average is 71.18 ± 0.0447%. By stabilizing the structure of the meat product, $Na_2H_2P_2O_7$ can influence the way fat is distributed in the product. This may lead to a more uniform dispersion of fat throughout the product, which could affect the fat content in the raw composition. The amount of fat in the control samples is 10.22 ± 0.1643%, compared to the experimental samples where the average is 7.58 ± 0.0447%. These results indicate a significant reduction in fat content in the experimental samples, suggesting that the addition of $Na_2H_2P_2O_7$ can positively influence the crude chemical composition of the pork product by reducing fat content and improving water retention.

Table 2. Analysis of the parameters fat, water, protein, collagen, and salt in the control and experimental samples

Parameters	Sample	N	Mean	Std. Deviation	Std. Error Mean
FAT (%)	**C**	5	10.22	0.1643	0.0735
	E	5	7.58	0.0447	0.0200
WATER (%)	**C**	5	69.02	0.1095	0.0490
	E	5	71.18	0.0447	0.0200
PROTEIN (%)	**C**	5	20.02	0.0447	0.0200
	E	5	21.52	0.1924	0.0860
COLLAGEN (%)	**C**	5	18.24	0.0548	0.0245
	E	5	19.01	0.0548	0.0246
SALT (%)	**C**	5	2.34	0.2702	0.1208
	E	5	3.00	0.1095	0.0490

Values are given as means, std. deviation and std. error mean from 5 repeated determinations; C-control samples, E- experimental samples.

Table 3. Results of the evaluation of the impact of $Na_2H_2P_2O_7$ on fat, water, protein, collagen, and salt in a heterogeneously structured meat product of *Sus scrofa domesticus*

Parameters	t	df	Significance		Mean Difference	95% Confidence Interval of the Difference	
			One-Sided *p*	Two-Sided *p*		Lower	Upper
FAT (%)	379	4	<0.001	<0.001	7.58	7.524	7.636
WATER (%)	3559	4	<0.001	<0.001	71.18	71.124	71.236
PROTEIN (%)	238.54	4	<0.001	<0.001	20.52	20.281	20.759
COLLAGEN (%)	773.22	4	<0.001	<0.001	18.94	18.872	19.008
SALT (%)	55.52	4	<0.001	<0.001	2.72	2.584	2.856

The significance level is 0.050. determined by the T-test.

T-test was used to determine if there was a significant difference between the two groups (C-control samples, E-experimental samples). According to Table 3, fat (%), water (%), protein (%) collagen (%), and salt (%) contents in the experimental samples were significantly different from the control samples, suggesting that the addition of $Na_2H_2P_2O_7$ significantly influenced these control parameters. For fat, we have a mean difference of 7.58%, and the 95% confidence interval for this difference is between 7.524% and 7.636%. The *p*-values for all tests are very small, $p<0.001$, indicating that the observed differences are highly unlikely to be the result of random variability and are therefore statistically significant. The mean difference is shown, along with the 95% confidence interval, which indicates how accurately the mean difference between the treated and control samples is estimated.

Depending on the fat and water content, the agent absorption may vary, which may affect the uniformity or intensity of the color of the finished product.

Figure 1 shows the analysis of the distribution of the means of the primary color coordinates L*(D65), a*(D65), and b*(D65) for two sets of samples: A (control) and B (experimental samples with $Na_2H_2P_2O_7$).

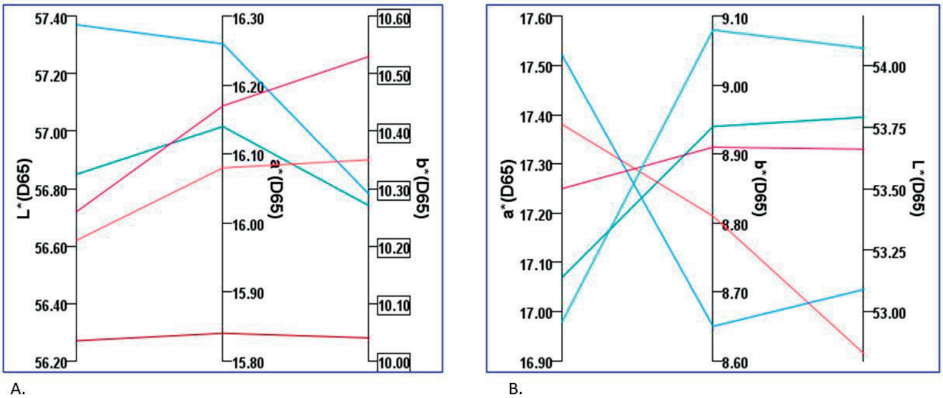

Figure 1. Distribution of the means of the primary color coordinates L*(D65), a*(D65), b*(D65) for A (control samples) and B (experimental samples)

Table 4. Results of linear regression analysis performed to determine the influence of $Na_2H_2P_2O_7$ on CIE(Lab) color coordinates: L*(D65), a*(D65), and b*(D65) in the samples studied

Parameters	Sample	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
						R Square Change	F Change	df1	df2	Sig. F Change
L*(D65)	C	.960[a]	.921	.894	.13017	.921	34.85	1	3	.010
	E	.308[a]	.095	-.206	.56299	.095	.315	1	3	.614
a*(D65)	C	.900[a]	.809	.746	.07975	.809	12.73	1	3	.038
	E	.552[a]	.305	.073	.21238	.305	1.31	1	3	.335
b*(D65)	C	.377[a]	.142	-.144	.18822	.142	.498	1	3	.531
	E	.722[a]	.521	.361	.12779	.521	3.26	1	3	.169

[a] Predictors: Constant

Table 4 shows for the experimental sample (E): R = 0.308, R Square = 0.095, Adjusted R Square = -0.206, Std. Error of the Estimate = 0.56299. This suggests that the influence of $Na_2H_2P_2O_7$ on L*(D65) in the experimental sample is not statistically significant. For the parameter a*(D65), R = 0.552, R Square = 0.305, Adjusted R Square = 0.073, Std. Error of the Estimate = 0.21238. In this case, the influence of $Na_2H_2P_2O_7$ on a*(D65) in the experimental sample is statistically significant. For the parameter b*(D65), R = 0.722, R Square = 0.521, Adjusted R Square = 0.361, Std. Error of the Estimate = 0.12779. In the experimental sample, the influence of $Na_2H_2P_2O_7$ on b*(D65) is statistically significant. These results indicate that $Na_2H_2P_2O_7$ has different influences on CIE(Lab) color coordinates.

Table 5. Results of analysis of variance (ANOVA) for linear regression applied for CIE(Lab) coordinates L*(D65), a*(D65), and b*(D65) in control and experimental samples

ANOVA[a]							
			Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
L*(D65)	C	Regression	0.59	1	0.59	34.851	0.010[b]
		Residual	0.051	3	0.017		
		Total	**0.641**	**4**			
	E	Regression	0.1	1	0.1	0.315	0.614[b]
		Residual	0.951	3	0.317		
		Total	**1.051**	**4**			
a*(D65)	C	Regression	0.081	1	0.081	12.736	0.038[b]
		Residual	0.019	3	0.006		
		Total	**0.1**	**4**			
	E	Regression	0.059	1	0.059	1.315	0.335[b]
		Residual	0.135	3	0.045		
		Total	**0.195**	**4**			
b*(D65)	C	Regression	0.018	1	0.018	0.498	0.531[b]
		Residual	0.106	3	0.035		
		Total	**0.124**	**4**			
	E	Regression	0.053	1	0.053	3.263	0.169[b]
		Residual	0.049	3	0.016		
		Total	**0.102**	**4**			

The significance level is 0.050. C-control samples, E- experimental samples; a. Dependent Variable: L*(D65), a*(D65), b*(D65);
b. Predictors: Constant

According to the results of the analysis of variance (ANOVA) for the linear regression applied on the coordinates CIE(Lab) - L*(D65), a*(D65), and b*(D65), presented in Table 5, we observe that there are significant differences between the control and experimental groups. For the parameter L*(D65), the regression is significant for the control group (p=0.010), suggesting a linear relationship between the sample means. In contrast, for the experimental samples, the regression is insignificant (p=0.614), indicating the absence of a linear relationship.

Regarding the parameter a*(D65), we observe that the regression is significant for the control group (p=0.038), while for the experimental group, the regression is not significant (p=0.335), suggesting the absence of a linear relationship.

In the case of parameter b*(D65), both control and experimental samples show insignificant regressions (p=0.531, p=0.169, respectively), indicating the absence of a significant linear relationship between these coordinates in both groups. These results highlight the variation in the significance of linear relationships between CIE(Lab) coordinates and the influence of $Na_2H_2P_2O_7$ in the heterogeneously structured meat product.

CONCLUSIONS

The results showed significant effects of fat and water content on the colorimetric profile. T-test was used to determine significant differences between the two groups (C - control sample, E - experimental samples). The fat, water, protein, collagen, and salt content of the experimental samples was significantly different from the control samples, indicating that the addition of $Na_2H_2P_2O_7$ significantly influenced these control parameters. Depending on the fat and water content, the absorption of the agent may vary, which may affect the uniformity or intensity of the color of the finished product.

The results of linear regression analysis performed to determine the influence of $Na_2H_2P_2O_7$ on CIE (Lab) color coordinates: L*(D65), a*(D65), and b*(D65) in the samples studied suggest that $Na_2H_2P_2O_7$ has different influences on CIE (Lab) color coordinates.

According to the results of analysis of variance (ANOVA) for linear regression applied on CIE (Lab) - L*(D65), a*(D65), and b*(D65) coordinates, significant differences are observed between the control and experimental groups.

Future studies could further explore the effects of $Na_2H_2P_2O_7$ addition on other aspects of the final product, such as texture and taste.

Research investigating how variations in the initial composition of the raw material affect the response of the product to this specific additive could also be useful. In addition, assessing the long-term impact of using $Na_2H_2P_2O_7$ in production processes and finished products could provide a more comprehensive understanding of its potential effects on food quality and safety. Research can also explore alternatives to $Na_2H_2P_2O_7$ and their comparisons in terms of efficacy and impact on finished product properties.

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IMPACT OF BONE BROTH ON PROTEIN CONTENT, COLOR, AND CONSUMER PREFERENCES IN EMULSIFIED CHICKEN AND TURKEY FRANKFURTERS

Marius Mihai CIOBANU, Mihai Cătălin CIOBOTARU, Ioana GUCIANU, Paul Corneliu BOIȘTEANU, Diana-Remina MANOLIU

"Ion Ionescu de la Brad" Iasi University of Life Sciences, 3 Mihail Sadoveanu Alley, 700490, Iasi, Romania

Corresponding author email: diana.manoliu@iuls.ro

Abstract

This study investigated the impact of incorporating beef bone broth into chicken and turkey frankfurters. Thus, diversification was achieved by the type of meat used and the level of bone broth introduced, resulting in the following samples: CC (control chicken frankfurter), C3% (chicken frankfurter with 3% bone broth), C6% (chicken frankfurter with 6% bone broth), C9% (chicken frankfurter with 9% bone broth), CT (control turkey frankfurter), T3% (turkey frankfurter with 3% bone broth), T6% (turkey frankfurter with 6% bone broth), and T9% (turkey frankfurter with 9% bone broth). After manufacture, the products were analysed in terms of chemical composition, instrumental colour and sensory perception. The addition of bone broth significantly increased moisture content and protein content (particularly at higher broth concentrations) compared to control sausages. Conversely, fat content decreased with increasing bone broth. The sensory evaluation revealed no significant changes in flavor, texture, or overall acceptability with broth addition, although some panelists perceived a sensory improvement. Instrumental color analysis showed minimal impacts on lightness and redness with broth addition, suggesting the broth's color did not substantially influence overall sausage color.

***Key words**: beef bone broth, chicken/turkey sausages, frankfurters.*

INTRODUCTION

Worldwide poultry meat consumption has steadily increased from 37,368.82 million tonnes in 2008, when it surpassed pork consumption, to 50,694.48 million tons in 2022 (OECD, 2023). In addition, according to the OECD/FAO projections 2023-2032, poultry meat consumption will increase the most globally, by 15% by 2032, accounting for 41% of animal protein intake (OECD/FAO, 2023).

Poultry meat consumption in the European Union is positioned as the second highest, following swine meat, at 1,003.33 million tons in 2022 (OECD, 2023). The pattern of pork consumption in Romania mirrors that of the European Union as a whole. On the contrary, poultry meat consumption exhibits a consistent upward trajectory, reaching an average of 27.9 kg per capita in 2022, as opposed to 20.1 kg per capita in 2014, according to Statista (2024).

Emulsified meat products, which consist of frankfurter sausages, bologna, Vienna sausage, and hot dogs, are among the most widely consumed meat products on a global scale. Primarily, this is attributed to their distinct flavor and nutritional composition. Furthermore, their affordability and convenience contribute to their widespread appeal among consumers (Sam et al., 2021; Cao et al., 2022).

Frankfurter sausages are widely consumed meat products that originate from various cultures. They are prepared using an emulsion method, are cooked-smoked, and consist of lean and fatty meat, water, and additional ingredients such as salts, ripening agents, and spices. The circumference of the sausage wrap is determined by the manufacturer and can be either natural pig membranes or collagen membranes (Feng et al., 2016; Bravo et al., 2020).

Frankfurter sausages possess a considerable lipid content, which comprises an estimated 20% to 30% of their overall composition. Fat is widely criticized and shunned due to the perceived dangers it presents and can be mitigated by choosing lean cuts of meat, removing fat entirely, modifying the diet to alter the composition of fatty acids, and regulating portions to decrease fat and calorie consumption. In recent years, there has been a notable emphasis on the development of meat products that incorporate various constituents that improve product quality and impart health benefits (Anchidin et al., 2023). Furthermore, the fat content accentuates the sensitivity to lipid oxidation, leading to degradation, rancidity, discoloration, a shorter shelf life, and the formation of hazardous chemicals (Bravo et al., 2020; Sam et al., 2021). To prevent this qualitative deterioration, intervention occurs in the actual manufacturing process of emulsified meat products through the use of a variety of additives, such as thickening agents, emulsifying agents, water/fat retention agents, and gelling agents. These additives have been used to improve the quality profiles of finished products and extend their storage life (Yuan et al., 2023).

In the case of emulsified varieties, the most important agents are binders and emulsifiers, as meat emulsion is considered a water-in-oil emulsion, heterogeneous composite materials composed of fat globules covered with proteins (oil droplets) dispersed in a matrix of myofibrillar protein gel (Santhi et al., 2015).

Emulsifying agents serve as an interface between immiscible components, responsible for forming stable emulsions. An emulsifier is a substance that enhances texture and palatability, inhibits separation of the food system, regulates rancidity processes, and either solubilizes or disperses flavors (Santhi et al., 2017; Surendran Nair et al., 2020).

Proteins derived from meat are excellent emulsifying agents. In order to avoid fat coalescence, the amino acids present in proteins interact with non-polar fat molecules and polar water molecules, thus establishing a continuous system in the meat mixture (Surendran et al., 2020). Additionally, the use of collagen leads to an increase in protein content within the emulsion, which in turn improves emulsion stability (Santana et al., 2011).

Bone broth is popular and appreciated for its distinctive flavor, delicious taste and multitude of water-soluble vital nutrients, including short peptides, minerals, vitamins and free amino acids. One of the primary nutrients found in bone broth is protein. As its primary protein components, bone broth primarily comprises chondroitin, collagen, and free amino acids (Zhang et al., 2014; Zhang et al., 2017; Meng et al., 2022).

In this context, the aim of the work was to obtain two assortments of meat products with emulsified structure, frankfurter type sausages from chicken and turkey meat, enriched with beef bone soup to improve protein intake. The experimental batches, as well as the control ones in each variety, were analyzed in terms of the proximate chemical composition and sensory perception, instrumental color, and also color changes that occurred during five days of storage at refrigerated temperature.

MATERIALS AND METHODS

The research was conducted at the "Ion Ionescu de la Brad" Iasi University of Life Sciences, where the frankfurter sausages were manufactured in the Meat Processing Workshop. The analytical procedures were carried out in the laboratories dedicated to meat and meat products technology, as well as sensory analyses.

In order to accomplish the intended objective, an experimental protocol was devised that resulted in the fabrication of two varieties of frankfurter sausages, a total of eight batches: four containing poultry meat and four containing turkey meat.

The frankfurter sausages were prepared as described by Boișteanu et al. (2022), with some adjustments. Except for the beef bone broth, which was produced in the meat processing facility before the sausages, all constituents (meat, salt, spices, membranes) utilized in the production of the sausage batches were procured from local suppliers.

The production process of the batches involved the introduction of the following raw materials: chicken and turkey thigh meat, beef bone soup, salt, spices, as described in Table 1. Thus, four frankfurter formulations were prepared for each variety in the ratios (chicken or turkey thigh meat/beef bone broth) of 100:0; 97:3; 94:6 and 91:9.

Table 1. Formulations of poultry frankfurters added with different concentrations of beef bone broth (kg)

	Control chicken (CC)	C3%	C6%	C9%	Control turkey (CT)	T3%	T6%	T9%
Lean chicken meat	3.5	3.392	3.277	3.154	-	-	-	-
Lean turkey meat	-	-	-	-	3.2	3.105	3.003	2.894
Beef bone soup	-	0.108	0.223	0.346	-	0.095	0.197	0.306

The other ingredients were added as follows: salt 20 g/kg, sweet paprika 2 g/kg, coriander 1 g/kg, garlic powder 5 g/kg, pepper 2 g/kg.

The manufacturing technology followed the steps described in a previous study (Manoliu et al., 2023), with some specific modifications for frankfurter sausages. Hence, the raw meat was first grinded in a machine (Grinder WP - 105) using a sieve of 3 mm diameter, then fine minced, using a machine only used in food industry for mincing chilled or frozen meat (with a maximum temperature of -7ºC) and obtaining emulsions containing meat (Cutter Titane V 45L), until a fine, homogeneous paste was obtained. In this stage, the bone broth was added, which due to the low temperature, also helped to stop the temperature of the paste from rising during the operation. Throughout the process, the temperature of the paste was checked with a vertical thermometer to prevent it from rising above 12°C.

After the meat batter had the required consistency, all other ingredients and spices were weighed and then mixed with the batter until evenly distributed in the paste structure. The resulting paste was placed in natural pork membranes of 38-40 mm diameter, which have been previously hydrated to form elasticity.

The heat treatment carried out was the same for the eight batches of frankfurter sausages; the steps are shown in Table 2.

Table 2. Heat treatment steps for the frankfurter sausages added with beef bone broth

Heat treatment stage	Time	Temperature inside the cell	Temperature in the thermal centre	Humidity
	minutes	°C	°C	%
Air drying	30	60	52	30
Smoking	20	70	65	30
Boiling	-	78	72	99
Hot air drying	5	80	72	30

The final products underwent physicochemical and sensory assessments in the Meat and Meat Products Technology Laboratory and the Sensory Analysis Laboratory.

The instrumental color characteristics of the frankfurters were determined using a Chroma Meter CR-410 colorimeter (Konica Minolta Inc., Japan) on the CIELAB scale. These parameters were represented as L*-values, a*-values, and b*-values. The instrument was equipped with a D65 light source, which had a 2° angle of observation and a measuring area with a diameter of 8 mm. The illuminating area had a diameter of 50 mm. The determination of chroma (C*) and hue (H*) values was conducted using equations (1) and (2), as outlined in the study conducted by Long et al. (2024). The calculation of the total color difference (ΔE) was performed using equation (3), as described by Yuan et al. (2023). The values of L*con, a*con, and b*con were obtained from the control samples, while the values of L*s, a*s, and b*s were measured from frankfurters prepared with different quantities of beef bone broth.

$$H(*) = tan^{-1}(\frac{b^*}{a^*}) \quad (1)$$

$$Chroma\ (C^*) = \sqrt{(a*)^2 + (b*)^2} \quad (2)$$

$$\Delta E = \sqrt{(L*s - L*con)^2 + (a*s - a*con)^2 + (b*s - b*con)^2} \quad (3)$$

The chemical evaluation encompassed the utilization of a FoodCheck analyzer, which is a spectrophotometer that operates on infrared light beams, to ascertain the moisture, fat, protein, collagen, and sodium contents, respectively.

Historically, the assessment of food products through sensory evaluation involved the investigation, examination, understanding, and interpretation of responses generated by individuals through the utilization of the primary senses (visual, olfactory, gustatory, tactile, and auditory) on said products (Ciobanu et al., 2022; Ruiz-Capillas et al., 2021). The sensory assessment was conducted using a modified version of the procedure outlined by Boişteanu et al. (2023). In summary, for the purpose of sensory evaluation, a cohort of twenty-five graduate students (consisting of sixteen females and nine males) was chosen from the meat processing technology laboratory at the Iasi University of Life Sciences. The panel participated in a vocabulary development session and a training session prior to sensory evaluation. On the basis of the following nine-point descriptive scales (low intensity to high intensity): interior color (1 = light brown; 9 = pink), uniformity (1 = low; 9 = high), flavour (1 = least intense, 9 = most intense), and juiciness (1 = extremely dry; 9 = extremely juicy), each frankfurter batch was assessed using sensory descriptive analysis. In addition, the panelists assessed the overall acceptability of each frankfurter assortment (1 = low; 9 = high).

The mean values obtained for the proximate composition, colour parameters and sensory evaluation were compared using analysis of variance (ANOVA) followed by Tukey's test at 5% significance level ($p < 0.05$), using IBM SPSS Statistics v.21.

RESULTS AND DISCUSSIONS

The results for proximate chemical composition expressed as mean value and standard deviation determined for the batches of frankfurter sausages produced are shown in Table 3. The moisture content of frankfurter sausage is an essential physicochemical attribute that has the potential to impact shelf life and sensory attributes, including texture. The alterations observed in the moisture content of the treated samples, which varied between 70.30% and 73.68%, were notably impacted by the incorporation of beef bone broth. Comparatively, the moisture content of the control products was considerably reduced ($p < 0.05$) in contrast to the enriched frankfurters formulated from chicken and turkey meat. The high moisture content observed in the beef bone broth-enriched samples was plausibly attributable to the inclusion that was introduced into the formulation. In general, bone broth comprises approximately 94.32% to 97.44% moisture (Yoon et al., 2015). This finding is consistent with earlier studies by Akullo et al. (2020), who reported a higher moisture content for tilapia and Nile perch sausages optimized with fish bone soup at 72.3% and 71.2%, respectively, compared to the control (69.7%).

The inclusion of beef bone broth in the chicken and turkey frankfurters formulation significantly affected the fat content with control samples from each group having the highest fat content, 8.72% (CC sample) and 5.24% (CT sample). The addition of bone broth therefore caused a decrease in lipid content, which was more pronounced in chicken frankfurters than in turkey frankfurters. Similar findings were reported by Akullo et al. (2020) for fish bone soup incorporated tilapia and Nile perch sausages; study that showed a decrease in crude fat content from 3.72% in the control sample to 2.13% in Talpia fish sausages.

As a recognized nutrient diluent, moisture tends to diminish the concentrations of numerous nutrients when present in excessive quantities. For example, there is a strong correlation between moisture and fat content in meat products, such that a high moisture content is indicative of a probable decrease in fat content (Sam et al., 2021). This trend was also present in the current study, as an inversely correlation between moisture content and lipid level was revealed.

While bone broth itself doesn't directly remove fat from sausages, its effect to lower overall fat content in the final smoked product may be due to the dilution effect, since bone broth is primarily water-based, containing very little fat. Adding broth to the meat batter dilutes the overall fat concentration in the mixture. This lowers the final percentage of fat in the finished sausage compared to one made solely with meat. Moreover, bone broth can contain gelatin, a protein derived from collagen in bones. Gelatin acts as an emulsifier, helping to disperse fat particles throughout the meat batter more evenly. This improves emulsification and allows for a more stable mixture with a lower overall fat content.

Protein is pivotal in determining the water-holding capacity of meat, as water molecules have a strong affinity for meat hemoproteins (Sam et al., 2021). A progressive rise in protein content was noted in frankfurter-type sausages with increasing levels of added beef bone broth. However, the increase was significant mainly for the higher level of addition (6% and 9%) for both chicken and turkey meat frankfurters. The collagen content (expressed as a percentage of total protein) was also directly proportional to the amount of protein. Thus, the highest protein level was reported for the 9% beef bone broth samples within each experimental group (chicken and turkey). These results can be explained by the intake of bone broth, which contains a certain amount of protein, and therefore collagen (from the raw material used to obtain it, i.e. bones with connective tissue and meat remains). Yoon et al. (2015) reported a protein content in beef bone broth between 2.08 and 4.04%, depending on the beef bone segmental (beef bone, woojok, tail). Furthermore, the collagen content can also be explained by the fact that about 90% of the protein in bovine bones is type I collagen, so the protein content of bone broth is also related to collagen solubilisation (Ma et al., 2023).

In addition to the differences impressed by the enrichment with bone broth in the two frankfurter sausages varieties, the prime material used (chicken and turkey meat) also impressed significant differences ($p < 0.05$) in the chemical composition of the finished products, in that turkey meat products showed higher water, protein and collagen content, and lower lipid content, compared to the corresponding chicken meat samples.

Table 3. Analysis of chemical composition and salt content of Frankfurt sausages

Parameters/ Sample	Moisture (%)	Lipid (%)	Protein (%)	Collagen (%)	Salt (%)
CC	70.30 ± 0.07 ^{F}	8.72 ± 0.11 ^{A}	20.38 ± 0.04 ^{E}	18.60 ± 0.07 ^{E}	2.52 ± 0.08 ^{B}
C3%	70.46 ± 0.05 ^{E}	8.50 ± 0.07 ^{B}	20.44 ± 0.08 ^{DE}	18.64 ± 0.05 ^{DE}	2.56 ± 0.08 ^{B}
C6%	70.88 ± 0.04 ^{D}	8.02 ± 0.04 ^{C}	20.50 ± 0.00 ^{CD}	18.76 ± 0.05 ^{CD}	2.34 ± 0.05 ^{C}
C9%	71.00 ± 0.07 ^{D}	7.86 ± 0.05 ^{D}	20.58 ± 0.04 ^{C}	18.78 ± 0.04 ^{C}	1.86 ± 0.05 ^{E}
CT	73.32 ± 0.04 ^{B}	5.24 ± 0.04 ^{E}	21.18 ± 0.09 ^{B}	19.48 ± 0.10 ^{B}	1.44 ± 0.05 ^{F}
T3%	73.16 ± 0.05 ^{C}	5.22 ± 0.04 ^{E}	21.22 ± 0.04 ^{B}	19.51 ± 0.04 ^{B}	2.62 ± 0.08 ^{B}
T6%	73.20 ± 0.01 ^{BC}	5.02 ± 0.09 ^{F}	21.24 ± 0.11 ^{AB}	19.60 ± 0.04 ^{AB}	3.24 ± 0.05 ^{A}
T9%	73.68 ± 0.11 ^{A}	4.60 ± 0.07 ^{G}	21.30 ± 0.14 ^{A}	19.62 ± 0.04 ^{A}	2.18 ± 0.08 ^{D}

Values are given as means ± SE from triplicate determinations; A-G in each column represent statistically significant differences ($p < 0.05$) determined by the Tukey test.

The assessment of frankfurters' quality heavily relies on the color determination, as the visual appeal of meat products has a direct impact on consumer preference. The comparison of the turkey meat and chicken groups' frankfurters indicates that the a*-values were significantly higher in the chicken group ($p < 0.05$), whereas the L* and b*-values were noticeably lower in the turkey meat group, as shown in Table 4. Conversely, the variation in bone broth quantity did not have a substantial impact on the L* and a*-values, although some variations were noted in the portions of turkey meat.

Furthermore, as the concentration of bone broth increased, the L*-values of chicken and turkey frankfurters exhibited a decline for the lower incorporations (3% and 6%). However, the lightness surpassed the control value at a 9% bone broth concentration.

All chicken sausages (CC, C3%, C6%, and C9%) retain considerably less redness than turkey frankfurter sausages (specifically CT and T6%). It is notable that the incorporation of beef bone broth into the chicken sausages (C3%, C6%, and C9%) did not result in a substantial augmentation of their coloration in comparison to the control chicken sausage (CC). This indicates that the beef bone fluid may not have significantly contributed to the red hue of these sausages. Frequently, paprika or nitrite are utilized to impart a red hue to sausages. Turkey meat may contain more red pigments by nature than poultry meat, which could account for the difference in inherent hue.

The b*-values of turkey frankfurters decreased with the increasing percentage of bone broth, while in the case of chicken sausages, the b*-value decreased until 6% bone broth added, and then increased at the higher broth addition (9%). Beef bone broth can have a slight yellow hue, but its impact on overall sausage color might be subtle, especially when added at lower concentrations (3%). Other ingredients in the sausages, like spices or fat content, could be contributing more significantly to yellowness variations.

The C* value indicates color saturation, which is the distance traversed by the central achromatic grey axis of the color space. The hue angle (H* value), on the other hand, signifies the chromaticity or tone of a color. The H* value ranges from 0° (redness)-90° (yellowness)-180° (greenness)-270° (blueness)-360° (redness) (Long et al., 2024).

The results for saturation index (C*) varied in the interval 18.91 - 19.93, the samples showing significant differences only between control turkey and 9% bone broth turkey frankfurters. The hue angle (h*) mean values varied in the interval 0.76-0.99, with significant differences only between turkey and chicken meat sausages. The low values registered for the hue angle places the products in the red quadrant (0-90°).

The ΔE-value is primarily utilized to detect color differences among various samples by considering combined changes in the L*, a*, and b* values, with a larger ΔE-value indicating a greater difference from the control group. As noted by Wibowo et al. (2015), the perceived color difference can be analytically classified as follows: not visible ($0 < \Delta E < 0.5$), slightly visible ($0.5 < \Delta E < 1.5$), visible ($1.5 < \Delta E < 3.0$), well visible ($3.0 < \Delta E < 6.0$), and great ($\Delta E > 6.0$). As presented in Table 4, the ΔE-values for all batches were below 1.5, indicating that the perceived color differences were slightly visible. However, the results obtained for the frankfurters made from chicken meat ranged from 0.58 to 0.78, which were smaller than those observed for the turkey meat batches (ranging from 1.36 to 1.44), suggesting that the addition of bone broth had a greater effect on color change in the case of frankfurters made with turkey meat.

Table 4. Color results for frankfurter sausages added with different concentrations of bone broth

Parameters/Sample	L*(D65)	a*(D65)	b*(D65)	Chroma (C*)	Hue (h*)	ΔE
CC	66.57 ± 0.46^{A}	10.73 ± 0.13^{C}	16.13 ± 0.22^{AB}	19.37 ± 0.25^{ABC}	0.98 ± 0.00^{A}	-
C3%	66.52 ± 0.38^{A}	10.99 ± 0.30^{C}	15.97 ± 0.32^{AB}	19.39 ± 0.42^{ABC}	0.97 ± 0.00^{B}	0.77 ± 0.61
C6%	66.25 ± 0.33^{A}	10.53 ± 0.10^{C}	15.79 ± 0.10^{B}	18.98 ± 0.11^{BC}	0.98 ± 0.01^{AB}	0.58 ± 0.48
C9%	66.90 ± 0.45^{A}	10.75 ± 0.51^{C}	16.40 ± 0.51^{A}	19.61 ± 0.71^{AB}	0.99 ± 0.01^{A}	0.72 ± 0.35
CT	62.79 ± 0.98^{BC}	14.43 ± 0.13^{A}	13.74 ± 0.18^{C}	19.93 ± 0.11^{A}	0.76 ± 0.01^{C}	-
T3%	61.61 ± 1.00^{BC}	13.82 ± 0.27^{B}	13.35 ± 0.24^{CD}	19.22 ± 0.28^{BC}	0.77 ± 0.01^{C}	1.44 ± 0.47
T6%	61.55 ± 0.55^{C}	14.04 ± 0.18^{AB}	13.23 ± 0.04^{CD}	19.29 ± 0.15^{ABC}	0.76 ± 0.01^{C}	1.43 ± 0.59
T9%	62.85 ± 0.22^{B}	13.58 ± 0.14^{B}	13.16 ± 0.14^{D}	18.91 ± 0.18^{C}	0.77 ± 0.00^{C}	1.36 ± 0.51

Values are given as means ± SE from five repeated determinations; A-G in each column represent statistically significant differences ($p < 0.05$) determined by the Tukey test.

The intensity of color changes that occur when products are stored at a cooling temperature and are influenced by air currents in the storage area is illustrated in Table 5. A notable reduction ($p < 0.05$) in the luminosity of L* was observed across all eight experimental groups throughout the five-day storage period. Conversely, the intensity of the red color increased significantly ($p < 0.05$) in the value of a*, with particular emphasis on the frankfurter samples with turkey meat. The value of b* exhibited a rising trend for the majority of samples until the third day of

storage, after which it declined for the following two days.
On the contrary, it was observed that the poultry frankfurter sample containing the greatest proportion of bone broth exhibited the least decrease in luminosity L* and the most minimal alteration in the intensity of the red (a*).
One of the variables that may influence the L*, a*, and b* color parameters of frankfurter samples stored in refrigeration currents is the oxidation of fats in the sausages' composition. This occurs when the fats react with oxygen in the air, resulting in the production of peroxides. The degradation of natural pigments in meat, such as myoglobin, by these peroxides can diminish its luminosity. Additionally, a reduction in luminosity may result from the release of moisture throughout the storage process. Because water reflects light, a reduced quantity of water will result in diminished light reflection. As a consequence, the samples containing the greatest quantities of bone broth exhibited a diminished degree of color change intensity.

Table 5. Color changes in five days storage at refrigerated temperature

Parameters	**Sample**	**Storage Period (Days)**				
		1	2	3	4	5
L*(D65)	CC	66.57 ±0.29 ^{ab,A}	61.08 ±0.37 ^{b,B}	53.96 ±0.70 ^{b,C}	47.38 ±0.96 ^{cd,D}	44.95 ±0.48 ^{c,E}
	C3%	65.94 ±0.27 ^{b,A}	57.85 ±0.87 ^{cd,B}	52.13 ±0.93 ^{c,C}	45.82 ±0.40 ^{de,D}	44.00 ±0.16 ^{cd,DE}
	C6%	66.84 ±0.30 ^{a,A}	62.45 ±0.61 ^{b,B}	57.52 ±0.61 ^{a,C}	50.31 ±1.42 ^{b,D}	47.24 ±0.59 ^{b,E}
	C9%	66.81 ±0.32 ^{a,A}	64.24 ±1.38 ^{a,B}	56.76 ±0.87 ^{a,C}	53.25 ±0.65 ^{a,D}	51.80 ±0.58 ^{a,DE}
	CT	61.37 ± 0.41 ^{d,A}	58.55 ± 0.36 ^{c,B}	51.67 ±0.96 ^{c,C}	46.13 ±0.55 ^{de,D}	44.04 ±0.63 ^{cd,E}
	T3%	61.53 ±0.33 ^{d,A}	54.78 ±0.71 ^{e,B}	49.85 ±1.00 ^{d,C}	44.74 ±1.55 ^{e,DE}	43.12 ±0.65 ^{d,E}
	T6%	61.92 ±0.10 ^{cd,A}	57.23 ±0.48 ^{cd,B}	52.26 ±0.30 ^{c,C}	46.96 ±0.54 ^{cd,D}	44.87 ±0.92 ^{c,E}
	T9%	62.38 ±0.40 ^{c,A}	56.64 ±0.76 ^{d,B}	52.30 ±0.76 ^{c,C}	48.86 ±1.38 ^{bc,D}	44.05 ±0.82 ^{cd,E}
a*(D65)	CC	11.72 ±0.24 ^{cd,D}	13.58 ±0.28 ^{d,C}	17.56 ±0.32^{abc,AB}	16.96 ±0.55 ^{c,B}	17.76 ±0.20 ^{c,A}
	C3%	11.79 ±0.20 ^{c,D}	14.85 ±0.53 ^{c,C}	17.15 ±0.54 ^{bc,B}	17.51 ±0.26 ^{bc,B}	19.63 ±0.26 ^{b,A}
	C6%	11.39 ±0.13 ^{d,E}	12.59 ±0.40 ^{e,D}	13.73 ±0.37 ^{e,C}	15.78 ±0.71 ^{d,B}	18.41 ±0.65 ^{c,A}
	C9%	11.43 ±0.24 ^{cd,C}	11.97 ±0.48 ^{e,C}	14.69 ±0.58 ^{d,B}	14.89 ±0.43 ^{d,B}	16.54 ±0.36 ^{d,A}
	CT	14.65 ± 0.15 ^{a,D}	15.13 ±0.26 ^{c,D}	18.01 ±0.35 ^{ab,C}	19.02 ±0.24 ^{a,B}	20.47 ±0.34 ^{a,A}
	T3%	14.78 ±0.10 ^{a,D}	17.15 ±0.27 ^{a,C}	18.42 ±0.37 ^{a,BC}	19.31 ±0.44 ^{a,B}	20.78 ±0.26 ^{a,A}
	T6%	14.21 ±0.18 ^{b,E}	15.51 ±0.36 ^{c,D}	17.05 ±0.23 ^{c,C}	18.63 ±0.29 ^{a,B}	20.44 ±0.50 ^{a,A}
	T9%	13.99 ±0.17 ^{b,E}	16.34 ±0.49 ^{b,CD}	17.38 ±0.50 ^{bc,BC}	18.42 ±0.70 ^{ab,B}	20.11 ±0.19 ^{ab,A}
b*(D65)	CC	18.23 ± 0.41 ^{a,D}	20.35 ±0.43 ^{a,C}	24.19 ±0.63 ^{a,A}	21.39 ±0.38 ^{a,BC}	20.74 ±0.38 ^{a,C}
	C3%	17.08 ±0.25 ^{c,C}	20.01 ±0.68 ^{ab,B}	21.20 ±0.58 ^{b,A}	20.73 ±0.33 ^{c,AB}	20.19 ±0.55^{ab,B}
	C6%	17.08 ±0.16 ^{c,C}	19.40 ±0.19 ^{bc,B}	20.41 ±0.22 ^{b,A}	19.47 ±0.47 ^{b,B}	19.21 ±0.55 ^{ab,B}
	C9%	17.72 ±0.29 ^{b,D}	18.74 ±0.50 ^{c,C}	20.74 ±0.43 ^{b,A}	19.25 ±0.20 ^{bc,BC}	19.86 ±0.37 ^{b,B}
	CT	13.40 ± 0.22 ^{de,BC}	14.28 ±0.36 ^{e,B}	15.20 ±0.15 ^{c,A}	14.26 ±0.17 ^{de,B}	14.22 ±0.23 ^{d,B}
	T3%	13.47 ±0.19 ^{de,C}	14.93 ±0.12 ^{e,AB}	15.33 ±0.29 ^{c,A}	13.99 ±0.42 ^{e,BC}	14.40 ±0.35 ^{d,B}
	T6%	13.18 ±0.10 ^{e,C}	14.88 ±0.10 ^{e,B}	15.54 ±0.24 ^{c,A}	14.29 ±0.19 ^{de,B}	15.53 ±0.39 ^{c,A}
	T9%	13.69 ±0.17 ^{d,C}	15.77 ±0.27 ^{d,A}	15.94 ±0.30 ^{c,A}	14.96 ±0.46 ^{d,B}	14.28 ± 0.23 ^{d,BC}

Values are given as means ± SE from five repeated determinations; means with different superscripts in a row orientation (uppercase alphabet) indicate significant ($p < 0.05$) differences between different storage days at the same bone broth addition level; means with different superscripts in a column orientation (lowercase alphabet) indicate significant ($p < 0.05$) differences between different bone broth addition levels at the same storage day.

Consumer behavior is influenced by psychological and environmental factors, including economic, social, cultural, and consumption context criteria. Neuroscience explains consumer satisfaction, leading to the concept of loyalty, which is defined into behavioral and attitudinal dimensions. The behavioral dimension refers to the frequency of repeated purchases, while the attitudinal dimension considers psychological commitment and beliefs about a product. These aspects influence consumers' decisions based on product characteristics and sensory quality (Ciobanu et al., 2023).
In this study, the sensory quality of the control samples and treatments C3%, C6%, C9%, T3%, T6% and T9% were evaluated after the production and cooling at the refrigeration temperature of 4°C. The data (Table 6) in the current study shows that adding beef bone

broth to chicken and turkey frankfurters did not cause significant changes in sensory perception ($p > 0.05$) of consumers, except for color and juiciness attributes, where the statistical differences were significant between all groups. Although for the attributes of flavor and texture, as well as for the general acceptability of the assortments, the differences noticed in the sensory perception of consumers were not significant, it was found that the addition of beef bone broth caused a sensory improvement. Thus, the sensory evaluation results described increasing average results correlated with the percentage of bone broth introduced for flavor, texture, juiciness attributes and overall acceptability, while sensory perception of color decreased with increasing the level of added broth.

Table 6. Sensory qualities of frankfurters sausages added with different concentrations of bone broth

Attribute	Color	Flavor	Texture	Juiciness	Overall acceptance
CC	6.92 ± 0.81^{AB}	6.28 ± 0.68	6.24 ± 0.88	5.88 ± 0.83^{B}	6.44 ± 0.51
C3%	6.68 ± 0.69^{AB}	6.36 ± 0.70	6.68 ± 0.75	6.40 ± 0.93^{AB}	6.64 ± 0.64
C6%	6.83 ± 0.83^{AB}	6.32 ± 0.80	6.88 ± 0.78	6.50 ± 1.08^{AB}	6.72 ± 0.54
C9%	6.58 ± 0.87^{B}	6.40 ± 0.76	6.72 ± 0.79	6.96 ± 1.00^{A}	6.52 ± 0.51
CT	7.44 ± 1.17^{A}	6.68 ± 0.63	6.60 ± 0.91	6.44 ± 0.77^{b}	6.88 ± 0.67
T3%	7.32 ± 0.84^{AB}	6.64 ± 0.81	6.68 ± 0.90	6.48 ± 1.08^{AB}	6.84 ± 0.47
T6%	7.04 ± 0.93^{AB}	6.44 ± 0.82	6.88 ± 1.01	6.80 ± 1.04^{A}	6.72 ± 0.54
T9%	7.04 ± 0.83^{AB}	6.56 ± 0.82	6.96 ± 0.93	7.00 ± 1.00^{A}	6.76 ± 0.44
p-value	0.007	0.579	0.076	0.001	0.088

Values are given as means ± SE; p-value expresses the statistically differences between groups determined by the ANOVA test; A-B represent statistically significant differences ($p < 0.05$) determined by the Tukey test.

CONCLUSIONS

This study explored the feasibility of incorporating beef bone broth into chicken and turkey frankfurter sausages. The findings demonstrate that bone broth addition significantly increased moisture content and protein content at the highest concentration of addition, compared to control sausages. Conversely, a decrease in fat content (up to 0.86% lower in chicken samples and 0.64% lower in turkey samples) was observed with increasing bone broth. Sensory evaluation revealed no significant changes in flavor, texture, or overall consumer acceptability. However, some panelists perceived a sensory improvement with broth addition regarding color and juiciness, attributes that were significantly affected by bone broth. Sensory evaluators reported a decrease in perceived color and an increase in juiciness. Instrumental color analysis showed minimal impacts of bone broth on lightness and redness, suggesting the broth's color did not significantly influence overall sausage color. Additionally, turkey sausages inherently had higher redness and lower yellowness compared to chicken sausages.

In conclusion, this study demonstrates the potential of using beef bone broth in frankfurters as a means to increase protein and collagen content and juiciness while maintaining consumer acceptance for flavor and texture. However, a slight decrease in perceived color might be observed. Further research could explore optimizing the formulation and processing techniques to address potential color changes associated with bone broth addition while maintaining the observed benefits in protein content and juiciness.

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QUALITATIVE DIFFERENCES CAUSED BY THE ADDITION OF LIQUID SMOKE IN MEAT PRODUCTS WITH DIFFERENT STRUCTURES COMPARED TO TRADITIONAL SMOKING

Mihai Cătălin CIOBOTARU, Diana-Remina MANOLIU, Mădălina MATEI, Răzvan Andrei CALCAN, Paul Corneliu BOIȘTEANU, Marius Mihai CIOBANU

"Ion Ionescu de la Brad" Iasi University of Life Sciences, 3 Mihail Sadoveanu Alley, 700490, Iasi, Romania

Corresponding author email: marius.ciobanu@iuls.ro

Abstract

The aim of this study was to compare the qualitative and sensory disparities between conventionally smoked meat products and those treated with liquid smoke. Conducted at the University of Life Sciences' meat micro-production workshop (IULS), the experiment yielded three batches of meat samples: compact, heterogeneous, and emulsion. While batches 2 and 3 received treatments of 0.1% and 0.2% liquid smoke, respectively, across all three product categories, the control batch was subjected to conventional smoking. Twenty semi-trained evaluators conducted both sensory and physicochemical analyses. Liquid smoke significantly altered the texture, flavour, and appearance of emulsion-type goods, favouring samples that had been traditionally smoked. The variations were less pronounced in the products with heterogeneous structures, where there was a slight preference for the control batch and a considerable appreciation for the 0.2% liquid smoke batch.

***Key words**: liquid smoke, meat products, sensory preferences, smoking methods.*

INTRODUCTION

The food industry is extensively researching and developing new products to meet the growing consumer demand for healthier options. Through reformulating food preparations, the aim is to enhance the physiological activity of natural nutrients or incorporate bioactive components to satisfy this continually expanding demand from consumers (Anchidin et al., 2023).

For decades, traditional smoking has been employed to preserve food, particularly meat. Smoke derived from wood burning not only aids in maintaining food quality through its antioxidant and antibacterial properties but also imparts appealing sensory characteristics to smoked preparations (Lingbeck et al., 2014).

The antimicrobial and antioxidant properties of smoke have been extensively researched by scientists from various countries over the years (Gucianu et al., 2023). Formaldehyde and phenols are emitted when wood is burned, imparting preservative characteristics to the resulting smoke. These chemical compounds hinder the growth of various microorganisms and limit oxidative reactions during the smoking process (Abou-Taleb et al., 2011).

Utilizing liquid smoke presents a quicker alternative to traditional smoking practices, offering environmental benefits and preserving the sensory attributes characteristic of traditional smoke, while simultaneously reducing the presence of potentially harmful compounds. Employing liquid smoke is a safer alternative to traditional smoking techniques due to the ability to eliminate residue molecules of polycyclic aromatic hydrocarbons (PAH) through re-distillation purification (Saloko et al., 2014).

Furthermore, the accumulation of tar residues and harmful air pollutant (HAP) chemicals, such as benzopyrene, in products can adversely affect consumer health. Thus, the use of the liquid smoke technique allows for a more convenient application of smoke flavor in food, as it can be simply immersed or introduced into food (Indiarto et al., 2020).

The purpose of this paper is to underscore the qualitative differences in meat preparations with various structures (compact, heterogeneous, and emulsified) processed by the addition of liquid smoke compared to the conventional smoking method. The research objectives are focused on analyzing the sensory and chemical parameters of the obtained batches.

MATERIALS AND METHODS

To achieve the set objectives, three distinct categories of meat products were developed, each with a specific structure: emulsified sausages, sausages with a heterogeneous structure, and smoked loin, with a compact structure.
The experimental materials were purchased from a local hypermarket and subsequently processed in the meat micro-production workshop of the "Ion Ionescu de la Brad" Iași University of Life Sciences, undergoing selection and processing operations resulting in pork products.
For each type of preparation, three different batches were formulated: one control batch smoked by the traditional method (L1), and two batches processed using liquid smoke flavoring (L2 and L3). The liquid smoke was purchased from ROCAS FDS SRL and composed of natural smoke extract and acetic acid. Concentrations of 0.1% and 0.2% were used for this study, as recommended by the manufacturer.
The formulation of control and experimental batches was based on a technological flow, utilizing raw materials, spices, and flavors as listed in Table 1. For preparations with a compact structure, after selection and preparation, the pork was brined with a 10% salt solution and liquid smoke, then immersed in the brine for 24 hours.
The technological process for obtaining preparations with a heterogeneous structure (sausages) followed the stages outlined in the study by Ciobanu et al. (2023), with optimal adjustments for the desired preparation. For preparations with an emulsified structure, the process followed the method outlined in the study by Manoliu et al. (2023), with specific modifications for this type of product. Initially, raw meat was coarse-ground using a WP-105 grinder with a 3 mm diameter sieve, then finely ground using a meat grinder (Cutter Titane V 45L) commonly used in the food industry for grinding cold or frozen meat. The mixture was homogenized until a fine and homogeneous meat paste was obtained. In the case of minced meat preparations, the liquid smoke was integrated into the paste during mixing.

Table 1. Formulation of experimental batches

	Ingredients	UM	Batch 1	Batch 2	Batch 3
Sausages	Pork meat	kg	2,4		
	Garlic	g/kg	15		
	Pepper		5		
	Coriander		3		
	Emulsifier		2		
	Liquid smoke		-	1	2
Emulsified sausages	Pork meat	kg	2,4		
	Garlic	g/kg	2		
	Pepper		2		
	Coriander		0,5		
	Emulsifier		4		
	Liquid smoke		-	1	2
Smoked loin	Pork loin	kg	2		
	Salt (in brine)	%	10		
	Liquid smoke	g/kg	-	1	2

UM - unit of measurement

The thermal treatment was adapted according to each type of preparation and each batch separately, so batch 1 represents the control batch for the resulting preparations, batch 2 and 3 are represented by the batches in which flavours of liquid smoke were used. Table 2 shows the times and temperatures used for preparation.

Table 2. Heat treatment applied to obtain formulations with different structures

Formulation	Batch	Air drying		Smoking		Hot air cooking	
		Time	*°C*	*Time*	*°C*	*Time*	*°C*
Sausages	SHL1	20	45	30	50	30	76
	SHL2			-	-		
	SHL3						
Emulsified sausages	SEL1	30	60	20	70	60	78
	SEL2			-	-		
	SEL3						
Smoked loin	SCL1	30	65	30	72	50	86
	SCL2			-	-		
	SCL3						

SHL1 - heterogeneous structure - control batch, SEL2 - emulsified structure - batch 0.1% liquid smoke, SCL3 - compact structure - batch 0.2% liquid smoke.

After obtaining the experimental batches, they underwent chemical and sensory analyses. The determination of the chemical composition of the experimental batches involved measuring the main components, including moisture, protein, collagen, fats, and salt. These determinations were conducted using the Food-Check automatic meat analyzer, which utilizes near-infrared (NIR) spectroscopy, referring to the region of the electromagnetic spectrum located near the infrared spectrum, with wavelengths between approximately 700 and 2500 nm.

The sensory analysis was conducted in the University's sensory analysis laboratory with a group of 20 semi-trained students, aged 20-23 years, without health problems, and with a diet that frequently includes meat products. At the beginning of the session, the evaluators were trained on the contents of the questionnaire and the terms used, to familiarize them with the descriptive terms. The samples were sliced using a professional slicer to ensure sample uniformity and presented to the evaluators in random batch order, coded with 3 randomly chosen digits to maintain sample anonymity.

For the sensory analysis, descriptive tests were conducted to asses the flavor and texture profiles, along with affective tests (hedonic test). The flavor profile followed the ISO 6564:1985 standard, considering sensory characteristics of smell (pork aroma, fat aroma, spice aroma, and smoke aroma) and taste (mouthfeel and aftertaste). For the texture profile, characteristics such as hardness, elasticity, juiciness, and masticability perception were analyzed, following the ISO 11036:2020 standard. The hedonic test employed a 9-point scale, following the method outlined by Manoliu et al. (2023).

Data interpretation utilized the Principal Component Analysis (PCA) method, a statistical technique used to reduce the number of dependent variables in a dataset by identifying underlying variables, known as factors, through analysis of correlation patterns between the original variables (Lawless, 2010).

The results obtained from the chemical and sensory determinations of the evaluated batches were compared using analysis of variance (ANOVA), followed by the Tukey test at a significance level of 5% ($p < 0.05$) using XLStat V.24.

RESULTS AND DISCUSSIONS

The data obtained for the three types of preparations with different structures are presented in Table 3. No significant differences were identified between batches of preparations with a heterogeneous structure (sausages) in terms of moisture, lipid, protein, and collagen content ($p > 0.05$). However, a significant difference in salt content ($p < 0.05$) was observed between batches in this product category. The salt content of the control sample (SHL1) was significantly higher than that of the SHL2 and SHL3 samples. Thus, the introduction of liquid smoke at different concentrations appears to have a significant effect on the salt content in pork sausages.

Regarding emulsified preparations, there were no significant differences between batches in terms of moisture, lipid, protein, collagen and salt content ($p > 0.05$).

Table 3. Chemical analysis results

Category	Moisture (%)	Lipid (%)	Protein (%)	Collagen (%)	Salt (%)
SHL1	$62.62^a \pm 1.68$	$15.14^a \pm 3.25$	$18.70^a \pm 0.04$	$16.80^a \pm 0.04$	$2.58^a \pm 0.16$
SHL2	$61.96^a \pm 0.36$	$17.20^a \pm 0.45$	$19.66^a \pm 1.77$	$18.12^a \pm 2.08$	$2.14^b \pm 0.09$
SHL3	$63.24^a \pm 1.14$	$14.60^a \pm 2.79$	$19.86^a \pm 1.59$	$18.24^a \pm 1.97$	$2.30^b \pm 0.12$
Pr > F(sig.)	***0.273***	***0.257***	***0.390***	***0.345***	***0.001***
SEL1	$61.96^a \pm 0.53$	$17.48^a \pm 0.18$	$18.28^a \pm 0.27$	$16.40^a \pm 0.28$	$2.28^a \pm 0.24$
SEL2	$62.70^a \pm 0.04$	$16.30^a \pm 0.09$	$18.60^a \pm 0.07$	$16.60^a \pm 0.04$	$2.40^a \pm 0.09$
SEL3	$62.80^a \pm 0.96$	$15.64^a \pm 2.30$	$19.28^a \pm 1.30$	$17.52^a \pm 1.61$	$2.28^a \pm 0.11$
Pr > F(sig.)	***0.112***	***0.128***	***0.15***	***0.178***	***0.382***
SCL1	$62.92^a \pm 0.81$	$14.46^a \pm 2.68$	$20.16^a \pm 1.98$	$16.86^b \pm 0.39$	$2.06^b \pm 0.09$
SCL2	$63.32^a \pm 1.54$	$14.00^a \pm 3.29$	$20.25^a \pm 1.84$	$18.80^{ab} \pm 2.20$	$2.42^a \pm 0.18$
SCL3	$64.10^a \pm 0.35$	$11.60^a \pm 0.52$	$21.92^a \pm 0.18$	$19.80^a \pm 1.51$	$2.38^a \pm 0.22$
Pr > F(sig.)	***0.221***	***0.187***	***0.242***	***0.032***	***0.011***

Values are expressed as means ± SE from triplicate determinations; letters a-b in each column represent statistically significant differences ($p < 0.05$) determined by Tukey's test. SH - heterogeneous structure, SE - emulsified structure, SC - compact structure.

The moisture, lipid, and protein content did not show significant differences between batches of preparations with a compact structure, SCL1, SCL2, and SCL3 ($p > 0.05$). However, there was a significant difference in collagen content between batches. The values for SCL1 were significantly lower than those of SCL2 and SCL3, and the value for SCL2 was significantly lower than that of SCL3. This could be related to the conventional smoking procedures applied. Khalid W. et al. (2023) confirm in their study that major meat proteins are denaturated by heat; denaturation of actin and myosin has been connected to harder meat; denaturation of collagen has been linked to a loss in firmness. Additionally, there was a significant difference in salt content ($p < 0.05$) between the control batch (SCL1) and the experimental batches (SCL2 and SCL3). Thus, the SCL2 and SCL3 samples appeared to have higher collagen and salt content compared to the control sample, SCL1.

Following the sensory analysis of the three types of preparations with different structures, the results were grouped according to the tests applied: descriptive tests (flavor and texture profile) and acceptance test (hedonic test).

Flavor profile

For the experimental batches of meat products with a heterogeneous structure, the sensory attributes followed in the flavor profile were represented by olfactory characteristics: pork smell (MCP), fat smell (MG), smoky aroma (MA); taste features: salty taste (GS), pork taste (GCP), fat taste (GG), spice taste (GC), and smoke taste (GA), along with mouthfeel (the general sensation of the product in the oral cavity). The sensory attributes pursued were chosen according to the specifics of each category of preparations.

Principal Component Analysis (PCA) revealed the direction and intensity of descriptive sensory attributes for batches of preparations with a heterogeneous structure with different percentages of added liquid smoke and allowed comparison of sensory profiling data with the conventional smoked control batch. In the first dimension (F1, 55.62%) of the variation, the aroma of smoke, fat taste, and fat odor were associated. The second dimension (F2, 44.38%) was mainly associated with the aroma of spices, pork, smoke, meat, salt, and a pleasant mouthfeel.

Figure 1 illustrates a distinct variation between batches of preparations with a heterogeneous structure, although the boundary between SHL2 (947) and SHL3 (503) is less defined, with this variation attributed to the technological processes used.

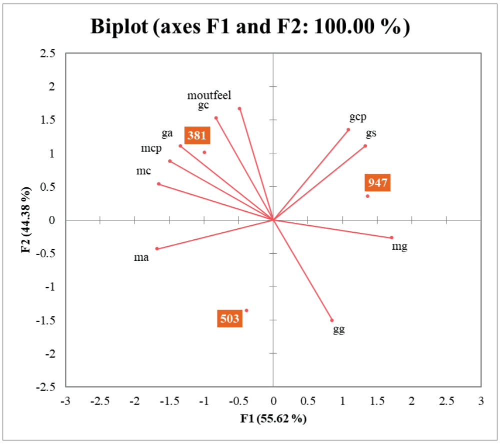

Figure 1. Principal Component Analysis (PCA) for consumer preference and sensory profile of 3 batches of heterogeneously structured products. Sample codes 381, 942 and 503 represent SHL1, SHL2 and SHL3 respectively

In the second dimension, the SHL3 batch (503), with 0.2% added liquid smoke, exhibited the most intense smoking aroma, while the SHL1 (381) and SHL2 (947) batches showed lower perception of this feature. Regarding taste, both the meat and salt were more pronounced in the batch with 0.1% liquid smoke (947).

For batches of products with an emulsified structure (Figure 2), the positioning of the batch with 0.1% liquid smoke (725) in the upper right quadrant of the biplot indicates high levels of olfactory characteristics (MCP, MG, MA) and moderate levels of taste characteristics (GS, GCP, GA). This suggests that 0.1% smoked emulsified sausages have a more intense taste and a moderate flavor.

Batch 492 (emulsified sausages with 0.2% liquid smoke) is located in the lower right quadrant of the biplot, indicating high levels of taste characteristics (GS, GCP, GA) and high levels of olfactory characteristics (MCP, MG, MA). This suggests that smoked emulsified sausages with 0.2% liquid smoke have an intense taste and flavor. Although batch 169 (conventional smoked emulsified sausages) is on the left side of the chart, it does not necessarily indicate negative characteristics. Its position is determined by its values on the F1 and F2 axes, reflecting a positive influence of olfactory, taste, and sensory characteristics.

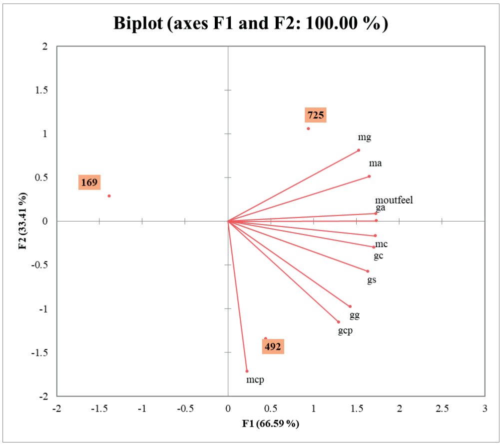

Figure 2. Principal Component Analysis (PCA) for consumer preference and sensory profile of 3 batches of emulsified structure products. Sample codes 169, 725 and 492 represent SEL1, SEL2 and SEL3, respectively

The experimental lots of cotlet were obtained by immersing the meat pieces in the brine with added smoke flavor. Conventional smoking involves directly exposing the product to natural smoke from wood burning. This process imparts a more complex and intense flavor characterized by notes of smoke, wood, and meat, which explains the presence of the control batch in the upper quadrant (Figure 3), where the smoky taste is predominant. Yin et al. (2021), in a study on the influence of industrial smoking on the aromatic profile of certain meat products, obtained similar results in sensory analysis regarding the smoky taste characteristic.

Figure 3 illustrates a distinct variation between batches of compact-structured preparations. The use of conventional smoking, as exemplified by the control batch (614), seems to result in a distinctive sensory profile characterized by a higher intensity of smoky taste.

In contrast, the application of the smoke flavor, represented by samples 270 and 836, seems to significantly influence the overall taste and sensation, with less impact on the latter batch. The subtle differences between samples with smoke flavor at different concentrations suggest the precise importance of the percentage of liquid smoke used, with sample 836 showing a better defined balance between taste and sensation, while sample 250 highlights a more temperate taste and a less sense of pleasure.

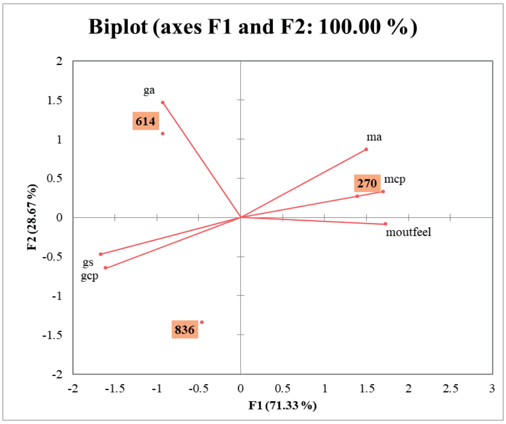

Figure 3. Principal Component Analysis (PCA) for consumer preference and sensory profile of 3 batches of compact structure products (pork loin). Sample codes 614, 270 and 83 represent SCL1, SCL2 and SCL3, respectively

Texture profile

The texture profile analysis encompassed the sensory characteristics of hardness, elasticity, juiciness, adhesiveness, and chewiness of the analyzed lots. The F1 axis primarily correlates with hardness and chewiness characteristics. Samples with higher values on the F1 axis are harder and more challenging to chew, whereas those with lower values are softer and easier to chew.

The F2 axis primarily relates to elasticity and succulence characteristics. Samples with higher values on the F2 axis are more elastic and juicy, while those with lower values are less elastic and drier.

The control batch of conventionally smoked sausages, SHL1 (503), is positioned at the top of the biplot (Figure 4), with higher values on the F1 axis and lower values on the F2 axis. This indicates that these sausages were perceived as harder, tougher to chew, and drier. For batches in which liquid smoke was utilized, specifically sausages with a concentration of 0.1% liquid smoke, SHL2 (381), positioning is observed in the center-left part of the biplot, with moderate values on both axes. This suggests that these sausages exhibit average hardness and chewiness, moderate elasticity, and moderate juiciness. Sausages formulated with a concentration of 0.2% liquid smoke, SHL3

(947), are situated on the lower left side of the biplot, with smaller values on both the F1 and F2 axes. This indicates that these sausages are soft, easy to chew, less elastic, and less juicy.

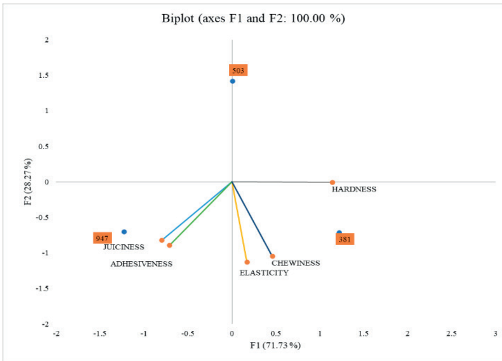

Figure 4. Principal Component Analysis (PCA) for consumer preference and sensory profile of 3 batches of heterogeneously structured products. Sample codes 503, 381 and 947 represent SHL1, SHL2 and SHL3, respectively

In this regard, a negative correlation has been highlighted between the amount of liquid smoke and the firmness of the sausages. An increased concentration of liquid smoke leads to softer sausages with improved masticability. Additionally, there is a direct relationship between the amount of liquid smoke and the flexibility and succulence of sausages. Thus, increasing the concentration of liquid smoke results in obtaining a more elastic and juicy texture of the products. Similar results were obtained by Yusnaini et al. (2012), who investigated the effect of different levels of dilution of liquid smoke in meat preparations, reporting after sensory evaluation that an increase in the amount of liquid smoke increases the tenderness of the meat.

For the batches of the emulsified structure product category (Figure 5), the results highlight a significant negative correlation between the amount of liquid smoke and the hardness of the emulsified sausages. The higher the concentration of liquid smoke, the softer and easier to chew the emulsified sausage become. Additionally, a significant positive correlation is observed between the amount of liquid smoke and the elasticity and juiciness of the emulsified sausages. Higher concentrations of liquid smoke lead to emulsified sausages that are more elastic and juicy.

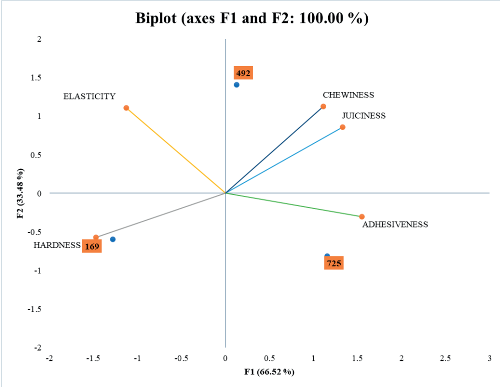

Figure 5. Principal Component Analysis (PCA) for consumer preference and sensory profile of 3 batches of products with emulsified structure. Sample codes 169, 492 and 725 represent SEL1, SEL2 and SEL3, respectively

The biplot indicates that conventionally smoked emulsified sausages (169) are situated in the lower left quadrant, denoted by modest values along both axes, F1 and F2. This validates the dry, tough, and difficult to mastic consistency of traditionally smoked emulsified sausages.

The biplot illustrates emulsified sausages containing 0.1% (492) liquid smoke, which are positioned in the center-right. The values along both axis are moderate. This position signifies an intermediate texture, as indicated by the mean values of succulence, firmness, chewiness, and elasticity.

The biplot illustrates emulsified sausages containing 0.2% (725) liquid smoke as the subject matter. The F1 axis represents small values, while the F2 axis represents large values. With a greater concentration of liquid smoke, this position verifies that smoked emulsified sausages have a tender, elastic, chewy, and squishy consistency. Probably as a result of the hydration of the emulsified sausages, liquid smokiness imparts a more appetizing texture to the sausages.

In regard to the samples falling under the compact structure product category (as illustrated in Figure 6), batch SCL1 (614) (conventional smoked cotlet), which is situated on the left-hand side of the chart, exhibited diminished levels of elasticity, succulence, and adhesiveness. In terms of firmness and chewiness, proximity to the F1 axis indicates that this sample is comparable to sample 836 (which contains 0.2% smoke flavor). Sample

270, which is a cotlet smoked with 0.1% liquid smoke, exhibits a greater degree of adhesion, succulence, and elasticity, as indicated by its position at the top of the chart.

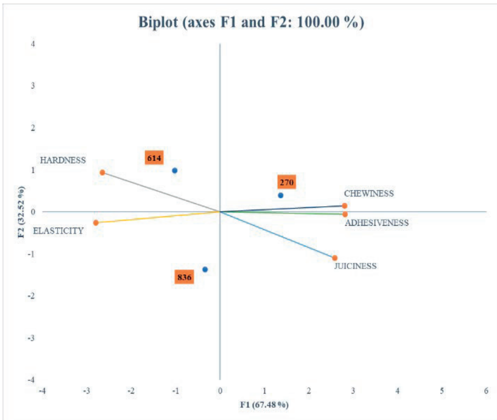

Figure 6. Principal Component Analysis (PCA) for consumer preference and sensory profile of 3 batches of products with compact structure. Sample codes 270, 614 and 836 represent SCL1, SCL2 and SCL3, respectively

In close proximity to the F2 axis, hardness and intense gnawing are less probable. Sample 836, which is a cotlet smoked with 0.2% liquid smoke, exhibits reduced elasticity, succulence, and adhesiveness, as evidenced by its position at the bottom of the graph. The PCA chart elucidates notable distinctions in texture between cotlets smoked conventionally (614) and those inhaled with liquid (270 and 836).

It appears that liquid smoke has a substantial effect on the succulence, adhesiveness, elasticity, and hardness of the smoked cotlet, but a lesser effect on its chewiness and hardness. The texture profile of the conventionally smoked cotlet (614) appears to be more preferable, as it is distinguished by increased succulence, superior adhesion, reduced hardness, and simplified chewing. The quantity of liquid smoke used did not exhibit a discernible correlation with the texture profile of the cotlet that had been smoked. The texture profile of the 0.1% liquid smoke sample (270) is comparable to that of the control sample (614), whereas the texture profile of the 0.2% liquid smoke sample (836) is less preferable.

Table 4 concentrates the data obtained from the hedonic test applied to the three types of meat preparations. The variance analysis (ANOVA) was used to determine whether there are statistically significant differences between the sausage samples (SHL1, SHL2 and SHL3) with regard to the variables analysed. The Tukey HSD test was used to individually compare the differences between smoked samples and to identify which samples differ statistically significantly from each other.

Table 4. Results of the acceptability test for samples with heterogeneous structure (hedonic test)

CATEGORY	APPEARANCE	COLOR	FLAVOR	TASTE	TEXTURE	GENERAL APPRECIATION
SHL1	6.35±1.60	6.95±1.54	7.05±1.57	7.40±1.35	6.75±1.65	8.00±0.97
SHL2	7.15±1.48	7.50±1.40	6.30±1.56	6.15±1.50	6.80±1.47	6.90±1.71
SHL3	7.10±1.48	7.10±1.48	6.45±1.54	7.45±1.47	6.80±1.40	7.45±1.05
Pr > F(sig.)	***0.007***	***0.479***	***0.098***	***0.008***	***0.993***	***0.033***

Values are expressed as means ± SE for a panel of 20 raters; $p > 0.05$ = non-significant differences, $p < 0.05$ = significant differences; $p < 0.01$ = distinctly significant differences; $p < 0.001$ = highly significant differences, determined by Tukey test. SH - heterogeneous structure.

The analysis of batch appearance revealed statistically significant differences between the control batches (SHL1) and the experimental batches (SHL3 and SHL2, $p < 0.05$), with the SHL1 batch obtaining a significantly lower score. Conversely, no statistically significant differences were observed between SHL2 and SHL3 lots ($p > 0.05$).

These results suggest a negative association between the use of traditional smoke and consumer perception of the visual appearance of smoked meat preparations. The SHL1 batch, which used traditional smoke, was perceived to be less visually attractive than the SHL2 and SHL3 batches, which contained liquid smoke (0.1% and 0.2%, respectively). However, it is important to point out that the score difference between the SHL1 and SHL2 lots is not practically significant.

Regarding the color of the batches, the results suggest that the use of liquid smoke may influence consumer perception of the color of smoked meat dishes. A low concentration of liquid smoke (0.1%) seems to be associated with a more intense and attractive color, while higher concentrations (0.2%) may have a lesser impact on the color.

The flavor analysis revealed insignificant variations ($p > 0.05$) in flavor, with the SHL2 sample receiving the lowest score. This suggests that the smell of the lot with liquid smoke is assessed as being less strong compared to those with traditional smoke. These results suggest a consumer preference for the more intense flavor of smoked meat preparations associated with traditional smoke.

Taste analysis revealed statistically significant differences between SHL1, SHL2, and SHL3 samples ($p < 0.05$). The SHL3 sample, which contained 0.2% liquid smoke, obtained a significantly higher score than the SHL1 (traditional smoke) and SHL2 (0.1% liquid smoke). The SHL1 sample obtained a significantly higher score than the SHL2. These results suggest a consumer preference for the stronger and richer taste of smoked meat preparations associated with traditional smoke. The SHL3 sample, with a higher concentration of liquid smoke, was also perceived to have a more intense taste.

The texture analysis did not identify statistically significant differences between the samples SHL1, SHL2, and SHL3 ($p > 0.05$). This result suggests that the texture of smoked meat preparations was not significantly influenced by the type of smoke used (traditional versus liquid). Similar reports in terms of general acceptability have also been made by Bhuyan et al. (2018).

The analysis of the results for appearance and color highlighted a significant preference of consumers for liquid smoke samples compared to the traditional smoke sample, while the attributes of flavor, taste, and overall appreciation were more appreciated in the traditionally smoked sample. These results suggest a dominant trend towards products containing liquid smoke, at a concentration of 0.2%. The harmonious combination of more intense color, stronger taste, and pleasant flavor of the SHL3 sample seems to have contributed to this preference.

Statistical analysis of sensory characteristics is found in Table 5, highlighting significant differences ($p < 0.05$) between the batches of products for appearance and taste attributes. The SEL1 batch, subjected to traditional smoking, obtained mean scores for appearance, significantly higher ($p < 0.05$) compared to the SEL2 and SEL3 batches treated with liquid smoke. No significant differences were observed between the batches in terms of color. These results suggest that the use of liquid smoke did not substantially influence the product's color perception.

The flavor analysis did not identify statistically significant differences ($p > 0.05$) between the batches of smoked meat products. This finding suggests that the use of liquid smoke did not significantly affect the olfactory perception of the product.

The taste analysis revealed statistically significant differences ($p < 0.001$) between lots of smoked meat products. The SEL1 batch containing traditional smoke obtained a significantly higher taste score compared to the SEL3 batches (0.2% liquid smoke) and SEL2 (0.1% liquid smoke). These results suggest a consumer preference for the stronger and richer taste of smoked meat preparations associated with traditional smoke. The SEL3 batch, with a higher concentration of liquid smoke, was perceived to have a more intense taste compared to the SEL2 batch.

The acceptability test showed no statistically significant variations between batches in terms of texture. This finding indicates that the use of liquid smoke did not exert a remarkable influence on the product's tactile perception.

The addition of liquid smoke had a significant impact on the sensory properties of emulsion-type preparations, mainly significantly influencing the appearance and taste of the products. These results suggest a significant preference of consumers for the classic smoked emulsion type preparations, appreciated for the more attractive appearance, stronger and richer taste. Although the liquid smoke significantly influenced the taste of the dishes, it failed to fully reproduce the complex and rich taste of traditional smoking.

Table 5. Results of the acceptability test for samples with emulsified structure (hedonic test)

CATEGORY	APPEARANCE	COLOR	FLAVOR	TASTE	TEXTURE	GENERAL APPRECIATION
SEL1	7.70±0.80	6.75±1.65	6.90±1.71	7.35±1.04	7.20±1.44	7.65±1.09
SEL2	6.25±1.07	7.50±1.40	6.45±1.54	6.00±1.45	6.75±1.65	6.90±1.71
SEL3	6.50±1.54	6.75±1.65	6.80±1.54	6.65±1.39	6.80±1.40	6.60±1.35
Pr > F(sig.)	***0.001***	***0.227***	***0.095***	***0.007***	***0.585***	***0.061***

Values are expressed as means ± SE for a panel of 20 raters; $p > 0.05$ = non-significant differences, $p < 0.05$ = significant differences; $p < 0.01$ = distinctly significant differences; $p < 0.001$ = highly significant differences, determined by Tukey test. SE - emulsified structure

The results of the statistical analysis of the acceptability test for compact-structured samples (Table 6) highlighted significant differences ($p < 0.05$) between batches in terms of taste, whereas for the attributes appearance, color, flavor, texture and overall appreciation no significant difference ($p > 0.05$) was identified.

Table 6. Results of the acceptability test for samples with compact structure (hedonic test)

CATEGORY	APPEARANCE	COLOR	FLAVOR	TASTE	TEXTURE	GENERAL APPRECIATION
SCL1	6.85±1.66	6.85±1.66	7.00±1.56	7.45±1.47	6.80±1.40	6.95±1.47
SCL2	6.35±1.60	7.50±1.40	6.45±1.54	6.15±1.50	6.75±1.65	6.90±1.71
SCL3	6.15±1.50	6.75±1.65	6.70±1.71	6.35±1.60	6.80±1.47	7.25±1.25
Pr > F(sig.)	***0.363***	***0.271***	***0.181***	***0.019***	***0.993***	***0.725***

Values are expressed as means ± SE for a panel of 20 raters; $p > 0.05$ = non-significant differences, $p < 0.05$ = significant differences; $p < 0.01$ = distinctly significant *differences*; $p < 0.001$ = highly significant differences, determined by Tukey test. SC - compact structure

The absence of significant differences in the perceptions of these sensory attributes suggests that the evaluation of these characteristics was not significantly influenced by the variables tested in this study.

The results indicated differences in evaluator preferences depending on the product structure. However, a significant preference was observed for the witness sample (SCL1), which did not contain liquid smoke, indicating an appreciation for the natural taste of the product. Liquid-smoked batches (SCL1 and SCL2) achieved moderate acceptability, suggesting a tolerance to the specific taste of liquid smoke.

CONCLUSIONS

The addition of liquid smoke caused significant differences in the qualitative and sensory characteristics of meat preparations, depending on their structural type.

In particular, emulsified preparations showed variations in perception of appearance, taste, and overall appreciation due to the use of liquid smoke.

Between batches, there was a distinct variation in the amount of collagen present; SCL1 values were much lower than SCL2 and SCL3 values. This might be explained by the use of conventionally smoking techniques.

For preparations with a heterogeneous structure, differences in sensory characteristics were less pronounced. There was a moderate preference for the control sample treated with traditional smoke, but also a significant appreciation for the batch treated with 0.2% liquid smoke.

Regarding compact-structured preparations, differences in sensory characteristics were less evident. There was a significant preference for the control sample, but also moderate acceptability for batches treated with liquid smoke.

In general, liquid smoke emerges as a viable alternative to traditional smoking for meat preparations with both heterogeneous and emulsified structures. It offers quality and sensory characteristics appreciated by consumers, such as an authentic texture and taste, suggesting an increased adaptability of this technique in the food industry.

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THE IMPACT OF BONE BROTH ADDITION ON THE SENSORY ACCEPTABILITY OF ASSORTED MEAT PRODUCTS WITH HETEROGENEOUS STRUCTURE

Mihai Cătălin CIOBOTARU, Diana-Remina MANOLIU, Mădălina MATEI, Beatrice Angela GHEORGHE, Paul Corneliu BOIȘTEANU, Marius Mihai CIOBANU

"Ion Ionescu de la Brad" Iasi University of Life Sciences, 3 Mihail Sadoveanu Alley, 700490, Iasi, Romania

Corresponding author email: marius.ciobanu@gmail.com

Abstract

A significant challenge in today's food industry is managing leftover bone waste, which is often disposed of as household or abattoir waste if it is not economically used. This study aims to integrate beef bone broth into a functional product with a diverse meat structure. The study seeks to explore sensory changes induced by cattle bone broth properties on product quality and assess consumer acceptance. Two sets of sausages were made, one from pork shoulder and the other from pork loin, each with four groups: a control sample and three variations with 3%, 6%, and 9% cattle bone broth. Sensory analysis involved 80 untrained evaluators. Acceptability tests showed a strong preference for batches with bone broth, with over 60% positive feedback. The batches with a high percentage of acceptability by evaluators are represented by batches SAU2, SAU4, and SAU7. Among the sensory attributes associated with these batches were juiciness, tenderness, overall aroma, and bone broth aroma. Adding bone broth significantly enhanced pork sausages' sensory attributes and appeal, offering a sustainable approach to waste utilization and consumer satisfaction.

Key words*: broth bone, functional product, sensory acceptability.*

INTRODUCTION

Meat consumption is influenced by living standards, diet, livestock production, and consumer prices, as well as by macroeconomic factors such as uncertainty and GDP (gross domestic product) shocks. Compared to other goods, meat is associated with high production costs and high prices. Meat demand is higher among those with higher incomes and is influenced by urbanization, which brings changes in food consumption, favouring an increase in protein intake of animal origin. While the global meat industry provides food and livelihoods for billions of people, it also has a significant impact on the environment and global health (OECD & FAO, 2023).

Slaughterhouses and meat processing plants are essential sectors in the food industry, which is continuously adapting to meet the demand of a growing population with diverse dietary needs (Ungureanu et al., 2023).

While health considerations are important, consumers also appreciate the sensory experience when consuming meat products. Food producers and manufacturers must find a balance between adhering to health recommendations, such as reducing saturated fats and sodium content, and achieving desired sensory characteristics. Furthermore, consumer demands are evolving, and there is increasing interest in meat alternatives or plant products. To meet these requirements, innovation in product and technology development is required (Rodrigues et al., 2023).

In modern times, as consumer preferences and nutritional requirements for food have evolved, meat recipes are increasingly focused on reducing fat and salt levels. Pre-sacrifice factors, including species, age, type of feed, slaughter conditions, and post-saffering parameters, such as the rate of occurrence of rigor mortis and the technique of bleeding, influence the final quality of the meat. Moreover, anatomical regions with varying qualities influence the physico-chemical quality of the final products and how this, in turn, affects consumer preferences (Manoliu et al., 2023).

In the modern food industry, a significant problem is the management of residual bone waste, which, in the absence of economic use, is often treated as household or slaughterhouse waste and disposed of in landfills. This practice not only creates difficulties in waste management, but can also have negative consequences for the environment and public health. Globally, animal bone waste is considerable, with an estimated annual output of over 130 million tons (Hart et al., 2022).

The development of effective strategies for the recovery of the remaining bones from the process of decoction of meat is essential for transforming them into value-added products by applying efficient strategies to ensure the sustainability of the meat industry. Promising approaches to bone recovery include producing concentrated bone soup, an alternative food product that offers remarkable nutritional benefits (Toldrá et al., 2021). Bone cells, which make up bone tissue, remain active throughout the life of an animal and produce the bone matrix. This matrix mainly contains various mineral salts, such as sodium, potassium, phosphorus, calcium, and magnesium, along with collagen, protein groups (such as proteoglycanes, glycoproteins, sialoproteines, etc.) and acid muco-polysaccharides (Aykın-Dinçer et al., 2021).

Generally speaking, distinctive sensory qualities have a significant impact on consumer perception. Customers' perceptions of the sensory attributes of meat vary depending on how familiar they are with the meat and how open they are to try new things (Ciobanu et al, 2023.

The aim of this study is to investigate the impact of bone soup additivation (in proportions of 3%, 6%, and 9% of the amount of meat used) on the sensory characteristics of a variety of heterogeneous meat preparations and to analyze the acceptability of products by consumers. By adding bone soup, we aim not only to improve the taste and texture of the products but also to harness food resources in a sustainable way, helping to reduce waste and increase the nutritional value of these preparations.

Given the wide variety of meat products available on the market, understanding and improving their sensory attributes is crucial for both producers and consumers.

MATERIALS AND METHODS

Obtaining experimental material

The research was carried out in the meat micro-production plant of the University of Life Sciences, where the technological process of obtaining sausages was performed. The raw and auxiliary materials were purchased from local producers, and the raw meat was subjected to selection and processing operations.

The beef-bone soup was purchased from the manufacturer ZWUP in a 1700 ml jar and stored at a cooling temperature. In order to obtain the assortments, an experimental protocol was prepared, resulting in 8 lots of products: 4 lots containing pork meat from pulp and 4 lots with pork from the back of pork (Table 1).

The process of making sausages involved specific steps, such as:

- preparation of the raw material for dry salting for a period of 24 h at a temperature of 2-4°C in the ripening room;
- after the salting period, grind the meat with a grinder (GRINDER WP - 105) with the size of the eyelids at a sieve of 6 mm,
- weighing and preparation of auxiliary materials and beef bone soup;- mixing the ingredients (cut meat and beef bone soup) until the composition is bound and homogeneous;
- the preparation of natural membranes and their filling with composition; the formation of sausages and their twisting and preparation for thermal treatment.

Table 1. Formulation of sausages added with bone broth in different percentages

	U. M.	Experimental batch of hind leg meat sausages				Experimental batch of pork shoulder sausages			
		SAU1	SAU2	SAU3	SAU4	SAU5	SAU6	SAU7	SAU8
Hind leg meat	kg	3	3	3	3				
Pork shoulder						3	3	3	3
Beef bone broth		0	0.09	0.18	0.27	0	0.09	0.18	0.27

The other ingredients were added in the following proportions: salt 25 g/kg, garlic 15 g/ Kg, coriander 3 g/ kg, pepper 5 g/Kg.
SAU1 – hind leg meat sausage control with 0% beef bone broth, **SAU2** – hind leg meat sausage with 3% beef bone broth, **SAU3** – hind leg meat sausage with 6% beef bone broth, **SAU4** – hind leg meat sausage with 9% beef bone broth, **SAU5** – pork shoulder sausage with 0% beef bone broth, **SAU6** – pork shoulder sausage with 3 % beef bone broth, **SAU7** – pork shoulder sausage with 6% beef bone broth, **SAU8** – pork shoulder sausage with 9% beef bone broth.

For the thermal treatment, the same specifications and stages (drying, smoking and cooking) were used for the experimental batches as shown in Table 2.

Table 2. Thermal treatment steps for added with bone broth

Heat treatment stage	Time	Temperature in the thermal centre	Humidity
	minutes	°C	%
Air drying	20	45	30
Smoking	30	50	30
Hot air drying	40	82	30

The sensory evaluation of the experimental lots was carried out in the IULS sensory analysis laboratory, which is equipped with the necessary equipment, such as individual tasting boxes, and the samples were served at a temperature of 16-18 degrees on white plates. The sensory panel was formed by a group of 80 naive evaluators, a group consisting of both female (60%) and men (40%) aged between 20 and 23 years, without health problems, and with a diet in which meat products are frequently consumed.

The samples were sliced with a professional slicer to ensure uniformity and were presented to the evaluators in batch order and coded with three randomly chosen digits to keep the samples anonymous. At the beginning of the session, the evaluators were explained the contents of the questionnaire and how to fill it out, as well as the explanation of the terms found in the questionnaire so that they could familiarise themselves with the terms encountered.

External Preference Mapping (EPM)

It is a technique used in sensory and market analysis to correlate consumer or respondent preferences with external product characteristics or contextual factors. This method is used to better understand how certain physical, chemical, sensory, or economic characteristics of products influence consumer perceptions and preferences.

For this test, evaluators were asked to rate the coded products (SAU1, SAU2, SAU3, SAU4, SAU5, SAU6, SAU7, and SAU8) on a scale from 1 to 10, depending on how much they liked the product, to find out from the total number how acceptable it is for the market.

The Hedonic Test

The attributes of visual appearance (whole piece and section), texture, smell, taste, freshness, and general acceptance were evaluated on a structured hedonic scale of 9 points (Dislike Extremely, Dislike Very Much, Dislike Slightly, Neither Like or Dislike, Like Moderately, Like Very Much, Like Extremely). To clean their palace, consumers were asked to wash their mouths with water between samples. All samples were presented in a sequential monadic test using a complete lock design. Consumers were not provided with any information about samples and received no financial incentive for their participation in order to prevent bias.

The check all-that-apply (CATA)

It is a method that aims to determine the sensory attributes characteristic of a specific product in a simplified way. The CATA method allows consumers to choose all possible attributes from a previously prepared list to describe the evaluated product. According to Henrique et al. (2015), terms can be generated by a trained sensory panel or by a group of consumers (for example, using discussion groups).

This differs from scaling tests because attributes are not evaluated in terms of intensities. In addition, terms are not limited to sensory attributes, but can also be linked to the use of the product or to the contexts in which they fall.

Participants started by evaluating the sensory profile of each sample using the CATA method. This approach involves providing consumers with a list of attributes intended for the sensory description of sausage products, and they indicate whether a particular attribute is relevant to describing those products. The list of attributes was established on the basis of previous studies and included 17 terms covering the appearance, smell, taste, and texture of sausages (Table 3).

Table 3. List of sensory attributes used for CATA evaluation

Sensory attributes	Characteristics
APPEARANCE	surface appearance, section appearance, red color
FLAVOUR	Spices flavour, bone broth flavour, pork meat flavour, smoked flavour, global flavour
TASTE	Spices taste, bone broth taste, pork meat taste, smoked taste, global taste
TEXTURE	hardness, elasticity, friability, cohesiveness, adhesiveness, succulence, chewiness, masticability

Hedonic responses can be used as additional data to help interpret the results, as CATA responses are directly related to consumer perceptions of product characteristics.
For CATA data, a contingency matrix for samples and attributes has been developed. Cochran's Q test was applied to this matrix to identify meanings between samples in terms of the frequency of attribute use (Meyners et al., 2013).

Statistical analyses
The data from the preference test were analyzed through variance analysis, taking into account consumers and sample causes of the variance. The Tukey test was used to check the significance of differences between averages ($p< 0.05$). The frequency of use of each term was determined for each sausage sample. The Multiple Factor Analysis (MFA) was carried out in the frequency table containing the answers for the CATA questionnaire. XLSTAT software was used for statistical interpretation (Addison V24).

RESULTS AND DISCUSSIONS

Following the start of the statistical analysis for the order of preferences, the results obtained are shown in Figure 1. Thus, the lots with the highest percentage of preference were SAU2 (the pork pulp lot with a bone soup ratio of 3%), SAU4 (the pig pulp batch with 9%), which obtained a percent range between 70 and 80%, and SAU7 (the back batch of pork with a 3% bone broth ratio). The remaining batches obtained percentages ranging from 40 to 60%.
Similar results by applying a similar method were obtained by Nguyen et al. (2023), who in their comparative study concluded that meat preparations (pork sausages) were preferred to sausages made from vegetable protein.

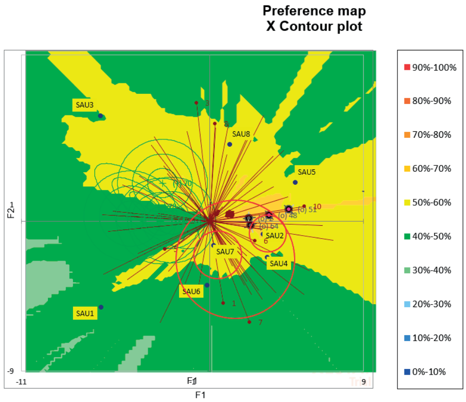

Figure 1. The order of preferences for the batches taken in the study

The check all-that-apply (CATA)
According to the results of the CATA test (Figure 2), significant differences in the frequency of attribute use between batches were identified. The rear sausages earned more appreciation than pulp sausages for most sensory characteristics. The differences between the average scores of pulp and back sausages were statistically significant for the following characteristics: bone broth flavour, juiciness, bone broth taste, fragility, chewingability, spice taste, overall taste, surface appearance, pork flavour, adhesiveness, and overall flavour. Factor analysis identified two groups of sensory characteristics: a group related to flavour and taste and a group linked to texture and appearance. The first dimension revealed a significant difference in the sensory profile of the SAU1 and SAU5 batches without bone soup additivation compared to batches with bone broth additivity. The sensory attributes that were distinct for the SAU6, SAU3, and SAU7 batches were "bone broth flavour", "spice flavour" and "suculence, fragility, and elasticity". For SAU8, SAU3, and SAU4, the attributes associated with these samples were "global flavour, smoked flavour and hardness", appreciations that can be proportional to the process of the soup added.

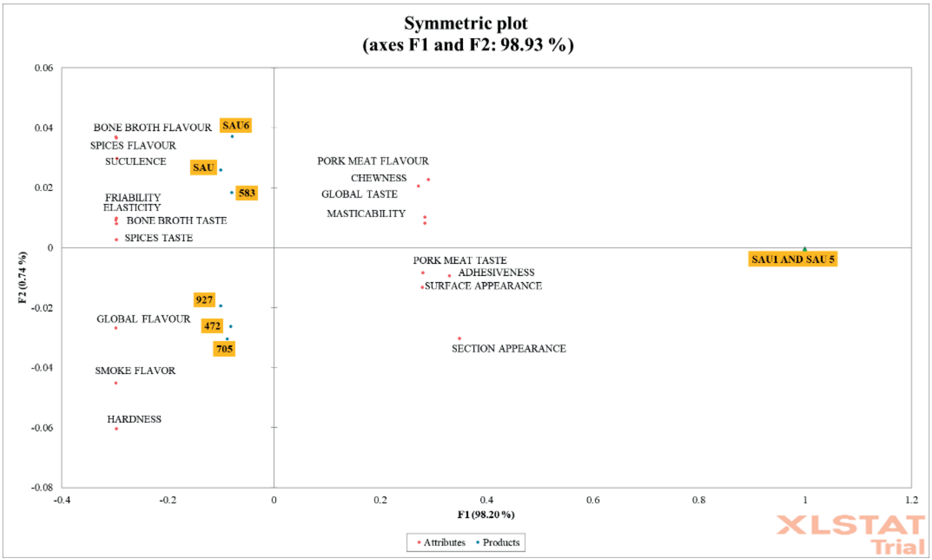

Figure 2. Illustration of the correlation of sensory attributes by evaluators by CATA test

The eight prepared sausage formulations were subjected to sensory analysis using a 9-point hedonic scale to select the panellists' preferred samples for each range. The hedonic scores for the sensory attributes (appearance, colour, texture, smell, taste, and general acceptability) of the batches of smoked sausages are presented in Table 4.

Following the study, the analysis of the hedonic test on experimental lots evaluated with the addition of different concentrations of bone broth indicates that attributes such as appearance, colour, smell, and taste were well appreciated by consumers, with values ranging from 7.2 to 7.9, regardless of the raw material used and the added concentration, the results were not differently significant. However, it can be noted that samples with higher concentrations of bone soup showed a less uniform texture, and the acceptability was slightly higher for samples that had a lower (3%) or average (6%) concentration of bones broth.

Table 4. Hedonic test results

SAMPLE	APPEARANCE	COLOR	TEXTURE	ODOR	TASTE	OVERALL ACCEPTABILITY
SAU1	7.4^{a}	7.2^{a}	7.2^{a}	7.4^{a}	7.4^{a}	7.5^{a}
SAU 2	7.4^{a}	7.4^{a}	7.3^{a}	7.5^{a}	7.8^{a}	7.9^{a}
SAU3	7.5^{a}	7.4^{a}	7.3^{a}	7.6^{a}	7.6^{a}	7.8^{a}
SAU4	7.6^{a}	7.4^{a}	7.9^{a}	7.5^{a}	7.5^{a}	7.6^{a}
SAU5	7.3^{a}	7.3^{a}	7.1^{a}	7.2^{a}	7.4^{a}	7.8^{a}
SAU6	7.3^{a}	7.4^{a}	7.3^{a}	7.4^{a}	7.3^{a}	7.4^{a}
SAU7	7.4^{a}	7.4^{a}	7.4^{a}	7.7^{a}	7.6^{a}	7.7^{a}
SAU8	7.6	7.5^{a}	7.7^{a}	7.5^{a}	7.3^{a}	7.5^{a}

CONCLUSIONS

In considering this investigation into the acceptability of various assortments of sausages that have been additively made with varying amounts of bone broth, the SAU2, SAU4, and SAU7 batches are those that showed a high percentage of acceptance by the assessors. These batches were characterized by a variety of sensory attributes, including succulence, fragility, overall smell, and bone broth flavour.

The goods created for this study have benefited from the addition of sausages with bone broth; nevertheless, concentrations should be carefully considered as an excessive concentration may affect the texture of the products from a sensory perspective.

The addition of bone broth was viewed favourably in light of the hedonic test's subjectivity, as the batches achieved high ratings for the desired qualities.

Further investigations on the concentrations of bone broth are recommended, and more studies are also needed on meat products with different structures.

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THE INFLUENCE OF COMMUNICATION AND MASS-MEDIA CAMPAIGNS ON CHOOSING FOOD ITEMS

Nicoleta DEFTA, Aurelia DEFTA (OSMAN), Nela DRAGOMIR, Ana-Maria SCRIPA

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: auraosman@gmail.com

Abstract

In recent years, healthy eating, the alarming problems of obesity and food advertising have become a topic of intense debate. These wide-ranging discussions explore how advertisements shape our perceptions of food, leading us to choose certain products. In this context, the aim of this study was to investigate whether there are significant gender differences in the influence of communication campaigns and media on food choice. Data were collected using a questionnaire (114 people) and the statistical procedures used were descriptive statistics and Pearson Chi-Square test (testing the significance of differences). Testing the significance of the differences between the values observed in the study and the expected values, according to the formulated hypotheses, revealed that these differences are not significant with regard to the respondent's gender variable, in relation to the analysed items. The results obtained are informative in terms of food purchasing behaviour of food products.

Key words: *food, healthy nutrition; consumer behaviour, socio-demographic factors.*

INTRODUCTION

The promotion of food products in the form of advertisement (publicity) is considered a commercial activity aimed at gaining the interest of the consumer. Vaughan (1980) and Jones (1998), in the advertising planning model, made a ranking of the effect in psychological terms for the interaction between psychology and advertising. This interaction is based on the three related processes: learning, emotions/feeling and doing/acting. Logical approach to thinking and high involvement in this process (Egger et al., 1997; 1999; Conner & Armitage, 2002) are important factors in the decision to choose and purchase food products.

Appropriate legislation exists to limit the way in which food products are promoted through communication and media campaigns. Advertisements are regulated by law (Caraher, 2001, 2003; Shepherd, 2009; Nestle, 2002) and due to the contradictions between the medical field and that of the food industry (producers). This conflict is supported by a number of studies. For example, Morgan (2003) produced a report concluding that the food industry needs to review its marketing practices and adapt them to concerns about healthy eating. Similar conclusions were drawn by UBS Warburg (2002). Both reports point to the potential of advertising in food choices. This aspect can lead to partnerships between the food industry and nutrition for public health. Also, numerous studies show a strong link between watching TV commercials and buying food products whose consumption leads to obesity (OMS/FAO, 2003; Taras & Gage, 1995; Lewis & Hill, 1998, Lindstrom & Seybold, 2003; Lewis & Hill, 1998; Hastings et al., 2003; Jarlbro, 2001; Robinson, 1998). There are also studies that demonstrate the positive aspect of promoting food products through advertisements (Klepp et al., 2007; Ind, 1993).

Calvert (2008) carried out a study on the implications of advertising in the choice of fast food, concluding that advertising has considerable effects on the choice of food by children and young people. In addition, there is evidence to indicate that young people influence their parents' buying patterns by requesting specific snacks or foods. Moreover, Hasting et al. (2003) argue that the effect of food promotion has been underestimated.

The aim of the study was to investigate a statistical population to find out the influence of advertising campaigns through mass media

channels, regarding the choice/purchase of food products.
As objectives to this end:
O1 Descriptive statistics regarding the organization and summary presentation of the questionnaire results
O2 Testing the significance of differences between certain types of variables, in relation to the variable gender of individuals. All variables are categorical.

MATERIALS AND METHODS

Materials

The investigation was carried out on a sample of 114 people, of which 73 were female and 41 were male, both from the urban environment (75 people) and from the rural environment (39 people). In order to identify whether the goals pursued in the study vary according to age, one of the items targeted this characteristic. The people participating in this study fall into the following categories: 20-30 years old (62 people), 31-40 years old (18 people), 41-50 (19 people), 51-60 (11 people) over 60 (4 people). Also, the educational level is an influencing factor of the objectives pursued in this study. For this item we had 4 answer options, respectively: high school level (23 people), professional level (17 people), university studies (52 people), postgraduate studies (19 people).

Study methodology

Methodology for making the questionnaire

To carry out this study, a questionnaire was designed, with 19 items.
The questionnaire was structured in 2 sections:
a. Socio-demographic data (4 items)
The personal, demographic and socio-economic attributes of the respondents were inferred based on the answers related to gender, age, level of education and domicile.
b. Perceptions of advertisements (15 items)
The questionnaire was designed in such a way as to meet the objectives pursued in the study. Most of the questions were closed, respondents could only choose from the options provided. Some of the questions were open-ended allowing the interviewees to express their opinions freely, precisely in order to gather as diverse a range of opinions as possible.

Data processing methodology

The interpretation of the questionnaire (Poșan, 2022) was achieved through the numerical (graphical) and summative presentation of the data.
Testing the significance of differences was done using the Pearson chi test.
The chi-square test (χ^2) of the association - is used for situations where the nature of the data collected for research is of the "counting" type, respectively it is characteristic of qualitative characters, being a non-parametric test.
The calculated χ^2 value was determined based on the calculation relationship:

$$\chi^2_{[GL]} = \frac{(O-A)^2}{A} \qquad (1)$$

where:
O - observed values;
A - expected values;
GL - degrees of freedom.
To achieve objective number 2, 3 hypotheses were formulated:
1. The frequency of choosing food products (A - *Always*, B - *Sometimes*, C - *Never*) is not influenced by the gender variable (female and male).
2. The way in which the choice of food products is made in relation to the influence that advertisements have: A* (*attracts my attention, but does not influence me*), B* (*makes me interested in the product*), C* (*makes me to buy that product*), D* (*I convince others to buy the product*), E* (*I don't give them importance*) is not influenced by the gender of the people (female and male).
3. The frequency of purchasing snacks in relation to viewing advertisements: A* (very often), B* (often), C* (sometimes), D* (never) is not influenced by the gender of people (female and male)

RESULTS AND DISCUSSIONS

Testing of hypothesis 1

Item tested: *Do ads help you choose the food you need?*
We were interested in checking whether the choice of food products is influenced by advertisements.
Of the 40 respondents who mentioned that they are *Always* influenced by advertisements when

purchasing food products, 24 are female and 16 are male. A large share of respondents (57.89%) stated that only *Sometimes* advertisements influence the choice of food products. From this category, 69.70% are female and 30.30% are male. There were only 8 people who stated that advertisements *Never* influence the purchase of food, respectively 3 female and 5 males.

To establish whether the observed differences are significant, the contingency table (Table 1) and the χ^2 test (Table 2) were developed.

Table 1. Correspondence table for gender characteristics and measurement scale levels

Features	Always	Sometimes	Never	Total columns
Female	24	46	3	**73**
Male	16	20	5	**41**
Total number of lines	**40**	**66**	**8**	**114**

Determination of statistical decision criteria:

• Critical level α = 0.05 (P = 95%), for significant differences

• GL = degrees of freedom = (2-1)*(3-1) = 2

• The tabular χ^2 value for these conditions is 5.99.

Since the calculated value (3.6476) of the test is lower compared to the tabular one (5.99), we accept the null hypothesis, concluding that there are no significant differences between the two genders regarding the influence of advertisements on the choice of food products (Table 2).

Table 2. Significance testing of differences

Characteristic	Observed frequency fO	Expected frequency fA	$f_O - f_A$	$(f_O - f_A)^2$	$\frac{(f_O - f_A)^2}{f_A}$
Female A*	24	25.616	-1.616	2.611456	0.101946
Female B*	46	42.2664	3.7336	13.93977	0.329807
Female C*	3	5.1232	-2.1232	4.507978	0.879915
Male A*	16	14.384	1.616	2.611456	0.181553
Male B*	20	23.7336	-3.7336	13.93977	0.587343
Male C*	5	2.8768	2.1232	4.507978	1.567011
Total number of lines	**114**	**114**			**3.6476**

Caption: A - Always, B - Sometimes, C - Never

Testing of hypothesis 2

The item tested: *How do you think you are influenced by advertisements when choosing food products?*

Most of the interviewees (40.35%) stated that advertisements attract my attention but do not influence me, of which 69.56% are female and 30.44% are male. The answer option *Make me interested in the product* was chosen by 35 of the respondents. The number of female respondents was almost double that of male respondents. Approximately equally, in terms of gender, the people who participated in the study mentioned that the ads *make me buy the product*. This category represented 8.77% of all respondents. A rather small number of interviewees (2 female and 5 male) chose as an answer that *they make me talk others into buying the product*. Some of the people interviewed (14.03% - 62.5% female and 37.5%) considered that advertisements are not important in choosing food products, choosing the answer option: *I do not consider them important* (Table 3).

Table 3. Correspondence table for the gender characteristic and item variants

Characteristics	**Female**	**Male**	**Total number of columns**
They draw my attention, but do not influence me	32	14	**46**
They make me interested in the product	23	12	**35**
They make me buy the product	6	4	**10**
They make me talk others into buying the product	2	5	**7**
I don't consider them important	10	6	**16**
Total number of lines	**73**	**41**	**114**

The research problem for this hypothesis consisted in testing the significance of the differences between the two genders regarding the perception of advertisements in the choice of food products.

Determination of statistical decision criteria:

• Critical level α = 0.05 (P = 95%), for significant differences

• GL = degrees of freedom = (2-1)*(5-1) = 4

• the tabular χ2 value for these conditions is 9.49

The statistical decision, in this situation, is that there are no significant differences between the two genders for the answer options A* (*attracts my attention but does not influence me*), B* (*makes me interested in the product*), C* (*makes me buy that product*), D* (*make me convince others to buy the product*), E* (*don't consider them important*) since the calculated value of the test (4.5637) is lower than the corresponding value in the distribution table χ2 (9.49) (Table 4).

Table 4. Significance testing of differences

Characteristic	**Observed frequency f_O**	**Expected frequency f_A**	$f_O - f_A$	$(f_O - f_A)^2$	$\frac{(f_O - f_A)^2}{f_A}$
Female A*	32	29.4584	2.5416	6.459731	0.219283
Female B*	23	22.414	0.586	0.343396	0.015321
Female C*	6	6.404	-0.404	0.163216	0.025487
Female D*	2	4.4828	-2.4828	6.164296	1.375099
Female E*	10	10.2464	-0.2464	0.060713	0.005925
Male A*	14	16.5416	-2.5416	6.459731	0.390514
Male B*	12	12.586	-0.586	0.343396	0.027284
Male C*	4	3.596	0.404	0.163216	0.045388
Male D*	5	2.5172	2.4828	6.164296	2.44887
Male E*	6	5.7536	0.2464	0.060713	0.010552
Total number of lines	**114**	**114**	**0**		**4.5637**

Caption: A* - They draw my attention, but do not influence me, B* - They make me interested in the product, C* - They make me buy the product, D* - They make me talk others into buying the product, E*- I don't consider them important.

Testing of hypothesis 3

The item for which the test was carried out: *How often did it happen in your case, after watching the advertisements for snacks, to buy that product*

For this item, four answer options were formulated (Table 5):

- *Very often*: 8.77% of respondents chose this answer option. Regarding the distribution between the two sexes, the proportions were approximately equal;
- *Often*: of all the interviewees who mentioned this aspect, 76.47% were females, and 23.53% were males. This category represented 14.91% of the total;
- *Sometimes*: the largest share of participants (52.63%) ticked this answer. Among those who chose that they sometimes buy snacks after watching ads, 57.57% are females and 33.33% are males;

- *Never*: approximately one fourth (59.26% of women and 40.74% of men) of the people participating in the study mentioned that they never buy snacks after watching an advertisement for this product category

Table 5. Correspondence table for gender characteristic and measurement scale levels

Characteristics	Very often	Often	Sometimes	Never	Total number of columns
Female	6	13	38	16	**73**
Male	4	4	22	11	**41**
Total number of lines	**10**	**17**	**60**	**27**	**114**

The research problem in this situation was represented by testing the significance of the differences observed between the two sexes in the purchase of snacks, after watching some advertisements for this product category (Table 6).

Determination of statistical decision criteria:
• Critical level α = 0.05 (P = 95%), for significant differences
• GL = degrees of freedom = (2-1)*(4-1) = 3
• the tabular χ2 value for these conditions is 7.81

Table 6. Significance testing of differences

Characteristic	Observed frequency fO	Expected frequency fA	$f_O - f_A$	$(f_O - f_A)^2$	$\frac{(f_O - f_A)^2}{f_A}$
Female A*	6	6.404	-0.404	0.163	0.025
Female B*	13	10.886	2.113	4.469	0.410
Female C*	38	38.424	-0.424	0.180	0.005
Female D*	16	17.290	-1.290	1.664	0.096
Male A*	4	3.596	0.404	0.163	0.045
Male B*	4	6.113	-2.113	4.465	0.730
Male C*	22	21.576	0.424	0.180	0.008
Male D*	11	9.709	1.290	1.667	0.171
Total number of lines	**114**	**114**	**0**		**1.490**

Caption: A - very often, B - often, C - sometimes, D - never

The calculated χ2 value (1.490) is lower than the tabular one (7.81), a situation in which the null hypothesis is accepted, concluding that there are no significant differences between the two sexes in terms of the persuasive power of information from advertisements in the purchase of snacks.

CONCLUSIONS

Promotion of advertisements and the impact they have on the consumer in terms of choosing and purchasing food products is an intense topic of discussion and debate.
The social environment in which food advertisements are promoted competes with the impact that advertisements have on food choice and purchase.

Testing the significance of the differences between the values observed in the study and the expected values, according to the formulated hypotheses, revealed that these differences are not significant with regard to the respondent's gender variable, in relation to the analysed items. As a result, it can be concluded that the frequency and manner of purchasing food products, respectively the purchase of snacks in relation to viewing advertisements, is not influenced by gender.
This study can be completed by testing the significance of the differences for the other demographic factors, namely residence, age, education level, monthly net income, in relation to the three items taken in this study. This represents one of the limitations of the study, because not taking into account all

demographic factors, an overall synthesis of how socio-demographic factors are or are not influenced by advertisements in the choice of food products was not achieved.

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FATTY ACID PROFILE AND QUALITATIVE EVALUATION OF THE FAT FRACTION IN WHITE BRINED CHEESE AT 24-HOURS OF PRODUCTION

Tsvetelina DIMITROVA[1], Silviya IVANOVA[2], Miroslav HRISTOV[1], Nikolay MARKOV[1]

[1]Agricultural Academy, Research Institute of Mountain Stockbreeding and Agriculture, 281 Vasil Levski Str., 5600, Troyan, Bulgaria
[2]Agricultural Academy, Institute of Cryobiology and Food Technology, 53 Cherni vrah Blvd, 1407, Sofia, Bulgaria

Corresponding author email: c.dimitrova@abv.bg

Abstract

The fatty acid composition of 24-hour white brined cheese produced from goat's milk from three groups of animals-Bulgarian White Dairy (BWD) breed and its crosses with Anglo-Nubian (BWD x AN) and Togenburg (BWD x TG) breeds during the lactation and the fatty acid composition of the milk fat of the product has been evaluated as a healthy source for human nutrition. Goat white brined cheese at the 24th hour after production, from the three groups of animals is characterized by a high level of saturated fatty acids from 75.52 g/100 g fat at BWD to 76.09 g/100 g fat at BWD x TG breed. MUFAs predominate in purebred goat cheese- 24.98 g per100 g fat and MUFA in the crosses of BWD x AN breed-3.32 g/100 g fat. The lipid preventive score is highest in BWD cheese - 53.85 g per 100 g cheese, and the atherogenic and thrombogenic index in BWD x TG breed cheese respectively 2.60 and 2.71. The analysed cheeses at the 24th hour from three goat groups is defined as having low content of TFA according to Regulation (EC) No1924/2006.

Key words*: conjugated linoleic acid (CLA), fatty acids, goat cheese, indices.*

INTRODUCTION

Milk and milk products have formed an essential part of the human diet since the beginning of the domestication of farm animals because together with other foods of animal and vegetable origin, they contain the necessary nutrients for the human body in an appropriate ratio.

Cheese as a source of fat and fatty acids is an integral part of a healthy diet, and the low lactose content makes it suitable for people with lactose intolerance (Hasler, 2000). Although the fat content, especially saturated and trans fatty acids, is associated with the occurrence of cardiovascular diseases, cheese consumption has not been proven to have a harmful effect, and the content of conjugated linoleic acid and oleic acid in cheese have health benefits (Gómez-Cortés et al., 2013).

The products of the decomposition of milk fat-fatty acids change the organoleptic indicators of cheese, which participate as substrates for oxidation to methyl ketones or as independent aromatic components, and on the other hand, as substrates for oxidation to methyl ketones. Minimum quantities of butyric, caproic, caprylic and capric acid give the cheese a pronounced taste (Senoussi et al., 2022).

Renna et al. (2012) traces the change of fatty acid profile upon a sudden change from indoor (hay and concentrate) to pasture grass-feeding (green grass). This change is mainly expressed in the increase of certain unsaturated fatty acids. From the point of view of healthy nutrition, changes in the content of vaccenic (C18:1t11), rumenic (C18:2c9t11) and Ω-3 fatty acids show that the profile improves significantly in the first days after switching from pasture to grazing. Scientists were working on the issue of improving the quality of milk by applying pasture feeding to ruminants, which in turn contributes to the production of high-quality cheese (Cismileanu et al., 2020; Dauber et al., 2022). Several scientists found that goat's milk is characterized by a higher content of short and medium-chain fatty acids (C4:0-C14:0) compared to cow's milk (Barłowska et al., 2011; Tziboula-Clarke, 2003; Park et al., 2017). The physicochemical and biochemical composition of processed milk is a determinant of its technological processing into dairy products and delicacies, which is determined by the breed and nutrition of different types of ruminants

(Bauman & Grinari, 2003; Cabiddu et al., 2005; Mihailova & Odjakova, 2006; Gosteva et al., 2017, Markov & Ivanova, 2020). The highest CLA values were determined in ewe's milk. Ivanova et al. (2017) studies showed that the level of CLA in sheep dairy foods was affected by the amount of concentrate feed, the type of roughage and the growth stage at harvest.

Cheeses made from the milk of pasture-raised compared to conventional ruminants were rich in unsaturated fatty acids, antioxidants and aromatic ingredients and have a low cholesterol content (Burgos et al., 2021; Nudda et al., 2021; Beltrão et al., 2022; Kavas et al., 2022; Galina et al., 2023; Shedeed et al., 2023; Thanh et al., 2023).

Rahmann et al. (2014) determined a lower concentration by long-chain and PUFAs, CLA and ratio from **Ω**-6 to **Ω**-3 fatty acids at indoor rearing goats.

Markova & Slavov (2019) determined an atherogenic index in milk from Srednostaroplaninska sheep breed a higher value (2.71) compared to Koprivshtenska breed (2.22). This makes the milk of Koprivshtenska breed a better product for healthy eating. The milk from Koprivshtenska breed had closer values of the lipid preventative score and the total fat content. The results are indicative of a better balance of the fatty acids in the milk of Koprivshtenska breed.

The fatty acid composition of white brined cheese, 24 hours after production from goat's milk from three groups of animals - Bulgarian White Dairy (BBM) and its crosses with Anglo-Nubian (BBM x AN) and Togenburg (BBM x TG) breeds was studied for a lactation period and it was evaluated that the fatty acid composition of the milk fat of the product is a healthy source in human nutrition.

MATERIALS AND METHODS

Nine samples of white brine cheese (3 x 3 pieces) were examined during the lactation period of the milk from three groups of animals - Bulgarian White Dairy (BWD) breed and its crosses with Anglo-Nubian (BWD x AN) and Togenburg (BWD x TG) breeds for fatty acid and evaluation of milk fat as a healthy source in human nutrition. The milk was taken in April, June and September and subjected to technological processing for cheese production. The white-bined goat's milk cheeses were examined at 24 hours after production.

The milk used is from experimental animals reared in one flock under the same production conditions in base at the Rimsa-Troyan which are 3 to 5 years old (second to fourth lactation with the indications being in February and the rearing system is indoor-pasture grass.

The extraction of total lipids was carried out by the Roese-Gottlieb method, using diethyl ether and petroleum ether and subsequent methylation with sodium methylate (CH_3ONa, Merck, Darmstadt) and drying with $NaHSO_4 \cdot H_2O$. Fatty acid methyl esters (FAME) were analysed using a Shimadzu-2010 gas chromatograph (Kioto, Japan) equipped with a flame ionization detector and an automatic injection system (AOC-2010i). The analysis was performed on a CP 7420 capillary column (100 m x 0.25 mm i.d., 0.2 μm film, Varian Inc., Palo Alto, CA). Hydrogen was used as the carrier gas, and as a make-up gas - nitrogen. Four-step furnace mode was programmed - the column's initial temperature was 80°C/min, maintained for 15 minutes, then increased by 12ºC/min to 170ºC and maintained for 20 minutes, followed by a further increase of 4ºC/min to 186ºC for 19 minutes and up to 220ºC with 4ºC/min until the process is complete.

The qualitative assessment of the fat fraction of the resulting samples includes the following: lipid preventive score (LPS), atherogenic (AI) and thrombogenic index (TI) (Ulbricht & Southgate, 1991), the ratio between hyper- and hypo-cholesterolemic (h/H) fatty acids, trans fatty acids (TFA) and the amount of saturated fatty acids (Regulation (EC) No 1924/2006).

LPS=FAT+2xSFA-MUFA-0.5PUFA,

AI=12:0+4×14:0+16:0/[ΣMUFAs+PUFA n-6+PUFAn-3]

TI=(14:0+16:0+18:0)/[0.5×ΣMUFAs+0.5×PUFAn-6+3×PUFAn-3+PUFAn-3/PUFA n-6]

h/H=(C18:1n-9+C18:1n-7+C18:2n-6+C18:3n-3+C18:3n-6+C20:3n-6+C20:4n-6+C20:5n-3+C22:4n-6+C22:5n-3+C22:6n-3)/(C14:0+C16:0).

The data was processed using the variation statistics methods using the statistical package of the EXCEL 2013 computer program.

RESULTS AND DISCUSSIONS

Different starters can change the total protein, fat and ash content, and the fatty acid composition of the cheese, due to the different activity and specificity of the proteolytic and lipolytic enzymes (Taboada et al., 2015; Lešić et al., 2016).

Saturated fatty acids (SFA) in white brined cheese at 24 hours from production with the highest amount were C-16: 0, followed by C-18:0, C-10:0 and C-14:0 (Table 1).

The contents of C-12:0, C-14:0 and C-16:0 that were associated with an increase in cholesterol levels in humans vary respectively: for C-12:0 from 3.52 g/100g fat from BWD to 4.16 g/100 g fat at BWD x TG, for C-14:0 from 9.40 g/100 g fat at WBD x AN to 10.02 g/100 g fat at WBD and for C-16: 0 from 26.90 g/100 g fat at WBD x AN to 28.50 g/100 g fat at WBD x TG. Similar results were obtained by Medeiros et al. (2013) for the cheese after seven days of ripening from the milk of Saan and Alpine goats in Brazil, fed with rations enriched with vegetable oils from different oilseed plants. The same authors also found stearic acid in different cheeses (C-18:0) - from 15.13% to 11.63% near to ours - from 11.56 at WBD x TG to 11.97 g/100 g fat at WBD x AN. Mihailova et al. (2004) were found in their studies on milk from the Bulgarian White Dairy breed and its crosses, that the levels of medium chain (C12:0 to C16:0) and long chain fatty acids over the C18:0 fatty acids in the analyzed milk were 38.5-39.8% and significantly higher than the short chain ones (C4:0- C10:0) - 20.5 and 21.5% respectively.

Table 1. Saturated fatty acids, g/100 g fat (n = 9)

Fatty acids	Breed group		
	BWD	BWD x TG	BWD x AN
	x ± Sx	x ± Sx	x ± Sx
C-4:0	3.90±0.555	3.98±0.207	4.28±0.182
C-6:0	3.30±0.296	3.30±0.138	3.59±0.069
C-7:0	0.01±0.004	0.01±0.0	0.02±0.005
C-8:0	3.17±0.202	3.15±0.182	3.39±0.106
C-9:0	0.03±0.005	0.03±0.005	0.03±0.008
C-10:0	10.62±0.368	10.70±0.677	11.11±0.644
C-11:0	0.04±0.007	0.04±0.012	0.03±0.007
C-12:0	3.52±0.311	4.16±0.677	3.70±0.387
C-13:0	0.05±0.012	0.05±0.009	0.04±0.011
C-14:0	10.02±0.581	9.66±0.652	9.40±0.737
C-15:0	0.60±0.088	0.64±0.099	0.57±0.080
C-16:0	28.15±1.913	28.50±1.497	26.90±2.030
C-17:0	0.55±0.033	0.56±0.048	0.55±0.046
C-18.0	11.90±1.463	11.56±1.781	11.97±1.712
C-20:0	0.24±0.007	0.24±0.014	0.24±0.012
C-21:0	0.05±0.010	0.06±0.010	0.05±0.011
C-22:0	0.07±0.005	0.07±0.005	0.06±0.012
C-23:0	0.02±0.005	0.02±0.014	0.03±0.005
C-24:0	0.01±0.011	0.03±0.005	0.03±0.005
C-26:0	0.01±0.004	0.06±0.031	0.02±0.008

White-brined cheeses are a good source of monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA). The largest amount was oleic acid (C-18:1c9), which ranges from 20.53 g/100 g fat at WBD x TG to 21.17 g/100 g fat at WBD (Table 2). These data were higher than those obtained by Markiewicz-Keszycka et al. (2013) - 18.65 g/100 g. Mihailova et al. (2004) determined the amount of trans isomers by oleic acid (C18:1) - 2.5%, and the concentration of conjugated linoleic acid (CLA) in goat milk from the Middle Balkan Mountain from 0.5 to 0.6 g/100 g fat.

In different types of cheese, polyunsaturated fatty acids range from 1.66 to 11.03% (Barac et al., 2016). In the batches of white brined cheese we studied, they were relatively close and varied within a narrow range - from 3.16 (WBD x TG) to 3.33 g/100 g fat (WBD x AN) (Table 3). The content of linoleic (C-18:2c9,12) acid was lowest-1.66 g/100 g fat by cheese from milk of WBD x TG and highest in cheese from milk of BWD - 1.79 g/100 g fat. The obtained results were lower than that found by Marinho et al. (2014) in "Kualo" cheese-2.05-2.53%.

Table 2. Monounsaturated fatty acids, g/100 g fat (n = 9)

Fatty acids	Breed group		
	BWD	BWD x TG	BWD x AN
	x ± Sx	x ± Sx	x ± Sx
C-10:1	0.18±0.025	0.18±0.033	0.19±0.025
C-12:1n1	0.01±0.003	0.02±0.003	0.02±0.005
C-14:1n5	0.05±0.010	0.06±0.012	0.05±0.014
C-16:1n7	0.33±0.018	0.38±0.040	0.36±0.035
C-17:1n7	0.19±0.018	0.17±0.015	0.19±0.022
C-18:1t4	0.01±0.003	0.01±0.0	0.01±0.0
C-18:1t5/6/7	0.16±0.014	0.17±0.026	0.16±0.026
C-18:1t9	0.20±0.020	0.17±0.012	0.19±0.018
C-18:1t10	0.17±0.009	0.17±0.010	0.18±0.022
C-18:1t11	0.92±0.235	0.95±0.287	0.92±0.248
C-18:1c9/C-18:1t12/13/	21.17±1.072	20.53±0.796	21.07±1.367
C-18:1t15	0.12±0.020	0.11±0.021	0.11±0.033
C-18:1c11	0.40±0.037	0.45±0.056	0.43±0.045
C-18:1c12	0.10±0.007	0.10±0.010	0.10±0.011
C-18:1c13	0.23±0.035	0.25±0.053	0.24±0.054
C-18:1t16	0.02±0.005	0.03±0.005	0.03±0.007
C-18:1c14	0.06±0.003	0.07±0.007	0.07±0.014
C-18:1c15	0.07±0.003	0.10±0.010	0.07±0.014
C-22:1n9	0.03±0.003	0.03±0.019	0.03±0.005

Table 3. Polyunsaturated fatty acids, g/100 g fat (n = 9)

Fatty acids	Breed group		
	BWD	BWD x TG	BWD x AN
	x ± Sx	x ± Sx	x ± Sx
C-18:2t9,12	0.19±0.017	0.17±0.014	0.18±0.009
C-18:2c9,12/19:0	1.79±0.014	1.66±0.125	1.72±0.083
gC-18:3n6	0.07±0.007	0.06±0.003	0.06±0.003
aC-18:3n3	0.48±0.152	0.51±0.129	0.54±0.131
CLA9c,11t	0.43±0.041	0.41±0.054	0.42±0.053
CLA9c,11c	0.02±0.011	0.02±0.005	0.04±0.010
C-20:2n6	0.03±0.008	0.04±0.012	0.04±0.010
C-20:4n6	0.03±0.005	0.03±0.007	0.04±0.012
C-20:3n3	0.15±0.028	0.17±0.023	0.17±0.035
C-22:5n3	0.06±0.039	0.07±0.024	0.09±0.015
C-22:6n3	0.01±0.007	0.02±0.007	0.03±0.003

The amounts of gC-18:3n6 and gC-18:3n3 vary slightly between the cheeses produced - 0.06 g/100 g fat in WBD x TG and WBD x AN and 0.07 g/100 g fat in WBD breed and 0.48 g/100 g fat at WBD and 0.54 g/100 g fat at WBD x AN, which is consistent with the results of Medeiros et al. (2013) for gC-18:3n6 - 0.08%, but they had lower values for gC-18:3n3 compared to those authors who receive concentration from 0.24 to

0.25% in cheese from milk of goat's rearing with supplements from spurge and ricin oil.

The present aspects concerning the composition of lactic fat in ruminants relate mainly to the content of conjugated linoleic acid (CLA), which can reduce the risk of many diseases such as obesity, atherosclerosis, cancer and more (Lawson et al., 2001). The CLA isomer 9c, 11t is the predominant form whose content is about 75-90% of the total CLA content in ruminant fat (Bauman et al., 2001). The CLA-containing products have been found to contribute to the reduction of body fat by inhibiting lipogenesis and stimulating lipolysis (Raff et al., 2009). The conjugated linoleic acid (CLA9c, 11t) is the highest in WBD cheese - 0.43 g/100 g fat and the lowest in WBD x TG-0.41 g/100 g fat, with no statistically significant differences. Mihailova et al. (2004) obtained a concentration of conjugated linoleic acid (CLA) in goat milk from the Middle Balkan Mountain from 0.5 to 0.6 g/100 g fat. Vieiteza et al. (2016) studied various types of goat cheese (fresh, ripe and storage) produced in Uruguay and determined the content of vaccenic acid from 1.4 to 4.9%, cis- isomers of oleic acid from14.9 to 25.4% and CLA - 0.4 to 1.5%.

Arachidonic acid (C-20:4n6), which is the other representative, besides the linoleic of the omega-6 group, has very low amounts between the batches of analyses cheese (0.03-0.04 g/100 g fat).

In recent years, there has been a growing interest in branched-chain fatty acids, as some of them have been shown to have health-promoting effects, and some dairy products can serve as a biomarker to evaluate the function of the abdomen (Vazirigohar et al., 2018).

The main representative of the studied cheese from the three batches was C-17iso, with values from 0.30 g/100 g fat at WBD to 0.31 g/100 g fat at WBD x TG and WBD x AN and C-17aiso, with a difference of 0.01% between batches, followed by C-15aiso, which is highest in WBD x TG - 0.29 g/100 g fat and lowest in WBD breed - 0.24 g/100 g fat (Table 4).

Table 4. Branched fatty acids, g/100 g fat (n = 9)

Fatty acids	Breed group		
	BWD	BWD x TG	BWD x AN
	x ± Sx	x ± Sx	x ± Sx
C-13iso	0.02±0.007	0.03±0.021	0.03±0.005
C-13aiso	0.01±0.012	0.01±0.012	0.02±0.024
C-14iso	0.05±0.022	0.06±0.031	0.05±0.024
C-15iso	0.21±0.036	0.21±0.102	0.21±0.021
C-15aiso	0.24±0.027	0.29±0.051	0.26±0.031
C-16iso	0.20±0.031	0.22±0.037	0.20±0.027
C-17iso	0.30±0.021	0.31±0.018	0.31±0.026
C-17aiso	0.30±0.021	0.32±0.031	0.31±0.028
C-18iso	0.04±0.0	0.03±0.003	0.03±0.0

The trans isomers in the investigated white brine cheeses ranged from 1.89 g/100 g fat at WBD to 1.94 g/100 g fat at WBD x TG, and the cis isomers from 21.05 at WBD x TG to 21.64 g/100 g fat at WBD breed (Table 5), while total content of CLA is in the range from 0.43 to 0.45 g/100 g fat.

Ivanova et al. (2018) found out for whole fat goat curd, CLA contains from 0.23 to 0.84 g/1000 g fat, omega-3 fatty acids from 0.26 to 1.12 g/100 g fat and from omega-6 fatty acids from 1.36 to 2.40 g/100 g fat.

Saturated fatty acids (SFA) and polyunsaturated (PUFA) have the lowest concentrations in WBD cheese - 75.52 g/100 g fat and 3.30 g/100 g fat, while monounsaturated (MUFA) were highest at WBD cheese - 24.98 g/100 g fat.

In recent years, there has been increasing interest in the role of Ω-6 and Ω-3 fatty acids in healthy nutrition. The appropriate ratio of Ω-6 and Ω-3 fatty acids for the prevention of cardiovascular disease is equal to or less than 4:1. In milk fat, this ratio is about 5:1 (much lower than in other foods), indicating that dairy products represent a good food for humans (Simopoulos, 2008).

Table 5. Fatty acid groups, g/100 g fat (n = 9)

Fatty acids	BWD	BWD x TG	BWD x AN
	x ± Sx	x ± Sx	x ± Sx
Σ CLA	0.45±0.042	0.43±0.057	0.45±0.052
Σ C-18:1 TFA	1.89±0.285	1.94±0.346	1.92±0.323
Σ C-18:1 CFA	21.64±1.079	21.05±0.825	21.54±1.402
SFA	75.52±0.725	76.59±0.989	76.01±1.879
MUFA	24.98±1.182	24.43±0.989	24.93±1.651
PUFA	3.30±0.156	3.22±0.297	3.32±0.210
Σ omega -3	0.71±0.155	0.82±0.130	0.82±0.116
Σ omega -6	2.25±0.050	2.08±0.108	2.14±0.105
Σ omega- 6/ omega -3	3.93±1.212	2.70±0.353	2.75±0.441
BFA	1.38±0.168	1.48±0.205	1.42±0.161
CLA	0.43±0.041	0.41±0.054	0.42±0.053

The **Ω**-3 fatty acids had the lowest concentration in cheese from WBD milk- 0.71 g/100 g fat and for **Ω**-6 by cheese from WBD x TG milk- 2.08 g/100 g fat. The ratio of **Ω**-6 and **Ω**-3 fatty acids ranges from 2.70 in cheese from WBD x TG to 3.93 in cheese from WBD, but remains below the recommended by The English Health Department (1994) is below 4.

Close to our results are obtained by Rahmann et al. (2014), in the production of goat cheese from the milk of goats reared under different feeding regimes, respectively saturated fatty acids - 75.1 and 75.8 g/100 g fatty acids, monounsaturated fatty acids - 20.0 and 19.7 g/100 g fatty acids, polyunsaturated fatty acids - 4.0 and 3.8 g/100 g fatty acids, **Ω**-3 fatty acids - 1.1 and 0.8 g/100 g fatty acids, **Ω**-6 fatty acids - 1.4 and 1.8 g/100 g fatty acids and CLA content - 1.0 and 0.8 g/100 g fatty acids.

The calculated lipid preventive score for the cheese at 24 hours after production (Table 6) is the lowest for the cheese produced from the milk of WBD x TG - 43.70 g/100 g cheese, and the highest for that from the WBD - 53.85 g/100 g cheese, with statistically significant results.

Table 6. Goat cheese indices

Indices	Breed group		
	BWD	BWD x TG	BWD x AN
	x ± Sx	x ± Sx	x ± Sx
LPS (g/100 g cheese)	53.85±1.43 a**, b*	43.70±1,18	46.46±0.60
AI	2.57±0.24	2.60±0.25	2.48±0.37
TI	2.56±0.24	2.71±0.12	2.62±0.24
h/H	0.63±0.06	0.61±0.05	0.67±0.08
TFA (g/100 g cheese)	0.45±0.05	0.38±0.07	0.40±0.06
SFA+TFA (g/100 g cheese)	18.57±0.45 a***, b*	15.08±0.39	16.06±0.18

a - BWD/BWDxTG; b - BWD/BWDxAN; *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$

The atherogenic index gives the correlation between the sum of the main saturated fatty acids and the unsaturated fatty acids, the former being considered proatherogenic (favoring the adhesion of lipids in the cells of the immune and circulatory system) and the second are anti-atherogenic (inhibit plaque aggregation and decrease levels of esterified fatty acids, cholesterol and phospholipids, thus preventing the occurrence of micro- and macro-coronary diseases).

The atherogenic index has the lowest values for cheese obtained from WBD x AN - 2.48 and the highest for WBD x TG - 2.60, which is in line with the one indicated by Cossignani et al. (2014) atherogenic index - 2.7÷2.4 in fresh and semihard cheeses from the Umbria trade network.

The thrombogenic index has the tendency to clot formation in blood vessels and is defined as the ratio between prothrombogenic (saturates) and antithrombogenic (monounsaturated and polyunsaturated Ω-3 and Ω-6 fatty acids) fatty acids (Ghaeni et al., 2013). The thrombogenic and atherogenic index, as indicators, should not exceed 1.00 while the cholesterol index is above 1.00 (Ivanova & Hadzhinikolova, 2015).

The thrombogenic index ranged from 2.56 in WBD cheese to 2.71 in WBD x TG, and the cholesterolemic index was low (below 1.0) in all three batches of cheese.

The values for trans fatty acids are in the range - 0.38 g/100 g cheese at WBD x TG to 0.45 g/100 g cheese at WBD, so the cheeses produced can be attributed to low TFA products according to Regulation 1924/2006.

Dimitrova et al. (2017) have obtained the following results for cheese produced during the lactation: lowest lipid preventive score in cheeses made from goat's milk WBD from 44.22 to 60.46 g/100 g product, lowest atherogenic and thrombogenic index in WBD x TG, respectively from 1.55 to 2.28 and from 2.03 to 2.56, and the highest ratio of hyper- and hypocholesterolemic fatty acids in cheese from milk of WBD x TG from 0.65 to 0.96, saturated fatty acids in cheeses ranges during the lactation to the following for WBD from 15.48 to 21.03 g/100 g cheese, at WBD x AH from 18.20 to 20.96 g/100 g cheese and in WBD x TG from 17.35 to 21.42 g/100 g cheese.

CONCLUSIONS

Goat white brined cheese at the 24th hour after production from the three groups of animals is characterized by a high level of saturated fatty acids from 75.52 g/100 g fat at Bulgarian White Dairy Breed to 76.09 g/ 100 g fat at Bulgarian White Dairy crosses with the Togenburg breed. MUFAs predominate in purebred goat cheese - 24.98 g/100 g fat and MUFA in the crosses of BWD x AN breed- 3.32 g/100 g fat. The lipid preventive score is highest in BWD cheese- 53.85 g/100 g cheese, and the atherogenic and thrombogenic index in BWD x TG breed cheese respectively 2.60 and 2.71. The analysed cheeses at the 24th hour after milk production of the three goat groups are defined as low content of trans fatty acid food - from 0.38 g/100 g cheese from Bulgarian White Dairy x Togenburg breed to 0.45 g/100 g cheese from the milk of Bulgarian White Dairy breed according to Regulation (EC) No 1924/2006.

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INFLUENCE OF SOME NATURAL SEEDS AND POWDERS ADDITION ON THE WHITE BREAD ANTIOXIDANT PROPERTIES AND OTHER CHARACTERISTICS

Delia-Gabriela DUMBRAVA[1], Diana-Nicoleta RABA[2], Camelia MOLDOVAN[1], Mirela-Viorica POPA[1], Corina Dana MISCA[1], Mariana-Atena POIANA[1], Carmen Daniela PETCU[3]

[1]University of Life Sciences "Regele Mihai I" from Timisoara, Faculty of Food Engineering, 119 Calea Aradului, 300645, Timisoara, Romania
[2]University of Life Sciences "Regele Mihai I" from Timisoara, Faculty of Management and Rural Turism, 119 Calea Aradului, 300645, Timisoara, Romania
[3]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Veterinary Medicine, 105 Independenţei Spl., District 5, 050097, Bucharest, Romania

Corresponding author email: cameliamoldovan@usvt.ro

Abstract

Bakery products industry occupies a very important place in people's major consumer products. Bread is a staple product that almost all of us consume every day. These foods provide the human body with an important part of the nutrients it needs for vital activity. A first objective of this work was to obtain two innovative assortments of white bread enriched with different additions: one with the addition of turmeric (Curcuma longa) radix powder and linseeds (Linum usitatissimum) (B1) and the second with the addition of sea buckthorn (Hippophae rhamnoides) fruits powder, sunflower (Helianthus annuus) and white sesame (Sesamum indicum) seeds (B2). A second objective was analysing the two enriched breads in terms of total polyphenols content, antiradical and antioxidant activity compared to the control (B0 - plain white bread). Porosity, proximate composition, energy value of the breads as well as their sensory characteristics were also determined. Total polyphenol content in B2, respectively B1 breads was with 66% and respectively 49% higher than the control (1.41±0.02 mg gallic acid/g). Anti-radical activity of the products was improved by 2.3 times in B2 and with 43% in B1 compared to plain white bread (38.76±0.19%). The porosity of B0 bread was the highest (68%), decreasing for B1 (60%) and B2 (53%) assortments. In terms of proximate composition, the additions of natural seeds and powders resulted in increased lipid and dietary fiber content, compared to the control. At the sensory analysis, B1 and B2 were rated higher than the control, with B2 scoring best (between 4.70 and 4.85).

***Key words**: antioxidant activity, bread, polyphenols, seeds, sea buckthorn, turmeric.*

INTRODUCTION

Bread, this basic food for human nutrition, has its origins since the Neolithic, and the art of bread making was initiated by the ancient Egyptians, then developed worldwide (Mondal & Datta, 2008; Arzani, 2019; Ghimpețeanu et al., 2022). The oldest evidence of bread making has been found at a 14,500-year-old Natufian site in the northeastern desert of Jordan (Arranz- Otaegui et al., 2018).

White bread commonly refers to bread made from wheat flour from which the bran and germ layers have been removed from whole wheat grains as part of the flour milling or grinding process, producing a light-colored flour. As plain white bread does not provide a high intake of antioxidants, there is now a growing trend for producers to make white bread with various seeds and other plant-derived additions with a high content of polyphenols and other antioxidants (Dziki et al., 2014; Sęczyk et al., 2017; Meral & Köse, 2019; Shori et al., 2021; Petcu et al., 2023; Ghimpețeanu et al., 2023).

Wheat flour is the main ingredient in bread making and consists mainly of starch (ca. 70-75%), water (ca. 14%) and protein (ca. 10-12%). In addition, non-starch polysaccharides (ca. 2-3%), especially arabinoxylans and lipids (ca. 2%) are minor constituents of flour, but relevant for bread production and quality (Goesaert et al., 2005; Petcu et al., 2019a), including the absence of contaminants (Petcu et al., 2019b).

Curcuma longa, or turmeric is an herbaceous evergreen plant member of the *Zingiberaceae* family and is cultivated extensively in Asia, especially in India and China. By grinding the boiled and dried rhizome a bright yellow powder of this spice is obtained. Turmeric has been used both in Asian cuisine for its flavor and color and in Chinese and Ayurvedic medicine. It is also officially included in the Pharmacopoeia of China and other Asian countries such as Japan and Korea (Laban, 2014; Verma et al., 2018). Chemical composition of *Curcuma longa* rhizomes includes a group of three curcuminoids: curcumin, demethoxycurcumin and bisdemethoxycurcumin, as well as volatile oils, sugars, protein and resins. Curcumin has been shown to be responsible for multiple therapeutic actions: antidiabetic, anticancer, hepatoprotective, cardiovascular protection neuroprotective, and others (Xu et al., 2018).

China's National Health Commission has classified sea buckthorn (*Hippophae rhamnoides* L.) berries as a "food medicine", as they provide numerous nutritional and therapeutic benefits. The fruits contain about 200 types of nutritive and bioactive compounds, such as: carotenoids, superoxide dismutase, polyunsaturated fatty acids, amino acids, vitamins, phytosterols, phenolic compounds, flavonoids. The importance of this fruit is underlined by its significant bioactivity, including anti-cancer, cardiovascular system improvement, anti-diabetic, antithrombotic and anti-obesity activity (Olas, 2016; Wang et al., 2022).

Linseeds (*Linum usitatissimum*), sesame (*Sesamum indicum* L.) seeds and sunflower (*Helianthus annuus* L.) seeds are key sources of phytochemicals in the functional food arena, being rich in polyunsaturated fatty acids, fiber, protein and phenolic compounds (Adeleke & Babalola, 2020; Brigante et al., 2020).

The primary purpose of this work was to obtain two varieties of white bread enriched with different additions of seeds and vegetable powders: one with the addition of turmeric powder and linseeds and the second with the addition of powdered buckthorn fruit, white sesame seeds and sunflower seeds. Another goal of the work was analysis the finished products in terms of total polyphenol content, antiradical and antioxidant activity, porosity, proximate composition, sensory properties, compared to the control (plain white bread).

MATERIALS AND METHODS

Bread preparation

Three types of bread were obtained: plain white bread (B0), white bread with added turmeric and flax seeds (B1), white bread with added buckthorn powder, white sesame seeds and sunflower seeds (B2), using the following raw and auxiliary materials: white wheat flour type 000, water, dry yeast, salt, turmeric powder, flax seeds, sunflower seeds, white sesame seeds, and buckthorn powder, all sourced from the domestic market. Table 1 shows the recipes used to bread assortments preparation.

Table 1. Recipes for bread assortment preparation

Bread type / Materials (%)	B0	B1	B2
White flour type 000	58.0	56.0	56.0
Water	40.0	40.0	39.0
Salt	1.4	1.1	1.1
Dried yeast	0.6	0.6	0.6
Turmeric powder		0.6	
Flax seeds		1.7	
Sea buckthorn powder			0.6
Sunflower seeds			1.7
White sesame seeds			1.0

To obtain the bread varieties, all the ingredients (flour, water, yeast, salt, turmeric and sea-buckthorn powders, seeds) were first weighed according to the recipes. Water was heated before being poured over the ingredients. The flour was placed in a bowl, then the yeast, salt and other ingredients were added, followed by the water. All these ingredients were kneaded well and the dough was left to rise for an hour at room temperature, after which it was divided and the loaves formed. Loaves were baked in an electric oven (Bosch HBG633NB1) at 180°C for 60 minutes. The baked loaves were removed from the oven and left to cool for at least 4 hours. Samples were taken from the obtained products for physical-chemical and sensory analysis (Figure 1).

B0 B1

B2

Figure 1. The three types of breads: B0 - plain white bread (control); B1- white bread with turmeric powder and linseeds, B2 - white bread with sea buckthorn powder, white sesame and sunflower seeds (Source: original photos)

Assessment of the total polyphenol content

Folin-Ciocalteu method was used for total polyphenol content (TPC) determination in breads as well as in turmeric and sea buckthorn powders, flax seeds, white sesame and sunflower seeds. For this, extracts in 70% ethanol were made from each sample (crust and crumb in mixture) and the same steps were performed as described by Dumbrava et al., (2020) with results expressed in mg gallic acid/g of sample.

Assessment of the antioxidant activity

For the three bread assortments, respectively for turmeric powder, sea buckthorn powder, flax seeds, white sesame and sunflower seeds, cupric ion reducing antioxidant capacity analysis method (CUPRAC) was applied, using the same ethanolic extracts as for the determination of TPC and the same working steps as Dumbrava et al., (2020).

Assessment of the antiradical activity

The antiradical activity of breads and added powders and seeds was evidenced by analysis of free radical 2,2-diphenyl-1-picryl-hydrazyl (DPPH) scavenging activity (RSA), using the same working method as described in our previous paper (Dumbrava et al., 2023).

Breads porosity assessment

To determine the breads porosity, in the middle of each type of bread, a slice was cut with parallel sides and a thickness of 60 mm. Using a perforator, previously greased with oil, a cylinder of core was removed from the middle of the slice. To obtain the core cylinder, the perforator was pressed and twisted into the mass of the core. The height of the core cylinder must be 60 mm and checked with a ruler. The core cylinder was weighed to two decimal places. Three determinations were made in parallel from the same sample (Petrusha et al., 2018).

Porosity was calculated using the relation (Petrusha et al., 2018):

$$\text{Porosity (\%)} = \frac{V-\frac{m}{\rho}}{V}\cdot 100$$

where:

V is the volume of the core cylinder (cm^3);

m - mass of the core cylinder (g);

ρ - density of the compact core (g/cm^3);

ρ = 1.31 (g/cm^3) for white flour bread and speciality breads.

Assessment of the proximate composition and energy value

The proximate composition of the finished products B0, B1 and B2 was determined using the following ISO methods: protein SR ISO 937:2007, total lipids SR ISO 1443:2008, mineral substances SR ISO 936:2009, sugar SR ISO 91-2007, and fiber according to ISO 13906:2007, moisture SR ISO 1442:2010, using FOOS Fibertec 2010 & M6, Sweden. The difference between 100 and the sum of protein, total fat, dietary fibre, minerals and moisture content was calculated to determine the carbohydrate content. Energy value of the three finished products was calculated by sum of the caloric intake of carbohydrates, lipids and proteins, considering that 1 g lipid provides 9 kcal, 1 g carbohydrate: 4 kcal and 1 g protein: 4 kcal.

Statistical analysis

Microsoft Excel 2010 software was used to process statistical data obtained from triplicate analyses for TPC, antioxidant activity, antiradical activity, porosity and proximate composition.

Sensory evaluation

The sensory evaluation of the three bread varieties was carried out according to ISO 4121:2002 by a panel of 20 evaluators (men and women) aged 20-55 years, using a hedonic rating scale from 1 to 5. Panelists assessed: external appearance, appearance in section, odor, taste, texture, chewing behavior, general acceptability. For sensory analysis, written informed consent was obtained from each evaluator, respecting the ethical requirements of the European Union Guidelines on Ethics and Food Research (Alfonsi et al., 2012). The evaluation was carried out under the same working conditions, acceptability levels, interpretation of score ranges as Dumbrava et al., (2020).

RESULTS AND DISCUSSIONS

Total polyphenols content

TPC results from the three bread assortments and the wheat white flour, added powders and seeds are shown in Table 2.

Table 2. TPC in the three breads assortments and in added powders and seeds

Sample	TPC (mg gallic acid/g)
B0	1.41±0.02
B1	2.10±0.08
B2	2.34±0.11
White wheat flour	2.11±0.06
Turmeric powder	22.11±0.23
Sea buckthorn powder	20.88±0.46
Linseeds	23.16±0.24
Sunflower seeds	19.52±0.12
White sesame seeds	85,46±0.44

Table 2. shows that between the natural powders and seeds used to obtain B1 and B2 bread assortments, white sesame seeds had the highest TPC (85.46±0.44 mg gallic acid/100 g), followed by linseeds (23.16±0.04 mg gallic acid/g) and turmeric powder (22.11±0.23 mg gallic acid/g). In the case of finished products, B2 was the richest in total polyphenols (23.35±0.19 mg gallic acid/g), the lowest concentration of these compounds being found in B0 (1.41±0.02 mg gallic acid/g). The addition of sea buckthorn powder, flax seeds, sunflower seeds, white sesame seeds and turmeric powder to plain white bread resulted in substantial increases in TPC of the finished product (by 66.19% for B2 and 49.04% for B1, respectively).

For different types of white wheat flour, depending on storage conditions and periods, Zhang et al. (2021) reported TPC values ranging from 1.91 to 2.84 mg gallic acid/g, and our results fall within this range. Elleuch et al., (2007) found for white sesame a TPC value (87.77 ± 3.15 mg gallic acid equivalents (GAE)/g) close to that in this paper. Also, Deme et al. (2021) reported close values of TPC for flax seeds of different varieties ranging from 20.53 mg GAE/g to 25.41 mg GAE/g. The range of values found by Romani et al., (2017) for different varieties of sunflower seeds (between 11.48 and 20.23 mg GAE/g), includes our results.

Lim et al. (2011) reported close values for TPC in turmeric (21.95 mg gallic acid/g) but much lower values in plain wheat bread (0.31 mg gallic acid/g) and turmeric-enriched bread (1.51 mg gallic acid/g), respectively, than in this paper. In bread with the addition of different proportions of sea buckthorn pomace powder Stanciu et al. (2023) found TPC values (between 1.70 and 2.30 mg gallic acid/g) comparable to those reported by us.

Antioxidant activity

The antioxidant activity for finished products as well as for white wheat flour, seeds and added powders, measured by the cupric ion reducing antioxidant capacity method, is presented in the Table 3.

The strongest antioxidant activity of the raw and auxiliary materials used in bread making, was reported in sea buckthorn powder (59.64 ± 0.90 mg Trolox/g), followed by turmeric powder (55.42 ± 0.81 mg Trolox/g) and white sesame seeds (50.16 ± 0.61 mg Trolox/g). In terms of finished products, B2 showed the highest antioxidant activity (29.85 ± 0.20 mg

Trolox/g), 93% higher than B1 and 9 times higher than B0.

Table 3. Antioxidant activity of the finished products, white wheat flour, seeds and added powders

Sample	Antioxidant activity (mg Trolox/g)
B0	3.31±0.08
B1	15.33±0.14
B2	29.85±0.20
White wheat flour	4.52±0.11
Turmeric powder	55.42±0.81
Sea buckthorn powder	59.64±0.90
Linseeds	49.28±0.64
Sunflower seeds	31.18±0.48
White sesame seeds	50.16±0.61

For white wheat bread, Meral & Köse (2019) determined (TEAC method) much lower values of antioxidant activity (between 0.17 and 0.25 mg Trolox/g) while and Yu et al. (2013), using the ORAC assay, found higher values (8.08 mg Trolox/g) than those reported in this paper, but for white wheat flour they determined a lower value (3.13 mg Trolox/g) than ours. Meral & Köse (2019) determined the antioxidant activity (TEAC method) for white wheat bread enriched with added pomegranate seeds and grape seeds, respectively, reporting values of 0.28-0.67 mg Trolox/g and 0.55-0.74 mg Trolox/g, respectively, much lower than those for enriched breads in this paper.

Free radical scavenging activity

The DPPH free radical scavenging activity (RSA %) determined for the finished products, white wheat flour, seeds and powders used is presented in the Table 4.

Of the additional ingredients added to B1 and B2, white sesame seeds had the strongest RSA (98.82 ± 0.41%). Sea buckthorn powder, although poorer in total polyphenols than turmeric powder, showed stronger RSA (95.14 ± 0.38%, compared to 93.86 ± 0.52% for turmeric powder). B2 bread had the highest RSA (88.00 ± 0.32%), followed by B1 (55.65 ± 0.29%). It is observed that the additions in B1 and B2 led to a significant increase in antiradical activity compared to control: with 43.58% in the case of B1, respectively by 2.27 times in the case of B2.

Table 4. Free radical scavenging activity (RSA) of the finished products, white wheat flour, seeds and added powders

Sample	RSA (%)
B0	38,76±0.19
B1	55,65±0.29
B2	88.00±0.32
White wheat flour	40.12±0.21
Turmeric powder	93,86±0,52
Sea buckthorn powder	95.14±0.38
Linseeds	91.18±0.26
Sunflower seeds	88.64±0.34
White sesame seeds	98.82±0.41

Breads porosity

The results obtained for the three bread assortments porosity are shown in Table 5.

Table 5. Porosity of breads

Sample	Porosity (%)
B0	68.27±0.42
B1	60,63±0.46
B2	53.12±0.58

The highest porosity was B0 (68.27 ± 0.42%), while breads with different additions showed decreases: 60.63 ± 0.46% for B1 and 53.12 ± 0.58% for B2. Ghendov-Mosanu et al. (2020) also found that porosity values of white bread decreased with increasing amount of sea buckthorn flour added (between 72.7 ± 1.3 and 59.7 ± 1.5%). The same authors reported a higher porosity for plain white bread (72.3 ± 1.4%), while Coțovanu and Mironeasa (2022) found lower value (64.33 ± 0.11%) than in this paper.

Proximate composition and energy value of finished products

The proximate composition and energy values determinations for breads led to the results presented in Table 6.

The three types of breads (B0, B1 and B2 respectively) had a very similar protein (6.24 ± 0.10, 6.32 ± 0.09 and 6.57 ± 0.11 g/100 g respectively) and total carbohydrates (46.05, 45.30 and 45.29 g/100 g respectively) contents. Sugars are almost non-existent in the products.

Total fats are better represented in the breads with different additions, so that in B1 the quantity is double, and in B2 is 3.24 times higher than in B0 (0.68 g/100 g). While in B0 saturated lipids are almost absent (0.01 g/

100 g), in B1 the amount reaches 0.31 g/100 g and in B2 is 0.22 g/100 g.

Table 6. Proximate composition and energy value of breads

Parameters	B0	B1	B2
Proteins (g/100 g)	6.24±0.10	6.32±0.09	6.57±0.11
Lipids (g/100 g)	0.68±0.02	1.36±0,04	2.20±0.03
Saturated fatty acids (g/100 g)	0.01±0.01	0.31±0.02	0.22±0.01
Carbohydrates (g/100 g)	46.05	45.30	45.29
Sugar (g/100 g)	0	0.02±0.01	0.07±0.01
Dietary fiber (g/100 g)	0.15±0.02	0.84±0.04	0.70±0.02
Moisture (g/100 g)	45.00±0.94	44.00±0.92	43.00±0.88
Mineral substances (g/100 g)	1.88±0.02	2.18±0.04	2.24±0.06
Energy value (kcal/100 g)	215.28	218.72	227.24

The additions in B1 and B2 breads also increased the dietary fiber content of B1 by 5.6 times and of B2 by 4.7 times compared to control (0.15 ± 0.02 g/100 g).

Sensory analysis

Sensory analysis of the breads led to the results shown in Figure 2.

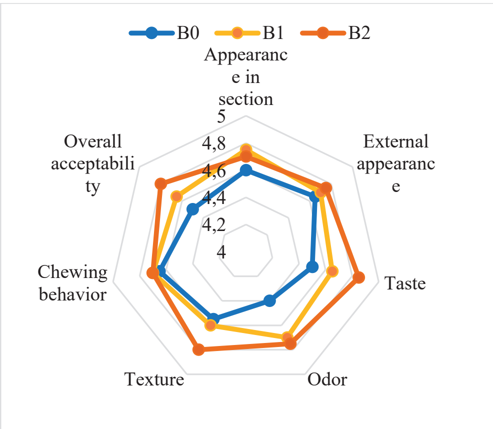

Figure 2. Global values of the sensory evaluation of breads using a 5-point hedonic scale

From organoleptic point of view, all three bread assortments were well evaluated by the tasters, obtaining scores above 4 for all characteristics. B1 and B2 breads were the best rated, with B2 scoring between 4.7 and 4.85 and B1 between 4.6 and 4.75. Apart from the appearance in section, where B1 scored the highest: 4.75 points, B2 had the best results in sensory analysis.

CONCLUSIONS

Obtaining bakery products enriched with various natural additives to enhance their antioxidant properties, nutritional value and sensory characteristics is a common trend nowadays.

This work aimed to obtain two innovative bread assortments: one enriched with added turmeric powder and flax seeds (B1), and the second with sea buckthorn powder, white sesame seeds and sunflower seeds (B2), using white wheat flour as principal raw material.

Of the natural ingredients added to B1 and B2, the highest content of total polyphenols was found in white sesame seeds, followed by flax seeds and turmeric powder. These natural additions resulted in a significant increase in the total polyphenol content of B1 and B2 compared to control.

The best antiradical and antioxidant activity was determined for white sesame seeds, followed by sea buckthorn powder and turmeric powder, and among the finished products, for B2. It was shown that the addition of sea buckthorn powder, sunflower seeds and white sesame seeds, respectively turmeric powder and flax seeds in the matrix of plain white bread led to a remarkable improvement of the antioxidant activity of the products obtained compared to the control.

The breads porosity was influenced by the ingredients added to the matrix of the white bread, thus showing a decrease in porosity in breads with additions, the lowest value being recorded for B2.

From a nutritional point of view, B1 and B2 provide significant additional dietary fiber and fat compared to plain white bread.

In the sensory analysis the enriched breads B1 and B2 were evaluated with very good scores, superior to the control, and of these, except the appearance in section, where B1 was the best evaluated, B2 recorded the highest scores, proving a very high level of acceptability.

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EVALUATION OF THE IMPACT OF ARTIFICIAL ADDITIVE ON PHYSICOCHEMICAL QUALITY PARAMETERS IN A FUNCTIONAL MEAT PRODUCT WITH HETEROGENEOUS STRUCTURE

Elena-Iuliana FLOCEA, Marius Mihai CIOBANU, Bianca Georgiana ANCHIDIN, Mihai Cătălin CIOBOTARU, Diana-Remina MANOLIU, Ioana GUCIANU, Mădălina MATEI, Paul Corneliu BOIȘTEANU

"Ion Ionescu de la Brad" Iasi University of Life Sciences, 3 Mihail Sadoveanu Alley, 700490, Iasi, Romania

Corresponding author email: paul.boisteanu@iuls.ro

Abstract

This work aims to evaluate the impact of sodium erythorbate ($C_6H_7NaO_6$) in different amounts (0.05%; 0.1%) on physicochemical quality parameters in a functional product with heterogeneous structure. We aimed to test two main anatomical areas used in the process of obtaining the finished product: Musculus gluteus maximus and Musculus longissimus dorsi from Sus scrofa domestica. Data distribution was evaluated using SPSS Statistics 26.0 software. Multivariate Analysis of Variance (MANOVA) is used to determine if there are significant differences between the amounts of $C_6H_7NaO_6$ and anatomical area on physicochemical parameters considering their interactions. Pearson correlation was used to analyse the degree of association between the amounts of $C_6H_7NaO_6$, the anatomical zone, and the physicochemical quality parameters of the finished product. Based on the results obtained, recommendations can be made on the optimal concentration of $C_6H_7NaO_6$ % to achieve the desired effects on physicochemical quality parameters without compromising consumer safety.

Key words: *artificial additive, functional product, physicochemical quality.*

INTRODUCTION

Meat a major basis in the human diet, challenges food industry specialists through consumer perception of meat quality (Boișteanu et al., 2024). Despite emerging dietary trends in Western societies promoting the reduction or replacement of meat in the human diet, global meat consumption is increasing (Bohrer, 2017; Manessis et al., 2020; Boișteanu et al., 2023).

Meat during shelf life is affected by several factors that have negative effects on sensory parameters and not least, nutritional quality (de Carvalho et al., 2019). Some changes related to sensory parameters lead to consumer uncertainty towards the product (Ciobanu et al., 2023a).

Lately, the implementation of strategies in line with a sustainable approach, such as improving lipid profile and nutritional indices has been extensively studied (Velázquez et al., 2022; Anchidin et al., 2023).

Research in this direction has increased since improving the lipid profile of meat products generates technological disadvantages, the main reason being the increase in the amount of monosaturated fatty acids and polyunsaturated fatty acids. These two factors lead to oxidative instability and significantly decrease the shelf life of products (Velázquez et al., 2022; de Carvalho et al., 2019; Domínguez et al., 2019).

Specialists in the food industry have found as a method of stabilization, the use of antioxidants that inhibit oxidation reactions of meat products, in particular, preserving the optimal characteristics for sensory testing. Antioxidants are divided into two categories, natural and synthetic (Ribeiro et al., 2019; Bellucci et al., 2022). The courses of action of antioxidants include inhibition of the chain reaction by scavenging radicals that initiate oxidation, breaking chain reactions that extract hydrogen for prolonged periods, lowering oxygen concentrations, decomposing peroxides, and preventing their conversion to initiating radicals (Manessis et al., 2020). Some researchers argue that the reaction of antioxidants occurs through electron donation to break and stop oxidation in the propagation step, thus preventing the formation of additional radicals, lipids, and proteins (Lorenzo, 2018; Bellucci et al., 2022). In 2021, Samantha Pfiffner and collaborators demonstrated the effects of tert-butyl hydroquinone (TBHQ) on the tumor suppressor gene p53 in breast cancer cells. TBHQ is one of the aromatic compounds used in food to prevent oxidation and extend shelf life. The present study examined the effects of TBHQ, alone and in combination with hormones and anti-hormones, on the expression of estrogen alpha (ERα) and tumor suppressor gene p53 in MCF-7 and T-47D breast tumor cell lines. Changes in ERα and p53 protein expression were shown after 24 h of treatment with different concentrations of TBHQ (0.005 to 1 mM). P53 levels show a continuous increase in expression by TBHQ concentrations (0.005 to 1 mM) in both MCF-7 and T-47D. Synthetic antioxidants are used due to their low cost and availability.

The study investigates the impact of $C_6H_7NaO_6$ analysed at different amounts (0.05%; 0.1%) on quality biomarkers and color parameters in a functional pork sausage product. Quality biomarkers and color parameters are analysed in the context of the use of this additive in the manufacturing process of the heterogeneous structure product. The results provide insight into how this additive can influence the properties and quality of the final product, providing relevant information for the food industry and the consumer.

MATERIALS AND METHODS

The samples used in this study were obtained in the Meat Microproduction Department (ULS IASI), following ISO quality standards. The raw materials, sourced from a local producer, were stored in cold storage for 24 hours before the start of the study. The samples used in the research consisted of muscle samples taken from *Musculus gluteus maximus* and *Musculus longissimus dorsi*, after the removal of excess fat and connective tissue. Two types of salt were used: NaCl (table/natural salt) for the control group and $NaNO_3$ (nitrate salt) for the experimental groups. These were mixed manually with the muscle samples and then subjected to a maturation period of 12 h at a controlled temperature of 2-4°C. After this stage, the samples were subjected to grinding. After maturation, the samples were coarsely grinded by Grinder WP - 105 using a sieve with a diameter of 3 mm, then the emulsion was obtained using the Cutter Titane V 45L. Subsequently, the samples were with the studied additive ($C_6H_7NaO_6$) in varying amounts depending on the experimental batches and were subjected to a specific heat treatment according to (Table 1).

Tabel 1. Heat treatment stage for meat product with heterogenous structure for the experiment

Heat treatment stage	Time	Temperature inside the cell	Temperature in the thermal center
	minutes	°C	°C
Drying	30	60	50
Smoking	25	64	58
Boiling	-	76	72
Drying	10	76	72

Samples were vacuum bagged in 2-layer vacuum bags, inner layer 60 µm polyethylene suitable for food contact, outer layer 15 µm polyamide, UV filtered using ATM Machinery, having 630W chamber, refrigerated at 2°C and separated from light for 12 h. After 12 hours separated from light, they were transported to the control laboratory for further analysis. The experimental configuration was designed in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives as regards heat-treated meat products. The amounts of $C_6H_7NaO_6$ taken in the study are shown in (Table 2).

Table 2. The amount of $C_6H_7NaO_6$ used in the control and experimental batch from two different anatomical areas *Musculus gluteus maximus* and *Musculus longissimus dorsi* from *Sus scrofa ferus*

Sample	$C_6H_7NaO_6$	*Musculus gluteus maximus*	*Musculus longissimus dorsi*
SC	0	3kg	3kg
S1	0.05%	3kg	3kg
S2	0.1%	3kg	3kg

SC-samples control, S1-sample experimental, S2-samples experimental

The final products were subjected to physico-chemical evaluations to test the influence of $C_6H_7NaO_6$ on the heterogeneously structured product. Instrumental color characteristics of the product were determined using a Chroma Meter CR-410 colorimeter from Konica Minolta Inc., Japan, on the CIELAB scale. These characteristics were expressed as L*, a*, and b* values. The primary color coordinates L* (brightness), a* (complementary red-green color coordinates), and b* (complementary yellow-blue color coordinates) were evaluated according to the LAB [CIE (Commission Internationale de l'Eclairage)] space over the sample area (presented as the average value of measurements taken at five locations equally distributed over the sample) and section using a Hunter Minolta CM-2600d colorimeter, with an observation angle of 2° and an 8 mm diameter measurement area, illuminating a 50 mm diameter surface, according to (Manoliu et al., 2023). Fat (%), Water (%), Protein (%), Collagen (%), and Salt (%) contents were also monitored using the Omega Bruins Food-Check near-infrared (NIR) spectrophotometer (Bruins Instruments GmbH, Puchheim, Germany). NIR (a region of the electromagnetic spectrum near the infrared spectrum, characterised by wavelengths between about 700 and 2500 nanometres. In this range, visible light is dominated by the colors red, orange, and yellow. These wavelengths allow absorption and emission by different substances, as well as interaction with different materials and biological systems).

Data distribution was evaluated using SPSS Statistics 26.0 software. Multivariate Analysis of Variance (MANOVA) is used to determine if there are significant differences between the amounts of $C_6H_7NaO_6$ and anatomical area on physicochemical parameters considering their interactions. Pearson correlation was used to analyse the degree of association between the amounts of $C_6H_7NaO_6$, the anatomical areas, and the physicochemical quality parameters of the finished product.

RESULTS AND DISCUSSIONS

The results of multivariate analysis (MANOVA) of variance for quality biomarkers in the study of the influence of $C_6H_7NaO_6$ in a product with heterogeneous structure from two anatomical areas *Musculus gluteus maximus* and *Musculus longissimus dorsi* from *Sus scrofa ferus* are presented in Table 3.

Table 3. Results of multivariate analysis of variance (MANOVA) for quality biomarkers (Fat, Water, Protein, Collagen, Salt) in the study of the influence of $C_6H_7NaO_6$ in a product with heterogeneous structure from two anatomical areas *Musculus gluteus maximus* and *Musculus longissimus dorsi* from *Sus scrofa ferus*

Anatomical Area	Effect		Value	F	Hypothesis df	Error df	Sig.
Musculus gluteus maximus	$C_6H_7NaO_6$	Pillai's Trace	1.981	188.86	10	18	<.001
		Wilks' Lambda	0	190.240[b]	10	16	<.001
		Hotelling's Trace	269.45	188.62	10	14	<.001
		Roy's Largest Root	198.33	356.988[c]	5	9	<.001
Musculus longissimus dorsi		Pillai's Trace	1.797	15.912	10	18	<.001
		Wilks' Lambda	0.002	39.089[b]	10	16	<.001
		Hotelling's Trace	129.44	90.61	10	14	<.001
		Roy's Largest Root	125.32	225.582[c]	5	9	<.001

[b] Exact statistic; [c] The statistic is an upper bound on F that yields a lower bound on the significance level. General linear model with significance level *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

Pillai's Trace, Wilks' Lambda, Hotelling's Trace, and Roy's Largest Root are analytical methods used to assess different aspects of the impact of the independent variable ($C_6H_7NaO_6$) on physicochemical quality parameters. Experimental samples from both the *Musculus gluteus maximus* and *Musculus longissimus dorsi* anatomical areas indicate a significant difference ($p < 0.001$), between the groups tested in these anatomical zones. These results show that $C_6H_7NaO_6$ influences physicochemical quality parameters in both anatomical zones studied, regardless of concentration (0.05% or 0.1%), and significant differences appear between control and experimental samples.

Table 4. Results of Tests of Between-Subjects Effects $C_6H_7NaO_6$ on Fat, Water, Protein, Collagen, and Salt in a product with heterogeneous structure from two different anatomical areas *Musculus gluteus maximus* and *Musculus longissimus dorsi* from *Sus scrofa ferus*

Anatomical Area	Independent Variable	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Musculus gluteus maximus	$C_6H_7NaO_6$	Fat (%)	64.025	2	32.013	108.887	<.001
		Water (%)	70.341	2	35.171	197.588	<.001
		Protein (%)	4.609	2	2.305	138.28	<.001
		Collagen (%)	8.1	2	4.05	418.966	<.001
		Salt (%)	0.497	2	0.249	10.971	0.002
Musculus longissimus dorsi		Fat (%)	51.6	2	25.8	323.849	<.001
		Water (%)	33.937	2	16.969	261.056	<.001
		Protein (%)	2.821	2	1.411	40.692	<.001
		Collagen (%)	2.981	2	1.491	159.714	<.001
		Salt (%)	0.485	2	0.243	16.93	<.001

Tests of Between-Subjects Effects; significance level *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

Table 4 presents the results of the Tests of Between-Subjects Effects carried out to determine the influence of $C_6H_7NaO_6$ on Fat%, Water%, Protein%, Collagen%, and Salt% in a heterogeneously structured product from two different anatomical areas *Musculus gluteus maximus* and *Musculus longissimus dorsi* from *Sus scrofa ferus*, recording significant differences (p <0.001) between the tested groups for all measured physicochemical quality parameters. $C_6H_7NaO_6$ is known to be used in the food industry for its antioxidant activity. The interaction with protein may be influenced by the ability of sodium erythorbate to protect protein structures against oxidative damage. Thus, it could contribute to maintaining or even increasing protein levels in the product. According to (ANS, 2015) depending on the processing and storage conditions, it could contribute to water retention in the product or reduce water loss by inhibiting fat oxidation.

Table 5. Correlation matrix analysis using Pearson correlation coefficient for $C_6H_7NaO_6$, Fat, Water, Collagen, Protein, and Salt variables in *Musculus gluteus maximus* samples subjected to the experimental protocol

		$C_6H_7NaO_6$ (%)	Fat (%)	Water (%)	Collagen (%)	Protein (%)	Salt (%)
$C_6H_7NaO_6$ (%)	Pearson Correlation	1	.931**	-.981**	-.993**	0.346	.613*
	Sig. (2-tailed)		<.001	<.001	<.001	0.206	0.015
	N	15	15	15	15	15	15
Fat (%)	Pearson Correlation	.931**	1	-.947**	-.925**	0.048	.681**
	Sig. (2-tailed)	<.001		<.001	<.001	0.865	0.005
	N	15	15	15	15	15	15
Water (%)	Pearson Correlation	-.981**	-.947**	1	.973**	-0.276	-.676**
	Sig. (2-tailed)	<.001	<.001		<.001	0.319	0.006
	N	15	15	15	15	15	15
Collagen (%)	Pearson Correlation	-.993**	-.925**	.973**	1	-0.343	-.591*
	Sig. (2-tailed)	<.001	<.001	<.001		0.211	0.02
	N	15	15	15	15	15	15
Protein (%)	Pearson Correlation	0.346	0.048	-0.276	-0.343	1	-0.182
	Sig. (2-tailed)	0.206	0.865	0.319	0.211		0.516
	N	15	15	15	15	15	15
Salt (%)	Pearson Correlation	.613*	.681**	-.676**	-.591*	-0.182	1
	Sig. (2-tailed)	0.015	0.005	0.006	0.02	0.516	
	N	15	15	15	15	15	15

**Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed).

According to (Table 5), correlation matrix analysis using Pearson correlation coefficient for the variables $C_6H_7NaO_6$%, Fat%, Water%, Collagen%, Protein%, and Salt% in samples from *Musculus gluteus maximus* subjected to the experimental protocol indicates a strongly significant positive correlation (p<0.001) between $C_6H_7NaO_6$ and fat (Pearson correlation = 0.931**), positively significant correlation with salt (Pearson correlation = 0.613*) and weakly significant with protein (Pearson correlation = 0.346). This indicates that an increase in $C_6H_7NaO_6$ concentration is associated with an increase in fat and salt percentage.

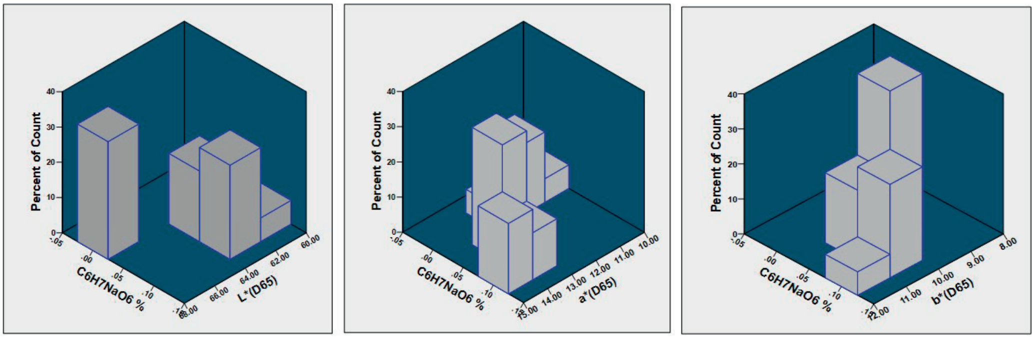
$C_6H_7NaO_6$ correlates negatively (p<0.001) with the amount of water (Pearson correlation = -0.981**) and the amount of collagen (Pearson correlation = -0.993**). Increasing the amount of $C_6H_7NaO_6$ decreases the amount of water and collagen.

Table 6. Correlation matrix analysis using Pearson correlation coefficient for $C_6H_7NaO_6$, Fat, Water, Collagen, Protein, and Salt variables in *Musculus longissimus dorsi* samples subjected to the experimental protocol

		$C_6H_7NaO_6$ (%)	**Fat (%)**	**Water (%)**	**Protein (%)**	**Collagen (%)**	**Salt (%)**
$C_6H_7NaO_6$ (%)	Pearson Correlation	1	0.785**	-0.794**	-0.808**	-0.791**	0.702**
	Sig. (2-tailed)		<0.001	<0.001	<0.001	<0.001	0.004
	N	15	15	15	15	15	15
Fat (%)	Pearson Correlation	0.785**	1	-0.999**	-0.931**	-0.997**	0.224
	Sig. (2-tailed)	<0.001		<0.001	<0.001	<0.001	0.422
	N	15	15	15	15	15	15
Water (%)	Pearson Correlation	-0.794**	-0.999**	1	0.929**	0.997**	-0.243
	Sig. (2-tailed)	<0.001	<0.001		<0.001	<0.001	0.382
	N	15	15	15	15	15	15
Protein (%)	Pearson Correlation	-0.808**	-0.931**	0.929**	1	0.928**	-0.240
	Sig. (2-tailed)	<0.001	<0.001	<0.001		<0.001	0.388
	N	15	15	15	15	15	15
Collagen (%)	Pearson Correlation	-0.791**	-0.997**	0.997**	0.928**	1	-0.234
	Sig. (2-tailed)	<0.001	<0.001	<0.001	<0.001		0.402
	N	15	15	15	15	15	15
Salt (%)	Pearson Correlation	0.702**	0.224	-0.243	-0.240	-0.234	1
	Sig. (2-tailed)	0.004	0.422	0.382	0.388	0.402	
	N	15	15	15	15	15	15

**Correlation is significant at the 0.01 level (2-tailed); *Correlation is significant at the 0.05 level (2-tailed).

In Table 6, using Pearson correlation coefficient for $C_6H_7NaO_6$, Fat%, Water%, Collagen%, Protein%, and Salt% variables in *Musculus longissimus dorsi* samples subjected to the experimental protocol compared to *Musculus gluteus maximus*, $C_6H_7NaO_6$ and protein were significantly negative correlations (Pearson correlation = -0.808**). Between $C_6H_7NaO_6$, and fat (Pearson correlation = 0.785**) and salt (Pearson correlation = 0.702**). The color intensity of the finished product can be influenced by the content of fat, water, collagen, protein, and salt (Ciobanu et al., 2023b). Figures 1 and 2 show the distribution of the means of the primary color coordinates L*(D65), a*(D65), and b*(D65) for control and experimental samples of *Musculus gluteus maximus* and *Musculus longissimus dorsi* from *Sus scrofa ferus*.



A. B. C.

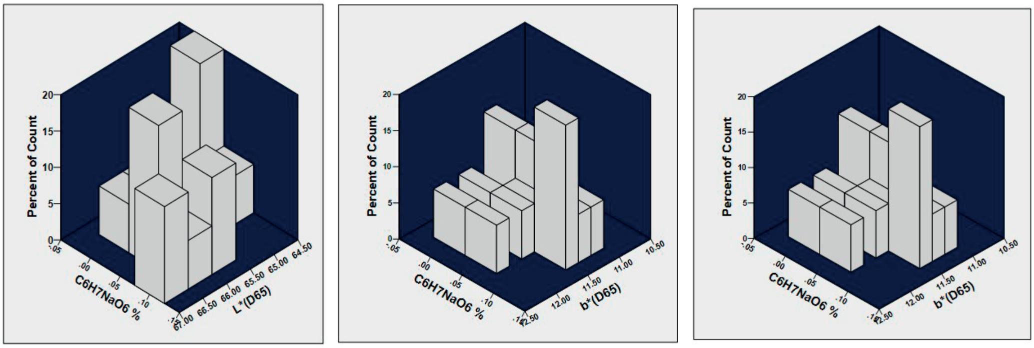
Figure 1. Distribution of mean primary color coordinates (**A**) L*(D65), (**B**) a*(D65), (**C**) b*(D65) for control and experimental samples (0.05% $C_6H_7NaO_6$, 0.1% $C_6H_7NaO_6$) from *Musculus gluteus maximus* of *Sus scrofa ferus*

Table 7. Correlation matrix analysis using Pearson correlation coefficient for $C_6H_7NaO_6$ variables L*(D65), a*(D65), b*(D65), in samples from *Musculus gluteus maximus* subjected to the experimental protocol

		***$C_6H_7NaO_6$* (%)**	**L*(D65)**	**a*(D65)**	**b*(D65)**
$C_6H_7NaO_6$ (%)	Pearson Correlation	1	-0.830**	0.897**	0.940**
	Sig. (2-tailed)		<0.001	<0.001	<0.001
	N	15	15	15	15
L*(D65)	Pearson Correlation	-0.830**	1	-0.873**	-0.886**
	Sig. (2-tailed)	<0.001		<0.001	<0.001
	N	15	15	15	15
a*(D65)	Pearson Correlation	0.897**	-0.873**	1	0.840**
	Sig. (2-tailed)	<0.001	<0.001		<0.001
	N	15	15	15	15
b*(D65)	Pearson Correlation	0.940**	-0.886**	0.840**	1
	Sig. (2-tailed)	<0.001	<0.001	<0.001	
	N	15	15	15	15

*Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed).

For Table 7, all *p*-values are below 0.01, showing that there are significant and very strong correlations between $C_6H_7NaO_6$ level (%) and all L*(D65), a*(D65), and b*(D65) color values. The correlation between $C_6H_7NaO_6$ and L*(D65) is negative and extremely strong, with a Pearson coefficient equal to -0.830. $C_6H_7NaO_6$ and a*(D65) are positively and very strongly correlated, Pearson coefficient is 0.897, and for $C_6H_7NaO_6$ (%) and b*(D65) are strongly correlated (Pearson coefficient = 0.940.) There are significant and very strong correlations between all L*(D65), a*(D65) and b*(D65). The correlation between L*(D65) and a*(D65) is negative and very strong, with a Pearson coefficient of -0.873. The correlation between L*(D65) and b*(D65) is negative and very strong, with a Pearson coefficient of -0.886. The correlation between a*(D65) and b*(D65) is positive and very strong, with a Pearson coefficient of 0.840.



A. B. C.

Figure 2. Distribution of mean primary color coordinates (**A**) L*(D65), (**B**) a*(D65), (**C**) b*(D65) for control and experimental samples (0.05% $C_6H_7NaO_6$, 0.1% $C_6H_7NaO_6$) from *Musculus longissimus dorsi* of *Sus scrofa ferus*

No significant correlation (p>0.05) between $C_6H_7NaO_6$ and b*(D65). The correlation between $C_6H_7NaO_6$ and L*(D65) is positive and moderate, with a Pearson coefficient of 0.579. The correlation between $C_6H_7NaO_6$ and a*(D65) is positive and strong, with a Pearson coefficient of 0.717. There is a significant correlation between a*(D65) and L*(D65) color values, but it is weaker and does not reach the significance level of 0.01. There is no significant correlation between L*(D65) and b*(D65) color values (Table 8).

Table 8. Correlation matrix analysis using Pearson correlation coefficient for $C_6H_7NaO_6$ variables L*(D65), a*(D65), b*(D65), in samples from *Musculus longissimus dorsi* subjected to the experimental protocol

		$C_6H_7NaO_6$ (%)	**L*(D65)**	**a*(D65)**	**b*(D65)**
$C_6H_7NaO_6$ (%)	Pearson Correlation	1	0.579*	0.717**	-0.151
	Sig. (2-tailed)		0.024	0.003	0.592
	N	15	15	15	15
L*(D65)	Pearson Correlation	0.579*	1	0.483	-0.372
	Sig. (2-tailed)	0.024		0.068	0.172
	N	15	15	15	15
a*(D65)	Pearson Correlation	0.717**	0.483	1	0.072
	Sig. (2-tailed)	0.003	0.068		0.799
	N	15	15	15	15
b*(D65)	Pearson Correlation	-0.151	-0.372	0.072	1
	Sig. (2-tailed)	0.592	0.172	0.799	
	N	15	15	15	15

*Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed).

CONCLUSIONS

The results of multivariate analysis of variance (MANOVA) for quality biomarkers (Fat, Water, Protein, Collagen, Salt) in the study of the influence of $C_6H_7NaO_6$ in a product with heterogeneous structure from two anatomical zones *Musculus gluteus maximus* and *Musculus longissimus dorsi* from *Sus scrofa ferus* indicate the influence of $C_6H_7NaO_6$ on physicochemical quality parameters in both anatomical zones studied, regardless of concentration (0.05% or 0.1%) and significant differences between control and experimental samples. For the colorimetric control profile of experimental samples from *Musculus gluteus maximus* the results suggest a strong correlation between $C_6H_7NaO_6$ content and color characteristics, and the color values L*(D65), a*(D65) and b*(D65) are also strongly correlated with each other. In the case of the colorimetric control profile of the experimental samples from *Musculus longissimus dorsi* the results indicate some significant correlations between $C_6H_7NaO_6$ and certain color values L*(D65) and a*(D65), as well as between a*(D65) and L*(D65). Compared to the experimental samples from *Musculus gluteus maximus*, there is no significant correlation between L*(D65) and b*(D65) color values.

Based on the results obtained, recommendations can be made on the optimal concentration of $C_6H_7NaO_6$ to achieve the desired effects on biomarker quality and colorimetric properties of the product without compromising its safety or acceptability. As we study the impact of artificial additives whereby the literature increasingly recommends their elimination, advanced research, acceptability, and sensory testing of natural versus artificially additivated products is needed.

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SENSORIAL EVALUATION OF PORK MEAT SAUSAGES ENRICHED WITH CHOKEBERRY (*Aronia melanocarpa*) AND BLUEBERRY (*Vaccinium myrtillus*) POWDERS

Oana Mărgărita GHIMPEȚEANU[1], Oana Diana MIHAI[1], Carmen Daniela PETCU[1], Raluca Aniela GHEORGHE IRIMIA[1], Carmen NEGOIȚĂ[1], Cristin BORDA[2], Paul Rodian TĂPĂLOAGĂ[3], Dana TĂPĂLOAGĂ[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Veterinary Medicine, 105 Splaiul Independentei, District 5, Bucharest, Romania
[2]University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, 3-5 Mănăștur Street, 400372, Cluj-Napoca, Romania
[3]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Animal Productions Engineering and Management, 59 Mărăști Blvd, District 1, Bucharest, Romania

Corresponding author email: carmen28petcu@gmail.com

Abstract

Sensory characteristics evaluation (appearance, colour, smell, taste, texture) represents the most important criterion in the purchase decision of meat products. The aim of the study was to produce and sensory evaluate seven batches of pork meat sausages (control, sausages+chokeberry powder in concentration of 1.2%, 2.5%, 5% and sausages+blueberry in concentration of 1.2%, 2.5%, 5%). The plant-based powders were added to improve shelf-life of the products and the sensorial assessment was used to choose the best concentration in terms of consumers´ acceptance. The evaluation involved completing an acceptability questionnaire using a 9-point hedonic scale, an acceptability test with scores on the general attributes of appearance, texture, aroma, taste, overall quality. The most favourable responses were recorded for the sausages with 2.5% chokeberry, followed by the sausages with 1.2% chokeberry. As an important feature, it is the colour change, appreciated by consumers. The most unfavourable responses were received following the evaluation of the sausage samples with 5% blueberry extract which gave a sweet taste uncharacteristic of meat products.

***Key words**: blueberry powder, chokeberry powder, pork meat sausages, sensorial evaluation.*

INTRODUCTION

Meat and meat products represent an important source of protein and the current trends in food safety are focused on preserving the nutritional quality by replacing the synthetic antioxidants with natural antioxidants (Petcu et al., 2023; Shah et al., 2014). Consumers are more and more interested in what they eat, so the meat industry has orientated towards using successful combinations of functional ingredients in order to obtain satisfactory sensory attributes (Hosseini et al., 2014; Miller, 2017). Sensory characteristics evaluation (appearance, colour, smell, taste, texture) represents the one of most important criterion in the purchase decision of meat products (Figure 1). Consumers´ acceptance is evaluated through hedonic tests to assess the overall liking and degree of liking for individual sensory attributes (Fiorentini et al., 2020). The aim of this study was to use the plant-based powders in order to improve shelf-life of the products and the sensorial assessment was used to choose the best concentration in terms of consumers´ acceptance.

Figure1. Multicriterial decision in consumer behaviour (adapted from Font-i-Furnols & Guerrero, 2014)

MATERIALS AND METHODS

The seven experimental batches of pork meat sausages were obtained in controlled conditions in the Faculty of Veterinary Medicine of Bucharest. The first batch (B1), considered being the control, contained pork, spices: coriander, garlic, basil, thyme, black pepper, white pepper, salt and sugar in different proportions. The other six batches had the same composition, but chokeberry and blueberry powders were added in different concentration as presented in Table 1.

Table 1. Composition of experimental batches

Batch	Specification
B1	control
B2	control+chokeberry powder 1.2%
B3	control+chokeberry powder 2.5%
B4	control+chokeberry powder 5%
B5	control+ blueberry powder 1.2%
B6	control+ blueberry powder 2.5%
B7	control+ blueberry powder 5%

The manufacturing process involved meat mincing, salting and seasoning, baking in the oven (T = 190°C, t = 40 min).

The sensorial evaluation was done by 37 students, aged between 22 and 24 years, trained in advance regarding specific sensory terms and organization of the tasting session.

The sausages were sliced in pieces of equal sizes, encoded and distributed to evaluators.

The evaluation involved fulfilling an acceptability questionnaire using a 9-point hedonic scale, with parameters between 1 (extremely pleasant) and 9 (extremely unpleasant) and an acceptability test with scores on the general attributes of appearance, texture, smell, taste (Meilgaard et al., 2016).

One-way ANOVA test was used to assess significant differences between independent experimental groups. This method helps in determining the existence of significant variations between groups depending on the independent variable being tested. The significance level chosen was 0.05. If the analysis was significant ($p<0.05$), the Tukey-Kramer test was used as a post-hoc test to compare the means of the groups and identify significant differences between them. In the situation in which the data did not show a normal distribution (by Shapiro-Wilk test) and/or did not meet the requirements for the application of the one-way ANOVA analysis, the non-parametric Kruskal–Wallis test was used and the multiple comparison graphs were made for a graphical representation of this test. It was followed by a post hoc analysis using the non-parametric Conover test.

RESULTS AND DISCUSSIONS

Hedonic analysis

Following the hedonic analysis with a 9-point scale (1-extremely pleasant, 2-very pleasant, 3-pleasant, 4-slightly pleasant, 5-neutral, 6-slightly unpleasant, 7-unpleasant, 8-very unpleasant, 9-extremely unpleasant), consumers' perception through the Kruskal–Wallis test (P = 0.000019) tends to consider B1 being very pleasant, followed by B2, B3, B5, and B6 as pleasant, and slightly pleasant B4 and B7 (Figure 2).

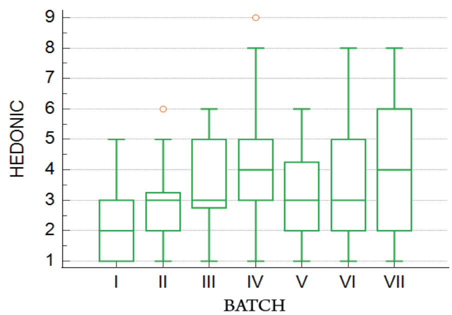

Figure 2. Multiple comparison graph - Hedonic analysis

The non-parametric Conover test reveals the differences among the batches as follows in Table 2.

Table 2. Differences among batches by hedonic analysis

Batch	n	Different(P<0,05) from batch no.
B1	37	B3, B4, B5, B6, B7
B2	37	B4, B7
B3	37	B1
B4	37	B1,B2,B5
B5	37	B1, B4, B7
B6	37	B1
B7	37	B1, B2, B5

Acceptability test

Assessing consumer perception of colour shade

According to the Shapiro-Wilk test, the data collected regarding the assessment of consumers' perception of colour-shade obtained in the experiment for the seven batches, were not normally distributed (P = 0.0216). At the same time, according to the significance level of the non-parametric Kruskal-Wallis test (P<0.000001), there are no significant differences between the researched batches. However, there is a variability in the data, pink shade is present at batches B1 and B2 followed by an increasing in colour intensity for B5, B3, and B6 and the most intense colour burgundy B4 and B7 (Figure 3).

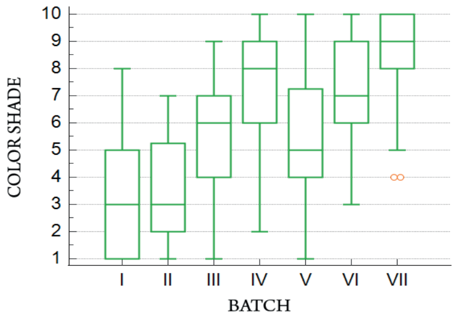

Figure 3. Multiple comparison graph - colour shade

The differences between batches using Conover test are presented in Table 3.

Table 3. Differences among batches colour-shade

Batch	n	Different (P<0.05) from batch no.
B1	37	B3, B4, B5, B6, B7
B2	37	B3, B4, B5, B6, B7
B3	37	B1, B2, B4, B6, B7
B4	37	B1, B2, B3, B4, B5, B7
B5	37	B1, B2, B4, B6, B7
B6	37	B1, B2, B3, B5, B7
B7	37	B1, B2, B3, B4, B5, B6

Assessing consumer perception - colour intensity

The data collected for this parameter were not normally distributed, Shapiro-Wilk test revealed P<0.0001.

On the colour intensity scale (1 - imperceptible, 2 - perceptible, 3 - intense), it was found that the samples belonging to B7 had the highest intensity (Figure 4).

According to the significance level of the non-parametric Kruskal-Wallis test (P = 0.003216), there are no significant differences between the researched batches.

The non-parametric Conover test reveals the differences among the batches as follows in Table 4.

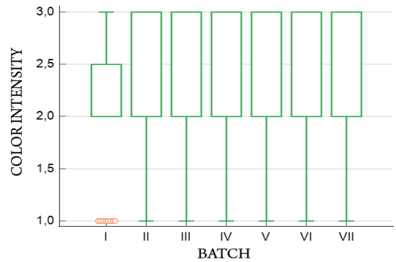

Figure 4. Multiple comparison graph - colour intensity

Table 4. Differences among batches colour-intensity

Batch	n	Different (P<0.05) from batch no.
B1	37	B4, B5, B6, B7 (4) (5) (6) (7)
B2	37	B6, B7
B3	37	B7
B4	37	B1
B5	37	B1
B6	37	B1, B2
B7	37	B1, B2, B3

Assessing consumer perception of taste intensity

The Shapiro-Wilk test revealed P<0.0001 and according to the significance level of the non-parametric Kruskal-Wallis test (P = 0.007257) there are no significant differences between the batches. B1 was considered to have the most pleasant intensity of the taste, meanwhile the other six batches obtained pleasant intensity taste (Figure 5).

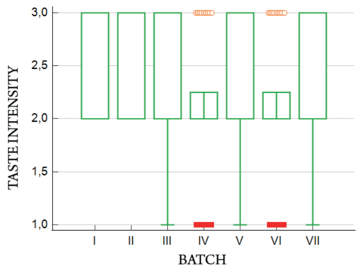

Figure 5. Multiple comparison graph - taste intensity

The non-parametric Conover test reveals the differences among the batches as follows in Table 5.

Table 5. Differences among batches taste-intensity

Batch	n	Different (P<0.05) from batch no.
B1	37	B4, B5, B6, B7
B2	37	-
B3	37	-
B4	37	B1
B5	37	B1
B6	37	B1
B7	37	B1

Assessing consumer perception - dominant taste
From the four perceptible tastes (1-fat, 2-meat, 3-spices, 4-other tastes), consumers specified that the dominant taste is of meat for batches 1, 2, and the other batches especially the taste of spice. According to the Shapiro-Wilk test, the data collected regarding the assessment of consumers' perception of dominant taste obtained in the experiment for the seven batches, were not normally distributed (P<0.0001). At the same time, according to the significance level of the non-parametric Kruskal-Wallis test (P = 0.030745), there are no significant differences between the researched batches (Figure 6).

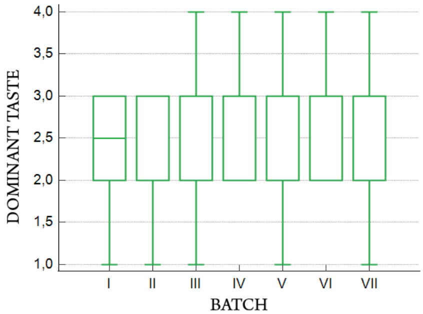

Figure 6. Multiple comparaison graph - dominant taste

The non-parametric Conover test reveals the differences among the batches as follows in Table 6.

Table 6. Differences among batches - dominant taste

Batch	n	Different (P<0.05) from batch no.
B1	37	B4
B2	37	B3, B4, B5
B3	37	B2
B4	37	B1, B2
B5	37	B2
B6	37	-
B7	37	-

Assessing consumer perception - dominant smell
By applying Shapiro-Wilk test the result for P was <0.0001 and according to the significance level of the non-parametric Kruskal-Wallis test (P = 0.522376), there are no significant differences between the researched batches. However, it can be seen that for batches 2, 3 and 4 the dominant smell was of meat and for batches 1, 5, 6 and 7 of spices (Figure 7).
The non-parametric Conover test showed that there were no differences between batches.

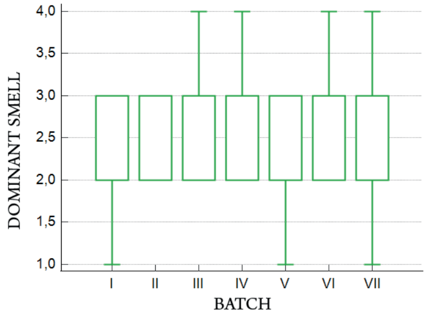

Figure 7. Multiple comparison graph - dominant smell

Assessing consumer perception - smell intensity
For this parameter, the Shapiro-Wilk test had a value of P<0.0001, so the seven batches had values not normaly distributed. At the same time, according to the significance level of the non-parametric Kruskal-Wallis test (P = 0.179158), there are no significant differences between the researched batches (Figure 8). The non-parametric Conover test showed that there were no differences between batches. All the batches had pleasant smell intensity.

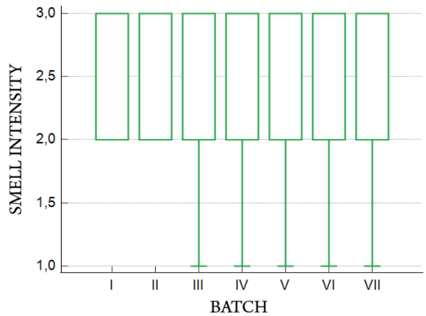

Figure 8. Multiple comparison graph - smell intensity

Assessing consumer perception - consistency friability
The scale used for this parameter was 1 -slightly crumbly, 2 - crumbly, 3 - very crumbly and the results showed that B1 and B2 were slightly crumbly and B3-B7 were crumbly.
The Shapiro-Wilk test showed an abnormal distribution (P<0.0001) and Kruskal-Wallis test (P = 0.064087), there are no significant differences between the researched batches (Figure 9).

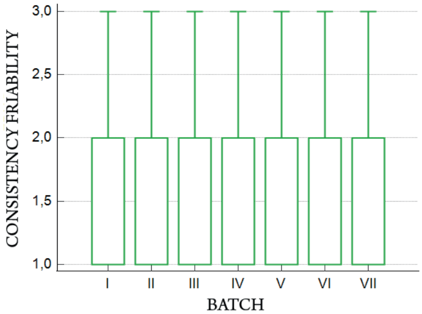

Figure 9. Multiple comparison graph - consistency friability

The non-parametric Conover test showed that there were no differences between batches.

Assessing consumer perception - juicy consistency

The batches B1, B3, B4, B6 and B7 were included in the slightly juicy category and B2 and B5 in juicy category. The Shapiro-Wilk test showed an abnormal distribution ($P<0.0001$) and Kruskal-Wallis test ($P = 0.111378$), there are no significant differences between the researched batches (Figure 10). The non-parametric Conover test showed that there were no differences between batches.

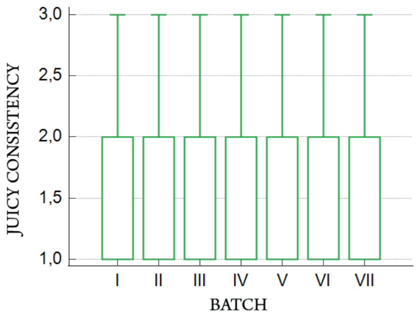

Figure 10. Multiple comparison graph - consistency friability

Assessing consumer perception - texture consistency

The Shapiro-Wilk test showed an abnormal distribution ($P<0.0001$) and Kruskal-Wallis test ($P = 0.000791$), there are no significant differences between the researched batches (Figure 10). The batches B1 and B2 had a smooth texture, meanwhile B3-B7 a hard consistency.

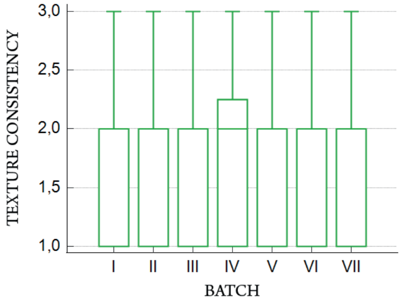

Figure 11. Multiple comparison graph - texture consistency

The non-parametric Conover test reveals the differences among the batches as follows in Table 7.

Table 7. Differences among batches texture consistency

Batch	n	Different (P<0.05) from batch no.
B1	37	B3, B4, B5, B6, B7
B2	37	B4, B6, B7
B3	37	B1
B4	37	B1, B2
B5	37	B1
B6	37	B1, B2
B7	37	B1, B2

CONCLUSIONS

After evaluating consumer perception using the 1-9 scale, although there were no significant differences between the tested treatments, there was an obvious variability in the results.

The most favourable responses were recorded for the sausages with 2.5% chokeberry, followed by the sausages with 1.2% chokeberry.

As an important feature, it is the colour change, appreciated by consumers.

The most unfavourable responses were received following the evaluation of the sausage samples with 5% blueberry extract which gave a sweet taste uncharacteristic of meat products.

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COMBATING FOOD WASTE BY MAKING A FUNCTIONAL PRODUCT FROM A SLAUGHTERHOUSE BY-PRODUCT AND PROFILING ITS NUTRITIONAL VALUE

Ioana GUCIANU, Florin-Daniel LIPȘA, Bianca Georgiana ANCHIDIN, Elena-Iuliana FLOCEA, Paul Corneliu BOIȘTEANU, Marius Mihai CIOBANU

"Ion Ionescu de la Brad" Iasi University of Life Sciences,
3 Mihail Sadoveanu Alley, 700490, Iasi, Romania

Corresponding author email: marius.ciobanu@iuls.ro

Abstract

The main objective of the current study was to use a by-product from the deboning process of poultry meat and use it to produce products with high nutritional value. Four product assortments (chicken and turkey soup concentrate and chicken and turkey stock respectively) were produced using bone tissue of the two species as biological material. It was found that there were no significant differences in the technological process to obtain them, the most varied differences being evident in the raw chemical composition, where chicken broth concentrate had the highest percentages of water (69.5%), protein (20.1%) and collagen (18.2%) compared to chicken stock where a higher rate of fat (17.6%) was evident. In the case of turkey backbone products, the highest percentage of fat was found in the concentrated broth, with the stock having the highest percentages of water (65.2%), protein (18.8%), and collagen (17%). The color characteristics are closely related to the Maillard reaction resulting from the cooking operation of the biological material, resulting in products with a positive sensory appearance and microbiological safety.

***Key words**: bone stock, circular economy, concentrated soup, food waste.*

INTRODUCTION

Food waste is a major concern for global food security because it has a significant economic, environmental, and social impact. The circular economy is a key solution to the problem of secondary flows, as it promotes the reuse of resources and minimization of waste. The circular economy is a system aimed at stabilizing economic growth through improved management and more efficient use of by-products while minimizing waste. In particular, the concept of 'end-of-life' is replaced by the reuse of waste from industrial production to create additional value. In this context, "industrial symbiosis" seems to be the best way to achieve a circular economy. One of the main problems is related to the lack of a homogeneous and standardized separate collection of biowaste at the national level, which makes the recovery of bones, skin, and food waste from the processing of chicken carcasses a difficult objective (Bux & Amicarelli, 2022).

Globally, meat consumption is on the rise due to population and income growth. The vertically integrated poultry meat rearing and slaughtering sector is the most used sector from processing to consumption, (Ciobanu et. al., 2019a). According to the National Institute of Statistics (INS), poultry meat production in Romania increased in 2023 by 5.5% compared to 2022, totaling 539,867 tons. In January 2024, 28.383 million birds were slaughtered, a significant increase compared to January 2023 with a total of 26.880 tons heads and compared to December 2023 when 24.963 million heads of poultry were slaughtered.

The meat supply chain generates waste and food losses at different stages. However, a considerable amount is produced during the slaughtering stage where the animal is transformed into edible and inedible parts, resulting in numerous animal offal (Karwowska et al., 2021; Ciobanu et al., 2019b). Slaughterhouses are a major contributor to the overall problem of food losses, and with population growth, industrialization, and urbanization, the management of animal by-products from these facilities becomes a huge challenge.

The OECD-FAO Agricultural Outlook projects an increase in global meat production, with pork leading the way as the second-largest meat category after poultry (OECD/FAO, 2021).

According to Song et al. (2016), after deboning and packing meat for retail sale, based on carcass weight, about 6-12% of bones remain. These bones are considered to be notable products to be transformed into high-value-added products by applying effective strategies to ensure sustainability (Toldora et al., 2021). Thus, one of the most effective strategies to valorize bones is the production of bone concentrates and bone stock. Concentrated bone broth is popular due to its rich nutrients and unique flavors (Meng et al., 2022).

MATERIALS AND METHODS

The stages of the present work were carried out at the Iasi University of Life Sciences (IULS) in Iasi, within the Department of Food Technologies, with the actual research activity being carried out in the Meat Processing Workshop, the Meat and Meat Products Technology Laboratory, and the Microbiology Laboratory. The biological material for analysis was purchased from different processing plants in Romania and consisted of poultry backbones from two different species *Gallus gallus domesticus* and *Meleagris gallopavo domesticus*. The main objective of the research was to obtain four functional products (chicken soup concentrate and turkey stock) from the two species, following characteristics related to the technological flow, nutritional, color, and microbiological characteristics of the products obtained. The technological flow applied included the stages of cooking, pressure cooking, the addition of vegetable mass (in the case of stock products), filtering, dosing, and sterilization of the product obtained. The heat treatment parameters applied are described in Table 1.

Table 1. Heat treatment parameters applied

Parameter	Temperature (ºC)	Time (hours)
Baking	140^0C	1
Boiling	110^0C	12

To observe the extraction capacity of organic compounds from the bone structure of the two bird species, the raw chemical composition was determined by monitoring the percentage of protein, collagen, and other indices such as moisture and fat. Chemical composition was determined by applying a versatile near-infrared (NIR) spectrophotometric method using the Food Check meat analyzer. A digital pH-meter for high-viscosity products (testo 206-pH2) was used to determine the pH of the four products obtained. The color characteristics were analyzed applying the CIEL*a*b* system using the Konica Minolta Chroma Meter CR-410 color analyzer, measuring the parameters of lightness (L*), red-green (a*), and yellow-blue (b*) color (Ciobanu et al., 2013). The calculation of losses incurred during the technological flow of obtaining was calculated according to the formula presented by Boișteanu et al. (2023).

$$\text{Cooking loss (\%)} = \frac{weight\ of\ raw\ sample - weight\ of\ cooked\ sample}{weight\ of\ raw\ sample} \times 100$$

The microbiological determinations applied to the four products consisted of determining the total aerobic bacterial count (TAPC), the microbial load of *Staphylococcus aureus*, *Escherichia coli,* and the presence or absence of *Salmonella*. Rapid culture media were used, with dilutions up to 10-3 with a 24-hour incubation period.

RESULTS AND DISCUSSIONS

Table 2 reports the losses incurred during the heat treatment steps applied to produce the four samples. It can be seen that in the cooking stage, the percentage losses were higher in the chicken concentrate with a percentage of 25%, and in the boiling stage, the highest percentages were found in the turkey concentrate (90.14%) and also in the turkey stock (56.6%).

The distribution of the numerical raw chemical composition data was determined using IBM SPPS software by applying an independent sample t-test to determine if there were significant differences between the means of the measured variables for the two independent groups (concentrate and stock), the test being applied to the generated results of the samples from the two species *Gallus gallus domesticus* and *Meleagris gallopavo domesticus*. (Table 3)

Table 2. Heat treatment losses

Parameter	CC	SC	TC	TS
Initial weight of biological material (kg)	13.1	5.75	13.9	5.3
cooking losses (%)	25	-	18	-
Vegetable meal additive (kg)	-	10	-	10
Water added (L)	60		72	
Boiling losses (%)	84.9	43.86	90.14	56.6

CC - Chicken concentrate; SC - Chicken stock; TC - Turkey concentrate; TS - Turkey stock.

Table 3. Descriptive Statistics for *Gallus Gallus domesticus*

	Product	N	Mean	Std. Deviation	Std. Error Mean
Fat %	CC	5	9.700	0.0707	0.0316
	SC	5	17.620	0.4919	0.2200
Moisture %	CC	5	69.580	0.0837	0.0374
	SC	5	63.140	0.4506	0.2015
Protein %	CC	5	20.100	0.0000	0.0000
	SC	5	18.300	0.1000	0.0447
Collagen %	CC	5	18.260	0.0548	0.0245
	SC	5	16.420	0.1304	0.0583
S.U %	CC	5	30.420	0.0836	0.0374
	SC	5	36.860	0.4505	0.2014

CC - Chicken concentrate; SC - Chicken stock

Table 3 illustrates that chicken bone products' highest percentages of protein and collagen are found in chicken concentrate, with values of 20.1% for protein and 18.26% for collagen in dry matter. It is also highlighted that the percentages of dry matter of 30.42% in chicken concentrate and 36.86% in chicken stock are inversely proportional to the two indices highlighted, while the percentage of fat is directly proportional.

Table 4. Averages between comparison tests (Levene's Test and Independent Sample Test) for *Gallus gallus domesticus*

		Levene's Test for Equality of Variances		t-test for Equality of Means							
						Significance				95% Confidence Interval of the Difference	
		F	Sig.	t	df	One Sided p	Two Sided p	Mean Diff.	Std. E. Diff.	Lower	Upper
Fat %	1	24.647	0.001	-35.634	8	<.001	<.001	-7.9200	0.2223	-8.4325	-7.4075
	2			-35.634	4.165	<.001	<.001	-7.9200	0.2223	-8.5276	-7.3124
Moisture	1	12.980	0.007	31.424	8	<.001	<.001	6.4400	0.2049	5.9674	6.9126
	2			31.424	4.276	<.001	<.001	6.4400	0.2049	5.8852	6.9948
Protein	1	16.000	0.004	40.249	8	<.001	<.001	1.8000	0.0447	1.6969	1.9031
	2			40.249	4.000	<.001	<.001	1.8000	0.0447	1.6758	1.9242
Collagen	1	4.356	0.070	29.093	8	<.001	<.001	1.8400	0.0632	1.6942	1.9858
	2			29.093	5.369	<.001	<.001	1.8400	0.0632	1.6807	1.9993
S.U. %	1	12.979	0.006	-31.423	8	<.001	<.001	-6.4399	0.2049	-6.9125	-5.9674
	2			-31.423	4.275	<.001	<.001	-6.4499	0.2049	-6.9948	-5.8851

1 - Equal variances assumed; 2 - Equal variances not assumed.

The Levene test was applied to identify the equality of variances between the two products obtained from *Gallus gallus domesticus*. F-values correlated with p-values indicate significant values for fat, water, and protein indices. In the case of collagen percentage

F-value = 4.356 and p-value = 0.070 noting that the two values are insignificant (Table 4). Regarding the T-test, it can be observed that there are significant values for all the determined indices (p-value being < 0.001).

Table 5. Descriptive Statistics for *Meleagris gallopavo domesticus*

	Product	N	Mean	Std. Deviation	Std. Error Mean
Fat %	TC	5	17.880	0.4764	0.2131
	TS	5	15.040	0.2793	0.1249
Moisture %	TC	5	63.000	0.3674	0.1643
	TS	5	65.260	0.2408	0.1077
Protein %	TC	5	18.180	0.1095	0.0490
	TS	5	18.880	0.0837	0.0374
Collagen %	TC	5	16.220	0.1643	0.0735
	TS	5	17.000	0.1000	0.0447
S.U. %	TC	5	37.000	0.3674	0.1643
	TS	5	34.740	0.2408	0.1077

TC - Turkey concentrate; TS - Turkey stock.

While in chicken bone products the highest values were found in concentrate, in turkey bone products the highest percentages of protein and collagen were found in turkey stock. Thus the value of the percentage of protein about dry matter (34.74%) is 18.88% and the value of collagen is 17%. In this case, too, the percentages of the indices analyzed are inversely proportional to the S.U. %, with only fat being directly proportional (Table 5).

Table 6. Averages between comparison tests (Levene's Test and Independent Sample Test) for *Meleagris gallopavo domesticus*

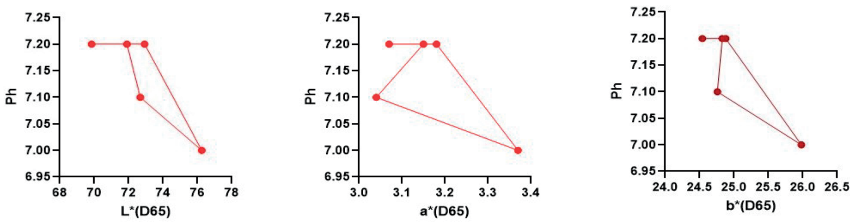
		Levene's Test for Equality of Variances		t-test for Equality of Means							
		F	**Sig.**	**t**	**df**	**Significance**		**Mean Diff.**	**Std.Error Diff.**	**95% Confidence Interval of the Difference**	
						One Sided p	**Two Sided p**			**Lower**	**Upper**
Fat %	1	7.910	.023	11.499	8	<.001	<.001	2.8400	.2470	2.2705	3.4095
	2			11.499	6.459	<.001	<.001	2.8400	.2470	2.2459	3.4341
Moisture %	1	5.744	.043	-11.503	8	<.001	<.001	-2.2600	.1965	-2.7131	-1.8069
	2			-11.503	6.901	<.001	<.001	-2.2600	.1965	-2.7259	-1.7941
Protein %	1	2.169	0.179	-11.355	8	<.001	<.001	-7.000	.0616	-.8422	-.5578
	2			-11.355	7.482	<.001	<.001	-7.000	.0616	-.8439	-.5561
Collagen %	1	6.649	.033	-9.067	8	<.001	<.001	-7.800	.0860	-.9784	-.5816
	2			-9.067	6.606	<.001	<.001	-7.800	.0860	-.9859	-.5741
S.U. %	1	5.743	0.04	11.503	8	<.001	<.001	2.260	.1965	1.8069	2.7130
	2			11.503	6.901	<.001	<.001	2.260	.1964	1.7940	2.7259

1 - Equal variances assumed; 2 - Equal variances are not assumed.

For the crude chemical composition of the batches obtained from turkey back, Table 6 shows that the F-values correlated with p-values indicate significant values for the fat, water, and collagen indices, and the F-value = 2.169 correlated with p = 0.179 of the protein index being insignificant. The t-test confirms (p <.001) that all the values of the indices taken in the analysis are significant.

Table 7. Correlation Pearson L*, a*, b* with pH for Chicken Concentrate

Pearson r	L*	a*	b*
r	-0.8473	-0.6346	-0.8552
95% confidence interval	-0.9897 to 0.1386	-0.9724 to 0.5628	-0.9903 to 0.1102
R squared	0.7179	0.4027	0.7314
P-value			
P (two-tailed)	0.0700	0.2501	0.0647
P-value summary	ns	ns	Ns
Significant? (alpha = 0.05)	No	No	No
Number of XY Pairs	5	5	5

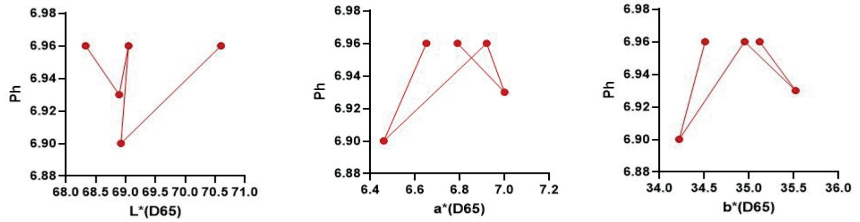


a. b. c.

To determine the correlation between color degree and pH index, the Person correlation was applied. For color determinations, 5 readings were taken for each product and one reading was taken for pH determination. From Table 7 it can be seen that the r-value of the brightness, color grade red-green, and yellow-blue show an insignificant correlation for the chicken concentrate sample (L* = -0.8473, a* = -0.6346, b* = -0.8552) (Table 7). It can be observed that concentrated soups (chicken and turkey) have higher brightness (L*) and pH values, while stocks (chicken and turkey) have lower brightness (L*) and pH values. Thus, the addition of vegetables improved the color of the product but led to a decrease in pH value.

Table 8. Correlation Pearson L*, a*, b* with pH for Chicken Stock

Pearson r	L*	a*	b*
r	0.2440	0.4820	0.3459
95% confidence interval	-0.8134 to 0.9268	-0.6964 to 0.9572	-0.7720 to 0.9410
R squared	0.05954	0.2324	0.1196
P-value			
P (two-tailed)	0.6924	0.4109	0.5685
P-value summary	ns	ns	ns
Significant? (alpha = 0.05)	No	No	No
Number of XY Pairs	5	5	5

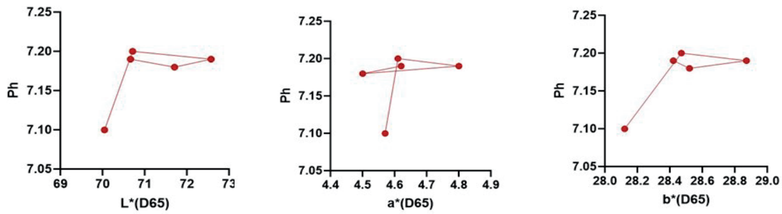


a. b. c.

The chicken stock showed significant correlation values between the two determinations, with R-values being positive for all determinants, as shown in Table 8.

Table 9. Correlation Pearson L*,a*,b* with pH for Turkey Concentrate

Pearson r	L*	a*	b*
r	0.5440	0.3083	0.7286
95% confidence interval	-0.6505 to 0.9637	-0.7884 to 0.9360	-0.4303 to 0.9805
R squared	0.2959	0.09503	0.5308
P value			
P (two-tailed)	0.3432	0.6138	0.1627
P value summary	ns	ns	Ns
Significant? (alpha = 0.05)	No	No	No
Number of XY Pairs	5	5	5

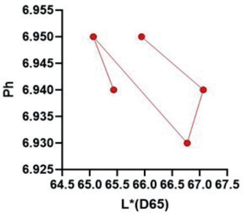


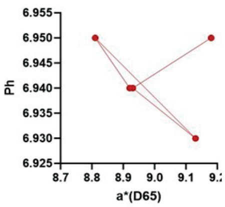
a. b. c.

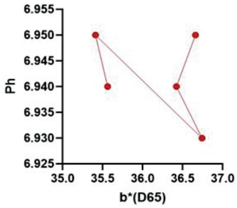
Concentrated turkey soup also (Table 9) shows significant values for the Person correlation coefficient (r) (L* = 0.5440, a* = 0.3083, b* = 0.7286)

Table 10. Correlation Pearson L*,a*,b* with pH for Turkey Stock

Pearson r	L*	a*	b*
r	-0.6377	-0.2578	-0.3941
95% confidence interval	-0.9727 to 0.5591	-0.9288 to 0.8083	-0.9471 to 0.7484
R squared	0.4067	0.06644	0.1553
P value			
P (two-tailed)	0.2470	0.6755	0.5115
P value summary	ns	ns	Ns
Significant? (alpha = 0.05)	No	No	No
Number of XY Pairs	5	5	5







Also in the turkey stock samples (Table 10), the Person correlation coefficient value (r) indicates insignificant values of the relationship between the color indices and the pH value of the analyzed sample (L= -0.6377, a*= -0.2578, b*= -0.3941).

Table 11. Results of microbiological determinations

Product	Dilution	PCA CFU/mL	*Staphylococcus aureus* CFU/mL	*Escherichia coli* CFU/mL	*Salmonella* (CFU/25mL)
			Logarithm number		
CC	10^{-2}	3.778151	2.30103	0	Negative
CC	10^{-3}	4.414973	3.60206	0	Negative
SC	10^{-2}	3.322219	0	0	Negative
SC	10^{-3}	4.30103	0	0	Negative
TC	10^{-2}	3.612784	0	0	Negative
TC	10^{-3}	3.477121	0	0	Negative
TS	10^{-2}	3.880814	2.477121	0	Negative
TS	10^{-3}	5.113943	3.477121	3.477121	Negative

The values of microbiological results for concentrates and stocks made (Table 11), expressed in logarithmic numbers, did not exceed the limits imposed by Ordin 976 of 29 December 2015, according to Manoliu et al. (2023).

CONCLUSIONS

Turning the bones obtained from the deboning process of poultry meat into a functional product with high nutritional value is based on the process of combating food waste and profiling the circular economy. These products can have a wide use both in the technological field, through their additive in different meat preparations presenting superior nutritional characteristics with a maximum value of 20.1% protein percentage evidenced in concentrated chicken soup and a minimum of 18.1% found in concentrated turkey soup. They can also be used in the HoReCa field as a cooking base thanks to the vegetable addition of 10%, this addition enhances the taste and sensory aspect of the cooked dishes. The sensations caused by different factors produce a complete perceptual experience resulting in the final consumer response (Ciobanu et al., 2023). At the same time, the collagen found in these products can easily replace thickening additives used in the meat processing industry as well as in various soups, sauces, etc., being safe products with low microbiological activity.

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EFFECTS OF MEAT CONSUMPTION ON CONSUMERS' HEALTH

Daniela IANIȚCHI, Paula POȘAN, Iuliu Gabriel MALOȘ, Lucica NISTOR, Marius Laurian MAFTEI, Carmen Georgeta NICOLAE, Ionela Florentina TOMA (ENACHE), Camelia HODOȘAN

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, Bucharest, Romania

Corresponding author email: paula.posan@usamv.ro

Abstract

Meat is a major part of human diets for centuries, a source of protein and essential nutrients, but concerns have been raised about the potential negative health impacts associated with high meat consumption. This review paper aims to provide an overview of the current scientific evidence on the effects of meat consumption on consumer health. The review covers the evidence on the relationship between meat consumption and various health outcomes, including cardiovascular disease, cancer, type 2 diabetes, and obesity. The review discusses the potential for promoting healthy meat consumption patterns and recommendations for consumers and policy-makers to reduce meat consumption and promote healthier dietary choices. Overall, the review highlights the complex relationship between meat consumption and consumer health, and the need for further research to better understand this relationship and inform public health policy and dietary guidelines.

***Key words**: healthy food, meat processing, nutritional quality.*

INTRODUCTION

Meat represents an important source of high-quality dietary protein for a large proportion of the global population. Meat consumption is a highly debated topic worldwide, with concerns about its impact on human health, the environment, and animal welfare. Meat is a major source of protein and micronutrients, including iron, zinc, selenium, vitamin D and vitamin B12, in many diets, but overconsumption has been linked to various health issues, including heart disease, stroke, cancer, and diabetes (Salter, 2018).

Meat consumption trends vary greatly across the globe. Furthermore, some individuals choose to either avoid meat altogether or certain types of meat for a variety of reasons, such as ethical or religious reasons, or because of socio-economic factors

For many in the developed world, meat is readily available and affordable, and represents a routine component of their diet. Consumption of even relatively small amounts of meat and other animal products can have a major impact on preventing protein-energy malnutrition, iron deficiency, anaemia and vitamin (Smith et al., 2013). At a global level, according to the Food and Agriculture Organization (FAO), global meat production has tripled over the past four decades, and it is projected to continue increasing in the coming years. This trend raises concerns about the environmental impact of meat production, including greenhouse gas emissions and water consumption, as well as the potential health consequences of increased meat consumption. Of the emission of greenhouse gas pollutants produced in the livestock sector, 80% comes from the growth of ruminants (Marin et al., 2020).

Meat consumption has been steadily increasing over the past few decades, with a significant increase reported in developing countries and is expected to double by 2050.

This trend is concerning given the potential health impact of consuming excessive amounts of meat, particularly fatty meat. As such, there is a growing need for research to examine the relationship between fatty meat consumption and health outcomes in order to inform public health policies and dietary guidelines

In Europe, meat consumption varies widely between countries and regions, with some countries consuming more than others.

According to Eurostat, the EU statistical office, in 2023, the average per capita meat consumption in the European Union was 66.13 kg, with the highest consumption reported in Luxembourg (107.9 kg) and the lowest in Albania (41 kg). In Romania, in 2021, cattle provided 4.69 kg of meat per capita, pigs provided 13.72 kg of meat per capita, while sheep and goat species contributed 5.84 kg of meat per capita, goats providing less than 2 kg of meat per capita (Maloş & Maloş, 2022).

Also, world carcass meat production that includes both commercial and farm slaughter has increased steadily from 1990 to the present (Figure 1).

Studying the effects of fatty meat consumption on consumer health is important because meat is a significant source of dietary fat, and excessive consumption of fatty meat has been linked to various health issues. The high fat content in meat, particularly saturated and trans fats, has been associated with increased risk of cardiovascular disease, obesity, type 2 diabetes, and certain types of cancer (Battaglia et al., 2015). A hypocaloric alternative to meat products can be obtained by including vegetable derivatives (soy, starch, chickpea, etc.) in the meat compositions, with the obtaining of nutritionally enriched products and sensorially close to the classic ones, at the same time capitalizing on secondary products of the food industry (Ianiţchi et al., 2023).

Meat can also be a source of contamination with microorganisms, the most important being raw meat, thermally processed meats having a much reduced microbial contamination (Şuler et al., 2021).

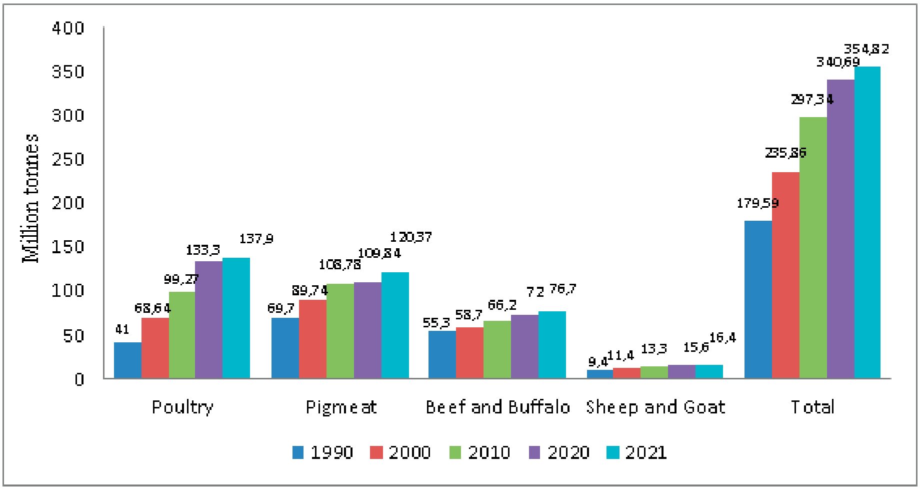

Figure 1. Meat production (carcass weight) in the period 1990-2021 (Ritchie et al., 2017)

MATERIALS AND METHODS

The paper aims to contribute to the review of various studies on the impact of meat and especially red and fatty meat consumption on humans' health. The study considered the follow-up of research published in the last years. Works published in open access scientific journals from databases such as Web of Science, ResearchGate and Google Scholar were studied.

The effects of the consumption of fatty meat on the health of consumers have been studied in order to find explanations for the various disorders that appear depending on the diet. Knowing these effects, many of them negative, it is necessary to look for alternatives in order to combat the associated diseases. In addition to some diets based on the predominant consumption of vegetables and fruits, an alternative can be obtaining an innovative preparation, made of minced beef, with added fiber. This low-calorie product, therefore with less fat, can contribute to reducing the occurrence of coronary diseases, obesity and diabetes.

The paper can be a basis for studies aimed at replacing conventional meat and meat products with alternative improved meat products.

RESULTS AND DISCUSSIONS

Following the bibliographic study carried out in the paper, it was established that the consumption of certain categories of meat and meat products is widespread throughout the world, both in developed and less developed countries, at any level and social status. Also, the consumption of these meat products can lead to the appearance of diseases under certain conditions and under the influence of certain factors.

Definition and types of meat

Fatty meat refers to types of meat that have a high fat content. These types of meat are often associated with a richer flavor and juicier texture, but can also have negative health effects if consumed in excess.

There are several types of fatty meat, including:

- Beef: Certain cuts of beef, such as ribeye, short ribs, and brisket, are high in fat content.
- Pork: Pork can be particularly fatty, especially cuts like bacon, pork belly, and sausage.
- Lamb: Lamb can also be high in fat, particularly the shoulder and leg.
- Poultry: While poultry is typically considered a leaner source of protein, some cuts like chicken wings and thighs can be high in fat content.
- Processed meats: Processed meats such as hot dogs, sausages, and deli meats are often high in fat content and may also contain added preservatives and chemicals.

It's important to note that not all fat in meat is unhealthy - some types of fat, like monounsaturated and polyunsaturated fats, can have positive health effects in moderation.

"Red meat" refers to beef, pork, lamb, goat meat that comes from domestic animals, including that contained in processed foods and most beef burgers. This category does not include poultry, wild game, or offal (however, the impact of organ consumption and wild game consumption on cancer risk is unknown). Although eating red meat in large amounts increases the risk of developing bowel cancer, red meat is also a good source of nutrients. In principle, it is recommended to avoid eating more than about 500 grams of red meat per week (500 grams of cooked meat, which is equivalent to about 700-750 grams of raw meat, depending on how is cut and cooked).

"Processed meat" refers to meat preserved by smoking, aging/maturing, salting or the addition of chemical preservatives. This category includes, for example, ham, bacon, salami and some sausages, such as frankfurters. As the consumption of processed meat is also positively associated with the risk of bowel cancer, even in smaller amounts, and does not provide any additional nutritional benefit over red meat, it is recommended to avoid processed meat as much as possible, for reducing the risk of cancer. (Linseisen et al., 2002; IARC, 2016).

The nutrient content and nutritional value of meat

The nutritional value of meat varies considerably, depending on the species, breed and even geographic region or country.

The chemical composition of meat is different within the same species, varying from one individual to another, depending on the ratio between different tissues (muscle tissue/adipose tissue), the age of the animal, the season of the year, the animal diet and state of fattening.

Also, differences appear depending on the particular cut of meat, the method of preservation and the method of cooking.

Some of the key nutrients found in meat include:

- protein: meat is a high-quality source of protein, which is important for building and repairing tissues in the body;
- iron: meat is a particularly rich source of heme iron, which is more easily absorbed by the body than non-heme iron found in plant-based foods. Iron is important for the production of hemoglobin, which carries oxygen in the blood;
- zinc: meat is also a good source of zinc, which is important for immune function and wound healing;
- B vitamins: Meat is one of the few dietary sources of vitamin B12, which is only found in animal-based foods and is essential for the proper functioning of the nervous system and the formation of red blood cells. Meat is a rich source of several B vitamins, such as thiamin, riboflavin, and niacin, which are important for energy metabolism.

Red meat contains high biological value protein and important micronutrients, all of which are essential for good health throughout life. Meat is a source of fat and contributes to intake of saturated fatty acids (SFAs). However, meat contains a range of fatty acids, including the essential omega-6 (n-6) and omega-3 (n-3) poly-unsaturated fatty acids (PUFAs) [linoleic and a-linolenic acids (ALNAs)] (Wyness et al., 2011).

The water content of meat varies inversely proportional to that of fat. In cattle it is 60-76%, in pigs 51-73%, in sheep 53-74%, in chickens 65.5-71%, in turkeys 60.0-69%, and in game between 69 and 74%.

The content of protein substances varies depending on the species and the state of fattening, being higher in poultry meat (12-24%) and moderate in the meat of slaughter animals (15-21%).

It was believed about meat that it would be the most protein food, but scientific data come to disprove these outmoded statements, as follows: meat contains 17-25% protein, while soy extract - 94%, tofu sheets - 54%, dried soy beans - 35%, seaweed - 35%, pumpkin seeds - 29%, chickpeas 28%, peanuts 26%, lentils 24%, sunflower seeds 24%, almonds 20%, barley 13%;

The lipid content varies depending on the state of fattening: cattle 3.0-20.0%; pigs 3.0-34%; sheep 3.7-26.0%; chickens 6.9-13.7%, ducks 23-37.0%, domestic rabbit about 10%. They are mostly composed of neutral glycerides and small amounts of phospholipids 0.5-0.9% (lecithins, cephalins, etc.) and steroids (cholesterol) about 0.8%.

The content of nitrogenous substances is 1-1.7% and consists of: amino acids, dipeptides (carnosine, anserine), tripeptides (glutathione), carnitine, nucleotides, purine bases (xanthine, hypoxanthine, and uric acid), creatine, and phosphocreatine.

The content of non-nitrogenous extractive substances is 2-3% and is mainly represented by glycogen, as a reserve of carbohydrates in the liver and in small quantities as a source of "immediate" energy in the muscles, followed by inositol, glucose, lactic acid, formic acid, malic acid.

The content of mineral substances in meat varies between 0.7-1.5%. The composition of mineral salts in meat includes: potassium (0.3-0.35%), iron (0.1-0.22%), phosphorus, sulfur, sodium (0.4-0.7%), magnesium and smaller amounts of calcium, etc.; the presence of easily assimilable iron from meat favors the formation of red blood cells.

The content of vitamins in meat is variable, being influenced by the same factors mentioned in the chemical composition.

Thus, vitamin A is found in the liver; vitamin B1 especially in the liver, heart, kidneys and muscles, vitamin B2 in the liver, kidneys, heart; vitamin B6 in cattle liver, pork and cow muscle, heart; vitamin PP in the liver, kidneys and muscles; pantothenic acid in the liver, kidneys and muscles, brain and heart; folic acid and vitamin B12 predominate in the liver. Vitamins C, D and E are found in meat in small quantities.

Table 1 provides some examples of the major macronutrient content of some common, relatively lean cuts of grilled meat, compared to a variety of non-meat, plant-based staples.

It is clear that, compared to carbohydrate-rich plant foods, all these meats are protein-rich. The red meats are also richer in total and saturated fats. By contrast, chicken is relatively low in fat. However, meat is not only rich in protein, but the protein is generally of higher quality (i.e. contains more essential amino acids) than plant equivalents. Perhaps unsurprisingly, as it comes from the muscle of other animals, it contains all the essential amino acids, in the appropriate proportions, to fulfil the nutritional requirements of humans.

Fatty meat is a type of meat that has a higher fat content compared to lean meat. Generally, it contains higher amounts of saturated fat and cholesterol compared to lean meat.

Saturated fat is a type of fat that is typically solid at room temperature and is commonly found in animal products. Consuming high amounts of saturated fat has been associated with an increased risk of heart disease and stroke. Fatty meat, particularly red meat, is a major source of saturated fat in the diet.

Cholesterol is a type of lipid or fat that is present in animal foods, including meat. It plays an important role in the body, but high levels of cholesterol in the blood have been linked to an increased risk of heart disease.

Fatty meat, particularly organ meats like liver and kidney, are particularly high in cholesterol. The nutrient content of different types of fatty meat can vary. For example, a 100-gram serving of cooked beef brisket contains about 14 grams of fat, while the same serving size of cooked pork belly contains about 30 grams of fat. Similarly, a 100-gram serving of cooked chicken thigh with skin contains about 10 grams of fat, while the same serving size of cooked duck with skin contains about 25 grams of fat.

In their paper Wyness et al., 2011 notes that while red meat is a good source of important nutrients such as protein, iron, and zinc, consuming high amounts of red meat, particularly processed meats, has been associated with an increased risk of several chronic diseases. For example, studies have found that consuming more than 100 grams of red meat per day is associated with an increased risk of colorectal cancer, as well as cardiovascular disease.

Table 1. Macronutrient content of various meats compared to some plant derived foods (Roe, 2015)

Meet	Energy, KJ/100 g	Protein	Fat	SFA	Carbohydrate	Reviewed
			g/100 g			
Beef	745	31.0	5.9	2.5	0	Reviewed 2013. LGC, Nutrient analysis of carcase beef, 1992-1993; ISO/IEC 17043:2010
Lamb	1,268	26.5	22.1	10.5	0	Reviewed 2013. LGC, Nutrient analysis of retail cuts of lamb, 1993-1994;
Pork	1,078	27.5	16.5	5.7	0	LGC, Nutrient analysis of retail cuts of pork, 1992-1993
Chicken	626	32.0	2.2	0.6	0	Reviewed 2013. LGC, Nutrient analysis of chicken and turkey, 1994-1995
Bread	931	7.9	1.6	0.3	42.7	LGC, Nutrient analysis of bread and morning goods, 1999; and industry data, 2013
Potatoes	413	2.5	0.2	0.1	21.2	DH, Nutrient analysis of fruit and vegetables, 2013
Rice	560	2.8	0.4	0.1	31.1	LGC, Nutrient survey of flours and grains, 2005

KJ: kilojoules
SFA: Saturated fatty acids
Values are per 100 g serving of grilled meat (beef rump steak, lamb loin chop, pork loin chop or chicken breast), white bread, baked potato or boiled rice.

Overall, the paper concludes that while red meat can be part of a healthy diet, it's important to consume it in moderation, and to choose lean cuts and healthy cooking methods. The paper also recommends increasing the consumption of plant-based foods, such as fruits, vegetables, and whole grains, which are associated with a range of health benefits.

It is also important to note that not all meats are equally nutritious. For example, processed meats, such as bacon, sausage, and deli meats, are often high in sodium, preservers, stabilizers and other food additives, which can contribute to negative health effects. Red meat, such as beef, pork, and lamb, has been associated with an increased risk of several chronic diseases, such as heart disease and certain types of cancer, when consumed in high amounts, but this also depends to a large extent on the way the meat is prepared (Pereira et al., 2013).

The consumption of red and fatty meat has been linked to several potential health hazards (Wok, 2016). Here are some of the most notable:

1. Cardiovascular disease: High intake of red meat, particularly processed meat, has been associated with an increased risk of cardiovascular disease, such as heart disease and stroke. The high levels of saturated fat and cholesterol in red meat can contribute to the build-up of plaque in the arteries, leading to reduced blood flow and increased risk of heart attack and stroke.
2. Colorectal cancer: Regular consumption of red meat has been linked to an increased risk of colorectal cancer. The potential mechanism for

this relationship may be related to the presence of harmful compounds formed during cooking or processing of meat, as well as the heme iron and saturated fat content of red meat.

3. Type 2 diabetes: Eating large amounts of red meat has been associated with an increased risk of type 2 diabetes, likely due to the high levels of saturated fat and heme iron in red meat, as well as its impact on insulin resistance and inflammation (Salter, 2013).

4. Obesity: A high intake of red meat, particularly processed meat, has been linked to an increased risk of obesity. Red meat is often high in calories and saturated fat, which can contribute to weight gain.

5. Inflammatory bowel disease: Some studies have suggested that high consumption of red meat may increase the risk of inflammatory bowel disease, including Crohn's disease and ulcerative colitis.

Some studies have found a positive association between meat consumption and these diseases, the evidence is not consistent and other factors, such as overall dietary patterns and lifestyle behaviors, may play a role (Williamson et al., 2005)

The articles also reviews the potential health benefits of consuming meat, such as improved cognitive function, and the importance of choosing lean cuts of meat and cooking methods that minimize the formation of harmful compounds.

Meat consumption and obesity and risk of nonalcoholic fatty liver disease

Meat consumption, including both unprocessed and processed red meat, was associated with significantly increased risk of developing NAFLD. NAFLD is a common condition in which excess fat accumulates in the liver, and it has been associated with an increased risk of liver damage and other health problems. This association was mediated largely by obesity

There are several mechanisms through which fatty meat consumption may contribute to weight gain and obesity:

1. High calorie density: Fatty meat is energy-dense and high in calories, meaning it provides a lot of calories in a small amount of food. This can lead to overconsumption of calories, which can contribute to weight gain.

2. Low satiety: Fatty meat may not be as satiating as other types of protein sources, such as lean meat or plant-based proteins. This can lead to overconsumption and increased calorie intake.

3. Impact on gut microbiota: Some research suggests that high intake of animal-based products, including fatty meat, can negatively affect the composition of the gut microbiota. This can lead to increased inflammation and insulin resistance, which can contribute to weight gain and obesity.

4. Hormonal changes: Fatty meat consumption may also impact hormone levels, including insulin and leptin. Insulin resistance can contribute to weight gain, while low levels of leptin, which regulates appetite, can lead to overconsumption of food.

5. Processed meat: Some types of fatty meat, particularly processed meats such as bacon, sausages, and hot dogs, may contain additives and preservatives that can contribute to weight gain and other negative health outcomes.

Overweight and obesity is an increasing public health problem worldwide, affecting people of all ages (both adults and children) and socio-economic groups. Globally, 57.8% of adults are estimated to have obesity by 2030 (Kelly et al., 2008).

Obesity is a condition in which an individual has an excess amount of body fat. It is typically defined as having a body mass index (BMI) of 30 or higher. BMI is a measure of body weight relative to height, calculated by dividing a person's weight in kilograms by their height in meters squared.

Obesity is a major public health concern, as it increases the risk of many serious health problems. These can include type 2 diabetes, high blood pressure, heart disease, stroke, certain types of cancer, and osteoarthritis. Obesity can also lead to psychological and social problems, such as low self-esteem and discrimination

Obesity is a complex disorder with a diverse range of causal factors, including genetics, lifestyle habits, and environmental factors (Katz, 2016). Diet, as an environmental factor, is one of the most important contributors to the obesity pandemic (Hill et al, 2000). Meats are a part of the human diet, which not only provide protein and high-quality nutrients, but also are a main source of saturated fatty acids and cholesterol (Dabbagh-Moghadam et al., 2017). Some of the most significant lifestyle factors that can contribute to obesity include consuming a high-calorie diet, engaging in physical inactivity, and getting insufficient sleep.

For an individual to become obese, energy intake must be higher than energy expenditure for an extended period of time. This means that either more energy than needed is consumed and/or that too little energy is used by the body because of a lack of physical activity. In general, weight gain seems to be a result of a combination of both increased energy intake and decreased energy expenditure. The wide range of etiological factors makes obesity both a complex and challenging disorder (Jebb et al., 2007).

The report of Jebb et al. (2007) found that obesity was a major public health issue in the UK, with around one-third of the adult population classified as obese. The authors highlighted the significant health and economic costs associated with obesity, including increased risks of cardiovascular disease, type 2 diabetes, and certain types of cancer, as well as increased healthcare costs and lost productivity and recommended a range of interventions, including promoting healthier diets and physical activity, improving the food environment, and increasing access to weight management services.

Treatment for obesity typically involves a combination of dietary changes, increased physical activity, and behavioral therapy. In some cases, medications or weight loss surgery may also be recommended. Preventing obesity is an important public health goal, and can be achieved through promoting healthy eating habits, encouraging physical activity, and addressing environmental factors that contribute to the development of obesity.

After controlling for potential covariates including energy intake, age, marital status, gender, physical activity, supplement use, house possession, education, family size, current smoking, night shift working, history of thyroid disease and depression, and intakes of vegetables, legumes, nuts, fruits, whole grains, and dairy, some studies suggests that a diet high in poultry and white meat is positively associated with the odds of general obesity, while a diet high in processed meat is related to elevated odds of central obesity (Khodayari et al., 2022, Dabbagh-Moghadam et al., 2017).

In one study, Kim et al. (2022) analyzed data from more than 43,000 women who participated in the Nurses' Health Study II, a large, long-term study of women's health.

The participants completed food frequency questionnaires to assess their dietary intake, including their consumption of red meat, and were followed up for a period of more than 20 years to track the incidence of NAFLD. The study found that higher red meat consumption was associated with an increased risk of NAFLD, and this association was stronger among women who were overweight or obese. Specifically, women who consumed more than one serving of red meat per day had a 20% higher risk of developing NAFLD compared to women who consumed less than one serving per week. The association between red meat consumption and NAFLD was independent of other dietary and lifestyle factors, such as alcohol consumption and physical activity.

In the study of Recaredo et al. (2019), the authors investigated the association between different animal protein sources and liver status in obese subjects with non-alcoholic fatty liver disease (NAFLD). The study included 59 subjects with NAFLD who were divided into two groups based on their intake of animal protein from red meat, poultry, or fish. The researchers measured liver enzymes, insulin resistance, and other markers of liver health in the study participants.

The study found that the intake of red meat was associated with higher levels of liver enzymes, insulin resistance, and worse liver health, while the intake of poultry and fish did not show significant associations with liver status. The authors suggest that reducing red meat intake and increasing the consumption of poultry and fish may be beneficial for individuals with NAFLD.

The study by Noureddin et al. (2020) aimed to investigate the associations between dietary factors and nonalcoholic fatty liver disease (NAFLD) in an ethnically diverse population. The researchers analyzed data from the Multiethnic Cohort, a large population-based study of adults in Hawaii and Los Angeles. The study included 4,227 participants who completed a food frequency questionnaire and underwent abdominal computed tomography to assess liver fat content.

The results showed that participants who consumed the most fatty/red and processed meat had a 45% higher odds of NAFLD compared to those who consumed the least amount. On the other hand, participants who consumed the most fruits and vegetables had a 30% lower odds of NAFLD compared to those who consumed the least amount. The study also found that the association between meat and processed meat consumption and NAFLD was stronger in women than in men, and in those with a higher body mass index (BMI). The association between fruits and vegetables and NAFLD was stronger in men than in women, and in those with a lower BMI.

Another study aimed to investigate the association between red and processed meat consumption and non-alcoholic fatty liver disease (NAFLD) and insulin resistance. The study was conducted in Israel and included 789 adults who underwent abdominal ultrasound and blood tests. The results showed that high consumption of red and processed meat was significantly associated with NAFLD and insulin resistance, even after adjusting for potential confounders such as age, sex, smoking, physical activity, and calorie intake. The study concluded that reducing red and processed meat consumption may be an important strategy for preventing NAFLD and related metabolic disor-ders (Zelber-Sagi et al., 2018).

A 2014 systematic review and meta-analysis of 29 observational studies found that high consumption of processed meat was associated with a higher risk of obesity than high consumption of unprocessed red meat (Schwingshackl & Hoffmann, 2014).

Another study conducted by Lutsey et al. (2008) examined the relationship between meat consumption and body mass index (BMI) in a sample of over 9,000 middle-aged adults. The study found that individuals who consumed the highest levels of red meat had higher BMIs than those who consumed the least amount of red meat. Similarly, other studies found that high intake of meat and processed meat is associated with a higher risk of weight gain and obesity (Vergnaud et al., 2010; Rouhani et al., 2014).

In addition to observational studies, several randomized controlled trials have investigated the effect of reducing meat intake on weight and body composition. One such trial by Barnard et al. (2009) found that a plant-based diet led to significantly greater weight loss and reduction in BMI compared to a control group consuming a standard American diet that included meat.

The mechanisms underlying the association between fatty meat consumption and obesity are likely multifactorial. As mentioned earlier, the high calorie and saturated fat content of fatty meat may contribute to weight gain and obesity when consumed in excess. Additionally, some studies have suggested that heme iron, a type of iron found in red meat, may also play a role in the development of obesity through its impact on gut bacteria and inflammation (Tang et al., 2013).

A study by Babio et al. (2014) in Spain found that a high consumption of red and processed meat was associated with a higher risk of obesity, as well as other cardiometabolic risk factors. Similarly, a study by Zhu et al. (2019) in China found that higher intake of red and processed meat was associated with a higher risk of abdominal obesity in both men and women.

Fatty meat consumption and cardiovascular health

A number of studies (mostly in Europe and the United States of America) have suggested a link between red and/or processed meat consumption and the risk of CVD and that the greatest consumers of meat had a 40% increased risk of dying from CVD (Rohrmann et al., 2013; Koeth et al., 2013).

CVD is a group of disorders that affect the heart and blood vessels and includes conditions such as coronary heart disease, stroke, and peripheral arterial disease. Studies have shown that consuming high amounts of saturated fat, which is found in fatty meat, can raise blood cholesterol levels and increase the risk of CVD.

A mechanisms through which the consumption of fatty meat may contribute to the development of cardiovascular disease (CVD) include the high levels of saturated and trans-fats. Fatty meat is often high in saturated and trans-fats, which are known to increase levels of LDL cholesterol (the "bad" cholesterol) in the blood. This can lead to the formation of plaques in the arteries, which can increase the risk of heart disease and stroke. Fatty meat consumption may also contribute to an inflammatory response in the body, which has been linked to the development of CVD. The excess fat and cholesterol in the diet can activate immune cells, leading to the release of inflammatory molecules that can damage the lining of blood vessels.

Numerous studies have investigated the relationship between fatty meat consumption and cardiovascular disease (CVD). A meta-analysis of 20 prospective cohort studies found that high consumption of meat was associated with a significantly increased risk of CVD, including coronary heart disease and stroke (Wang et al., 2016). The study also showed that replacing meat with plant-based protein sources was associated with a lower risk of CVD.

Another study that analyzed data from over 400,000 participants found that high intake of meat was associated with an increased risk of CVD mortality, particularly among men (Zhong et al., 2020).

It is important to note that not all studies have found a clear link between fatty meat consumption and CVD. Some studies have suggested that the quality of the meat consumed, as well as the cooking method, may play a role in its potential health effects. However, overall, evidence suggests that consuming high amounts of fatty meat can increase the risk of CVD, and reducing its consumption may be beneficial for cardiovascular health.

The research article by Astrup et al. (2011) reviewed the available evidence on the relationship between saturated fat intake and the risk of cardiovascular disease (CVD) and concluded that reducing intake of saturated fat can lower the risk of CVD. The authors discussed the findings of several studies, which provided evidence for the role of saturated fat in the development of CVD. The authors also discussed the potential mechanisms by which saturated fat intake may increase the risk of CVD, such as raising LDL cholesterol levels and promoting inflammation. The article emphasized the importance of replacing saturated fat with healthy unsaturated fats, such as those found in nuts, seeds, vegetable oils, and fatty fish.

The study conducted by Appel et al. in 1997 was a randomized clinical trial that investigated the effects of dif-ferent dietary patterns on blood pressure. The participants were 459 adults with prehypertension or stage 1 hypertension, who were randomly assigned to one of three dietary groups: a control group that followed a typical American diet, a fruits and vegetables group that consumed a diet rich in fruits and vegetables but low in fat and dairy products, and a combination group that followed the fruits and vegetables diet as well as the DASH (Dietary Approaches to Stop Hypertension) diet, which is rich in fruits, vegetables, and low-fat dairy products and low in saturated and total fat. The study found that the combination group had the greatest reductions in blood pressure, followed by the fruits and vegetables group, compared to the control group. The study suggests that dietary patterns that emphasize fruits, vegetables, and low-fat dairy products can have beneficial effects on blood pressure, which is a risk factor for cardiovascular disease.

While many studies have found a link between fatty meat consumption and increased risk of cardiovascular disease (CVD), some studies have not found a clear association. A systematic review and meta-analysis of prospective cohort studies found that high consumption of red and processed meat was associated with increased risk of coronary heart disease (CHD) but not stroke (Micha et al., 2010). Study by Guasch-Ferré et al. (2019) found no significant association between meat intake and incident cardiovascular disease, coronary heart disease, or stroke in women.

Fatty meat consumption and diabetes

A number of studies have reported a link between red and processed meat consumption and the risk of devel-oping T2D. Type 2 diabetes is strongly associated with obesity and, as a high consumption of meat is frequently asso-ciated with energy-dense diets, often in combination with low physical activity levels, this may explain at least part of the association. Obesity frequently leads to resistance to the action of the hormone insulin and, in a proportion of obese people, this can develop further into T2D (Wok, 2016).

As with CVD, the high saturated fat and cholesterol content of fatty meat is thought to play a role in the devel-opment of insulin resistance and impaired glucose metabolism, which are key contributors to type 2 diabetes.

The mechanisms underlying this association are likely related to the high saturated fat and cholesterol content in fatty meat, which can lead to insulin resistance and impaired glucose metabolism. Another mechanism is the formation of advanced glycation end-products (AGEs) during the cooking of fatty meat. AGEs are formed when proteins and fats react with sugars at high temperatures, and they have been shown to increase oxidative stress and inflammation in the body, both of which are associated with insulin resistance and diabetes. In addition, fatty meat consumption has been linked to increased inflammation in the body, which is also associated with insulin resistance and diabetes. Finally, consuming high levels of saturated fat has been shown to contribute to the development of obesity, which is a major risk factor for type 2 diabetes.

Sanchis et al. (2016) study investigated the association between meat consumption and the risk of developing type 2 diabetes in a highly educated middle-class population using data from the SUN project. The authors found that a higher intake of processed meat was associated with an increased risk of developing type 2 diabetes, while no significant association was observed for total meat consumption or unprocessed red meat intake.

Another prospective cohort study by Ericson et al. (2013) found that higher consumption of processed meat was associated with an increased risk of developing type 2 diabetes, while higher consumption of poultry was associated with a lower risk. A study by Pan et al. (2011) found that higher consumption of red meat and processed meat was associated with an increased risk of developing type 2 diabetes, while higher consumption of nuts, whole grains, and dairy was associated with a lower risk.

Data from cross-sectional and prospective studies suggest that individuals who regularly consume meat products may have up to twice the risk of developing diabetes, compared with individuals who avoid meat entirely (Snowdon & Phillips, 1985; Fraser, 1999; Vang et al., 2008; Tonstad et al., 2009, 2013)

Individuals who eat meat regularly also tend to have higher plasma total and low density lipoprotein cholesterol concentrations (Barnard et al., 2009), higher blood pressure values, higher risk of hypertension (Berkow & Barnard, 2005), and higher body weight (Berkow & Barnard, 2006), all of which contribute to cardiovascular risk, the principal danger in diabetes. All of these conditions improve when meat is no longer consumed (Barnard et al., 2005; Ornish et al., 1990; 1998; Barnard et al., 2006). Meat consumption is consistently associated with diabetes risk. Dietary habits are readily modifiable, but individuals and clinicians will consider dietary changes only if they are aware of the potential benefits of doing so. The foregoing review indicates that the identification of meat consumption as a risk factor for diabetes provides helpful guidance for clinicians and at-risk individuals, and sets the stage for beneficial behavioral changes (Barnard et al., 2014).

The Women's Health Study was a prospective cohort study conducted in the United States that aimed to investigate the relationship between lifestyle, dietary factors, and the incidence of chronic diseases in middle-aged and elderly women. The study followed 37,309 female health professionals aged 45 years or older for an average of 8.2 years. The results of the study showed that women who consumed higher amounts of meat had a significantly increased risk of developing type 2 diabetes compared to those who consumed less meat. After adjusting for age, BMI, physical activity, smoking, alcohol intake, and other dietary factors, the study concluded that a high consumption of meat was associated with an increased risk of type 2 diabetes in middle-aged and elderly women. The findings supported the notion that dietary factors play a significant role in the development of type 2 diabetes and highlighted the importance of promoting healthy dietary habits to prevent this chronic disease (Song et al., 2004).

Insulin and insulin-like growth factor (IGF)-1 signaling is a proposed mechanism linking dietary protein and major chronic diseases. However, it is unclear whether animal and plant proteins are associated with biomarkers of insulin and IGF axis. In the study of Lee at al. (2022), higher animal protein intake was associated with higher IGF-1 and lower IGFBP-1 and IGFBP-2, whereas higher plant protein intake was associated with higher IGF-1 and IGFBP-1.

Fatty meat consumption and cancer

Studies on the relationship between fatty meat consumption and cancer risk have yielded mixed results. Some studies have found a positive association between red and processed meat consumption and increased risk of certain cancers, while others have not found a significant association.

Over recent years, a number of epidemiological studies have demonstrated a link between red meat, and in par-ticular processed red meat, and the risk of cancer. In reviewing the existing evidence, the International Agency for Research on Cancer (IARC) concluded in 2015 that processed meat is carcinogenic to humans and that fresh red meat is probably carcinogenic (Bouvard et al., 2015). The mechanisms by which meat contributes to this increased cancer risk have largely been attributed to chemical carcinogens produced during curing or smoking of processed meats or through cooking meat at high temperatures (Neuman et al., 2007).

The mechanisms through which fatty meat consumption may contribute to cancer risk are not fully understood and are still being studied, but several. Some hypotheses have been proposed and there are some possible ways in which this relationship may exist. Some studies suggest that cooking meat at high temperatures, such as grilling or frying, can produce carcinogenic compounds, such as heterocyclic amines (HCAs) and polycyclic aromatic hydrocar-bons (PAHs). These compounds can damage DNA and increase the risk of cancer.

Some studies suggest that the gut microbiome may play a role in the relationship between fatty meat consumption and cancer. Consumption of high levels of saturated fat and cholesterol found in fatty meat may increase levels of certain hormone, may increase the production of bile acids and may alter the balance of gut bacteria, which can affect the immune system and inflammation levels which are associated with the development of certain types of cancer, such as breast and prostate cancer (Cross et al., 2010).

Several meta-analyses have found that high consumption of meat is associated with an increased risk of colorectal cancer. For example, a meta-analysis of 27 cohort studies found that high intake of meat was associated with a 28% increased risk of colorectal cancer (Chan et al., 2011). Similarly, a meta-analysis of 14 studies found that high consumption of processed meat was associated with a 20% increased risk of colorectal cancer (Larsson & Wolk, 2006).

A study published in 2018 reported that high intake of red and processed meat was associated with an increased risk of breast cancer (Farvid et al., 2014) and another meta-analysis published in 2014 found that high consumption of red and processed meat was associated with a modestly increased risk of breast cancer in premenopausal women.

Some studies have found a positive association between meat consumption and increased risk of pancreatic, prostate, and lung cancer, while other studies have not found a significant association. Other types of cancer have also been studied in relation to meat consumption, but the evidence is less consistent.

Overall, the evidence suggests that high consumption of red and processed meat is associated with an increased risk of colorectal cancer, but the evidence for other types of cancer is less consistent. Further research is needed to better understand the relationship between fatty meat consumption and cancer risk, and to identify the underlying mechanisms.

Alternative protein sources to fatty meat

Some article discusses the role of meat in the human diet, with a focus on the balance between the nutritional benefits of meat and its potential health risks. The authors highlight the importance of promoting a balanced diet that includes adequate amounts of meat as well as other nutrient-rich foods, such as fruits, vegetables, whole grains, and legumes, promoting a more sustainable and healthful diet (DeSmet & Vossen, 2016).

Lean meats: Chicken breast, turkey breast, lean beef, and pork tenderloin are all good sources of protein and are lower in fat than fattier cuts of meat.
Fish: Fish such as salmon, tuna, and trout are rich in protein, omega-3 fatty acids, and other important nutrients. They are also generally lower in fat than red meat.
Beans and legumes: Lentils, chickpeas, black beans, and kidney beans are all great sources of protein and fiber. They are also low in fat and can be used in a variety of dishes.
Soy products: Tofu, tempeh, and edamame are all good sources of protein and are lower in fat than fatty meats.
Nuts and seeds: Almonds, walnuts, sunflower seeds, and pumpkin seeds are all high in protein and healthy fats. They can be added to meals or eaten as a snack.
By incorporating these alternative protein sources into the diet, it can be reduced the intake of fatty meats and promote overall health and well-being. Populations following plant-based diets, particularly vegetarian and vegan diets, are at lower risk for ischemic heart disease mortality (Ferdowsian & Barnard, 2009).
There are plants with a high content of bioactive compounds, such as fruits, vegetables, herbs, spices, which can also be studied as vegetable sources with antioxidant action, to combat the action of fatty meat (Baldin, et al., 2016; Fernandes et al., 2017, Schilling, et al., 2018, Menegali, et al., 2020).
An advantage of using vegetable antioxidants would be also, in addition to reducing fats and improving the profile of fatty acids, the extending of shelf life of meat products (Selami et al., 2022).
It is possible to create a hypocaloric product made of minced beef with added fiber, but it is important to note that simply adding fiber to a product does not necessarily make it healthier or beneficial for preventing or managing coronary diseases, obesity, or diabetes. It is essential to consider the overall nutrient composition of the product, including its fat content, saturated fat content, sodium content, and added sugars, among other factors. Additionally, it is important to consider the potential environmental impact of producing such a product, as well as the ethical implications of using animal products. Therefore, any product claiming to fight these health conditions should be carefully evaluated based on its overall nutritional profile and potential impact on health and the environment.
An innovative hypocaloric product made of minced beef with added fiber could have several potential health benefits. Here are a few examples:
- Weight management: A hypocaloric product means that it contains fewer calories than regular minced beef, which could help with weight management. Additionally, the added fiber could increase satiety and help with portion control.
- Reduced risk of chronic diseases: Minced beef is a good source of protein, iron, and other essential nutrients, but it is also high in saturated fat. By reducing the fat content and adding fiber, the product could be a healthier alternative and may help reduce the risk of chronic diseases like heart disease, obesity, and diabetes.
- Improved gut health: Adding fiber to minced beef could improve gut health by promoting the growth of bene-ficial gut bacteria and increasing stool bulk, which can help with regularity and reduce the risk of constipation.
- Convenience: Minced beef is a versatile ingredient that can be used in a variety of recipes. An innovative hy-pocaloric product made of minced beef with added fiber would provide a convenient and healthy option for people who want to incorporate beef into their diet while also watching their calorie and fat intake.
However, it is important to note that the effectiveness and safety of such a product would depend on the quality of the ingredients and the processing methods used to produce it. It would also be important to ensure that the product is properly labeled and marketed to prevent any misunderstandings about its nutritional content and health benefits.

CONCLUSIONS

Some general conclusions based on existing research are:
1. High consumption of red and processed meat is associated with increased risk of several

chronic diseases, in-cluding cardiovascular disease, type 2 diabetes, and some cancers.
2. Some studies suggest that substituting red and processed meat with plant-based protein sources may have health benefits.
3. The risks associated with meat consumption may depend on factors such as the type and cut of meat, the method of cooking, and individual differences in genetics and metabolism.
4. It is important to consider the general dietary pattern and lifestyle factors, such as physical activity and smoking, in addition to meat consumption when assessing its impact on health.
Overall, the evidence suggests that reducing meat consumption, especially of red and processed meat, may have health benefits. However, more research is needed to better understand the complex relationships between meat consumption and health outcomes, and to identify specific factors that may modify the risks associated with meat consumption.

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THE IMPACT OF FRUIT FIBER ON MEAT PRODUCTS: A MINI REVIEW

Diana-Remina MANOLIU, Marius Mihai CIOBANU, Mihai Cătălin CIOBOTARU, Bianca Georgiana ANCHIDIN, Paul Corneliu BOIȘTEANU

"Ion Ionescu de la Brad" Iasi University of Life Sciences, 3 Mihail Sadoveanu Alley, 700490, Iasi, Romania

Corresponding author email: paul.boisteanu@iuls.ro

Abstract

The meat industry is making technological advancements in order to provide food that is both healthier and more sustainable. The purpose of this study is to provide an overview of the impacts that may be attributed to the incorporation of whole fruits or byproducts in a variety of forms into different meat products. The review investigated the effects that these incorporations have on physicochemical and technological features, sensory characteristics, and the potential to improve shelf life. The incorporation of fruit fiber into meat products led to an increase in cooking yield, emulsion stability, capacity to bind water and fat. However, it also resulted in a reduction in shrinkage, cooking losses and pH, with variations depending on the concentration, type, initial pH, and storage period. The addition of dietary fiber led to an increase in hardness and chewiness, despite the fact that it enhanced the water-holding capacity and reduced the cooking loss. It was found that the effect on the instrumental color characteristics and color sensory perception differed depending on the source of the addition and its color.

Key words: *byproducts, fruit fiber, meat products, quality parameters.*

INTRODUCTION

In general, dietary fiber includes carbohydrates that the human body is incapable of digesting in the small intestine, but undergo fermentation in the large intestine. Although there exist multiple methods for categorizing this group of components, the most common system of classification involves determining its solubility in water. Soluble fiber undergoes fermentation more readily than insoluble fiber (Bajcic et al., 2019). The most representative exemples are: structural polysaccharides including cellulose, hemicellulose, and pectin; oligosaccharides including inulin and oligofructose; lignin found in the cell wall of plants; lignin derivatives including wax and suberin; seaweed polysaccharides such as carrageenan, agar, and alginate, as well as gum agents devoid of structural constituents like gum arabic and guar gum (Arslan et al., 2021; Mishra et al., 2023).
Given their content in vital nutrients including vitamins, minerals, and fibers, fruits and vegetables influence positively the health of individuals, those components serving diverse physiological functions within the body and provide advantages to human organism (Cassiano et al., 2024). However, following sanitation, fruit and vegetable processing is one of the most substantial industries contributing to environmental waste. Fruits and vegetable processing wastes comprise approximately 30% to 50% of the overall fresh product. These wastes, including peel and seeds, contain significant amounts of high-value materials that are capable of being repurposed. Consequently, they possess considerable economic value. The utilization of these by-products in the manufacturing of dietary supplements or food additives that are nutritionally significant has garnered growing interest; consequently, their economic viability is enhanced by their recovery (Sakr et al., 2023).
Meat and meat products are versatile and nutritionally dense due to the presence of bioactive compounds, fat-soluble vitamins, minerals, trace elements, and proteins with high biological value. Meat, despite its considerable nutritional value, is notably lacking in dietary fiber (Das et al., 2020) and is a significant source of cholesterol and saturated fatty acids, both of which have been associated with health complications (Arslan et al., 2021; Younis et al., 2022).

Nowadays, consumers become more aware of the correlation that exists between nutrition and health. This highlights the need to design functional products by improving their quality with dietary fibers, essential oils, or various plant extracts (Anchidin et al., 2023; Lungu et al., 2023). Due to the health benefits, technological enhancements and the improvement on shelf life associated with the incorporation of dietary fiber into meat and meat products, this has become a topic of increased interest among scientists (Dragomir et al., 2023). Ratulangi et al. (2022) demonstrated that the addition of purple sweet potato flour (*Ipomoea batatas* L.) to chicken nuggets (at levels of 10, 20, 30, and 40 grams) resulted in a decrease in water content and an increase in fat, protein, and dietary fiber content. Additionally, it improved the antioxidant activity of the nuggets.

Therefore, besides their economic value and nutritional benefits, dietary fiber sourced from vegetable sources also have a structural benefit on the meat products, improving cooking parameters, such as cooking loss, cooking yield, diameter reduction (Boișteanu et al., 2023), water holding capacity (Sakr et al., 2023), expressible water (Al et al., 2024; Barbut, 2023). Dietary fiber is a vital component of a balanced diet, promoting digestive health, weight control, and reducing the risk of chronic illnesses (Arslan et al., 2021). However, consumption often falls below recommended amounts due to widespread consumption of refined and processed foods. Understanding consumer preferences regarding dietary fiber content is crucial for market segmentation in the food industry.

Lungu et al. (2023) reported in a study evaluating the incorporation of different vegetable derivatives (such as extracts or powders from walnut leaves and cherry stems, pumpkin seed protein, chickpea protein isolate, mushrooms) in meat products that these supplements have a positive effect on overall quality and that the sensory attributes are accepted by consumers, if not even perceived as improved.

Therefore, this review aims to investigate the effect of different dietary fibers found in fruits and their impact on the qualitative attributes of meat and meat products, including their nutritional addition, technological and physical quality, effect on shelf life and sensory perception.

MATERIALS AND METHODS

The present literature review includes studies summarizing the outcomes of incorporation of fruit fiber in different forms (powder, paste, flour, pomace) into meat products (for exemple, burgers, sausages, nuggets, meat patties, meatballs). The main variables followed to highlight the effects of the additives applied to the products were the physico-chemical and technological properties, the potential to extend shelf life (through the antioxidant properties of the added fibre) and sensory properties.

The literature search was performed utilizing Web of Science, ResearchGate and Google Scholar as search platforms. The inclusion criteria for this study extended to English-language articles and had full-text accessibility. Research examining the impact of fruit fiber on the supplementation in animal feed was similarly disregarded. The eligibility criteria included only research that examined the use of whole fruit, fruit byproducts or fruit fiber in meat products. For the search, the subsequent word combinations were implemented: fruit fiber in meat products, fruit byproducts in meat products, meat products enriched with fruit fiber.

A total of 68 publications were returned by the initial search; the results were imported via Mendelay Desktop (by Elsevier Limited, version 1.19.8) and duplicates were removed. After removing duplicate articles from the databases, 61 studies remained. Following that, abstracts from the remaining articles were assessed in order to identify those that were pertinent to the subject of the study. After conducting a comprehensive examination of the complete papers to verify adherence to the inclusion criteria, 30 articles remained. Further analysis was conducted on the reference lists of the chosen papers in order to identify publications that might have been pertinent. After conducting an exhaustive evaluation of complete texts, a total of 23 publications were deemed suitable for inclusion in this review.

RESULTS AND DISCUSSIONS

Impact of adding dietary fiber on the physical and chemical characteristics of meat products

The incorporation of fiber into meat products has altered their overall composition, resulting in the emergence of novel fiber sources and presenting promising opportunities for their utilization in many industries. Table 1 shows the effect of fruit fiber on technological and nutritional quality of different meat products.

The incorporation of blanched date pulp in camel meat burgers had a significant impact on the moisture, protein, and fiber levels of the cooked samples. In contrast, the ash and fat content were not affected by the date pulp addition. The moisture content of the raw burgers decreased from 58.32% in the 0% pulp formula to 55.82% in the 15% date pulp formula. Additionally, there was a considerable rise in the fiber levels and a decrease in the protein content. Moreover, the inclusion of date pulp resulted in a substantial rise in the levels of K, Ca, Zn and Mg, while simultaneously reducing the levels of Fe and Cu (Abd El-Hady et al., 2022). A similar increase in mineral content (calcium, magnesium, potassium, and iron), described by Kęska et al. (2023), was produced by the addition of lyophilized dragon fruit in baked pork meat. The observed rise in mineral content can be attributed to the high concentration of vital nutrients present in dates and lyophilized dragon fruit pulp, as they serve as a valuable reservoir of iron, cobalt, zinc, and calcium, while also exhibiting higher-than-average levels of potassium and magnesium (Ayad et al., 2020; Kęska et al., 2023). Despite the decrease in water content in the raw burgers, the cooking characteristics showed an improvement, namely the shrinkage and cooking loss of the patties decreased from 26.67% and 36.98% (in control samples) to 9.17%, respective 31.76% (in the 15% formulation).

The research of Sánchez-Zapata et al. (2011) showed that the addition of date paste in bologna sausages resulted in an increase in moisture, ash, and total dietary fiber content, while causing a decrease in protein and fat content. However, these changes were only statistically significant when the date paste was introduced at a concentration exceeding 5%. The only parameter that exhibited significant changes across all treatments was the total dietary fiber content, the increase being directly proportional to the concentration of added date paste. The results of the proximate analysis revealed that bolognas with 10% and 15% date paste exhibited the maximum moisture content. This observation may be attributed to the water content and water-holding ability of the date paste.

Similar results were reported by Besbes et al. (2010), who found a decrease in dry matter (wt.%) in beef burgers formulated with 1.5% date fiber concentrate. The authors explain the increase of water content by the fact that adding a higher percentage of date fiber concentrate, the formulation needed a higher quantity of water.

In a particular study, the inclusion of dried apple pomace in chicken sausages resulted in a decrease in moisture and protein content, and an increase in crude fiber, while the fat content did not differ significantly (Yadav et al., 2016). Incorporating dry apple pomace (DAP) into sausages resulted in notable improvements in cooking yield and emulsion stability across all treatments, with a significant enhancement observed at the 6% level of fiber incorporation. These improvements can be attributed to the fiber's capacity to retain water and fat within the treated sausages. Interestingly, there was a significant decrease in pH values noted. This decrease in pH was attributed to the inherently acidic nature of DAP, which has a pH of 4.80.

Ahmad et al. (2021) reported that apple peel powder determined a decrease in pH values of buffalo meat fillets observed within the first 7 days of storage, followed by a significant increase thereafter. The initial decrease in pH can be ascribed to microbial activity that converts accessible carbohydrates into organic acids, causing this shift. Following this, on the seventh day, a significant rise in pH is observed, potentially attributed to a microbial transition towards protein utilization as a food source; as a result, the product contains a greater concentration of alkaline protein-based degradation metabolites.

The overall findings suggest that the inclusion of DAP in sausages not only enhances their physical properties but also influences their acidity levels, potentially affecting flavor and texture profiles (Yadav et al., 2016; Ahmad et al., 2021).

The moisture of chicken meat nuggets decreased with the addition of orange albedo, due to the low water content of orange albedo. This aligns with previous studies indicating lower moisture content in beef burgers enriched with orange albedo flour (Silva et al., 2020). Protein and ash content do not significantly differ, but slightly increase with fiber addition from citrus albedo, while the fiber content increase significantly. The highest fiber content was found in the meat products containing the higher citrus albedo powder (Fernández-Ginés et al., 2004; Ammar, 2017; Silva et al., 2020).

Table 1. Effect of fruit fiber on technological an nutritional quality

Meat product	Type of fiber added	Dosage used	Technological effect	Nutritional modifications	Reference
Camel Meat Burger	Date fruit pulp	2.5%, 5%, 7.5%, 10%, and 15%	↓ Shrinkage; Cooking loss ↑ Cooking yield	↓ Moisture and protein ↑ Total carbohydrate; fiber; K and Ca levels NSD Fat	Abd El-Hady et al., 2022
Bologna sausages	Date paste	5%, 10% and 15%	ND	↑ Moisture; ash; total fiber content ↓ Protein and fat content	Sánchez-Zapata et al., 2011
Chicken sausages	Dried apple pomace (DAP)	3, 6 and 9%	↑ Cooking yield, emulsion stability	↓ Moisture, protein ↑ Crude fiber NSD Fat	Yadav et al., 2016
Chicken meat nuggets	Orange albedo powder	5% and 10%	↓ pH; frying shrinkage ↑ Frying yield, moisture retention	↓ Moisture ↑ Protein, fat, ash and fibre content	Ammar, 2017
Beef burgers	Orange albedo flour	2.2%; 4.3%; 6.5% and 8.7%	↑ Shrinkage ↓ Cooking yield, WHC	↓ Moisture, fat ↑ Protein, fiber, ash content	Silva et al., 2020
Emulsified Alpaca Sausages	Red dragon fruit peel powder	3.29%, 6.57%, and 9.86%	↓ Cooking yield ↑ Frying loss	↑ Moisture, total fiber ↓ Total lipids and protein contents	Corimayhua-Silva et al., 2024
Spam-like products	Passion fruit albedo flour	2.5%, 5%, 7.5% and 10%	↓ Cooking losses, water exudations ↑ Fat exuded	↓ Moisture, protein ↑ Carbohydrates and TDF	dos Santos et al., 2021
Chicken meat patties	Pomegranate peel (PPP) and bagasse powder (PBP)	0.01% and 2%	↑ Water holding capacity; emulsion stability, cooking yield	↓ Moisture, protein (in the PPP addition) ↑ Fat, ash, crude fibre	Sharma & Yadav, 2020
Pork meatballs	Kiwi fruit pomace fiber	0.5%, 1%, 3%, 5% and 7%	↑ Cooking yield, emulsion stability and water / fat binding abilities	↑ Moisture, carbohydrate ↓ Fat NSD Protein	Zhao et al., 2021

↑ - the addition of fiber increased the value of the parameter; ↓ - the addition of fiber decreased the value of the parameter; NSD - no significant difference; ND - not determined.

In one study the moisture and fat content of spam-like products was not substantially impacted by the concentration of passion fruit albedo flour (PFF). Conversely, the protein and total carbohydrate contents exhibited variability in response to the quantity of PFF introduced, with a tendency for carbohydrate content to increase and protein content to decrease. Physicochemical characteristics of the spam-like products such as weight loss on cooking, water and fat exuded registered a decrease with the increase of PFF added (dos Santos et al., 2021). Similar results were described by López-Vargas et al. (2014), where the addition of passion fruit albedo (PFA) in pork burgers induced a decrease in moisture and protein content, while improving the cooking characteristics in terms of yield, fat and moisture retention. Those findings are related to the fibers characteristics that function as emulsion-stabilizing agents, thereby contributing to the retention of water and lipids in the food matrix by increasing water retention via their jellifying capacity (dos Santos et al., 2021).

The impact of dietary fiber inclusion on the color, texture, and sensory attributes of meat products

Modern consumer perception encompasses both pre-purchase and post-purchase value of a product, leading to the concept of loyalty as a general attitude towards a product. In general, loyalty is demonstrated through three stages: conative intention, attitude, and belief. As each phase is completed, loyalty and commitment improve. A complete perceptual experience is formed by summing all of the component sensations caused by various factors such as organoleptic sensations, affective sensations and perceptions, or sensations associated with environmental conditions, all of which constantly interact to produce the final acceptability (Ciobanu et al., 2023). Customer acceptability of meat products is determined by several critical sensory attributes, namely aroma, flavor, color, appearance, tenderness, and juiciness.

The incorporation of kiwi fruit pomace fiber into pork meatballs (increasing levels, from 0.5% up to 7%) resulted in enhanced tenderness while leaving the meat juiciness unaffected, but only with a maximum level of 3% kiwi fruit pomace fiber. At higher levels of kiwi fruit pomace fiber (5 and 7%) a decline in appearance, flavor and texture scores was registered, while the instrumental determined hardness increased significantly. The overall acceptability decreased, the closest to the control sample in sensory perception being the formulation with 3% addition (Zhao et al., 2021).

Adding lemon albedo to bologna sausages did impart the acidic flavor, the perception increasing for doses above 5% of albedo. Conversely, the perceptual impact of the color red and also the instrumental reading of color was negatively affected through the incurporation of fresh lemon albedo. However, samples with albedo also showed higher lightness values and were perceived as shinier (Fernández-Ginés et al., 2004). The sensory acceptance of beef burgers with a low addition of orange albedo flour (2.2% and 4.3%) did not differ from the control. However, the scores for color, appearance, flavor, texture, and overall liking decreased with the addition of 6.5% and 8.7% orange albedo flour. This may have led to the perception of fruity flavors (Silva et al., 2020).

The incorporation of fiber extracted from pumpkin into frankfurter sausages at a concentration of 2% yielded final products of exceptional quality taking into account that the addition of pumpkin fiber was made with the purpose of reducing the fat content. The color, flavor, tenderness, juiciness, and overall acceptability scores of frankfurters showed a tendency to decrease by reducing added pork fat levels without adding the pumpkin fiber, while treatments with pumpkin fiber added received higher sensory scores (Kim et al., 2016).

The extent to which fiber is incorporated into the meat product can alter its ultimate properties. In this sense, the sensorial perception of chicken meat balls enriched with grape pomace powder and pomegranate pomace powder (GPP/PPP; 1%/0.5%; 2%/1%; 3%/1.5%; 4%/2%) decreased in all samples compared to the control group, the 1% / 0.5% formulation was evaluated the closest to the control sample in terms of appearance and color, texture, tenderness and overall acceptability (Santhi et al., 2020). Mango peel pectin (MPP) incorporated in dried Chinese sausages at 5%, 10%, and 15% levels indicated that as the concentration of MPP applied increases so does the pigmentation and yellowness of the sausage in all formulated products, as well as the intensity of the color, while the lightness value decreases. The findings revealed that the hardness of the formulated samples did not vary substantially. The control sample achieved the greatest hardness value of 15.87 N, that decreased slightly with the addition applied. Nevertheless, interventions incorporating pectin fibers resulted in reduced levels of springiness, cohesiveness, gumminess, and chewiness. The inclusion of MPP resulted in sausages with a noticeably softer consistency. This may be related to the gel strength of the quantity of pectin compressed (Wongkaew et al., 2020). The variations in hardness profiles observed may be attributed to the fat and MPP mixture's capacity to bond and retain water.

The impact of dietary fiber addition on the shelf-life of meat products

Preserving the quality and retail stability of a meat product throughout its storage period is critical. Diverse effects of incorporating various types of fiber sources into meat products have been observed to affect their preservation quality. A frequently employed method for assessing oxidative stress in foods is the analysis of TBA (thiobarbituric acid) metabolites generated during preparation. The method measures the MDA (a substance that undergoes a reaction with thiobarbituric acid) content of meat products, serving as an indicator of their quality (Mazur et al., 2021).

Table 2. Effect of fruit fiber on oxidative stability, sensorial and physical quality

Meat product	Type of fiber added	Dosage used	Effect on oxidative stability	Sensorial modifications	Physical quality	Reference
Homogenized meat products	Guelder rose (*Viburnum opulus*)	0.05%, 0.1%, 0.15%	↑ oxidative stability microbiological quality ↓ TBARS levels	↑ Sensory acceptance in overall appearance and color ↓ acceptability of taste and smell by increasing the addition of GRFP	↓ pH, L*, a* (on a 14 days storage period)	Mazur et al., 2021
Chicken Meat Nuggets	Drumstick (*Moringa oleifera*) flower	1% and 2%	↑ Total phenolic content (mg GAE/g), oxidative stability ↓ TBARS values (mg MDA/kg), microbial count (log cfu/g) on a 20 days storage time	↓ Sensory attributes (especially after 15 days of storage) The control group received a lower taste score and a rancid odour on day 15, which may have reduced taste and acceptability scores	↑ L*, b* ↓ a*, pH, hardness (N/cm^2), gumminess, chewiness	Madane et al., 2019
Pork burgers	Passion fruit albedo (PFA)	2.5% and 5%	↓ Lipid oxidation (raw and cooked sample)	↓ Color intensity; fatness perception ↑ Flavor perception; meaty flavor, taste intensity score	↑ L* (raw burgers), a*, b* (raw burgers), hardness, chewiness ↓ L* (cooked burgers)	López-Vargas et al., 2014
Chicken meat nuggets	Orange albedo powder	5% and 10%	*NSD* TBARS values on day 0 ↓ TBARS values for a 20 days storage time ↑ control in lipid oxidation	↑ Color and appearance perception ↓ Flavor, texture and juiciness perception; overall acceptability	↑ a*, b*, Hardness, Chewiness, Gumminess ↓ L*, Cohesiveness, *NSD:* Springiness	Yadav et al., 2016
Pork meatballs	Kiwi fruit pomace fiber (KWIDF)	0.5%, 1%, 3%, 5% and 7%	↓ TBARS values (indicating that the oxidation stability of low-fat meatballs could be improved by the addition of KWIDF)	↓ Appearance scores, flavor and texture; overall acceptability ↑ Juiciness scores (in the 3% KWIDF)	↑ a* and b*, hardness, gumminess, chewiness (by adding 5% and 7%). ↓ pH, L*, hardness, gumminess and chewiness (by adding 0.5%, 1%, 3%)	Zhao et al., 2021

↑ - the addition of fiber increased the value of the parameter; ↓ - the addition of fiber decreased the value of the parameter; NSD - no significant difference

The inclusion of 1% pomegranate seed and peel extract in chicken patties determined a rise in TBA concentrations during storage, the control sample C demonstrated the most substantial increase, culminating in a maximal value of 1.221 mg MA/kg after a duration of 12 days in refrigeration. Samples added with pomegranate seed and peel extract exhibited marginal increases in MA content (0.637 and 0.632 mg/kg, respectively). This observation illustrates the effect that these extracts have on the oxidative stability of the product, possible cause of the decreased TBA concentrations can be the phenolic compounds and natural antioxidant components in pomegranate seeds and skins (Al et al., 2024). Similar results were reported by Sharma & Yadav (2020) for chicken meat patties enriched with pomegranate peel powder (PPP, 2%) and pomegranate aril bagasse powder (PABP, 4%). In this study, TBA values substantially increased for both treated and control patties during refrigeration storage. However, during the storage period, the TBA values of chicken patties treated with PPP and PABP remained substantially lower than those of the control group. The increase in TBA value was from 0.56 mg malonaldehyde/kg for the control sample on day 0 to 1.95 mg MA/kg on day 16 of storage; while in the samples with added PPP and PABP the TBA value increased from 0.24 mg MA/kg and 0.30 mg MA/kg respectively to 0.81 mg MA/kg and 0.84 mg MA/kg, respectively on day 16 of storage.

Kęska et al. (2023) reported across the entire duration of storage of 21 days, products enriched with lyophilized dragon fruit pulp exhibited, on average, greater TBARS values. An anomaly was observed in the variant containing the least amount of lyophilizate, specifically 0.5%. In proportion to the dose administered, the extent of the difference in TBARS levels is directly notable. Specifically, during the entire storage period, the formulation with 4% variant demonstrated a greater quantity of secondary fat oxidation products in comparison to the control. The mean values of mg MDA/kg as determined for homogenized meat products enriched with freeze–dried guelder rose fruit powder (GRFP), during the initial experimental phase (post-production), exhibited notable distinctions between control sample and prepared formulations with 0.05, 0.1, 0.15 g/100 g of GRFP. The formulation lacking GRFP contained a greater quantity of MDA compared to the groups supplemented with GRFP. Furthermore, it was observed that sample with the greatest concentration of GRFP (0.15 g/ 100 g) had the least quantity of MDA, and the greatest resistance to lipid peroxidation (Mazur et al., 2021). During the fourteen-day storage period at 4°C, elevated MDA concentrations were observed in all samples relative to those determined on day zero; this was attributed to lipid peroxidation. Samples containing 0.05 and 0.1 g/100 g of GRFP exhibited a comparable antioxidative effect, as evidenced by the average TBARS values of the experimental samples.

CONCLUSIONS

The incorporation of dietary fiber into meat products has shown diverse effects on their physical, chemical, sensory, and shelf-life attributes. Various fiber sources, such as blanched date pulp, lyophilized dragon fruit, and dried apple pomace, have demonstrated significant alterations in moisture, protein, fiber, and mineral content, presenting novel opportunities for enhancing product quality and nutritional value. Additionally, fiber addition has influenced cooking characteristics, such as shrinkage and cooking loss, while also impacting pH levels. Sensory evaluations have revealed changes in aroma, flavor, color, texture, and overall acceptability, depending on the type and concentration of fiber incorporated. Furthermore, fiber inclusion has shown potential in improving oxidative stability and extending shelf life by reducing lipid peroxidation. However, careful consideration of fiber type, concentration, and processing methods is essential to optimize product quality and consumer acceptance. Overall, the findings underscore the multifaceted benefits and challenges associated with incorporating dietary fiber into meat products, highlighting the need for further research to harness the full potential of this approach in the food industry.

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VARIATION IN MINERAL OIL HYDROCARBONS CONTENT OF MILK DURING PROCESSING

Mădălina MATEI, Silvia-Ioana PETRESCU, Elena-Iuliana FLOCEA, Dragoş Mihai LĂPUŞNEANU, Daniel SIMEANU, Ioan Mircea POP

"Ion Ionescu de la Brad" Iaşi University of Life Sciences, 3 Mihail Sadoveanu Alley, Iaşi, Romania

Corresponding author email: mircea.pop@iuls.ro

Abstract

MOSH/MOAH in a food product may result from unintentional contamination occurring at various levels of the supply chain or migration from packaging. This preliminary research aims to evaluate the differences and variations in contamination levels between raw and processed milk samples to assess the contamination risk associated with milk processing. Using LC-GC-FID, mineral oil hydrocarbons (MOSH; MOAH) were quantified in eight milk samples. A T-test was applied to evaluate the differences in the distribution of carbonic sub-fractions and total levels of MOSH and MOAH contamination (n-C_{10-50}) in experimental samples of unprocessed milk and lyophilized milk. All statistical analyzes were performed using SPSS Statistics 26.0 and GraphPad Prism 9 software packages. The results of the comparative analysis revealed that milk processing operations had a significant impact on the variation of the MOH contamination level in the freeze-dried milk. MOSH and MOAH content varied significantly between initial samples (0.8-8.7 mg/kg MOSH; 0-2.8 mg/kg MOAH) and final samples (4.5-13.1 mg/kg MOSH; 3.2-5.35 mg/kg MOAH). These results are relevant for evaluating the impact of the freeze-drying process on these contaminants.

Key words: *contamination, milk, mineral oil hydrocarbons, processing.*

INTRODUCTION

Mineral oil hydrocarbons (MOH) come from petroleum distillation or are produced synthetically by coal and natural gas extraction (Bratinova & Hoekstra, 2019; Menegoz Ursol et al., 2023). These substances form a major category of contaminants of petrogenic origin, present in the environment as a result of pollution or due to contamination during production processes (Bruhl, 2016; Canavar et al., 2018).
MOH can reach the environment through various means, and their presence can be linked to multiple sources (Srbinovska et al., 2023; Purcaro et al., 2016). The major concerns about MOH contamination stem from the approved use of mineral oils in various products. Certain mineral oils have approved uses, including additives and processing aids in food (Van Heyst et al., 2019; Hoccheger et al., 2021).
Essential technological steps for the packaging and transport of raw materials can play a significant role in MOSH (Mineral Oil Saturated Hydrocarbons) and MOAH (Mineral Oil Aromatic Hydrocarbons) contamination, as these hydrocarbons can be present in paper and cardboard materials (Biedermann & Grob, 2015; Van Heyst et al., 2019; Menegoz Ursol et al., 2023), all with specific food migration properties (EC, 2011).
Currently, only food-grade lubricating mineral oils that are unrefined or partially refined can be used in food processing and related industries (EFSA, 2012; Menegoz Ursol et al., 2022).
The effects and toxicity of MOH are still unclear, but ongoing research shows that MOSH can accumulate in human organs and tissues (Hidalgo Ruiz et al., 2021), and MOAH, due to their structural similarity to PAHs, have carcinogenic potential (Nestola, 2022).
As a result of the non-polar character given by their chemical structure, MOSH and MOAH show a strong affinity for fatty substrates. Amounts of MOH have been found in a variety of foods, including those of animal origin (Foodwatch, 2021).
The occurrence and possible sources of contamination have been discussed in detail in the specialized literature (Moret et al., 2009; Gharbi et al., 2017; Matei & Pop, 2023).
Food safety of milk is an important issue given its essential role in providing nutrients, especially for vulnerable consumer groups (Matei & Pop, 2022; Singh, 2022).

Industries that want to be competitive develop new products for the market (Mierliță et al., 2024). As the industry develops new products, it is essential to consider tailoring them to consumer neuro-perception, balanced with their safety and healthiness. This involves the integration of sensory characteristics according to food safety issues (Lăpușneanu et al., 2021; Ciobanu et al., 2023).

To date, MOH in milk has been addressed mainly in the context of potential risks associated with packaging, and a few studies have reported the presence of mineral oils in infant milk products (Biedermann–Brem et al., 2012; Zhang et al., 2019).

In relation to the environment, MOH represent a new class of contaminants. MOSH and MOAH can be present in food from various sources, such as food packaging or environmental contamination. Because these substances can migrate into food and accumulate in the human body, there are concerns about their potential impact on human health.

The purpose of this preliminary research is to highlight the differences in the level of contamination between raw and processed milk samples, as well as to analyze the variation in contamination levels following processing. This aims to assess the risk of contamination associated with milk processing methods.

Using LC-GC-FID, saturated and aromatic mineral oil hydrocarbons (MOSH; MOAH) were quantified in eight milk samples. A T-test was applied to evaluate the differences in the distribution of MOSH and MOAH structured by carbon chain lenghts and the total level of contamination (n-C_{10-50}) in experimental samples of unprocessed milk and lyophilized milk. All statistical analyses were performed using SPSS Statistics 26.0 and Graph Pad Prism 9 software.

The information available to date and the results of this study can be integrated to develop future models for monitoring food contamination with MOH during processing.

MATERIALS AND METHODS

Samples

As part of the experimental monitoring proposed for the analysis of MOSH/MOAH content, four samples of cow's milk were collected from farms located in the northeastern Romania. Before conducting the actual testing, the milk samples, which were unprocessed and not previously packaged, were analyzed to determine the content of crude fat and dry matter for standardization, compatibility, and accuracy of the determinations.

To highlight the potential influence of processing on the level of sample contamination, the four samples were coded according to the type of processing applied:

A/B/C/D–1: experimental samples of unprocessed milk;

A/B/C/D–2: experimental samples of processed milk (lyophilization).

The processing applied to the experimental samples (2) involved staged lyophilization of frozen samples (-18°C), at temperatures ranging from -30°C to -75°C, with a pressure of 1.65 mbar and a vacuum of 0.200 mbar, over a period of 7 days using a Christ Epsilon 2-4 LSC plus lyophilizer.

During processing, the frozen milk samples were covered with classic, bleached, waxed food paper purchased from a supermarket.

Details regarding the characteristics of the studied samples are included in Table 1.

Table 1. Specifications for experimental milk samples

	Code & sample no.			
	A	**B**	**C**	**D**
DM %	12.3	11.9	13.1	12.5
Fat (% DM)	37.3	35.4	29.6	29.6

DM = dry matter.

MOSH/MOAH analysis

MOH analysis was performed using the advanced coupled LC-GC-FID technique. The determination method was originally developed by Biederman et al. (2009) and later detailed by Bierdemann & Grob (2012), concerning the process of extraction, separation, and quantification of MOH. The current working protocol was adapted and optimized by Moret et al. (2016) and has been successfully applied in more studies, including those conducted by Menegoz Ursol et al. (2022), Srbinovska et al. (2022); Srbinovska et al. (2023). This method has been validated according to the analytical performance criteria set out in the JRC Guideline (Bratinova & Hoekstra, 2019).

Table 2. Infrastructure for MOH analysis

Sample preparation and analysis	Equipment	Performance, integration and quantification	Data collection and processing
n-hexane (≥ 95 %; CAS: 110-54-3)	[1]LC-GC 9000*: HPLC Phoenix 9000 coupled to GC model Trace 1310	internal standard (IS) #31070 (150-600 µg/ml in toluene 99 %)	Chromeleon
methanol (≥ 99,9 %; CAS: 67-56-1)			
saturated KOH (CAS: 1310-58-3)			
metachloroperoxybenzoic acid (70–75 %; CAS: 937-14-4); 200 mg/ mL ethanol			
anhydrous sodium thiosulfate (CAS 7772-98-7)	HPLC column 25 cm×2.1 mm i.d. with Lichrospher Si 60.5 µm particle size		
aluminum oxide (CAS: 1344-28-1)			
sodium sulfate (CAS: 7757-82-6)			
Supplier			
Merck Millipore (Massachusetts, SUA) Sigma-Aldrich Supelco Acros Organics, Thermo Fisher Scientific (Waltham, Massachusetts, SUA)	Brechbuhler (Zurich, Elveția) Thermo Fisher Scientific (Waltham, Massachusetts, SUA) DGB (Germany)	Restek, Bellefonte, PA, SUA	Thermo Fisher Scientific (Waltham, Massachusetts, SUA)

[1]configured with a dual channel allowing simultaneous analysis of MOSH and MOAH.

Data distribution was assessed using GraphPad Prism 9 and SPSS Statistics 26.0 software. A T-test was applied to evaluate the differences in the distribution of MOSH and MOAH, structured by carbonic sub-fractions and the total level of contamination (n-C_{10-50}).

RESULTS AND DISCUSSIONS

Milk is highly susceptible to exogenous contamination regardless of its form.

The fat and dry matter content influenced the analytical methods used to detect contaminants. Precision and accuracy of measurements were ensured by adjusting the analytical components based on the fat and dry matter content of the four analyzed milk samples.

Standardization of the samples based on the lipid fraction and dry matter content enable comparability of results across the samples.

MOSH and MOAH tend to accumulate more in the lipids of foods. The distribution of contaminants based on fat content showed varying concentrations depending on the composition of each milk sample.

The levels of MOSH and MOAH varied significantly between experimental samples of raw milk (0.8-8.7 mg/kg MOSH; 0-2.8 mg/kg MOAH) and freeze-dried milk (4.5-13.1 mg/kg MOSH; 3.2-5.35 mg/kg MOAH). Almost all milk samples exceeded the recommended limits set by the Standing Committee for Plants, Animals, Food and Feed, Section for Novel Foods and Toxicological Safety of the Food Chain (ScoPAFF) of the European Commission: 0.5 mg/kg for products with fat content < 4% (samples C, D); 1.0 mg/kg for products with fat content > 4% (samples A, B).

The MOSH/MOAH concentrations measured in lyophilized milk (A_2; B_2; C_2; D_2) were significantly higher than those in liquid form (A_1; B_1; C_1; D_1) within the same category.

The test results indicate significant differences in MOH contamination levels. A T-test was applied to evaluate the differences in MOSH and MOAH distribution based on carbonic sub-fractions and total contamination levels (n-C_{10-50}) between raw milk and freeze-dried milk.

Table 3 presents the results of MOSH distribution based on carbon sub-fractions and total contamination levels (n-C_{10-50}) in experimental samples of unprocessed milk, aiding in the understanding of MOSH contamination levels in unprocessed samples. The Skewness coefficient for n-C_{10-16}, indicates a slight left skewness of the data, but not significant, while for the total level of contamination (n-C_{10-50}) it suggests a positive skewness, indicating values both lower and higher than the mean (3.73) in the dataset.

Table 4 shows the results of MOSH distribution structured based on carbon chain lenghts and total contamination levels (n-C_{10-50}) in experimental samples of freeze-dried milk.

For n-C_{10-16}, the Skewness coefficient of -0.75 indicates a slight left skewness in the distribution, suggesting that there are several values lower than the mean of 0.53.

Table 3. Distribution of MOSH mg kg^{-1} structured by carbonic sub-fractions and the total level of contamination ($n\text{-}C_{10\text{-}50}$) in experimental samples of unprocessed milk (A, B, C, D – 1)

	n-C_{10-16}	**n-C_{16-20}**	**n-C_{20-25}**	**n-C_{25-35}**	**n-C_{35-40}**	**n-C_{40-50}**	**n-C_{10-50}**
Mean	0.58	0.83	0.70	1.30	0.23	0.10	**3.73**
Standard Error	0.22	0.25	0.50	1.00	0.13	0.07	**1.73**
Median	0.60	0.85	0.25	0.35	0.10	0.05	**2.70**
Standard Deviation	0.44	0.51	1.00	2.00	0.25	0.14	**3.46**
Sample Variance	0.20	0.26	1.01	4.01	0.06	0.02	**11.97**
Kurtosis	(4.77)	(0.10)	3.87	3.97	4.00	1.50	**2.58**
Skewness	(0.13)	(0.26)	1.96	1.99	2.00	1.41	**1.52**
Range	0.90	1.20	2.10	4.10	0.50	0.30	**7.90**
Minimum	0.10	0.20	0.10	0.20	0.10	0	**0.80**
Maximum	1.00	1.40	2.20	4.30	0.60	0.30	**8.70**
Sum	2.30	3.30	2.80	5.20	0.90	0.40	**14.90**
Count	4.00	4.00	4.00	4.00	4.00	4.00	**4.00**
Confidence Level (95.0%)	0.70	0.80	1.60	3.19	0.40	0.23	**5.51**

For the total contamination (n-$C_{10\text{-}50}$), values in dataset vary below or above the mean of 8.83. Figure 1 and the results in Table 5 indicate significant differences between raw milk and freeze-dried milk ($p \leq .015$) for the following carbon sub-fractions: n-$C_{10\text{-}16}$, n-$C_{16\text{-}20}$, n-$C_{20\text{-}25}$, n-$C_{25\text{-}35}$, n-$C_{35\text{-}40}$ and n-$C_{40\text{-}50}$.

Table 4. Distribution of MOSH mg kg^{-1} structured by carbonic sub-fractions and the total level of contamination ($n\text{-}C_{10\text{-}50}$) in experimental samples of freeze-dried milk (A, B, C, D – 2)

	n-C_{10-16}	**n-C_{16-20}**	**n-C_{20-25}**	**n-C_{25-35}**	**n-C_{35-40}**	**n-C_{40-50}**	**n-C_{10-50}**
Mean	0.53	4.28	2.18	1.35	0.30	0.20	8.83
Standard Error	0.09	0.88	0.33	0.33	0.09	0.07	1.76
Median	0.55	4.30	2.15	1.40	0.30	0.15	8.85
Standard Deviation	0.17	1.76	0.66	0.66	0.18	0.14	3.51
Sample Variance	0.03	3.09	0.43	0.43	0.03	0.02	12.34
Kurtosis	0.34	1.44	1.41	1.61	(3.30)	1.50	1.46
Skewness	(0.75)	(0.08)	0.23	(0.45)	0.00	1.41	(0.04)
Range	0.40	4.30	1.60	1.60	0.40	0.30	8.60
Minimum	0.30	2.10	1.40	0.50	0.10	0.10	4.50
Maximum	0.70	6.40	3.00	2.10	0.50	0.40	13.10
Sum	2.10	17.10	8.70	5.40	1.20	0.80	35.30
Count	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Confidence Level (95.0 %)	0.27	2.80	1.04	1.04	0.29	0.23	5.59

Significant differences ($p = .002$) were observed for the total contamination level (n-$C_{10\text{-}50}$) of MOSH between raw milk (1) and freeze-dried milk (2). Freeze-dried milk (2) exhibited significantly higher contamination levels compared to raw milk (1), particularly evident in the values of n-$C_{10\text{-}50}$.

The effect size, Cohen's d was estimated at 0.3117 with a confidence interval ranging from 0.604 and 2.883, indicating significant results for n-$C_{10\text{-}16}$. For the total contamination level (n-$C_{10\text{-}50}$) of MOSH in raw milk and freeze-dried milk, Cohen's d was calculated as 4.2250, and with Hedges correction, it was 4.7568.

The 95 % confidence interval for Cohen's d ranged from 0.433 to 2.493, and for Hedges correction, it ranged from 0.385 to 2.214. These values suggest a significant and consistent difference in the effect of MOSH across various carbon chain lengths.

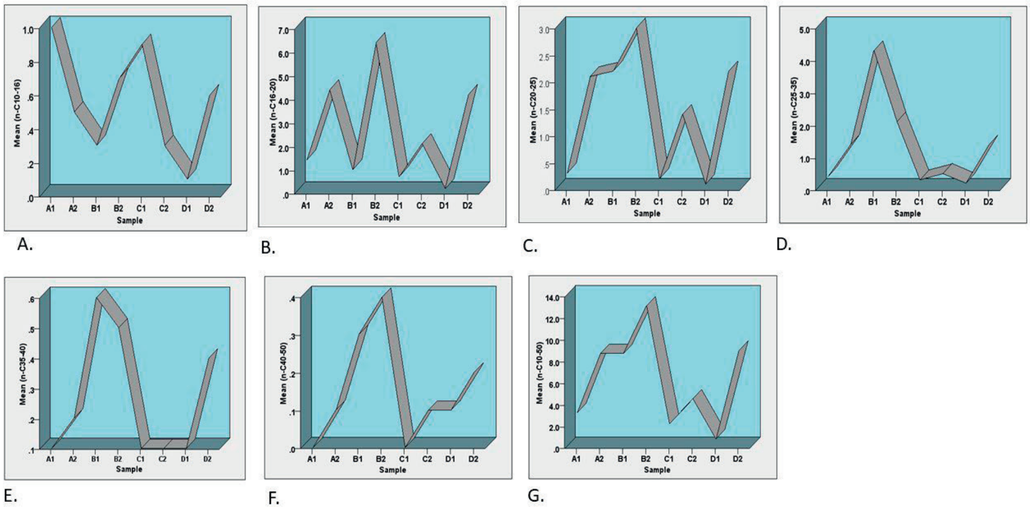

Figure 1. Distribution of structured MOSH averages at different carbon sub-fractions A (n-C_{10-16}), B (n-C_{16-20}), C (n-C_{20-25}), D (n-C_{25-35}), E (n-C_{35-40}), F (n-C_{40-50}) and total contamination level G (n-C_{10-50}) in experimental raw milk samples (A_1, B_1, C_1, D_1) and lyophilized milk samples (A_2, B_2, C_2, D_2)

Table 3. Differences in the distribution of MOSH mg kg^{-1} structured by carbonic sub-fractions and the total level of contamination (n-C_{10-50}) in experimental unprocessed milk samples (1) and freeze-dried milk samples (2)

	t	Df	Significance		Mean Difference	95 % Confidence Interval of the Difference	
			One-Sided *p*	Two-Sided *p*		Lower	Upper
n-C_{10-16}	4.991	7	<.001	.002	.5500	.289	.811
n-C_{16-20}	3.280	7	.007	.013	2.5500	.712	4.388
n-C_{20-25}	3.656	7	.004	.008	1.4375	.508	2.367
n-C_{25-35}	2.717	7	.015	.030	1.3250	.172	2.478
n-C_{35-40}	3.594	7	.004	.009	.2625	.090	.435
n-C_{40-50}	3.000	7	.010	.020	.1500	.032	.268
n-C_{10-50}	4.201	7	.002	.004	6.2750	2.743	9.807

T-test, significance level *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

Table 4. Cohen's d and Hedges' correction results for evaluating the effect of MOSH mg kg^{-1} between experimental samples of unprocessed milk (1) and lyophilized milk (2), with 95 % confidence intervals for carbonic sub-fractions n -C_{10-16}, n-C_{16-20}, n-C_{20-25}, n-C_{25-35}, n-C_{35-40}, n-C_{40-50} and total contamination level (n-C_{10-50})

		Standardizer[a]	Point Estimate	95 % Confidence Interval	
				Lower	Upper
n-C_{10-16}	Cohen's d	.3117	1.765	.604	2.883
	Hedges' correction	.3509	1.567	.537	2.561
n-C_{16-20}	Cohen's d	2.1987	1.160	.224	2.051
	Hedges' correction	2.4754	1.030	.199	1.821
n-C_{20-25}	Cohen's d	1.1122	1.292	.311	2.229
	Hedges' correction	1.2522	1.148	.276	1.980
n-C_{25-35}	Cohen's d	1.3792	.961	.088	1.789
	Hedges' correction	1.5528	.853	.078	1.589
n-C_{35-40}	Cohen's d	.2066	1.271	.296	2.199
	Hedges' correction	.2326	1.129	.263	1.954
n-C_{40-50}	Cohen's d	.1414	1.061	.157	1.920
	Hedges' correction	.1592	.942	.139	1.705
n-C_{10-50}	Cohen's d	4.2250	1.485	.433	2.493
	Hedges' correction	4.7568	1.319	.385	2.214

a. The denominator used in estimating the effect sizes. Cohen's d uses the sample standard deviation. Hedges' correction uses the sample standard deviation, plus a correction factor.

Cohen's d is a standard measure of effect size, and Hedges' correction is a modification of Cohen's d designed to account for potential overestimation in small sample sizes. Both measures are valuable for interpreting the magnitude of differences between groups in research (Table 4).

Table 5 shows the results of MOAH distribution structured by carbon sub-fractions and total contamination level (n-C_{10-50}) in experimental unprocessed milk samples, aiding in the understanding of MOAH contamination levels in these samples. For n-C_{10-16}, the Skewness coefficient of 2.00 indicates a significant right skewness in the data distribution, suggesting a trend towards higher values in the dataset. The total contamination level (n-C_{10-50}) exhibits positive skewness in the distribution, indicating that the dataset contains values lower and higher than the mean (0.70).

Table 5. Distribution of MOAH mg kg^{-1} structured by carbonic sub-fractions and the total level of contamination (n-C_{10-50}) in experimental samples of unprocessed milk

	n-C_{10-16}	**n-C_{16-25}**	**n-C_{25-35}**	**n-C_{35-50}**	**n-C_{10-50}**
Mean	0.15	0.43	0.13	0	0.70
Standard Error	0.15	0.43	0.13	0	0.70
Median	0	0	0	0	0
Standard Deviation	0.30	0.85	0.25	0	1.40
Sample Variance	0.09	0.72	0.06	0	1.96
Kurtosis	4.00	4.00	4.00	0	4.00
Skewness	2.00	2.00	2.00	0	2.00
Range	0.60	1.70	0.50	0	2.80
Minimum	0	0	0	0	0
Maximum	0.60	1.70	0.50	0	2.80
Sum	0.60	1.70	0.50	0	2.80
Count	4.00	4.00	4.00	4.00	4.00
Confidence Level (95.0 %)	0.48	1.35	0.40	0	2.23

The results of the distribution of MOAH (mg/kg) structured by the total contamination level (n-C_{10-50}) in experimental samples of freeze-dried milk (2) indicate a positive skewness in the distribution to the right (Table 6). This suggests that there are values in the dataset higher than average (4.25).

The distribution of MOAH structured by carbonic sub-fractions and the total contamination level (n-C_{10-50}) in experimental samples of unprocessed milk (1) and freeze-dried milk (2), did not reveal significant differences for n-C_{10-16} and n-C_{35-50} carbon sub-fraction (p = 0.175) (Figure 2).

Significant differences (p = 0.008) were observed between unprocessed milk and freeze-dried milk for the carbon sub-fractions n-C_{16-25}, n-C_{25-35} and n-C_{10-50} (Table 7).

Table 6. Results of the distribution of MOAH mg kg^{-1} structured by carbonic sub-fractions and total level of contamination (n-C_{10-50}) in experimental samples of freeze-dried milk

	n-C_{10-16}	**n-C_{16-25}**	**n-C_{25-35}**	**n-C_{35-50}**	**n-C_{10-50}**
Mean	0	2.96	1.28	0.01	4.25
Standard Error	0	0.40	0.08	0.01	0.45
Median	0	2.90	1.30	0	4.23
Standard Deviation	0	0.80	0.15	0.03	0.90
Sample Variance	0	0.64	0.02	0.00	0.80
Kurtosis	0	(0.92)	(3.90)	4.00	0.31
Skewness	0	0.38	(0.37)	2.00	0.15
Range	0	1.85	0.30	0.05	2.15
Minimum	0	2.10	1.10	0	3.20
Maximum	0	3.95	1.40	0.05	5.35
Sum	0	11.85	5.10	0.05	17.00
Count	4.00	4.00	4.00	4.00	4.00
Confidence Level (95.0 %)	0	1.27	0.24	0.04	1.43

Table 7. Differences in the distribution of structured MOAH by carbon sub-fractions and the total contamination level ($n\text{-}C_{10\text{-}50}$) in experimental samples of unprocessed milk and freeze-dried milk

	t	df	Significance		Mean Difference	95 % Confidence Interval of the Difference	
			One-Sided *p*	Two-Sided *p*		Lower	Upper
$n\text{-}C_{10\text{-}16}$	1.000	7	.175	.351	.0750	-.102	.252
$n\text{-}C_{16\text{-}25}$	3.078	7	.009	.018	1.69375	.3927	2.9948
$n\text{-}C_{25\text{-}35}$	3.076	7	.009	.018	.7000	.162	1.238
$n\text{-}C_{35\text{-}50}$	1.000	7	.175	.351	.00625	-.0085	.0210
$n\text{-}C_{10\text{-}50}$	3.200	7	.008	.015	2.47500	.6461	4.3039

T-test, significance level *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

Table 8. Cohen's d and Hedges' correction results for evaluating the effect of MOAH mg kg^{-1} between raw milk and experimental freeze-dried milk samples; 95 % confidence intervals for $n\text{-}C_{10\text{-}16}$ carbon sub-fractions, $n\text{-}C_{16\text{-}20}$, $n\text{-}C_{20\text{-}25}$, $n\text{-}C_{25\text{-}35}$, $n\text{-}C_{35\text{-}40}$, $n\text{-}C_{40\text{-}50}$ and total $n\text{-}C_{10\text{-}50}$ contamination level

		Standardizer[a]	Point Estimate	95 % Confidence Interval	
				Lower	Upper
$n\text{-}C_{10\text{-}16}$	Cohen's d	.2121	.354	-.374	1.058
	Hedges' correction	.2388	.314	-.333	.940
$n\text{-}C_{16\text{-}25}$	Cohen's d	1.55619	1.088	.176	1.956
	Hedges' correction	1.75207	.967	.156	1.737
$n\text{-}C_{25\text{-}35}$	Cohen's d	.6437	1.088	.175	1.955
	Hedges' correction	.7247	.966	.156	1.736
$n\text{-}C_{35\text{-}50}$	Cohen's d	.01768	.354	-.374	1.058
	Hedges' correction	.01990	.314	-.333	.940
$n\text{-}C_{10\text{-}50}$	Cohen's d	2.18763	1.131	.205	2.013
	Hedges' correction	2.46298	1.005	.182	1.788

[a]The denominator used in estimating the effect sizes. Cohen's d uses the sample standard deviation. Hedges' correction uses the sample standard deviation, plus a **correction** factor.

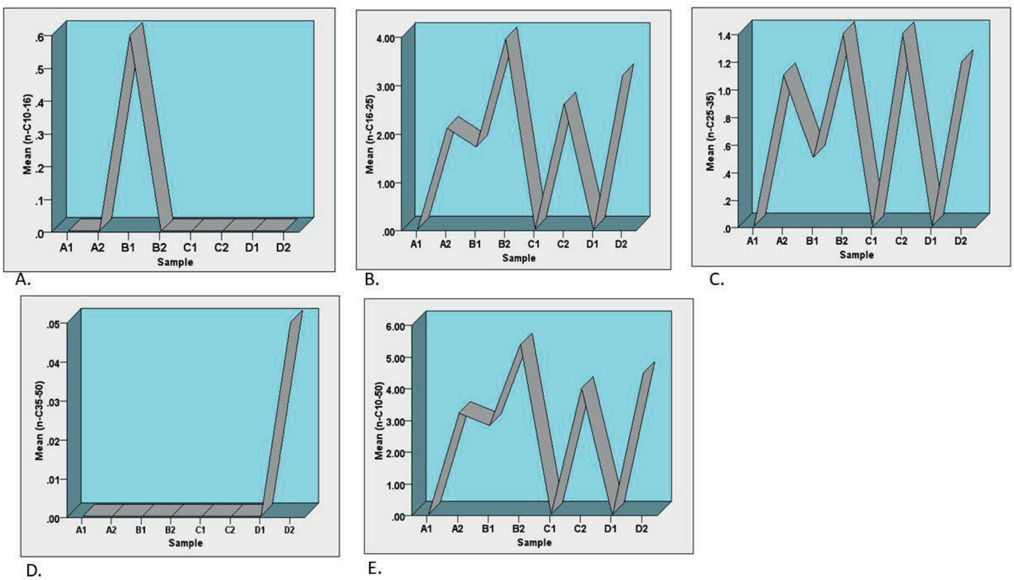

Figure 2. Distribution of structured MOAH media at different carbon sub-fractions A ($n\text{-}C_{10\text{-}16}$), B ($n\text{-}C_{16\text{-}25}$), C ($n\text{-}C_{25\text{-}35}$), D ($n\text{-}C_{35\text{-}50}$) and total contamination level E ($n\text{-}C_{10\text{-}50}$) in experimental raw milk samples (A_1, B_1, C_1, D_1) and freeze-dried milk samples (A_2, B_2, C_2, D_2)

For the total contamination level (n-C_{10-50}) of MOAH in raw milk (1) and freeze-dried milk (2), the point estimate of Cohen's d is 2.18763, and for the Hedges correction it is 2.46298. The 95 % confidence interval, for Cohen's d ranges from 0.205 to 2.013, and for the Hedges correction it ranges from 0.182 to 1.788 (Table 8). These results highlight a significant trend in the MOAH effect across the carbon fraction length scale, as indicated by the point estimates and confidence intervals for Cohen's d and Hedges correction.

This study underscores the potential for food contamination with MOH during food processing, particularly in the context of packaging. Different components of food contact material (FCM) can migrate into food, particularly following volatilization under the influence of temperature (Groh et al., 2021; Sonego et al., 2023).

Waxed paper (paraffin paper) which consists of cellulose and silicon, is an example of FCM that poses contaminations risks due to its chemical composition and production processes (Fellows, 2022). Previous research (Lorenzini et al., 2010), has shown significant migration of MOH from FCM to food products during processing. Studies have also highlighted the transfer of MOH from packaging materials to food (Pack et al., 2020; Conchione et al., 2020; Pan Jing et al., 2021; Fengler & Gruber, 2022). However, concrete scientific guidance on this matter is lacking in current legislation, leaving the possibility and risk of MOH contamination through packaging a subject of debate. Due to their higher toxicity (EFSA et al., 2019), MOAH remains a priority for monitoring contaminants in the food chain.

CONCLUSIONS

The difference in contamination levels between raw and processed milk samples, and the variation in contamination levels following processing, were evaluated to assess the contamination risk associated with different milk processing methods. A T-test was applied to assess the differences in the distribution of MOSH and MOAH, structured by carbon sub-fractions and the total contamination level (n-C_{10-50}), in experimental samples of unprocessed milk (1) and lyophilized milk (2).

Milk is highly susceptible to exogenous contamination regardless of its form. The MOSH/MOAH concentrations measured in lyophilized milk (A_2; B_2; C_2; D_2) showed significantly higher values than liquid samples from the same category (A_1; B_1; C_1; D_1). These test results are essential for evaluating the impact of the freeze-drying process on these contaminants.

This study highlights the potential for food contamination with MOH during food processing. Various components of FCM can migrate into food products, particularly following volatilization under the temperature.

The findings indicate that milk processing operations significantly influenced the variation in contamination levels of freeze-dried milk. However, precise identification of the causes of contamination is challenging due to milk's increased susceptibility to contamination and the involvement of multiple factors.

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TECHNOLOGICAL ADVANCES AND SOCIO-ECONOMIC IMPLICATIONS IN THE POULTRY INDUSTRY - AN ANALYSIS OF CURRENT TRENDS IN POULTRY MEAT PRODUCTION AND CONSUMPTION

Andrada-Elena MOISE, Minodora TUDORACHE, Ioan CUSTURĂ, Dănuţ Nicolae ENEA, Aurelia OSMAN, Dumitru DRĂGOTOIU

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: eandrada62@yahoo.com

Abstract

This paper investigates the evolution of the poultry industry in recent decades, highlighting technological advances and socio-economic changes that have influenced the production and consumption of poultry meat. By analysing data on genetic selection, feeding technologies and impact on animal health, the paper highlights how the poultry industry has become a key pillar of food security. Consumer preferences and consumption trends, including demand for poultry semi-finished products, are also examined. This analysis provides a comprehensive insight into the evolution of the poultry industry and its impact on society, highlighting the importance of continuous research and innovation to ensure sustainable and high-quality poultry meat production.

Key words: *chicken feeding, poultry industry, quality standard, semi-preparation, technological processes.*

INTRODUCTION

Currently, poultry farming is considered one of the most stable branches of the agri-food complex, playing an essential role in guaranteeing food security of the state. (Macari, 2015).

After a transition period, this industry quickly managed to revitalize egg and meat production, registering a vertiginous development (Macari et al., 2014).

In the period between 1967 and 1971, Romania laid the modern foundations of poultry farming, developing specialized programs for their selection and hybridization.

In 1971, the Central Poultry Plant in Bucharest was established, marking a crucial moment in the development of the Romanian poultry sector (Văcaru-Opriş, 2004).

Broiler breeders have observed that feeding chicks to saturation can lead to a decrease in reproductive performance, but can achieve optimal hatching egg production by applying appropriate dietary restrictions (D'Eath et al., 2009). Limiting the diet to broilers is a significant concern, given the need to maintain a high level of feeding motivation (Van Krimpen & De Jong., 2014).

Reducing feed consumption by 25% to 33% during the growth phase and by 50% to 90% during production is a common practice to minimize metabolic disorders and improve productivity in the poultry industry (Bruggeman et al., 1999; Chen et al., 2006). With the improvement of the growth rate of broilers, birds reach slaughter weight at younger and younger ages.

There is ample evidence indicating that restricting food intake can provoke physiological responses such as stress, boredom, aggression, and other abnormal behaviors in poultry (Mench, 2002; Van Krimpen & De Jong, 2014). In a 1983 experiment, it was found that chickens fed variable amounts of fodder in hot conditions experienced an 8.5% increase in body weight, compared to the thermoneutral environment where the increase was 9.6% (Smith, 2002).

As animal feed costs rise, total production costs are on an upward trend, with feeding accounting for between 60% and 70% of them. Most people are familiar with the concept of the three "R's" (reduce, reuse, recycle). Adding a fourth "R", responsibility, could be the key to a sustainable society. The use of by-products as feed ingredients is one of the most effective ways to dispose of them, but this practice can sometimes be restricted by legislation and the specific nature of the by-product (Vlaicu et al., 2017).

In recent years, chicken meat production has seen a significant increase, thanks to outstanding productive performance, improvements in feeding technology and advances in animal health, biosecurity and welfare (Custura et al., 2019). In most countries, poultry meat is obtained within industrial production systems, but there are significant differences in the level of efficiency and especially in economic profitability (Curea et al., 2023). The main elements of the energy flow through the body of a broiler, used in the analysis of quantitative data on energy efficiency, include the following: the constant energy content of proteins and lipids, while other components, such as the mass of proteins and lipids in the bird's body, may vary depending on the breed (Tallentire et al., 2016). Variations in locomotor activity of young chickens are strongly influenced by genetic factors, and research has shown that this is reduced by 6% in fast-growing breeds compared to slow-growing broiler breeds (Bokkers & Koene, 2003).

There are a variety of natural or synthetic feed additives that can be used as alternatives to antibiotic-based growth promoters. They aim to improve technical and economic performance. Among these supplements are probiotics, which have shown the ability to improve production parameters such as body weight, specific consumption and reduction of animal mortality (Nawaz et al., 2016). The normal microbiota in the digestive tract of birds plays an essential role in maintaining the health and functionality of the digestive tract (Miles, 2002). Fast-growing broilers also demonstrated lower levels of physical activity compared to slow-growing broilers, both in terms of behaviors such as trickling, stretching, and pecking on the ground, as well as other aspects of their behavior (Siegel et al., 1997). The ability to identify and remedy problems in the environment in which birds live, as well as in their diet, can have positive effects on their health and performance (Miles & Butcher, 2002).

MATERIALS AND METHODS

The paper is based on the examination of various reports and articles related to the production and quality of poultry, especially chicken, and analyzes the correlation with products derived from it. Scientific and genetic advances over the past five decades, together with the unique biological characteristics of birds, have facilitated the production of high-quality chicken meat hybrids and led to improved technical standards (Cărătuș, 2020). The livestock industry supplies a diverse range of products, which include not only milk and meat, but also goods with high added value, such as leather or pre-cooked food.

Economic development and individual income growth have contributed to increased demand and changes in diets (Henning et al., 2006).

Among the factors that have contributed to the evolution of consumer education is the significant influence of media reports on public perception of the relationship between diet and health (Lungu et al., 2023). The quality standard of products is one of the key criteria taken into account by consumers of animal products when deciding on their purchase (Custura et al., 2013). Among consumers aged 50 years and older, an increased interest in the quality of chicken meat is observed. As for the active population, people who have preferences regarding certain parts of chicken predominate and appreciate chicken for its nutritional benefits. For example, Serbia has the highest proportion of consumers showing disinterest in chicken meat (Dubravka et al., 2017).

RESULTS AND DISCUSSIONS

Global poultry production increased by approximately 46 million tonnes between 2003 and 2018 (WATT executive guide to world poultry trends, 2018), and their breeding process is becoming increasingly centralised (Schibabaw, 2019). It is estimated to increase from around 123 million tonnes in 2018 to 139 million tonnes in 2027. At the same time, pork production, its main competitive product, is estimated to increase from around 121 million metric tons to 130.9 million tons in 2027 (WATT executive guide to world poultry trends, 2018).

Figure 1 shows significant differences in mixed sex size and body weight between birds aged 0, 28 and 56 days. Thus, the development over the years is illustrated.

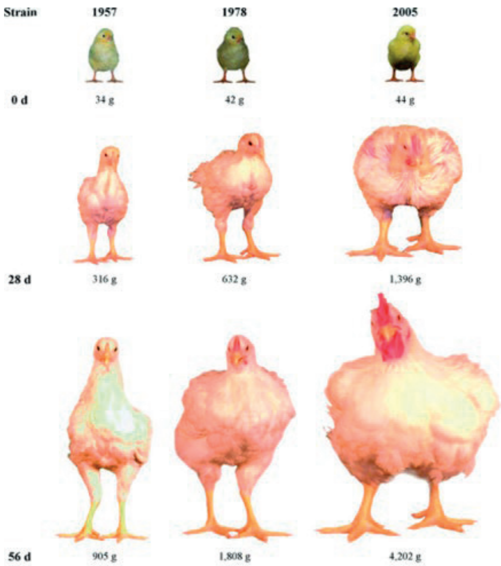

Figure 1. A front profile pictorial record at 0, 28, and 56 d of age along with average mixed-sex BW for each strain (Zuidhof et al., 2014)

The average meat yield per carcass, expressed in kilograms per chicken, has changed over time with an increase in body weight from one year to the next (Figure 2).

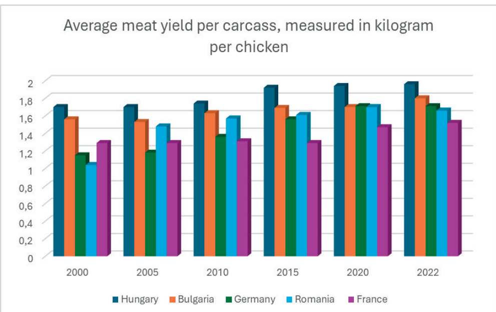

Figure 2. Average meat yield per carcass (Source: Our World in Data - Meat and Dairy Production, 2000-2022)

The present data in Figure 2 indicate a higher yield of meat per carcass in Hungary compared to France, suggesting significant variations in farming practices and poultry farming conditions between the two countries.

After examining the data collected from "Our World in Data - Meat and Dairy Production" regarding the yield of meat per carcass in the five countries of the European Union, according to Table 1, we find that there were no significant differences in the amount of raw material during over 22 years.

Table 1. Appendix (Source: Our World in Data - Meat and Dairy Production)

	Hungary	Bulgaria	Germany	Romania	France
2000	1.70 kg	1.56 kg	1.15 kg	1.04 kg	1.29 kg
2005	1.70 kg	1.53 kg	1.18 kg	1.48 kg	1.29 kg
2010	1.74 kg	1.63 kg	1.36 kg	1.57 kg	1.31 kg
2015	1.92 kg	1.69 kg	1.56 kg	1.61 kg	1.29 kg
2020	1.94 kg	1.70 kg	1.71 kg	1.70 kg	1.47 kg
2022	1.96 kg	1.80 kg	1.71 kg	1.66 kg	1.52 kg

On the other hand, in accordance with the data in Table 2, poultry meat production recorded a significant increase in Germany and Romania, while France recorded a decrease during the same period of time. These trends can be attributed to the conditions of poultry breeding and welfare in each country. In terms of people's nutrition, food is not only a provider of essential nutrients and energy, but also a carrier of information.

Table 2. Poultry production (Source: Our World in Data - Meat and Dairy Production)

Poultry production, 2000 to 2022. Expressed in tons				
	Hungary	Germany	Romania	France
2000	470,028	790,202	259,414	2,147,673
2022	485,157	1,506,688	475,450	1,478,476
Absolute Change	+15,129	+716,486	+216,036	-669,198
Relative Change	+3%	+91%	+83%	-31%

Human nutrition is fundamental to an individual's well-being. The link between health and personal balance is closely linked to food choices. Adopting a proper diet, along with other beneficial behavioral practices, contributes to a healthy and balanced life (Hodoșan et al., 2023).

One study shows that the frequency of chicken consumption for a person is 2 to 3 times a week, with a dietary intake of at least 90-100 g at each meal, within a daily meal (Hessel et al., 2019).

Meat processors give high priority to the diversified assortment of chicken meat products, which is considered the main attribute of quality. In the context of retail strategies, special attention is paid to issues such as adequate shelf lighting and strategic product placement in order to maximise visibility and attract consumer attention (Djekic et al., 2018). As for the consumption of chicken meat products, they are in high demand around the world.

According to own study on preferences for semi-finished products, as presented in Table 3, we found that in Romania there is a significant demand for Milanese schnitzel, mainly attributed to its savory taste and aroma, generated by the presence of Parmesan cheese in the crust. In Germany and Croatia, preferences go to Viennese products, which also include flour in the composition, while in England they opt for double bread crumbled schnitzel. In Croatia and France, ready-to-eat chicken cubes are preferred, they only require the defrosting process and can later be added as a topping on various dishes.

Table 3. Chicken semi-finished products according to the preferences of that country (own source)

Country	Preferred product
Croatia	Viennese chicken cordon
Finland	Cooked chicken cubes
Romania	Milanese
Germany	Viennese Schnitzel
England	Schnitzel double crumbed
France	Cooked chicken stripes

As detailed in Table 4, the target values and limit for colony forming units concentration (CFU/g) and presence of Salmonella for poultry meat products shall be determined according to ISO method 6579, with the objective of achieving absence in 25 grams for both measurements, according to specific standards and food safety requirements established for assessing food quality.

Table 4. Specific Product Patterns, Parameters of biological testing (Moise, 2023)

Testing parameter	Testing method	Target value CFU/g	Limit value CFU/g
Salmonella	ISO 6579	Absent in 25 g	Absent in 25 g

In order to comply with quality standards, a trace metal check is carried out using a special detector in accordance with the test procedures. The purpose of this procedure is to ensure the conformity of the packaged product with the quality standards, by identifying and eliminating possible traces of metal present in the packaged product. This reduces the risk of contamination and guarantees the safety and integrity of the product, protecting all consumers, according to the information in Table 5.

Table 5. Parameters of physical testing (Moise, 2023)

Testing of metal traces	Purpose	Max 3.5mm Fe, Max 3.5mm NonFe, Max 3.5mm StSt
	Testing requirements	Every package to be checked by metal detector.

Based on the Table 6, more than 50% of leading manufacturers and brands of chicken products occupy an established position in the market.

Table 6. Semi-prepared food, market share July 2019-June 2020 (Source: revistaprogresiv.ro)

Top producers:
Frosta, Macromex, Hochland, Europrod, Transavia - 56.3%.
Own brand owners - 25.4%
Others - 18.3%
Top Brands:
Edenia, Hochland, Agricola, Papane, Frosta - 52.8%
Own brand owners - 25.4%
Others - 21.8%

As per the Gierlinger-Holding, the characteristics leading to the purchase of ready-to-eat products include:
- Speed of preparation;
- Pleasant taste;
- Favorable price-quality ratio;
- Offering an alternative to serving in restaurants or dining out;
- Pleasant appearance of the product;
- The advantage of a meal at an affordable price;
- It is a product appreciated by both adults and children.

CONCLUSIONS

After a careful analysis of the data on the consumption behaviour of the population regarding semi-prepared products, it is observed that they are spread to a significant extent, especially in the urban environment. There is an obvious preference for these products among busy people who do not have time to cook, but have higher incomes. It is also found that the interest in semi-prepared products is more pronounced in the urban environment compared to the rural one. In terms of consumption behaviour according to days of the week and seasons, no significant differences are observed, and search preferences go mainly to supermarkets and local shops. These observations underline the important role of the poultry industry in ensuring food security and adapting to market needs.

Also, the focus on the responsible use of resources in the poultry industry is related to the concern for product quality and safety. The introduction of natural additives and alternatives to antibiotic-based growth promoters is an important direction to meet the health demands of consumers and to maintain high quality standards in poultry production.

In conclusion, previous observations on consumption preferences, purchasing behaviour and the importance of the poultry industry for food security are complemented by findings on technological evolution and innovations in this field. These aspects are essential to ensure a sustainable and high-quality production of poultry meat, in accordance with the demands and expectations of consumers.

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INFLUENCE OF THE ADDITION OF APICULTURE PRODUCTS ON THE CHARACTERISTICS OF FORTIFIED YOGHURT

Camelia MOLDOVAN[1], Mirela-Viorica POPA[1], Corina Dana MISCA[1], Diana-Nicoleta RABA[2], Aurica-Breica BOROZAN[3], Mariana-Atena POIANA[1], Carmen Daniela PETCU[4], Delia-Gabriela DUMBRAVA[1]

[1]University of Life Sciences "King Mihai I" from Timisoara, Faculty of Food Engineering, Calea Aradului 119, Timisoara 300645, Romania
[2]University of Life Sciences "King Mihai I" from Timisoara, Faculty of Management and Rural Tourism, Calea Aradului 119, Timisoara 300645, Romania
[3]University of Life Sciences "King Mihai I" from Timisoara, Faculty of Engineering and Applied Technologies, 119 Calea Aradului, 300645 Timisoara, Romania
[4]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Veterinary Medicine, 105 Independenţei Spl, District 5, 050097, Bucharest, Romania

Corresponding author email: deliadumbrava@usvt.ro

Abstract

Dairy products represent food appreciated by a large number of consumers, of all ages. The aim of this work was to fortify yoghurt with bee products and to characterize sensory, physico-chemical and microbiologically the products obtained. We used for fortification: honey, pollen and royal jelly and propolis. Sensory evaluation showed good acceptability of all products, but the most appreciated were those with honey in combination. The addition of honey as such or in combination reduces the acidity of the fortified yoghurt. Antioxidant activity was greatly enhanced as a result of fortifying the yoghurt, with most antioxidants found in the royal jelly yoghurt sample. Antimicrobial activity was substantially inhibited by the presence of bee products.

Key words*: acidity, antimicrobial activity, antioxidant activity, yogurt, sensorial.*

INTRODUCTION

The popularity of dairy pods is due to both sensory and nutritional characteristics, with well-known consumer benefits of immune stimulation and digestibility (Oprea et al., 2019; Prokisch et al., 2022). From the category of acidic dairy products, yogurt is at the top of consumers' preferences, both in the form of natural yogurt and in the form fortified with various additions (fruits, vegetables, seeds and nuts, aromatic/spice/medicinal plant, bee products) in the form of: pieces as such, purees, flours, juices, jams, oils or extracts) (Moldovan et al., 2021). The diversity of the assortment range of yogurt was achieved due to the consumers demands/needs. Literature studies on the benefits of yogurt consumption are very numerous. Yogurt is considered a functional food, and the latest studies confirm its antidiabetic and hypoglycemic effects (Khorraminezhad & Rudkowska 2021, cited by Prokisch et al., 2021), given that diabetes is in the top 10 diseases that cause death (WHO, 2021). The multitude of benefits of bee products have been known since ancient times. Honey and bee products have been used throughout time, both for their sensory and nutritional properties, and for therapeutic purposes (Ammar et al., 2015; Fratini et al., 2016). The presence in honey of bioactive compounds such as phenolic compounds, flavonoids, carotenoid-like derivatives, organic acids, Maillard reaction products, catalase, ascorbic acid and other antioxidant compounds (Bogdanov et al., 2008, cited by Ammar et al., 2015) gives honey antibacterial, antimutagenic, antiproliferative, hepatoprotective, hypoglycemic and antioxidant effects (Erejuwa et al., 2010; Ghashm et al., 2010, cited by Ammar et al., 2015). Thus, the sensory characteristics and increased nutritional value of honey have determined its incorporation into various food products. The combination of yogurt and honey has been the subject of numerous research studies. The results show that the addition of honey had beneficial effects on the sensory characteristics (Pereira, 2003; Varga, 2005; Ammar et al., 2015; Machado et al., 2017; Zlatev et al., 2018; Coskun & Karabulut Dirikan, 2019; Mohan et al., 2020; Camacho-Bernal et al., 2021), physico-chemical (Ammar et al., 2015, Coskun & Karabulut Dirikan, 2019) and microbiological (Coskun & Karabulut Dirikan, 2019; Mohan et al., 2020; Camacho-Bernal et al., 2021). It was also observed the reduction of the syneresis phenomenon (Ammar et al., 2015; Mohan et al., 2020; Camacho-Bernal et al., 2021), the increase of viscosity (Camacho-Bernal et al., 2021), acidity (Ammar et al., 2015; Coskun & Karabulut Dirikan, 2019; Mohan et al., 2020; Camacho-Bernal et al., 2021) and the total polyphenol content (Camacho-Bernal et al., 2021). Due to the presence of the carbohydrate substrate in yogurt, an increase in the viability of bifidobacteria was observed (Ammar et al., 2015; Camacho-Bernal et al., 2021), which is why honey could be used not only as a sweetener, but also as a prebiotic in the production of bio-yogurt (Ammar et al., 2015; Mohan et al., 2020; Camacho-Bernal et al., 2021). The presence of honey in yogurt improved the antioxidant activity compared to control yogurt (Camacho-Bernal et al., 2021),

The specialized literature includes pollen on the list of functional foods due to its nutritional characteristics, having applicability, both in the food industry and in apitherapy. Pollen contains proteins (20%-60%), lipids (1-32%), carbohydrates (40%-60%), fibers, mineral salts (3%), vitamins (A, B, C, D, E), but the therapeutic effects are mainly owed to the presence of antioxidant phenolic compounds (Atallah, 2016; Karabagias et al., 2018). Studies have attributed antifungal, antimicrobial, antiviral, anti-inflammatory, hepatoprotective, anticancer immunostimulatory and local analgesic properties to pollen (Karabagias et al., 2018). The addition of pollen to yogurt significantly improved the flavor, body, texture (Atallah, 2016; Karabagias et al., 2018) and color of yogurt (Özcan et al., 2020). At 0.5% pollen in yogurt, a good level of acceptability was reported; at 2.5-20 mg/ml pollen in yogurt, the taste was negatively affected, but the texture was improved (Camacho-Bernal et al., 2021). Antioxidant activity and polyphenol content of yogurts were significantly higher due to pollen addition (Karabagias et al., 2018), and acidity was lower (Atallah, 2016). The results of the studies by Karabagias et al. (2018) show that the addition of pollen to yogurt decreased the population of microorganisms during storage.

The chemical composition of royal jelly is remarkable. This is a colloidal compound consisting of water (60-70%), carbohydrates (11-23%), proteins (9-18%), lipids (4-8%), vitamins and some mineral salts (08-3%) (Fratini et al., 2016; Atallah, 2016). In specialized literature, royal jelly is also included in the category of functional foods due to its high nutritional value, its antioxidant activity and the beneficial effects on the immune system and memory, its hypoglycemic, hypocholesterolemic, hypotensive, antitumor effects (Kavas, 2022). Probiotic supplements with the addition of royal jelly have proven to be products with good acceptability (Metry et al., 2009; Atallah, 2016; Camacho-Bernal et al., 2021; Kavas, 2022). The addition of 2% (w/v) royal jelly resulted in the significant improvement of the physicochemical, rheological, sensory and microbiological properties of yogurt (Kavas, 2022). It was observed that the syneresis phenomenon was significantly reduced in the yogurt with royal jelly compared to the control yogurt (Atallah & Morsy, 2017). It has also been reported that royal jelly acts against Gram-positive bacteria, but the effectiveness against Gram-negative ones is less (Fratini et al., 2016). Metry et al. (2009) found that the addition of royal jelly up to 0.6% improved the sensory characteristics of yogurt with no negative effects on lactic acid bacteria and the lowest number of yeasts and molds.

Propolis, another bee product, is known in alternative medicine for its antimicrobial, anti-inflammatory, antitumor, immunomodulatory and antioxidant effects (Camacho-Bernal et al., 2021). Studies have shown that the optimal level of propolis in yogurt would be up to 0.3%, above this level the acceptability of yogurt decreases (unpleasant sensation and bitter taste), the presence of an aftertaste and the change in taste, color and smell are noted (Camacho-Bernal et al., 2021). The propolis extract caused a considerable increase in the content of phenolic compounds, flavonoids and considerably improved the antioxidant activity, about 50% more than in the control yogurt (Camacho-Bernal et al., 2021). The presence of propolis in yogurt acted as an inhibitor of lactobacilli, but stimulated the activity of *Streptococcus thermophylus* (Gunes-Bayi et al., 2021). Studies show that bee products provide major benefits to the yogurts they are added to. The present study comes to complete the existing results until now, being known that bee products have variable chemical composition, depending on the geographical area of origin, on the diverse flora with unique particularities. Therefore, our experiment aimed at the fortification of yogurt with bee honey, pollen, royal jelly and propolis - all being products of the beehive in the western part of Romania.

MATERIALS AND METHODS

Preparation of fortified yogurt

For fortification, Greek yogurt 10% fat, purchased from a supermarket, was used. Polyflora bee honey, pollen, royal jelly and propolis were added to this type of yogurt according to Table 1.

Table 1. The constitution of yogurt samples with addition

Samples	wavering
yogurt control	C
yogurt + honey 2%	YH
yogurt + pollen 1%	Yep
yogurt + propolis 0.5%	YPr
yogurt + royal jelly 1%	YRJ
yogurt + honey 2% + pollen 1%	YHPo
yogurt + honey 2% + propolis 0.5%	YHPr
yogurt + honey 2% + royal jelly 1%	YHRJ

The bee products used were incorporated into the yogurt and kept at refrigeration temperature for 24 hours, after which the sensory and chemical analysis was performed. During this time the samples were kept in the refrigerator.

Titrable acidity, sugar content, free radical 2,2-diphenyl-1-picryl-hydrazyl (DPPH) scavenging activity (RSA) and total polyphenols content were determined by chemical examination.

The titratable acidity was determined by titrating the samples with NaOH in the presence of alcoholic phenolphthalein, up to a pink color change. The results were expressed in Thorner degrees.

The sugar content was determined by the refractometric method using the KRUSS DR301-95 device, and the results were expressed in degrees Brix.

Antioxidant activity (AA). Aqueous extracts (1:10) were prepared for AA evaluation of yogurt samples with addition of bee products, which were left to rest for 15 minutes. In a 50 ml beaker, 2 ml of aqueous extract were added to which 1 ml of H_2SO_4 solution was added 20% After one minute, a drop (0.04 ml) of 0.1N potassium permanganate solution was added to the acidified solution. With the help of a timer, the disappearance of the pink color (discoloration time) of the solution was monitored. The results were expressed in seconds.

The DPPH (2,2-diphenyl-1-picryl-hydrazyl) free radical scavenging activity was evaluated by the spectrophotometric method, according to the method described in the previous work (Dumbrava et al., 2023). The results were expressed in percentages.

Total polyphenols content was determined colorimetrically with the Folin-Ciocalteu reagent, as was done in the previous work (Moldovan et al., 2023), the results being expressed in mg gallic acid equivalents / g.

Sensory characteristics were: appearance, color, consistency, smell and taste, their assessment being made in accordance with ISO 4121:2002. The sensory examination was performed by 16 evaluators, who awarded between 1-9 points (1-dislike extremely; 2-dislike very much; 3-dislike moderately; 4-dislike slightly, 5-neither dislike nor like, 6-like slightly; 7-like moderately; 8-like very much; 9-like extremely).

The antimicrobial activity of yogurt samples with bee products was tested against *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922) according to the protocol presented by Borozan et al., (2023).

The Microsoft Excel software was used for the ***statistical analysis*** of the obtained results. Each determination was performed in triplicate, calculating the arithmetic mean of the obtained values.

RESULTS AND DISCUSSIONS

The acidity

The results regarding acidity are presented in Figure 1.

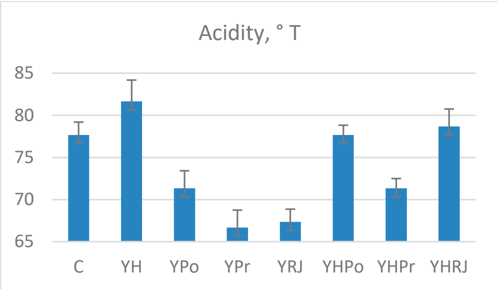

Figure 1. Acidity of yogurt samples

The results show that the addition of bee products influenced the acidity of the fortified yogurts differently. Thus, honey increased the acidity of YH yogurt by +5.15% compared to Y control. This variation was also reported by other authors (Metry & Owayss, 2009; Ammar et al., 2015; Bakr et al., 2015; Das et al., 2015; Machado et al., 2017; Coskun & Dirican, 2019; Camacho-Bernal et al., 2021; Darwish et al., 2022) with a direct correlation observed between acidity and the percentage of honey added to yogurt. They claim that honey, through its compounds, favors the activity of lactobacilli and this would be the main reason for the increase in acidity. This also explains the lower syneresis of honey-fortified products, which at refrigeration temperature behaves like a pseudo-plastic fluid, offering a better stabilization of the physical structure of the yogurt (Pereira, 2003).

Contrary to those mentioned, the studies of Zlatev et al. (2018) report that the presence of honey in yogurt causes a reduction in acidity compared to the control, but show that there is still a direct correlation of acidity with the percentage of honey added.

The addition of pollen decreased the acidity of YPo yogurt by -8.15% compared to the Y control, a trend also reported by Atallah (2016), Zlatev et al. (2018), Özcan et al. (2020). The acidity of yogurts with YPr propolis was much lower (-14.16%) than that of control yogurt Y. These results correlate directly with those obtained by Gunes-Bayi et al. (2021) and Korkmaz et al. (2021). In the studies carried out by Chon et al. (2020) and Taşdemir & Gölge (2023) no significant statistical differences were found in the acidity of yogurts with propolis compared to the control, while Darwish et al. (2022) claim that the acidity of yogurts with added propolis increased. The acidity of YRJ royal jelly yogurts was lower than the Y control by -13.3%. Similar results were obtained by Atallah (2016) and Kavas (2022).

Contrary to what was stated, Darwish et al. (2022) reported the increase in the acidity of yogurts with RJ and explains this either through the synergistic effect of bee products on the microbial fermentation of the starter cultures, or as result of the enzymatic activity behind which amino acids and fats acids that could affect acidity are produced.

The addition of honey along with pollen in YHPo yogurts recorded an acidity value of -0.43% lower than the control yogurt Y. For yogurts with honey and propolis YHPr, compared to control Y, an acidity of -8.15% was found, and the addition of honey and royal jelly determined an acidity value of +1.28% higher than control Y.

The mixture of pollen, RJ and bee bread (mixture of pollen and nectar or honey) used as an addition to yogurt increased the acidity of the respective samples, compared to those of the control group Darwish et al. (2022).

Sugar content

The results regarding the sugar content of the yogurt samples are presented in Table 2.

Table 2. Sugar content of yogurt samples

Samp	average sugar content, °Brix	the variation compared to control, %
C	7.66 ±0.06	-
YH	19.46 ±0.07	+153.91
Yep	8.93 ±0.11	+16.52
YPr	8.12 ±0.03	+5.87
YRJ	7.81 ±0.08	+1.95
YHPo	21.41 ±0.19	+166.30
YHPr	19.08 ±0.03	+148.91
YHRJ	19.53 ±0.12	+154.78

As expected, the highest content of sugars was recorded in the yogurt samples with honey and pollen (YHPo), followed by those with honey (YH) and its combinations with royal jelly (YHRJ) and propolis (YHPr).

The contents of sugars in the samples without honey (YPo, YPr and YRJ) were lower than those without honey, but higher than in the yogurt sample without additions (Y).

Antioxidant activity (AA) and DPPH free radical scavenging activity

The antioxidant activity of the yogurt samples was strongly influenced by the nature of the additives. The results of AA and RSA are shown in Table 3.

Table 3. AA and RSA results

Samp	fade time, s	RSA, % (average)
C	521.67 ± 8.66	10.84 ± 0.08
YH	273.67 ± 8.62	16.36 ± 0.26
Yep	316.33 ± 9.61	51.27 ± 0.07
YPr	125.33 ± 7.77	85.97 ± 0.03
YRJ	501.67 ± 12.50	18.66 ± 0.29
YHPo	285.33 ± 5.51	52.05 ± 0.11
YHPr	144.67 ± 5.69	86.85 ± 0.04
YHRJ	478.33 ± 9.45	15.34 ± 0.18

Compared to control yogurt Y, all yogurts examined had better AA. Among the yogurts with additions, the best AA was observed in the samples of yogurt with propolis YPr (by 75.97% compared to Y), respectively propolis and honey YHPr (by 72.27% compared to Y), and the weakest AA it was for the variants of yogurt with royal jelly YRJ (by 3.83% compared to Y), respectively YHRJ (by 8.31% compared to Y).

With reference to RSA, it can be noticed that the samples with propolis had the best antiradical activity (YPr 85.97% and YHPr 86.85%), being followed by those with pollen (YPo 51.27% and YHPo 52.05%). The correlation coefficient between the two variables is -0.88.

	fade time	*RSA*
fade time	1	
RSA	-0.8801834	1

All yogurt samples with the addition of bee products showed antiradical activity superior to the sample without additions (Y). These results are also supported by the data from the specialized literature. Thus, by supplementing yogurt with propolis, substantial improvement of AA was found (Remeňová et al., 2018; Camacho-Bernal et al., 2021; Elkassas et al., 2023). Likewise, the addition of pollen to yogurt increased the AA value (Karabagias et al., 2018; Camacho-Bernal et al., 2021). Following the addition of 5% honey in yogurt, it was observed a 5-fold improvement in their AA (Camacho-Bernal et al., 2021). Similar results were reported by Mercan & Akın (2017). Supplementation with RJ increased the AA value of the YRJ sample by 72.19%, respectively the YHRJ sample by 41.54% compared to the Y control, which is also supported by Hassan et al. (2022).

Total phenolic compound

The results obtained from the analysis of total phenolic compounds (TPC) were presented in Table 4.

Table 4. Total phenolic content of yogurt samples

Samp	TPC, mg GAE/g
C	0.340 ± 0.032
YH	3.168 ± 0.033
Yep	3.632 ± 0.057
YPr	7.964 ± 0.439
YRJ	6.830 ± 0.087
YHPo	2.455 ± 0.089
YHPr	6.433 ± 0.033
YHRJ	6.507 ± 0.099

According to the results obtained, it can be seen that the yogurt samples with additions of bee products had a higher TPC than the control sample Y. The highest TPC was recorded in the yogurt sample with YPr propolis, 7.96 mg GAE/g, 23 times more than control Y, 0.34 mg GAE/g. Also, the mix of honey with propolis (YHPr) in yogurt increased TPC 18.92 times compared to control Y. Elkassas et al. (2023) reported that following propolis supplementation, TPC of yogurts increased significantly.

The samples with the addition of RJ recorded increased TPC values: in YRJ, 6.83 mg GAE/g were found, and in YHRJ, 6.5 mg GAE/g.

Pollen from the YPo and YHPo samples respectively recorded TPC values 10.68 times and 7.22 times higher than the Y control. This situation was also reported by Karabagias et al. (2018), and Darwish et al. (2022), which claims that TPC from functionally fermented dairy samples with added pollen is potentiated by the addition of RJ.

The presence of honey in yogurt (YH) increased the TPC content by 9.31 times compared to the Y control. A similar situation was reported by Bakr et al. (2015), who found 5 times more TPC in yogurt samples with 20% honey, but the step of adding honey to the yogurt was different from the one presented in this paper.

Sensory characteristics

The results of the sensory examination are presented in Figure 2.

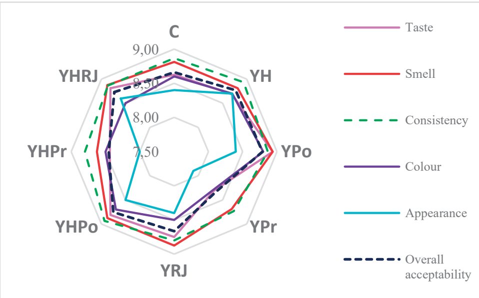

Figure 2. Sensory evaluation results

The sensory examination showed an excellent ***acceptability*** of the yogurt samples with the addition of honey, pollen, propolis and RJ with scores between 8.38 (YPr) and 8.78 (YPo) out of 9 possible. Pollen, honey and RJ samples had better acceptability than the control (Y). Although the yogurts with propolis (Ypr and YHPr) had lower scores than the control (Y), they were well accepted by the evaluators. This fact is also supported by other studies: Ammar et al. (2015); Machado et al. (2017); Santos et al. (2019); Mohan et al. (2020); Camacho-Bernal et al. (2021) and Taşdemir & Gölge (2023).

The appearance of yoghurts with addition of honey (YH), pollen (YPo), their mix (YHPo) and YHRJ mix was better appreciated than the control yoghurts (Y). Machado et al. (2017), Coskun & Karabulut Dirican (2019) and Mohan et al. (2020) confirmed better scores for yogurts with honey, but Ammar et al. (2015) found no differences. Also, Karabagias et al. (2018) confirmed that the addition of pollen determined a better appreciation of the appearance of the respective yogurts, but Atallah (2016) found no differences. Compared to control Y, yogurts with propolis (YPr and YHPr) were less appreciated in terms of appearance, a result also reported by Gunes-Bayir et al. (2022). These results are contradicted by Elkassas et al. (2023) who support the improvement of sensory scores. Yogurts with honey and RJ (YHRJ) obtained scores or higher than the control (Y), results correlated with those supported by Atallah (2016) and Kavas (2022).

The color of yogurts with honey (YH), with pollen (YPo) and the combination of these additions (YHPo) obtained higher scores than the control (Y), while the scores of yogurts with propolis (YPr), with RJ (YRJ) and with their mix with honey (YHPr and YHRJ) had lower scores. Following honey supplementation, Machado et al. (2017) and Mohan et al. (2020) reported similar results. Zlatev et al. (2018) claim that in yogurts with honey, color differences could be observed only from 10-15% addition, and those with pollen did not present color differences compared to the control ones. Karabagias et al. (2018) reported that the addition of pollen led to improvement in the color of yogurts. While Özcan et al. (2020) found color differences between control and pollen yogurts, Atallah (2016) reported no color differences in either pollen or RJ yogurts compared to control. When adding honey, Ammar et al. (2015) found no color differences between control and fortified yogurts.

The additions of honey to yogurts (YH) and pollen (YPo and YHPo) determined the improvement of their ***consistency*** compared to the control (Y), probably as a result of the increase in the content of total solids, which was also reported by Machado et al. (2017) and Camacho-Bernal et al. (2021). On the other hand, Mercan & Akin (2016) did not find influences of the addition of honey between 5-7% on the consistency of yogurts, and Zlatev et al. (2018) argue that the addition of honey or pollen in yogurt decreases their consistency. The addition of RJ in YRJ reduced the consistency compared to the control (Y) and the mix of honey with RJ (YHRJ) had the same consistency value as Y. These results contradict the results of Kavas (2022), who reported the better consistency of yogurt with RJ than control during the storage period, respectively the results obtained by Atallah and Morsy (2017) who found that the addition of pollen and RJ in yogurts improves their consistency. The addition of propolis had the effect of reducing the consistency of YPo and YHPo yogurts compared to the Y control. The same results were obtained by Taşdemir & Gölge (2023).

When judging ***the smell***, the yogurt samples with pollen (YPo) were the best rated (8.94), followed by yogurts YHPo, YRJ, YHRJ, all of which exceeded the score obtained by control Y (8.81), equal to YH. Karabagias et al. (2018) noted the observations of some panelists regarding the addition of pollen in yogurt: "wonderful", "nice smell".

With reference to ***the taste***, except for the yogurts with propolis, all other samples with additions were better appreciated than the control (Y), the highest score being recorded for the yogurt with pollen (YPo). The panelists in the study by Karabagias et al. (2018) appreciated yogurts with pollen as "sweet and pleasant taste". Zlatev et al. (2021) show that the addition of up to 0.5% pollen in yogurt would determine a high degree of acceptability, but at 1%, the taste would be affected. The negative influence of pollen at the concentration of 2.5–20 mg/mL in fermented dairy products was also reported by Yerlikaya (2014). Pollen added in a proportion of up to 1% improved the taste of yogurts obtained from cow's and sheep's milk, but in the case of goat's milk, at 1% a lower score was observed than the control (Karabagias et al., 2018). In a market study, Machado et al. (2017) reported that yogurts with honey would be preferred over plain ones. Also, the results obtained by Camacho-Bernal et al. (2017) confirm the better taste appreciation for yogurts with honey, even after 14 days of storage. The addition of 2-6% honey in yogurt determined a better appreciation of them (Ammar et al., 2015). However, Zlatev et al. (2018) claim that the addition of honey to yogurt would improve its taste and aroma only from a concentration of 10%, but for the aftertaste characteristic, the addition of 5% honey almost doubled the score compared to the control. Varga (2006) claims that the percentage of 5% honey in yogurt gives it a too sweet taste and recommends the percentage of 3%. Among the observations collected by Mohan et al. (2021), when sweetening with various variants of Manuka honey 5% of yogurts was evaluated as "astringent tastes", "bitterness", "dry aftertaste", "unpleasant mint flavor", "strange honey flavor". However, the yogurts sweetened in this way received better scores for sweet and sour taste than the control. At the same time, the addition of honey and pollen in yogurt gave it a sweet and fruity taste, the addition of RJ - spicy taste, and the one of propolis - a toasted, sweet, nutty taste Camacho-Bernal et al. (2017). In our experiment, the addition of RJ (YRJ and YHRJ) was also very well scored. Darwish et al. (2023) reported better scores for yogurts with RJ than the control. In fresh yogurts, Kavas (2022) reported no changes in the taste of yogurts with the addition of 2% RJ. The addition of propolis from the YPR and YHPr samples determined the poorer scoring of these yogurts compared to the control (Y). The same was observed by Gunes-Bayir et al. (2022). Elkassas et al. (2023) found that by adding 1% aqueous extract of propolis (20%) to yogurt, it was appreciated better than the control, but at 3% addition, the score decreased a lot compared to the control.

Antimicrobial activity

The obtained results are presented in Figure 3.

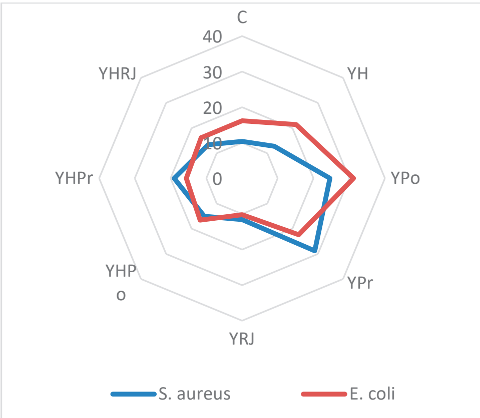

Figure 3. Antimicrobial activity

The results of this study show the effective antibacterial activity of propolis (YPr) on *S. aureus* and pollen (YPo) on *E. coli*. These results correlate with those of the study carried out by Kacániová et al. (2012), showing that *E. coli* manifested the highest sensitivity to the methanolic pollen extract and the ethanolic one. The study also highlights the increased sensitivity of the *S. aureus germ* to the 70% propolis ethanolic extract. The antimicrobial activity of propolis on strains of *Staphylococcus sp.* and *E. coli* was also observed by Rahman et al. (2010) (at the propolis concentration of 5.48

mg/ml) and Castaldo & Capasso (2002), who reported that the propolis extract has better antimicrobial activity than the of pollen. The antimicrobial activity of the pollen extract was also confirmed by the studies carried out by Khider et al. (2013). The addition of honey to yogurt exerted reduced bactericidal activity on the *S. aureus species*, and more effective antibacterial activity on the *E. coli species*, but both superior to that of the control yogurt (Y) on both microbial species. The results obtained by Mothershaw & Jaffer (2007) show that yogurt with honey does not exert bactericidal activity on the *S. aureus species*, but only on the *E. coli species*. It is interesting that, taken separately, both yogurt and honey show antimicrobial activity on the studied strains of *S. aureus* and *E. coli*. Therefore, the yogurt-honey mix does not exert a synergistic antimicrobial effect. Rahman *et al.*, (2010) confirm that honey exerts an inhibitory effect on *S. aureus* and *E. coli species*, but the efficiency is much reduced compared to propolis on these microorganisms. The minimum inhibitory concentration of propolis and honey on *S. aureus* and *E. coli germs* is 5.48 mg propolis/ml, respectively 375 mg honey/ml - dilution method, or 3.5 mg propolis/ml, respectively 350 mg honey/ml - by the gradient-plate technique (Rahman et al., 2010). The addition of RJ in yogurt (YRJ) did not have the effectiveness of propolis on the *S. aureus species*, but it was higher than the control group (Y). The effectiveness The RJ on the *E. coli species* was, however, reduced compared to the control (Y). Similar results were also reported by Hassan et al. (2022), who show that the addition of 0.5-1.5% RJ in dairy products improves their antibacterial activity. Thus, in the control sample it was found that the inhibition zone for *E. coli* was 13 mm, while with 1.5% RJ added, it was 19 mm, and that for 100% RJ was of 22 mm. At the same time, no inhibition was reported for *S. aureus in the control and the sample with 0.5% RJ*, but at 1 and 1.5% RJ the inhibition zone was 10 and 11 mm, respectively. Bactericidal effects on *S. aureus* and *E. coli* were also confirmed by Garcia et al. (2010).

It is noted, in the specialized literature, the precariousness of the studies related to the antimicrobial activity of combinations of the type yogurt - honey - other beekeeping product. Numerous studies reports that bee products show inhibitory activity for many pathogenic microorganisms, while protecting the activity of lactobacilli, bifidobacteria or streptococci from the production cultures used to obtain yogurts.

CONCLUSIONS

The results obtained in this paper and the literature study allow several conclusions.

By using bee products as additives in yogurts, functional products with outstanding sensory, physical-chemical and nutritional properties were obtained.

The addition of bee products determined the significant improvement of the antioxidant activity and the total polyphenol content of fortified yogurts, which includes them in the category of foods with immense health benefits.

The sensory characteristics of yogurts with bee products showed a very good acceptability of them, which would increase the potential for sales. Moreover, these yogurts being dietary products can be recommended to any category of consumers, from children to the elderly, from healthy people to people with various pathologies. However, it is not recommended for people with allergies to bee products or lactose.

Bee products added to yogurts demonstrated in the present study the effectiveness against *S. aureus* and *E. coli*, but the specialized literature mentions antimicrobial activity against other species as well.

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CHANGES IN THE SENSORY PROFILE OF NITRITE-FREE SALAMI FORMULATED WITH THE ADDITION OF FRUIT POWDER MIXTURES

Adriana-Ioana MORARU MANEA[1], Ioana-Alina POP[1], Diana MOIGRADEAN[1], Carmen Daniela PETCU[2], Delia-Gabriela DUMBRAVA[1], Viorica-Mirela POPA[1], Camelia MOLDOVAN[1], Diana-Nicoleta RABA[3], Mariana-Atena POIANA[1]

[1]University of Life Sciences "King Mihai I" from Timisoara, Faculty of Food Engineering, 119 Aradului Street, 300645, Timisoara, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Veterinary Medicine, 105 Splaiul Independentei, District 5, 050097, Bucharest, Romania
[3]University of Life Sciences "King Mihai I" from Timisoara, Faculty of Management and Rural Turism, 119 Aradului Street, 300645, Timisoara, Romania

Corresponding author email: marianapoiana@usvt.ro

Abstract

This study evaluated the effects of incorporating fruit powder mixtures, consisting of sour cherries powder (SCP), blackcurrants powder (BCP) and cranberries powder (CP), as natural sodium nitrite substitutes on the sensory attributes of nitrite-free salami. Four powder mixtures (SCP+BCP, BCP+CP, CP+SCP and BCP+CP+SCP) have been included in the salami formulas at a dose to provide a total phenol content (TPC) of 90, 200 and 300 mg gallic acid equivalent (GAE)/kg meat, with the minimum dose set according to the level of nitrite content per kg of processed meat (90 mg nitrite/kg). Before use, the fruits were slowly convective dehydrated at a temperature of 60°C. The results revealed that the addition of fruit powder mixtures impacted on the appearance of sample, while other sensory characteristics did not show any significant change. The highest overall acceptability was recorded for sample with BCP+CP+SCP in a dose that provided a TPC of 300 GAE/kg meat. Thus, fruit powders could become an attractive option in the formulation of nitrite-free meat products due to their high total phenol content.

***Key words**: fruit powder mixtures, nitrite-free salami, sensory profile, sodium nitrite substitute.*

INTRODUCTION

An important part in human diet are meat and meat products, therefore their quality and safety are very important (Custură et al., 2012; Lelieveld, 2015). The meat industry has been seriously affected by the discovery of carcinogenic and genotoxic N-nitroso compounds (NOCs) in processed meat products. Nitrite is used to improve flavor and color and to prevent lipid oxidation in the curing of various meat products (Savu & Petcu, 2002; Petcu, 2006; Sebranek & Bacus, 2007).

Currently, there is a noticeable concern for methods to reduce the use of nitrites and nitrates in meat or meat products (Cadariu et al., 2022; Lee et al., 2020; Zhang et al., 2023).

To improve the health benefits and shelf life of meat products, the meat industry is keen to find cost-effective natural additives (Abd El-Khalek et al., 2013; Marin et al., 2015; Abdel-Naeem et al., 2022; Bariya et al., 2020). Nowadays, consumers are becoming increasingly aware of what they eat and the possible health implications of using synthetic additives, which has led them to set restrictive requirements for their food, which should be nutritious, high quality and free of any antioxidants or chemical presservatives (Aziz & Karboune, 2018; Manihuruk et al., 2017; Sharma & Yadav, 2020).

As an alternative to using synthetic additives, the meat industry has been encouraged to use plant-based additives in meat systems (Grispoldi et al., 2022; Munekata et al., 2020; Shah et al., 2014). Therefore, one of the most common alternatives is the use of fruits, vegetables, herbs and other plant extracts or powders as natural preservatives to improve the quality of meat products and extend their shelf life (Beya et al., 2021; Manessis et al., 2020; Nikmaram et al., 2018; Reddy et al., 2018).

Natural nitrate is considered to be more functional for this purpose for two reasons: first of all, the amount of nitrate that enters and remains can be lower than that of its synthetic counterparts, and secondly, it can be approved for biological use (Nicorescu et al., 2018; Pennisi et al., 2020).

In the meat processing, nitrite is considered to be a multifunctional food additive (Petcu, 2013). Due to its potential carcinogenic effects in humans, several studies have suggested that nitrite intake should be limited. On the other hand, some studies have demonstrated the favorable effects of nitrite on human health, if legal requirements and food safety are implemented (Mitrea et al., 2003; Goncearov et al., 2004; Petcu et al., 2007; Ianițchi et al., 2007). Nevertheless, consumer interest in natural or nitrite-free meat products remains significant (Cadariu et al., 2022).

In the meat-processing industry, fruit and vegetable powders can replace sodium nitrite. Liang et al. (2023) investigated the effects of different addition levels and marinating times on the properties of roast beef patties using Chinese cabbage, celery and cranberry powders. With regard to the different marinating times using cranberry powder on the properties of roasted beef patties, the marinating time of 12 hours showed the best effect. The analysis of this study revealed a possible solution for the replacement of nitrites in roast beef meatballs, also the research results indicated that cranberry powder could effectively increase the redness in roast beef meatballs and could potentially replace and reduce the number of synthetic nitrites.

The use of tomato processing by-products in smoked and burnt sausages compared to smoked and dried sausages was also analyzed as a nitrite replacement. Results analyzed it was noted a stronger inhibitory effect against lipid oxidation, with the addition of tomato processing byproduct as a natural antioxidant in reformulated nitrite-free sausages.

Abdel-Naeem et al. (2022) highlighted the effect of fruit powder specifically fruit peels namely lemon, orange, grapefruit, and banana (1% each), on the oxidative stability, microbial quality, physicochemical properties and sensory attributes of chicken meatballs during storage. As a result, in comparison with control samples all fruit peel powders showed significant antioxidant and antibacterial activities. The sensory characteristics were also improved in all samples analyzed; therefore, this study shows once again that the use of fruit peel powders can be used as a natural source of antioxidants.

Nour (2022) used fruit mixes such as sour cherries and plums in the form of marinades, used with pork pulp, to study the effect of fruit on quality characteristics and oxidative stability. Following the results, it was found that cherry and plum juice can be used as a marinating ingredient, as it increases the sensory properties and improves the storage stability of pork pulp.

In another study, the sensory evaluation, residual nitrite and oxidation levels of Chinese-style sausages were investigated, which were produced by both the reduction of nitrite levels and the addition of tomato powder (0%, 2% and 4%). Analysis of the results showed that nitrite reduction increased and oxidation levels decreased with the addition of 2% tomato powder, a result that suggests a potential solution for replacing nitrite in meat products (Xu et al., 2013).

The high content of bioactive compounds in fruits and fruit processing by-products has led them to be seen as an attractive possibility to slow down or inhibit lipid oxidation reactions, acting in the direction of preventing the formation of free radicals or as scavengers of free radicals (Doaa & Refaat, 2017; Shui & Leong, 2006). A recent study on the replacement of nitrates with a natural additive addressed the use of cranberry powder in the manufacturing process of fermented sausages, leading to improved quality characteristics (Yang et al., 2023).

Research on the effect of incorporating fruit powder (sour cherries, blackcurrants and cranberries) into salami formulas on sensory characteristics was conducted by Moraru Manea et al. (2022). Based on the observed findings, the research was continued by incorporating fruit powders into salami in the form of SCP+BCP, BCP+CP, CP+SCP and BCP+CP+SCP mixtures, following the impact of their addition on the sensory properties of the designed nitrite-free salami formulations.

MATERIALS AND METHODS

Fruit powder production

Three species of fruits were used, such as: sour cherries (SC), blackcurrants (BC) and cranberries (C), which were incorporated as powder in the manufacturing recipe of salami formulas. For this purpose, the fruits were dehydrated and ground. The dehydration process lasted 15 hours (5 h per day, 3 days in a row) at a moderate temperature of 55-60°C in a forced-air oven (Froilabo AC60/France, 1000 W) to preserve the bioactive compounds of the fruits. Dehydrated fruits were ground with a laboratory mill (Grindomix Retsch GM 2000). The fruit powders obtained (SCP, BCP and CP), in the form of mixtures, two-by-two and all three, were used to replace sodium nitrite in salami formulas.

Determination of total phenolic content of fruits powder

Since the doses of fruit powder in the form of mixtures were calculated according to their total phenolic content (TPC), the corresponding TPC was determined by Folin-Ciocalteu colorimetric method (Singleton et al., 1999). The results were expressed in mg gallic acid equivalents (GAE)/g dry weight (d.w).

Salami formulas preparation

The stages of the salami manufacturing process are as follows: weighing of raw material, weighing of auxiliary material, mixing of raw material by adding auxiliary material, filling of membranes, heat treatment and storage. In order to replace sodium nitrite, the mixture fruits powder is used in three doses, to ensure a level of total phenolic content (TPC) of 90, 200, 300 mg GAE/kg in salami recipe in the mixing stage with raw material and other auxiliary materials. The added nitrite content in 1 kg of raw minced meat is 90 mg/kg, from this value was chosen a minimum dose of total phenolic compounds from dehydrated fruit powder. The amounts of powder from each fruit in the mixtures were calculated to make an equal contribution to the TPC doses. Nitrite-free salami formulations were analyzed in comparison with control salami samples prepared with added nitrite and without nitrite, respectively.

Heat treatment of salami samples with the addition of fruit powder mixes takes place in a smoking cell with closed smoke flap, the samples are smoked with beech sawdust and steamed until they have reached 62°C in the technological center of the product. The last step in the technological process of salami manufacturing is the storage of the product at 2-4°C. After 21 days of storage, the sensory analysis is performed.

The sample were labeled, as follows:

- SC: Salami control sample;
- SCN: Nitrite-free Salami;
- S(SCP+BCP)90: Salami formula with mixture of SCP and BCP to ensure a TPC of 90 GAE/kg of raw processed meat;
- S(SCP+BCP)200: Salami formula with mixture of SCP and BCP to ensure a TPC of 200 GAE/kg of raw processed meat;
- S(SCP+BCP)300: Salami formula with mixture of SCP and BCP to ensure a TPC of 300 GAE/kg of raw processed meat;
- S(BCP+CP)90: Salami formula with mixture of BCP and CP to ensure a TPC of 90 GAE/kg of raw processed meat;
- S(BCP+CP)200: Salami formula with mixture of BCP and CP to ensure a TPC of 200 GAE/kg of raw processed meat;
- S(BCP+CP)300: Salami formula with mixture of BCP and CP to ensure a TPC of 300 GAE/kg of raw processed meat;
- S(CP+SCP)90: Salami formula with mixture of CP and SCP to ensure a TPC of 90 GAE/kg of raw processed meat;
- S(CP+SCP)200: Salami formula with mixture of CP and SCP to ensure a TPC of 200 GAE/kg of raw processed meat;
- S(CP+SCP)300: Salami formula with mixture of CP and SCP to ensure a TPC of 300 GAE/kg of raw processed meat;
- S(BCP+CP+SCP)90: Salami formula with mixture of BCP, CP and SCP to ensure a TPC of 90 GAE/kg of raw processed meat;
- S(BCP+CP+SCP)200: Salami formula with mixture of BCP, CP and SCP to ensure a TPC of 200 GAE/kg of raw processed meat;
- S(BCP+CP+SCP)300: Salami formula with mixture of BCP, CP and SCP to ensure a TPC of 300 GAE/kg of raw processed meat.

Sensory characteristics evaluation

Sensory analysis of the designed salami formulas was carried out using the hedonic scale method (STAS 12656-88) by a group of 20 tasters (10 men and 10 women) which were recruited from the staff and students of the University of Life Sciences “King Mihai I” from Timisoara, Romania.

The evaluators, aged between 20 and 50, were regular consumers of meat products. The participation of evaluators in this study was voluntary.

Prior to the sensory analysis, panel members were trained to identify the characteristics that will be assessed. Evaluators assessed the sensory attributes of salami samples including appearance, flavor, texture, taste and overall acceptability according to their rating and awarded points according to the five-point hedonic scale as follows: 5 - highly appreciated; 4 - moderately appreciated; 3 - neither liked nor disliked; 2 - slightly disliked; 1 - extremely disliked (Cadariu et al., 2023).

By adding up the maximum points allowed for each sensory attribute, a maximum of 20 points can be achieved. Each taster assigns a score to one sensory characteristic of a sample. The average score is then calculated. The sum of the average scores of the 4 sensory attributes analyzed for a test will result in the overall average score. Each sample has an overall average score, which helps us to identify which concentration level of the added fruit powder is more effective to ensure an optimal level of natural antioxidant, but also which is the best fruit powder among the three analyzed (SCP, CP, BCP) as a substitute for sodium nitrite.

By analysing the average total score of each salami sample, it is possible to identify which concentration of fruit mixture powder has a similar effect on sensory characteristics as the nitrite dose, in order to be recommended as its substitute.

RESULTS AND DISCUSSIONS

Total phenolic compound of fruit powder

The results obtained from the analysis of total phenolic compounds (TPC) were presented in Table 1.

Table 1. Total phenolic content of fruits powder

Fruits powder	Total phenolic content (mg GAE)/g d.w)
BCP	14.68
CP	11.49
SCP	9.55

Based on the values obtained, the amounts of fruit powder providing particular levels of TPC in the designed salami formulas were determined.

Sensory characteristics of salami formulas

Sensory evaluation of the salami formulas was carried out to identify the acceptability of the designed products. Figure 1 shows the salami formulations obtained by adding the SCP+BCP mixture to ensure a TPC level of 90, 200 and 300 GAE/kg processed raw meat, compared to the SCN and SC control samples.

SCN

SC

S(SCP+BCP)90

S(SCP+BCP)200

S(SCP+BCP)300

Figure 1. Control samples and salami formulas with addition of SCP+BCP mixture (own source)

Figure 2 shows the results obtained from the sensory analysis of salami formulations. It can be observed that the sample with the highest score was S(SCP+BCP)300 with 21.8 points on the total average score, followed by S(SCP+BCP)200 with 18.8 points and S(SCP+BCP)90 with 16.9 points, which is the lowest total average score of the salami samples. In terms of appearance, the average scores awarded by assessors increased in the order S(SCP+BCP)300 > S(SCP+BCP)200 >

S(SCP+BCP)90 suggesting that the addition of fruit powder mixture in a dose to provide a TPC of 300 GAE/ kg of raw processed meat with mixture of SC powder and BC powder is the most appreciated sample by the evaluators.

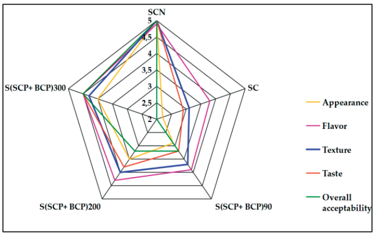

Figure 2. Sensory evaluation scores of salami with SCP+BCP mixture versus control samples

The mixtures contain sour cherry powder which slightly influences the taste that had decreasing values, the other sensory characteristics flavor texture did not change significantly.
The salami formulas with BCP+CP mixture are presented in Figure 3.


SCN


SC


S(BCP+CP)90


S(BCP+CP)200


S(BCP+CP)300

Figure 3. Salami control samples and salami formulas with addition of BCP+CP mixture (own source)

From the results of sensory analysis depicted in Figure 4, it can be seen that the highest scores have been registered for salami sample S(BCP+ CP)300 with 23.4 points for overall acceptability, followed by S(BCP+CP)200 with 22.7 points and S(BCP+CP)90 with 20 points.

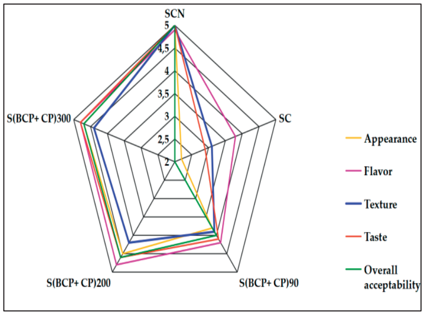

Figure 4. Sensory evaluation scores of salami with BCP+CP mixture versus control samples

As regard the appearance, the highest value was registered for the salami sample S(BCP+CP)300 (4.7) respectively S(BCP+CP)200 (4.5) and the lowest value for sample S(BCP+CP)90 (3.8) as it can be observed in Figure 5. The other sensory features did not undergo significant changes.
The salami formulas obtained by incorporating the CP+SCP mixture are shown in Figure 5, and the results of the sensory analysis of the developed formulas against SC (control sample) and SCN (nitrite-free sample) are shown in Figure 6.
The sensory ratings of salami formulations containing a mixture of CP and SCP with different TPC levels increased in the following order: S(CP+SCP)90 with 20 points, S(CP+SCP)200 with 21.7 points and S(CP+SCP)300 with 22.7 points.
A sensory characteristic that has undergone slight changes is the appearance, with 3.9 points for S(CP+SCP)90 sample, 4.2 points for S(CP+SCP)200 sample and 4.4 points for S(CP+SCP)300 sample the highest scores. No significant changes were observed for other characteristics.

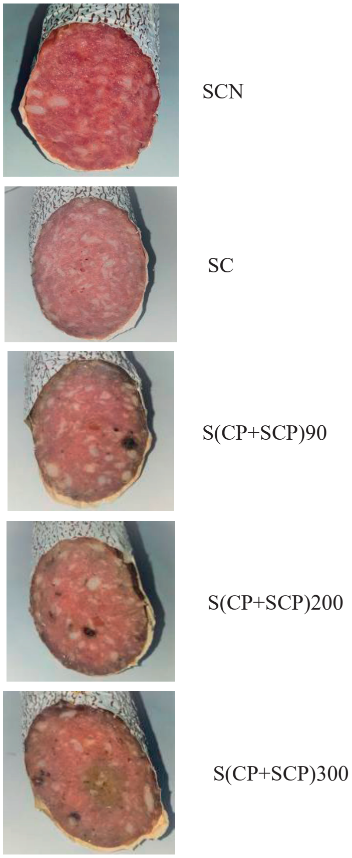

Figure 5. Salami control samples and salami formulas with addition of CP+SCP mixture (own source)

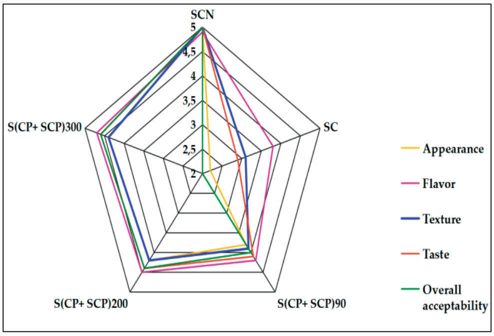

Figure 6. Sensory evaluation scores of salami with CP+SCP mixture versus control samples

The fourth fruit powder blend consists of all three fruit powders (BCP, CP and SCP). This blend is incorporated as a natural substitute for sodium nitrite in the salami recipe to provide the three levels of TPC. The obtained salami formulations are presented in Figure 7.

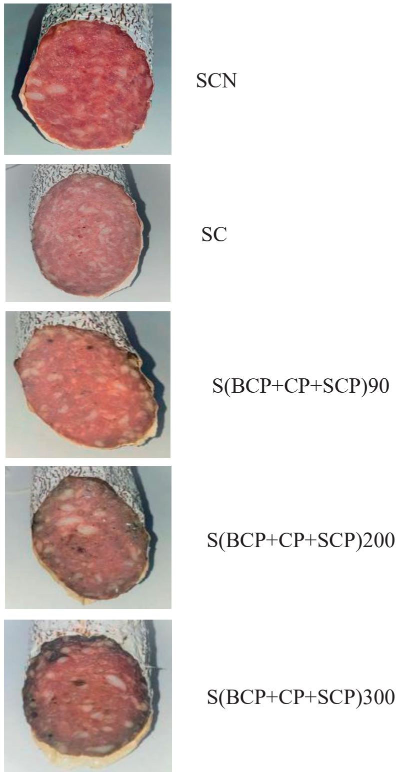

Figure 7. Salami control samples and salami formulas with addition of BCP+CP+SCP mixture (own source)

Results obtained from the sensory analysis of the salami formulas with addition of BCP+CP+SCP mixture are shown in Figure 8.

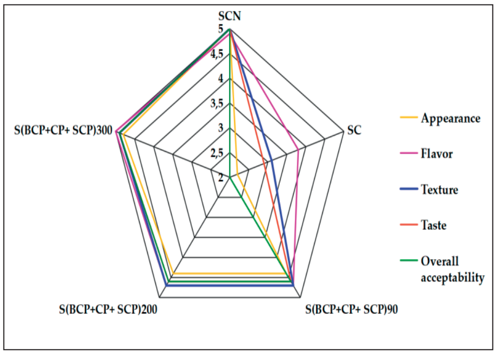

Figure 8. Sensory evaluation scores of salami with BCP+CP+SCP mixture versus control samples

It can be seen that in terms of appearance of the samples, the score increased significantly as the level of TPC provided by the fruit powder mixture increases from 4.4 points S(BCP+CP+SCP)90 sample, to 4.7 points

S(BCP+CP+SCP)200 sample, to the highest score of 4.8 points S(BCP+CP+SCP)300 sample. The other sensory characteristics did not change significantly. The data represented in Figure 8 revealed that the highest value for overall acceptability was obtained for salami formulas S(BCP+CP+SCP)300, with 24.5 points, followed by S(BCP+CP+SCP)200 sample with 23.4 points, while the lowest value with 23 points was obtained for sample S(BCP+CP+SCP)90.

CONCLUSIONS

Fruit powder, as a natural antioxidant, is used as a substitute for sodium nitrite in salami formulas.

In order to determine the most favorable fruit powder mixture, as well as the dose that does not impair sensory attributes, an analysis of the sensory characteristics of nitrite-free salami formulas was presented.

The results showed that, compared to control samples with or without added sodium nitrite, there were changes in the appearance of salami formulas with added fruit powder mixtures, while other sensory characteristics did not show a significant influence.

Higher scores were obtained for all salami formulas than for the nitrite-free control sample, but the highest overall acceptability was recorded for the salami formula with BCP+CP+SCP, which provided a total phenol content of 300 GAE/kg raw meat with 4.9 points, and an average total score of 24.5 points.

Analyzing the changes in the sensory profile of salami formulas without nitrites but with the addition of fruit powder blends, it can be concluded that fruit powders can successfully replace nitrites in boiled and smoked salami formulas, therefore being recommended as a natural additive for this purpose.

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THE VALORIZATION OF PLANT AND ANIMAL BY-PRODUCTS FOR FOOD SUSTAINABILITY

Adina NICHITA, Mona Elena POPA

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, Bucharest, Romania

Corresponding author email: nichitaadina1979@gmail.com

Abstract

This review is based on a bibliographic study of over 70 articles published between 2001-2023 and tried to highlight the most important valorization directions, as well as the most used valorization methods of waste from the food industry, for the purpose of environmental sustainability. The recycling of waste in the cascade helps to solve problems related to the environment, economic, social, ethical, etc. Waste recovery methods are diversified depending on the type of waste, requiring in-depth studies, innovative technologies, an appropriate legislative framework, as well as alternative solutions, etc., so as to obtain zero waste. Current perspectives come in the face of finding alternative solutions, such as meat analogous and food industry sustainability approaches.

Key words*: animal by-products, sustainability, vegetable by-products.*

INTRODUCTION

In recent decades, the food industry has become a focal point in discussions about environmental protection and sustainability. (Zioga al., 2022).

The earth is an important source of natural resources which, in connection with the activities of operators in different sectors, generate a wide range of waste. (Srivastava et al., 2023).

Agriculture and the food industry are key branches of the European economy (Palvic et al., 2023), but they bring with them significant challenges related to the management of waste and economic losses (Ilyas et al., 2021).

The lack of effective waste management directions resulting from the processing of raw materials and the processing of products leads to the formation of significant amounts of waste that affect the environment in quantitative and qualitative terms (Awasthi et al., 2021).

To address this problem, improving waste management in agriculture and the food industry through recycling and recovery of by-products has become crucial globally (Lamolinara et al., 2022).

This approach promotes the sustainable use of resources by valorizing by-products and residues in a variety of ways (Ubando et al., 2020).

An example of an effective way of using agricultural waste is anaerobic digestion, which transforms organic materials into biogas and other valuable products (Nagarajan et al., 2023).

As awareness of the negative impact of animal production grows, an increased direction of technological development towards plant proteins is observed (Szpincer et al., 2022; Estel et al., 2021; Lai et al., 2017).

Proteins are found in higher amounts in animal products (Singh & Krishnaswamy, 2022), but the shift to plant proteins is a significant trend in the food industry, with benefits for both human health and the environment (Sabater et al., 2021).

Inefficient management of waste from the primary processing of plant raw materials, as well as waste from product processing, results in the quantitative formation of waste and implicitly environmental pollution (Awasthi et al., 2021).

The review aims to identify the main directions for the valorization of various by-products of plant and animal origin resulting from agriculture and industrial food processing, for the purpose of environmental sustainability, etc.

MATERIALS AND METHODS

For this research we have analyzed Scopus Elsevier data base and SpringerLink Journals by specific key words like, “vegetable by-products”; “animal by-products”; “sustainability”. We have also used official information of different states.

With an estimated world population of approximately 10 billion people by 2050, there is a growing demand for food and biomass to meet this increased need (Vicente et al., 2023). This increase in food production and consumption also results in a significant amount of agri-food waste that requires effective management and recovery (Saratale et al., 2021). One of the significant aspects is the massive production of plastic materials globally, which reaches about 40 billion tons per year (Li & Wilkins, 2020).

This high production of plastic generates serious problems related to environmental pollution, risks to human health and toxic impact on ecosystems. In this context, the management of agri-food waste can contribute to addressing this problem through the processes of valorization and transformation of this waste into sustainable alternatives to plastic. A notable example is the use of agro-food waste as raw materials to obtain fermentable sugars and subsequently biofuels (Kumar et al., 2022).

This process involves steps such as pre-treating raw materials to obtain fermentable sugars, fermenting these sugars to produce biofuels, and purifying these biofuels, such as ethanol, through distillation. This approach contributes not only to the recovery of agri-food waste, but also to the reduction of dependence on fossil fuels, with significant benefits for the environment and climate change. At the same time, fruit and vegetable by-products, which contain high concentrations of bioactive compounds, represent a valuable source for obtaining bioactive substances with health benefits (Renard, 2018).

The extraction and valorization of these compounds from co-products contributes to the minimization of waste and the development of food products with added value. For the processing of agricultural waste, such as melon peels or peanut shells, it is essential to pre-treat them to obtain value-added products (Ajayi & Lateff, 2023). The full recovery of eggplant waste, through the extraction of anthocyanins and pectin, is carried out by different methods and techniques developed and improved, with ultrasound and microwaves (Karimi et al., 2021).

Table 1. Schematic presentation of the use of agri-food waste for the production of value-added products (Source: Kumar et al., 2022)

Waste material	**Pre-treatment Method**	**Value Added Products**
-Fruit & Vegetable processing waste -Edible oil waste -Coffee processing waste -Kitchen waste -Meat & poultry waste -Brewery waste	-Microwave radiation -Ultrasound method -Steam explosion -Liquid hot water treatment -Ammonia fiber explosion -Pyrolysis -Microfluidics	-Biofuel -Biosurfactant -Bio composites - Biofertilizer -Biopolymer -Na-ion batteries

Although many research has been done in the valorization of food industry waste, many challenges are still need to be further addressed. Few challenges are described in the Figure1.

An interesting aspect addressed in these researches is fermentation, which is being investigated as an efficient and economical way of valorizing indigenous African leafy vegetables. Controlled fermentation involves the use of lactic acid bacteria, representing one of the promising approaches in this context. This fermentation process significantly improves the nutritional content of vegetables and extends their shelf life (Misci et al., 2022).

The pretreatment of melon skins, peanut skins, etc., is a necessary step in obtaining products with added value (Ajayi & Lateff, 2023).

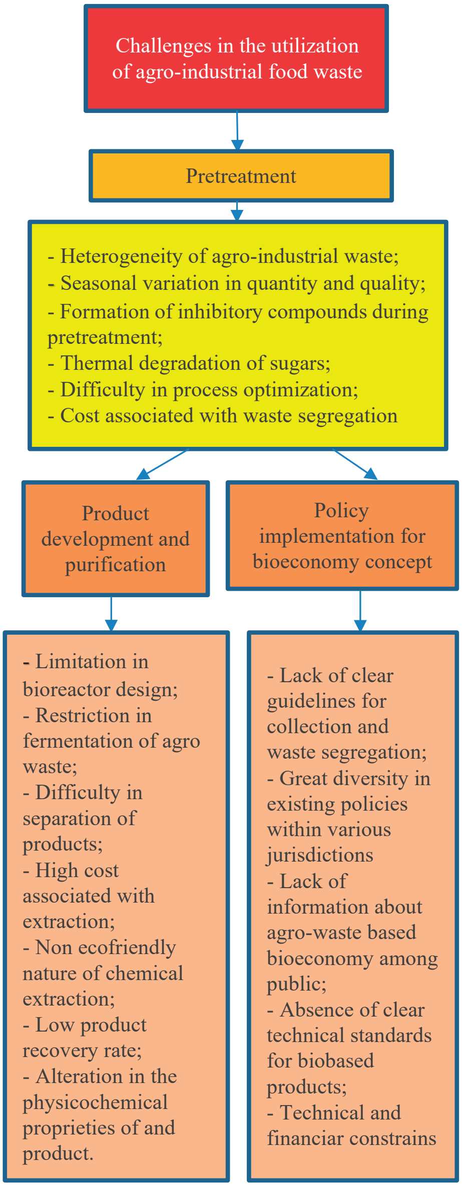

Figure 1. Challenges in the sustainable utilization of agro-industrial waste
(Source: Kumar et al., 2022)

Pressed drupes resulting as waste from oil extraction could be exploited by hydrolysis to obtain therapeutic benefits that have a higher value than the current value as animal feed (Sari et al., 2022).

Anaerobic digestion technology is becoming more and more important due to its contribution to environmental sustainability and the circular bioeconomy (Tavera-Ruiz et al., 2023), anaerobic digestion being a way of valorizing agricultural waste (Nagarajan et al., 2023).

RESULTS AND DISCUSSIONS

Directions for valorization of plant & animal by-products

Annually, approximately 1.3 billion tons of waste and by-products are generated globally, and 38% of this comes from food processing (Gottardi et al., 2021).

The waste resulting from the slaughter of animals is rich in proteins and lipids and represents a valuable resource for the cosmetic and medical industry (Javourez et al., 2021).

By-products from various processing steps, such as whey, molasses, starch, fresh fruits and vegetables, can be redirected to various food matrices, producing edible bioproducts such as lactic acid and cellular proteins. Contaminated, altered or expired waste can be used in anaerobic digestion processes (Awasthi et al., 2022; Awasthi et al., 2022b; Sar T. et al., 2021).

The valorization of these wastes and other agro-food by-products aims to stimulate innovative technologies, thus contributing to a more efficient management of resources (Javourez et al., 2021). Another important aspect in the recovery of waste and by-products is their use to replace plastic materials with ecological bioplastics in the form of polyhydroxyalkanoates, which are biodegradable and biocompostable (Saratale et al., 2021).

The dairy industry generates significant amounts of waste in the form of whey. This whey contains important nutrients like lactose, lipids, soluble proteins and others. The use of this whey can contribute to the reduction of environmental impact and the development of value-added products (Gutierrez-Hernandez et al., 2022).

Agro-industrial wastes can be used for fermentation, thus contributing to the improvement of economy and productivity (Arya et al., 2022).

Industrial biotechnologies and the use of microbes in various applications, such as the production of bioplastics, food additives, cosmetics and valuable chemicals, have become key components of the global bioeconomy (Pfeifer et al., 2021).

In addition, organic waste from the food and agricultural industry represents an important resource of recoverable biomass for the purpose of producing renewable energy (Akbi et al., 2017), contributing to reducing dependence on traditional energy sources and combating climate change (Usmani et al., 2023).

It is clear that this approach has significant potential to bring about considerable improvements in the food industry and in the fight against climate change (Stadler & Chauvet, 2018; Surendra et al., 2020).

In addition to energy production, agro-food by-products and waste can also serve as raw materials for the food industry. Fermentation and bioconversion processes can convert these wastes into value-added products such as lactic acid or cellular proteins (Awasthi et al., 2022; Arya et al., 2022).

This approach not only reduces wastage of resources but also helps increase the productivity of the economy. In addition, the development of biorefineries represents a promising direction in the exploitation of renewable resources (Kasani et al., 2022).

These biorefineries can be used to extract various valuable chemicals from agri-food waste, thereby helping to reduce pressure on natural resources. Valorization of waste and agri-food by-products has a significant impact on the food industry and food safety. With a growing population, it is essential to develop strategies for the use of resources that are sustainable in the long term, which involves the transformation of waste from milk production into valuable products such as lactic acid or soluble proteins (Gutierrez-Hernandez et al., 2022).

A review indicates the industrial processing of sugar beet to obtain sugar, resulting in significant quantities of sugar beet pulp, which in the past was mainly used as animal feed. However, recent developments have opened new perspectives, suggesting that this sugar beet pulp can be efficiently exploited to obtain biofuels, biohydrogen and other valuable chemicals such as alcohols, microbial enzymes, lactic acid, citric acid, single-cell proteins and biodegradable materials (Usmani et al., 2022). In addition, fruits, vegetables and other food products are rich sources of dietary carbohydrates and bioactive phytochemical compounds that, in addition to their basic nutritional intake, also provide significant health benefits (Dranca & Oroian, 2018; Liu, 2013).

These directions for the valorization of plant by-products demonstrate the enormous potential of transforming waste into valuable resources within the food and chemical industries. Pectin, known for its traditional use as a gelling and thickening agent in food industry, now has an expanded role as a functional agent or fat substitute that promotes health (Dranca & Oroian, 2018).

Moreover, the isolation of pectin from plant materials or agro-food by-products has opened applications in the pharmaceutical and medical industry, due to its bioactive benefits (Dranca & Oroian, 2018).

A concrete example is the use of the by-products resulting from the processing of melons (*Cucumis melo* L.), which generate seeds and peel rich in antioxidants. These melon peel extracts contain phytochemical compounds with antimicrobial, antiviral, antioxidant, anti-inflammatory and antidiabetic properties, opening new perspectives for the development of functional foods (Gómez-Garcia et al., 2020).

Another example is the industrial processing of eggplant, which generates by-products such as eggplant skins. These peels are rich in anthocyanins, with antioxidant, antimicrobial and antitumor properties. The use of these eggplant peels can be a promising alternative to synthetic additives in the food industry (Karimi et al., 2021).

Avocado processing results in the generation of impressive amounts of pits that represent 13-18% of the fruit's mass. Avocado seeds represent a significant proportion of the mass of the fruit, which can be exploited in various fields, from the production of avocado oil to their use as biofertilizers (Tesfaye et al., 2022).

These examples highlight that agri-food by-products can have significant value if properly managed and exploited. Biorefineries represent a promising approach for this valorization, contributing to a circular and sustainable economy (Kasani et al., 2022; Rodriguez-Martinez et al., 2022).

The secondary products resulting from the wine and olive oil industry are rich in bioactive compounds (Balli et al., 2021).

Olive waste contains phenolic compounds, their concentration being influenced by the growing area and variety. The phenolic fraction consists of different groups: phenolic acids, phenolic alcohols, flavonoids and secoiridoids (Veneziani et al., 2017).

Grape seeds contain polyphenols, such as flavanols, flavonols, anthocyanins and stilbenes, being mainly located in the skin, while others, such as catechins and procyanidins, are only present in the seeds in concentrations that vary according to terrain, variety, etc. (Moro et al., 2021).

Olive waste contains phenolic compounds, their concentration being influenced by the growing area and variety. The phenolic fraction consists of different groups: phenolic acids, phenolic alcohols, flavonoids and secoiridoids (Veneziani et al., 2017). The fructification of waste from the food industry aims to stimulate innovative technologies to improve the use of resources in the cascade (Javourez et al., 2021).

Due to the presence of carotenoids and implicitly their bioactive properties, waste of vegetable origin offers wide opportunities for sustainable productions, being able to be used to color fruit juices, pasta, meat, etc. (Cassani et al., 2022). Vegetable and fruit by-products contain high concentrations of bioactive compounds, which makes the extraction of bioactive substances a capitalization strategy (Renard, 2018).

Anthocyanins from eggplant peel are an important alternative to synthetic additives that can be used to extend the shelf life of food products (Karimi et al., 2021).

A review indicates the deepening of studies for the blood root vegetable (*Haemodorum spicatum*) which is part of the family Haemodoraceae, which grows especially in Western Australia. The bulbs, stem, leaves and seeds of the vegetable produce natural dyes: red, pink, purple and green. Red pigments (Hemocorina) are responsible for the spicy and hot flavor of the bulbs (Macintyre & Dobson, 2017).

The red pigment of *Haemodorum spicatum* can be seen as an alternative substitute for replacing artificial dyes with natural dyes to increase the demand for organic, non-toxic and healthy food (Liang et al., 2023).

Trends and perspectives

The evolution of anaerobic digestion technology is becoming increasingly important in the context of environmental sustainability and the circular economy (Tavera-Ruiz et al., 2023). This technology contributes significantly to reducing the negative impact on the environment and to the efficient use of resources. Waste management thus becomes a key solution in the transition to a circular economy in the food sector (Lavelli, 2021; Jurgilevich et al., 2016).

Industrial waste and by-products from the food industry can serve as sustainable and renewable sources of biomass that can be used to produce electricity, bioliquids, biofuels and more (Gill, 2022). This approach can contribute to the diversification of biomass sources and the development of renewable energy sources.

The circular economy, with an emphasis on food traceability, is a key element in promoting a sustainable food system. This approach aims to produce healthy and environmentally friendly food (Tesfaye et al., 2022).

The current trends regarding the recovery of waste from the food sector, but also the finding of innovative solutions regarding the partial or total replacement of animal proteins with vegetable proteins are evaluated by scientists from the food sector, which contribute to the need to find answers to some ethical requirements, pro-health and, last but not least, environmental sustainability (Kotecka-Majchrzak et al., 2020).

The current trend in the valorization of peas, soybeans, peanuts is to obtain meat alternatives due to their excellent gelling properties and the potential to form fibrous structures (Zhang et al., 2021).

Also, the use of natural dyes in food products aims to improve their sensory quality and give them attractive colors (Cassani et al., 2022). Natural pigments from plant wastes, such as carotenoids, can provide significant benefits through their bioactive properties, including antioxidant and antitumor roles (Cassani et al., 2022).

Industrial biotechnologies have an important role in the future of the European and global bioeconomy. Current studies and advances have indicated that bioconversion or fermentation generates a wide range of food products, chemicals, energy and even hydrocarbons (Stadler & Chauvet, 2018).

Finally, plant-based dairy and meat alternatives have the potential to contribute to environmental sustainability by reducing greenhouse gas emissions and improving food systems (Giacalone et al., 2022). Technological innovations in this field focus on optimizing the quality of food products according to consumer requirements and environmental sustainability objectives (Abecassis et al., 2018).

CONCLUSIONS

The article emphasizes the importance of cascading recovery of raw materials for environmental sustainability. Research and innovative technologies hold promise for finding sustainable alternatives, such as meat analogs, that can meet market needs and reduce reliance on traditional resources. Soy foods are considered to have a prosperous future on the international market, highlighting the increased interest in vegetable proteins.

The concept of waste recovery from agriculture and the food industry is essential in the development of a circular bioeconomy. It includes steps such as the transformation of food by-products into new products, the implementation of innovative technologies, the use of ecological methods and the creation of model biorefineries. These efforts have the potential to contribute to efficient use of resources and environmental protection.

The main conclusions and key aspects derived based on the studied materials are the following:

Organic agriculture: It represents an important alternative for the cascading use of raw materials and for ensuring a sustainable production of organic products. This can meet the growing demand for such products.

Innovations in food production: Research and innovative technologies play a crucial role in developing alternatives, such as meat analogs, to meet consumer demands and contribute to environmental sustainability.

Circular bioeconomy: The concept of animal and plant waste recovery is part of a circular bioeconomy approach. It involves the use of food by-products to create new food products, the development of innovative technologies and the application of ecological methods to reduce the impact on the environment.

In conclusion, cascading resources, promoting organic agriculture, developing sustainable alternatives in the food industry and adopting ecological practices are important steps towards a more circular and environmentally friendly economy.

Organic waste and by-products can be used as a sustainable, renewable, unlimited source of biomass to generate electricity, bioliquids, biofuel, etc.

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RESEARCH ON THE PHYSICO-CHEMICAL PROPERTIES OF MANGALITSA PORK DERIVED FROM PIGS RAISED IN THE NORTHEASTERN REGION OF ROMANIA

Constantin NISTOR, Bianca-Petruța TIHINIUC-POPA, Benone PĂSĂRIN, Dragoș Florin MANEA

"Ion Ionescu de la Brad" Iasi University of Life Sciences,
3 Mihail Sadoveanu Alley, Iasi, Romania

Corresponding author email: ncm9393@gmail.com

Abstract

Mangalitsa represents a breed of pigs specialized in fat production, which was exploited in our country until the 1950sHowever, the swine population of this breed decreased significantly thereafter due to the emergence of breeds and crossbreeds with much better productive performances, the advent of modern pig farms, and changes in the population`s consumption habits. This paper presents data obtained from the analysis of the physicochemical properties of Mangalitsa pork, derived from pigs raised on a pig farm located in the northeastern region of the country, in Neamț County. The biological material required for this study consisted of Mangalitsa pigs: the red variety and the swallow-belly variety. To achieve the purpose of the study, samples were taken from 3 distinct body regions - neck, thigh, and loin - which were processed in a specialized laboratory. The pH of Mangalitsa pork measured 24 hours post-slaughter ranged between 5.74 and 5.84, with the lowest dry matter values recorded for the batch of pigs from the red Mangalitsa variety. The protein content in the meat varied between 15.47% and 20.90%, depending on the anatomical region, while the lipid content showed wide variations, with the highest values in the Mangalitsa neck. The average values obtained for pH, dry matter, protein, and fat in Mangalitsa pork meat were within the optimal range for this breed, according to specialized literature.

Key words: *loin, Mangalitsa breed, neck, physicochemical properties, thigh.*

INTRODUCTION

Mangalitsa is one of the old-type pig breeds, formed centuries ago through the crossbreeding of European and Asian primitive pigs. The Mangalitsa pig breed was introduced to Romania from Serbia in the 19th century (Ciobanu et al., 2001; Paraschivescu et al., 2010).

Mangalitsa is a breed of pigs specialized in fat production, with a fat percentage in the carcass of 65-70%, which was exploited in our country until the 1950s. After this period, the pig population of this breed greatly decreased due to the rise of other breeds and hybrids with much better productive performances, the appearance of modern pig farms and changes regarding the consumption habits of the population. (Hoha et al., 2018; Egerszegi et al., 2003; Hollo et al., 2003).

This breed is among the ancient pig breeds formed in antiquity from the crossing of primitive European pigs with primitive Asian pigs brought by the Romans to the territory of today's Europe (Nagy, 2013).

In Romania, the Mangalitsa pig breed has been documented since 1830, with its distant origins tracing back to the domestication of the wild boar "*Sus vittatus*". As domestication progressed and human intervention in breeding intensified, several varieties with approximately the same morphological characteristics were developed: baris, blonde, red, black, and a "swallow" belly (Ciornei, 2015).

The ruggedness of the Mangalitsa breed has led to the development of a pig population with high resistance to diseases, harsh macroclimate and microclimate conditions, and grazing, especially on wet terrain. It is also noteworthy that in semi-intensive farming conditions, feeding does not require the administration of animal protein, as fruits such as acorns and beech mast are very well utilized (Ciornei, 2015).

After being neglected for several decades, the Mangalitsa pig breed, thanks to its exceptional meat qualities, has become fashionable again. In recent years, numerous studies on this breed have been conducted abroad, especially in Hungary. The population dynamics of the Mangalitsa pig breed have been sinuous, significantly influenced by market demands.

Although not originally created in our country, the Mangalitsa is considered a local breed because it has been raised on Romanian territory since ancient times, during which it adapted perfectly to our natural conditions and rearing practices. Within the breed, several varieties have emerged (blonde, red, black, 'swallow belly,' and 'baris'), each differing primarily in color, but with nearly common morpho-productive characteristics. Among these, the blonde variety has been the most widespread and represents the breed's prototype.

Pigs of the Mangalitsa breed, regardless of the color variety, have a carcass meat percentage of less than 40% (Szabó, 2006; Zăhan et al., 2009).

The meat from Mangalitsa pigs has superior taste qualities, is juicy, marbled, lending itself very well to the preparation of winter salamis (Habeanu et al., 2011).

Pork meat represents a very important source of energy and essential nutrients for humans. Due to its balanced chemical composition in nutrients with high biological value (proteins, lipids, minerals and vitamins) and its increased digestibility, meat is a food that cannot be absent from the human diet.

The chemical composition of pork is crucial for the composition of the products obtained after processing. Pork meat has a relatively constant chemical composition, very close to that of muscular tissue, but the chemical composition of fatty pork meat can vary widely (Ionescu et al., 2009).

MATERIALS AND METHODS

The biological material under investigation consists of the Mangalitsa pig population, raised on a livestock farm located in the northeastern part of the country, categorized as type A exploitation, specialized in the breeding, rearing and commercialization of Mangalitsa pigs. This population comprises specimens of Mangalitsa pigs, including the red variety and the swallow belly variety.

The zootechnical farm began its activity in 2018. Initially, the biological material came from a sow with 4 piglets of the red variety. Later, biological material was acquired from the blonde and swallow belly varieties. Young males that are not selected for reproduction are castrated, and then they are moved to the growth and fattening areas, alongside females that are not used for breeding1. This approach ensures efficient farm management and optimal utilization of available resources.

To achieve the established goal, 2 experimental batches of Mangalitsa breed pigs were studied, raised in a zootechnical farm specialized in the breeding of this pig breed in the NE area of the country. Each of the 2 experimental batches consisted of 10 individuals, castrated males and females of the red variety and swallow belly variety. In the zootechnical farm under study, a semi-free-range breeding system is practiced, with the pigs being fed with concentrated feed mixtures, having permanent access to pasture. Individuals from the 2 experimental batches were slaughtered in a specialized slaughterhouse at about one year old and a weight of approximately 100 kg.

The determination of the average slaughter weight of the biological material (castrated males and females) was conducted at the slaughterhouse prior to slaughter. At the slaughterhouse, the pigs were housed in individual pens in preparation for slaughter, during which they were not fed, only provided with small amounts of water (waiting period of 12-24 hours).

The basic component of meat is represented by muscle tissue, which is composed of all the striated muscles. These muscles consist of morphological and structural units known as striated muscle fibers. These fibers are made up of numerous cylindrical cells containing sarcolemma and sarcoplasm, within which myofibrils of myosin and actin are found. The striated appearance of muscle results from the alternating presence of these two types of myofibrils, each with different diameters (Multon et al., 1991).

In order to determine the physico-chemical properties of Mangalitsa pork, samples were collected from three distinct body regions: neck, leg and loin.

The pulp samples were represented by the musculature from the pelvic region, from which the bones, soft fat, ligaments, and vascular formations on the processed surfaces were removed.

The neck samples were represented by the deboned, defatted, well-trimmed neck muscle, which exhibited an intermittent layer of up to 0.5 cm of fat on the surface, with the fascia (covering membrane) still adhering to the muscle. These samples had straight and well-defined edges, without any cuts in the muscle mass, fraying, or bone remnants.

The pork chop samples were obtained by processing the muscle from the dorsal region, which was well-trimmed. On the surface, it exhibited a thin layer of fat, no more than 0.3 cm thick, with straight and well-defined edges. There were no cuts in the muscle mass, no fringes, or small bone fragments.

These samples were processed in a specialized laboratory equipped with modern equipment and advanced instrumentation, allowing for high-precision analyses using current methodologies for these determinations (Figure 1).

pork leg pork neck pork chop

Figure 1. Sampling for Physico-chemical analysis (own source)

The pH determination of Mangalitsa meat was performed in accordance with the current standards (SR ISO 2917:2007; SR 2443:2008).

The acidity of meat is determined by the content of organic acids in pork meat, as well as by all substances in meat that have an acidic character. This property of meat is expressed through the pH value (the logarithm with reversed sign of the hydrogen ion concentration).

pH influences the quality of pork meat in terms of organoleptic, hygienic and technological aspects. Thus, a gradual decrease in pH to a value of approximately 5.4 results in a brighter color of the meat, while a sudden decrease in pH leads to a pale pink hue, characteristic of exudative meat, frequently encountered in the case of pigs (Păsărin et al., 2007).

The knowledge of pH evolution in pork meat during the period after slaughter is of paramount importance. This parameter directly influences the organoleptic properties of the meat, including its tenderness, consistency, aroma, and taste. Additionally, it affects the meat's water retention capacity and its suitability for preservation.

In terms of chemical composition, pork meat consists of the following components: water, nitrogenous protein and non-protein substances, carbohydrates, lipids, enzymes, vitamins and mineral substances.

The water found in meat constitutes the largest proportion of its total weight. However, its proportion in meat can vary depending on numerous factors, including breed, age of the animal, level of fatness, physiological condition, sex, and diet.

The fatness level of pigs significantly influences the water content in their meat. Consequently, leaner pigs have a higher water content in their meat compared to fatter pigs, where the water content is lower.

The determination of moisture content in Mangalitsa meat was carried out according to the ISO 1442:2010 standard - Meat and meat products.

The water and dry matter content of pork meat can vary widely depending on the type of meat, breed, sex and age of the slaughtered pigs, the fattening condition, the rearing system, the provided diet, the anatomical region, etc.

The determination of protein content in Mangalitsa meat was conducted according to the SR ISO 937:2007 standard - Meat and meat products - Determination of nitrogen content.

The protein content of pork meat is influenced by the fattening condition of the animal, the water content of the meat, the breed, the age of the slaughtered animal, as well as the type of muscle.

The determination of lipid content in Mangalitsa meat was conducted in accordance with the SR ISO 1444:2008 standard - Meat and meat products - Determination of free fat content.

The amount of lipids in pork meat varies greatly depending on the type of muscle, the breed and age of the animal and its fattening condition. The lipid content of meat is inversely proportional to its water content.

RESULTS AND DISCUSSIONS

The physico-chemical properties of pork were determined on samples of leg, loin, and neck collected from 10 carcasses, for each of the two varieties of Mangalitsa breed pigs studied (the red variety, the swallow belly variety) (Figure 1).
The laboratory analyses performed allowed the determination of the pH, the dry matter, protein, and fat content of the pork.

Figure 1. Mangalitsa pigs, swallow belly variety (own source)

To obtain a good yield at slaughter and meat with optimal physico-chemical and organo-leptic qualities, within the unit, Mangalitsa breed pigs were slaughtered at weights exceeding 100 kg live weight, thus obtaining a higher percentage of lard in Table 1.

Table 1. Medium weight at slaughter

Lot	n	Sex	Live weight MEAN ± SEM (kg)
Mangalitsa, the red variety	10	♀+♂	105.51±1.43
Mangalitsa, swallow belly variety	10	♀+♂	108.14±1.31

The pH values of the analyzed meat samples were determined in a specialized laboratory 24 hours after slaughtering the pigs according to current standards. The determinations were made on samples collected from the leg, neck and loin for the two varieties of Mangalitsa pigs, according to the laboratory protocol.
The recorded values for pH 24 hours after slaughtering ranged between 5.80±0.02 and 5.84±0.03 in the case of neck samples, between 5.74±0.01 and 5.77±0.03 in the case of leg samples, and between 5.76±0.04 and 5.78±0.02, as recorded in the case of loin samples (Table 2).

Table 2. The pH values of the pork from the Mangalitsa breed

Lot	n	Sex	pH MEAN ± SEM		
			neck	leg	loin
Mangalitsa, the red variety	10	♀+♂	5.84±0.03	5.74±0.01	5.78±0.02
Mangalits, swallow belly variety	10	♀+♂	5.80±0.02	5.77±0.03	5.76±0.04

Understanding the evolution of pH in pork meat during the post-slaughter period is of particular importance because this parameter directly influences the organoleptic properties of the meat and its water retention capacity, as well as the meat's suitability for preservation.
Regarding the dry matter content of the analyzed samples, the average values obtained are presented in Table 3.

Table 3. The dry matter of pork from the Mangalitsa breed

Lot	n	Sex	D.M. MEAN ± SEM		
			neck	leg	loin
Mangalitsa, the red variety	10	♀+♂	41.44±0.30	30.94±0.52	32.87±0.51
Mangalitsa, swallow belly variety	10	♀+♂	42.05±0.38	32.67±0.43	33.38±0.51

According to the data in Table 3, the dry matter content of the meat samples from the two experimental batches varied depending on the anatomical region. Thus, the highest values were recorded for pork neck, ranging between 41.44% and 42.05%, while the lowest were recorded for pork leg, situated in the range of 30.94% to 32.67%.
Comparing the average values obtained for dry matter, it can be observed that the lowest

values were recorded in the case of pigs from the Mangalitsa breed, red variety.

The protein content of the samples studied, collected from Mangalitsa breed pigs, varied depending on the anatomical region, so for pork neck the recorded values were the lowest, being between 15.47% in the case of the red variety and 15.69%, as recorded in the case of the swallow belly variety (Table 4).

Table 4. The protein content of pork from the Mangalitsa breed

Lot	n	Sex	Proteins MEAN ± SEM		
			neck	leg	loin
Mangalitsa, the red variety	10	♀+♂	15.69±0.28	20.90±0.31	20.31±0.37
Mangalitsa, swallow belly variety	10	♀+♂	15.47±0.22	20.33±0.24	19.74±0.31

For pork leg and loin, the average values obtained for protein content were around 20%, the highest values being determined in the case of the red variety.

In terms of protein content in Mangalitsa loin, we can observe that the highest value for this indicator was calculated for the red variety (20.31±0.37%), while the lowest was determined for the swallow belly variety (19.74±0.31%).

The lipid content of Mangalitsa pork varied within a wide range, thus the lowest average values were between 8.85±0.25% and 8.94±0.20% and were recorded for Mangalitsa leg, while the highest values were 24.55±0.35% for the neck from the red variety, respectively 25.32±0.47% for the neck from the swallow belly variety (Table 5).

Table 5. Lipid content of Mangalitsa pork

Lot	n	Sex	Lipids MEAN ± SEM		
			neck	leg	loin
Mangalitsa, the red variety	10	♀+♂	24.55±0.35	8.85±0.25	12.05±0.12
Mangalitsa, swallow belly variety	10	♀+♂	25.32±0.47	8.94±0.20	12.22±0.10

The lipid content of Mangalitsa meat varied within quite wide limits, both depending on the variety of Mangalitsa breed pigs and especially on the type of meat, with Mangalitsa neck presenting the highest lipid content.

The average values obtained from the laboratory analyses for pH and the content of dry matter, protein, and fat in Mangalitsa pork meat fell within the optimal range for this breed of pigs, being close to those reported in the specialized literature (Stănescu et al., 1987; Egerszegi et al., 2003; Hollo et al., 2003; Szabó, 2003; Szabó, 2006; Lugasi et al., 2006; Petrović et al., 2007; Zăhan et al., 2009; Petrović et al., 2010; Nistor et al., 2012; Nagy, 2013).

CONCLUSIONS

The values obtained for the pH of Mangalitsa meat determined at a 24-hour post-slaughter interval varied between 5.74, as recorded in the case of pork leg samples from pigs of the red variety, and 5.84, as recorded in the case of pork neck samples, also collected from pigs of the red variety.

The dry matter content of the meat samples from the two experimental groups ranged between 30.94% and 32.67% for pork leg, between 41.44% and 42.05% for pork neck and between 32.87% and 33.38% for pork loin.

The values recorded for pH 24 hours after slaughter were close for all 6 experimental batches, being around the value of 5.7.

Comparing the average values obtained for dry matter, it can be observed that the lowest values were recorded in the case of the batch consisting of pigs from the Mangalitsa red variety.

The protein content of the samples from the two experimental groups collected from Mangalitsa pigs ranged between 15.47%, as recorded in the case of pork neck collected from the swallow belly variety, and 20.90%, as obtained in the case of pork leg collected from pigs of the red variety. Thus, the percentage of protein in the samples taken in the study varied depending on the anatomical region, with the lowest values recorded for pork neck. Regarding the lipid content of Mangalitsa meat, it varied within quite wide limits, both depending on the variety of Mangalitsa pigs and especially on the type of meat, with Mangalitsa neck showing the highest values. Regarding the lipid content of Mangalitsa meat, it varied within quite wide limits, both depending on the variety of Mangalitsa breed pigs and especially on the type of meat, with Mangalitsa neck presenting the highest values.

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DYNAMICS OF CERTIFICATION OF TRADITIONAL DAIRY PRODUCTS AT EU LEVEL AND SPECIFICALLY FOR ROMANIA

Aurelia DEFTA (OSMAN)[1], Gratziela Victoria BAHACIU[1], Monica MARIN[1], Nicoleta DEFTA[1,*], Dănuț ENEA[1], Mirela Aurora STANCIU[2], Livia VIDU[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]University Lucian Blaga of Sibiu, 10 Victoriei Blvd, Sibiu, Romania

Corresponding author email: nicoleta.defta@usamv.ro

Abstract

Certified traditional products are an essential part of every country`s cultural and gastronomic heritage. The quality and authenticity of these products are ensured by the authentic ingredients and artisanal methods of preparation. Consumers` growing interest in healthy eating makes the certification of traditional products a necessity. In this study, we have carried out a comprehensive statistical analysis of the current number of food products certified under quality schemes such as PDO, PGI, TSG, and also labelled as `Traditional Product` and `Traditional Romanian Recipe`, both in Romania and in the European Union (EU). We also looked in detail at the current number of dairy products certified according to these standards. The data were obtained from the EU`s eAmbrosia electronic register and the Ministry of Agriculture and Rural Development`s CPAC electronic catalogue. The results provide a valuable and up-to-date perspective on certified and labelled food products, contributing to the development and promotion of the gastronomic heritage in Romania and the EU.

***Key words**: dairy products, quality scheme, registered Romanian traditional recipe, traditional product.*

INTRODUCTION

In the current context, healthy eating plays a crucial role in maintaining our overall health and well-being. Moreover, consumers are increasingly opting for sustainable foods that promote sustainability and are characterized by superior quality (Chifor & Arion, 2023; Brons & Oosterveer, 2017; Bublitz et al., 2013; Smith-Spangler et al., 2012). They are interested in the origin of food products, especially for food products with certain geographical regions where culinary traditions are respected (Florek & Gazda, 2021; Vandecandelaere et al., 2018). 82% of consumers attach importance to the origin of food accordingly to the Swiss Federal Office of Public Health study that (Luykx & Van Ruth, 2008).

In order to respond to consumer demands, the EU has created the geographical indication quality systems (short, quality schemes) that aim to create added value by regulating and protecting agri-food products, ensuring that they respect and highlight their unique physical characteristics, impact on the environment and traditions specific to the region of origin (Poetschik et al, 2021; Giovannucci et al, 2009). The first regulation was adopted by the EU in 1992, and that created the basis for the development of these quality schemes, with the aim of protecting quality and promoting products with geographical indication (Pieniak et al., 2009; Council of the European Union, 1992). In 2012, the European Parliament and the Council of the European Union adopted a new regulation that establishes the requirements for quality schemes with geographical indication, asking that most production stages take place in a well-defined geographical area, the manufacturing recipe be authentic, the raw materials must be original, the production process must be traditional and/or contain characteristics specific to the respective region, and they must pass on part of the gastronomic heritage of a community (European Commission, 2023; Glogovețan et al., 2022; Gellynck et al., 2008). According to the European Union, these quality schemes are considered an important tool in protecting and promoting high-quality agri-food products, ensuring that farmers and producers benefit from a fair competitive environment, correctly informing consumers and promoting rural development in the respective areas (Chifor et al., 2022; Arfini et al., 2019). Various researchers have highlighted that these quality schemes make a valuable contribution to the sustainable development of rural areas, including by conserving local plant varieties, supporting diversity and social cohesion and creating employment opportunities (Crescenzi et al., 2022; Blakeney et al., 2021; Connelly et al., 2015).

In Romania, all products certified as PDO, PGI, TSG, and Mountain Product quality schemes, as well as those certified as Traditional Product and Established Romanian Recipe are included in the Certified Products and Activities Catalogue (CPAC), which is an online platform launched by the Ministry of Agriculture and Rural Development (Ministry of Agriculture and Rural Development, 2023). Through this platform, all certified products are organized by product class, making it easy to navigate and search for information and allowing the consumer to quickly identify certified products. This classification is based on categories such as: milk products; meat products; fish products; bee products; vegetable-fruit products; bakery products; drinks; wineries; others (Catalogue of certified products and activities, 2023).

Romanian culinary heritage and the variety of traditional products represent a significant component of local culture and identity, influencing the socio-economic development of rural areas in a unique and valuable way (Soare et al., 2023).

The aim of this paper is to present the dynamics and evolution of PDO, PGI, TSG, Traditional Product and Established Romanian Recipe certified agri-food products in Romania, with special emphasis on certified dairy products. To achieve this goal, the following objectives were formulated:

- analysis of the distribution of these products by region;
- highlighting the close links between the areas that have preserved local traditions and practices and the distribution of the agri-food products studied.

MATERIALS AND METHODS

Materials

For the research we have used some specific Romanian and EU databases like:

- Legislation regulating the licensing conditions of agri-food products certified according to PDO, PGI, TSG quality schemes at the European Union level and Traditional Product, Established Romanian Recipe at a national level.
- eAmbrosia online platform: for monitoring the current status of products certified under the PDO, PGI and TSG quality schemes;
- The online platform Catalogue of certified products and activities (CPAC): access information about the status of PDO, PGI, TSG, traditional products and Established Romanian Recipe certified products in Romania;
- The National Registry of Traditional Products (RNPT) and the National Registry of Certified Traditional Recipes (RNRC): relevant information on food products certified as traditional products in Romania, as well as on producers who have certified and registered products according to an Established Romanian Recipe.

The monitoring of the data from the accessed materials led to results showing accuracy and precision in terms of the certification/licensing of traditional Romanian food products, contributing to the development of knowledge in this important field of Romania's cultural and gastronomic heritage.

Methods

1. ***Identification of the certification methods of Romanian traditional products***

Products certified under the quality schemes benefit from specific logos, which confirm their quality and authenticity (Figure 1). These distinctive logos help consumers easily identify them on the shelf, differentiating them from other products in the same category (Somicu & Vladu, 2023).

At the level of the European Union there are several protected, recognized and officially regulated quality schemes (Table 1).

Table 1. Protected quality schemes and allotted logos

Description	Specific logo*
Protected Designation of Origin (PDO) This certification applies to agri-food products and wines, provided that all stages of production, processing and packaging take place in the respective region.	
Protected Geographical Indication (PGI) This certification applies to agri-food products and wines, provided that at least one of the stages of production, processing and packaging takes place in the respective region.	
Geographical Indication of Spirit Drinks (GI) This certification applies to spirits and aromatized wines, provided that one of the distillation or processing steps takes place in the respective region.	
Traditional Speciality Guaranteed (TSG) This certification applies to agri-food products, provided that the product is made according to a traditional recipe, which highlights traditional aspects and is not less than 30 years old	
Mountain product This certification applies to agri-food products, provided that the animal feed and raw material come from mountain areas. For processed products, it is necessary that the processing process takes place exclusively in the mountain area (European Commission, 2023).	

*Extracted from: European Commission, https://agriculture.ec.europa.eu/farming/geographical-indications-and-quality-schemes/geographical-indications-and-quality-schemes-explained_ro (accessed on 14 November 2023).

In Romania, in addition to the PDO, PGI, GI, TSG and Mountain Product quality schemes, there are two main ways by which agri-food products are protected and valued, which preserve their authenticity and respect traditional recipes and methods (Table 2).

Table 2. Official logo for Traditional product and the established Romanian recipes

Description	Specific logos*
Traditional product This certification applies to food products, provided that the raw material is local, does not contain food additives, is based on a traditional recipe, and the production and/or processing process is traditional, products that will be registered in the National Registry of Traditional Products (Ministry of Agriculture and Rural Development, Food Industry, 2023).	
Established Romanian recipes This certification applies to food products, provided that the recipe according to which the product is manufactured is more than 30 years old from the date of entry into force of "Decree no. 394/2014", recipes that will be registered in the National Registry of established recipes (Ministry of Agriculture and Rural Development, 2023).	

*Extracted from: Ministry of Agriculture and Rural Development,
https://www.madr.ro/industrie-alimentara.html (accessed on 14 November 2023).

2. Data processing methods

Descriptive statistics was used for data processing through techniques of organization and summative presentation of data (representation in the form of graphs and tables).

RESULTS AND DISCUSSIONS

The distinctive feature of these products is their superior quality. The products stand out for their strict adherence to traditional manufacturing methods, the recipes being inspired by traditional culinary practices (Soare, 2023; Sproesser, 2022).

Based on the synthesis and statistical processing of the data taken from the official platforms, it was concluded that at the end of December 2023 there were 1527 certified products, of which 57 TSG, 659 PDO and 810 PGI (Figure 3). In the case of PGI products, the certification procedure does not involve carrying out all the stages of obtaining the respective product in the geographical region characteristic of the product.

In contrast, PDO certification restricts the production, processing and packaging stages to take place in the geographical area to which the product is assigned.
This restriction is what makes PDO-certified products so valuable, as they guarantee authenticity and a close connection with the specific traditions and resources of the respective geographical region.
For the certification of TSG products, the requirements refer to strict adherence to tradition and authentic production methods. This aspect led to a smaller range of products, but highly appreciated by consumers (Figure 1).

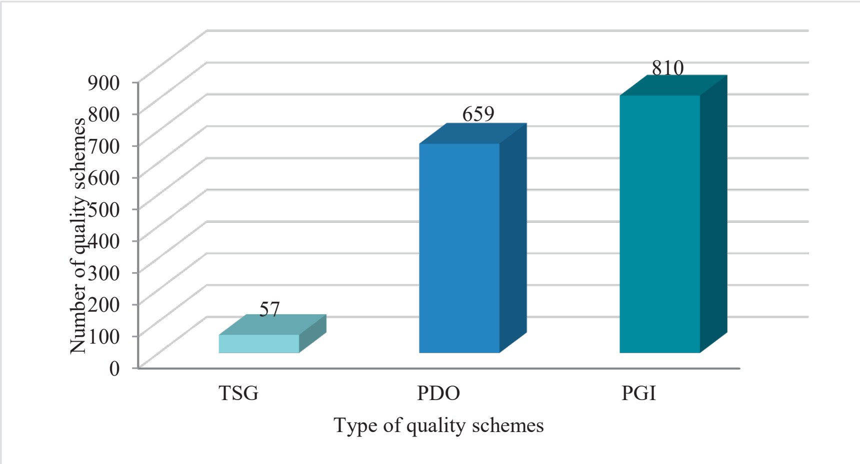

Figure 1. Number of food products certified through PDO, PGI and TSG quality schemes within the EU (December 2023)

The countries for which gastronomy is valued as an art, respectively the countries where tradition is a cult, stand out for the majority of products that are part of the PDO, PGI, TSG quality schemes. The feeling of belonging and attachment to the territory, respect for traditions and culinary recipes contributed to obtaining certification for many local products. For example, Italy is known for its respect for traditions and its culture rich in gastronomy, strongly anchored in tradition and with a strong sense of territorial identity. Each region of Italy has its own traditional specialties and specific production methods, which has led to a remarkable diversity of certified food products. Italian locals and producers are proud of their products and traditions and are dedicated to protecting and promoting them. Italy also has a long history of protecting and promoting local products through the Italian Association of Geographical Indications Consortia (AICIG), which works constantly and rigorously to ensure product quality and authenticity. France (famous for its cheeses and pastries) and Spain (known for its olive oils and traditional hams) have a rich history of culinary traditions and are considered leaders in high-quality gastronomy (Figure 2).

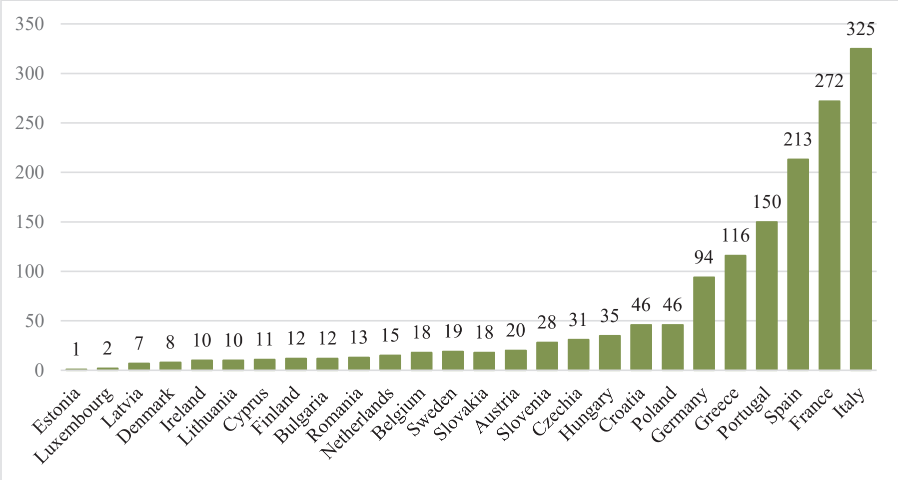

Figure 2. Number of food products certified through PDO, PGI and TSG quality schemes in EU member states (December 2023)

In Romania, there are 13 products certified at the European level through the corresponding quality schemes, among which PDO, PGI and TSG. In addition, at the national level, there are its own certification and recognition mechanisms for traditional agri-food products: Established Romanian Recipe (25 products) and Traditional Product (765 products), Figure 3.

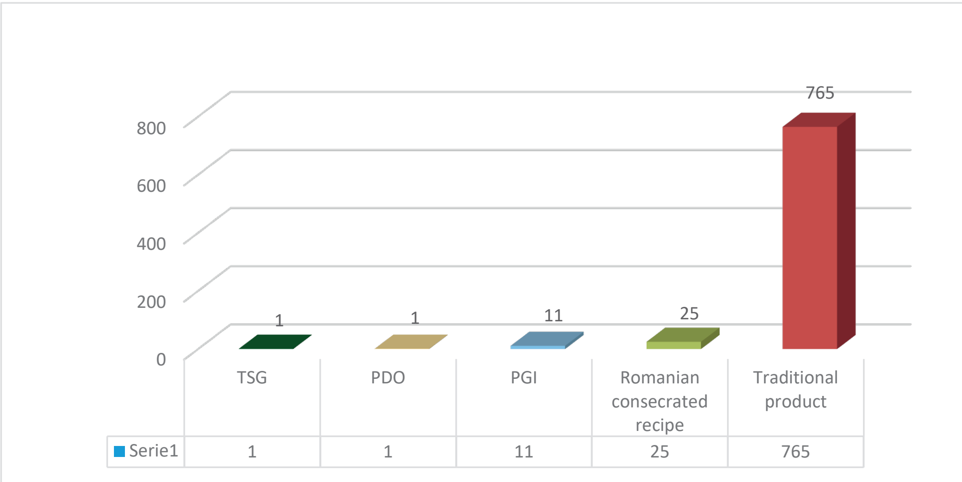

Figure 3. Number of food products certified in Romania as Traditional Product and Established Romanian Recipe (December 2023)

Regarding the PDO, PGI and TSG certified dairy products, the same countries stand out at the European level, whose products are in accordance with the strict standards of quality, provenance and tradition, respectively (Figure 4):

- France (62 products) - French cheeses are famous for their traditional recipes and refined taste (Roquefort, Camembert and Brie);
- Italy (58 products) - known for its experience in the production of fine cheeses (Parmigiano Reggiano, Gorgonzola and Mozzarella di Bufala)
- Spain (33 products) - offers a diverse range of authentic and delicious cheeses, recognized for their superior quality and their specific origin (Manchego, Queso de Tetilla or Queso de Cabra Majorera).

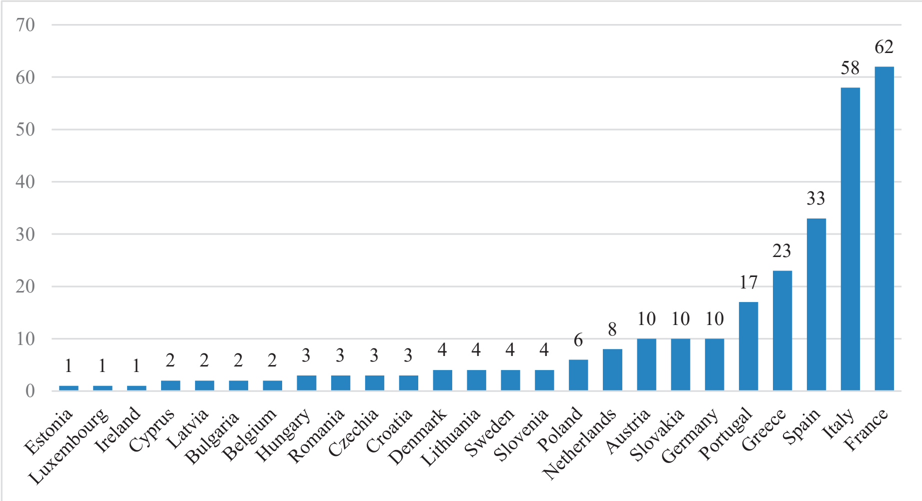

Figure 4. Number of PDO, PGI and TSG quality schemes certified dairy products in EU member states (December 2023)

In Romania there are 3 dairy products certified at European level, of which 1 is PDO (*Telemeaua de Ibănești* - certified in 2016) and 2 PGI (*Telemeaua de Sibiu* - certified in 2019 and *Cașcaval de Săveni* - certified in 2021) (Figure 5 and Table 3).

In addition, traditional dairy products from Romania benefit from various attestations and certifications, recognized both at the national level and at the level of the European Union, respectively Traditional Product (138 products) and Established Romanian Recipe (8 products) (Figure 5 and Table 3).

The evolution of the attestation of dairy products in Romania, within the PDO, PGI, Traditional Product and Established Romanian Recipe quality schemes, is significant and highlights the increased interest in valorising and protecting authentic dairy products and the close connection between the distinct characteristics of the products and the geographical region specific (Figure 5 and Table 3).

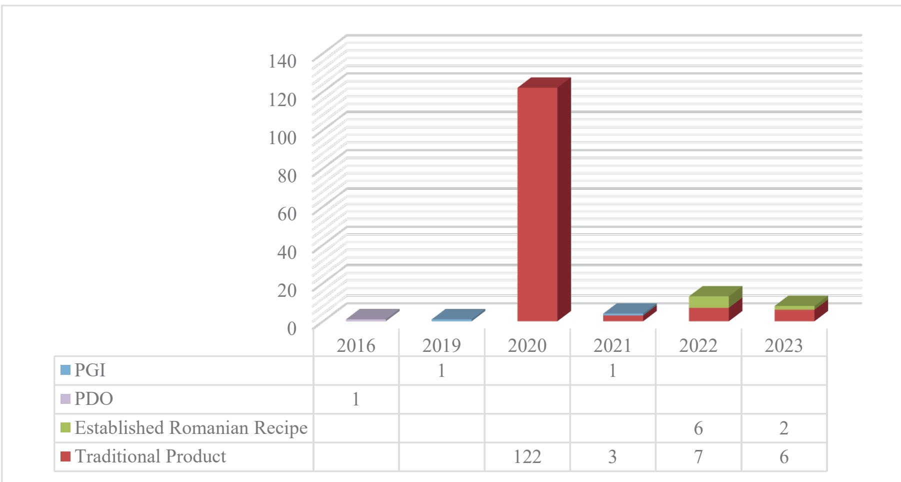

Figure 5. Evolution of dairy products' PDO, PGI, Established Romanian Recipe and Traditional Product certification in Romania (December 2023)

Increased interest and efforts in the valorisation and promotion of authentic Romanian products have led to the certification of a large number of dairy products in the Traditional Product category, of which 122 dairy products in 2020, 3 dairy products in 2021, 7 in 2022 and 6 in 2023.

The certification of the first dairy products (6 products) as Established Romanian Recipe was achieved in 2022. In the following year, 2 more products from this category were certified (Figure 5 and Table 3).

Table 3. Dynamic of certification/licensing of dairy products in Romania

No. of products	Product type	Certification year
	Dairy products certified as Protected Designation (PDO) within Romania and the EU	
1	Telemea de Ibănești	2016
	Dairy products certified as Protected Geographical Indication (PGI) within Romania and the EU	
1	Sibiu cheese (Telemea de Sibiu)	2019
2	Săveni yellow cheese (Cașcaval de Săveni)	2021
	Dairy products certified as Established Romanian Recipe in Romania	
1-6	Lapte bătut 2% grăsime; Chefir 3,3% grăsime; Sana 3,6% grăsime; Unt 80% grăsime; Cașcaval Dalia; Cașcaval Rucăr	2022
7-8	Brânză telemea de vacă; Brânză telemea de oaie	2023
	Dairy products certified as Traditional Product in Romania	
1-122	Cașcaval de Covasna Roby; Cașcaval afumat / afumat împletit de Covasna Roby; Urdă dulce/sărată Bangălă Fundata; Brânză de burduf Bangălă Fundata; Telemea de vacă "Ferma Istrate" Fundata; Brănză de burduf "Ferma Istrate" Fundata; Caş "Ferma Istrate" Fundata; Cașcaval "Ferma Istrate" Fundata; Telemea de oaie "Ferma Istrate" Fundata; Urdă "Ferma Istrate" Fundata; Caș Drăgoiu; Brânză de burduf Drăgoiu; Urdă Drăgoiu; Brânză telemea Drăgoiu;Cașcaval Poponeci; Brânză telemea Poponeci; Urdă Poponeci; Brânză de burduf Poponeci; Caș proaspăt/afumat Poponeci; Cașcaval "Baci Nicolae"; Urdă "Baci Nicolae"; Brânză de burduf "Baci Nicolae"; Cașcaval afumat Mănăstiresc; Telemea de vacă Mănăstirească; Unt de vacă Mănăstiresc;Cașcaval afumat Baciu Fane; Cașcaval afumat Ciobănașul; Brânză de burduf Ciobănașul; Telemea de vacă Ciobănașul; Telemea de oaie Ciobănașul; Cașcaval afumat Moș Onica; Caș proaspăt/afumat Ferma Cățean; Brânză de burduf Ferma Cățean; Telemea de oaie Ferma Cățean; Cașcaval Ferma Cățean; Urdă Ferma Cățean; Telemea de vacă Ferma Cățean; Cașcaval Izvorul Alb; Urdă dulce Suhardo; Caș proaspăt/afumat Mihnea; Brânză de burduf Mihnea; Brânză telemea Mihnea; Urdă Mihnea; Telemea de vacă Lițoi; Caș afumat Lițoi; Urdă sărată Lițoi; Cașcaval opărit și afumat Lițoi; Brânză de burduf Lițoi; Brânză de burduf Dorin; Urdă Dorin;Telemea de oaie Dorin; Caș neafumat/afumat Dorin; Cașcaval de casă Dorin; Cașcaval Petrovan de Valea Stejarului; Brânză frământată Petrovan de Valea Stejarului; Brânză telemea Petrovan de Valea Stejarului; Cașcaval Cicos; Cașcaval Lelița; Cașcaval Sofica de Arad; Telemea de oaie/vacă Bangală Fundată; Brânză telemea Stărp Ioan; Caș dospit Stărp Ioan; Urdă dulce Stărp Ioan; Brânză de burduf Stărp Ioan; Smântână dulce Suhardo; Telemea de vacă Suhardo; Cașcaval Suhardo; Caș de oaie Raul; Caș de capră Raul; Brânză telemea de oaie Raul; Cașcaval Genys; Smântână Botiș de Cărpiniș; Urdă Botiș de Cărpiniș; Cașcaval Botiș de Cărpiniș; Caș "Cami" de la Loman; Cașcaval afumat Deluț; Caș Deluț; Brânză frământată Deluț; Urdă Deluț; Brănză Produs de Țaga; Brânză Năsal; Brânză de burduf în coajă de brad "Ferma Istrate"; Caș de oaie Căciulă; Telemea de oaie Căciulă; Cașcaval Căciulă; Telemea de vacă Căciulă; Caș afumat Căciulă; Brânză de burduf Căciulă; Urdă Căciulă; Brânză tradițională Peșteană din Fermele ADO; Cașcaval "Curtea Brăneană"; Caș "Curtea Brăneană"; Urdă "Curtea Brăneană"; Brânză de burduf "Curtea Brăneană"; Telemea de vacă "Curtea Brăneană"; Telemea de oaie "Curtea Brăneană"; Urdă Bran-Spârchez; Brânză telemea de oaie Bran-Spârchez; Brânză de burduf Bran-Spârchez; Caș afumat Bran-Spârchez; Brânză de oaie Burebista; Unt tradițional din Ozunca; Telemea de Nicolae Ciobanul; Cașcavea de Doftana "Crăița"; Urdă dulce de vacă Baciul Brănean; Brânză de burduf în bășică Baciul Brănean; Brânză de burduf în coajă de brad Baciul Brănean; Caș afumat Baciul Brănean; Cașcaval afumat de oaie Baciul Brănean; Brânză frământată Viofanny; Caș Viofanny; Urdă Viofanny; Telemea Viofanny; Telemea de capră transilvăneană din Moiad; Caș de capră transilvăneană din Moiad; Telemea de capră Picurariu din Stejaru; Brânză burduf de capră transilvăneană din Moiad; Cașcaval de capră transilvănean din Moiad; Brânză frământată de Sângeorz Băi; Urdă de capră transilvănean din Moiad; Cașcaval tradițional Vlăsie; Cașcaval Viofanny	
1-3	Cașcaval afumat Vâlsănesc; Cașcaval Bangălă Fundata; Caș proaspăt/afumat Bangălă Fundata	
1-7	Brânză de vaci Romfulda din Beclean; Telemea de vacă Romfulda din Beclean; Brânză frământată în burduf Romfulda din Beclean; Brânză de vacă făcută la Sălaș; Iaurt de bivoliță –lapte acru din rădești; Caș de vacă-proaspăt din rădești; Brânză puțin sărată din Rădești	
1-6	Cașcaval văcuță ICA; Caș văcuță ICA; Urdă văcuță ICA; Telemea văcuță ICA; Brânză frământată; Sinelli de Teaca; Cașcaval de Lactomac	

Source: ec.europa.eu/agriculture/eAmbrosia

In order to achieve the second aim of the study, the distribution of the producers of products from the PDO, PGI, Traditional Product and Established Romanian Recipe categories was carried out on the map of Romania. A strong connection was noticed regarding the diversity of certified dairy products and how traditions are preserved in the respective regions (Figure 6).

According to the data obtained from the Catalogue of Certified Products and Activities (CPAC), Transylvania is the region with the largest variety of producers of traditional dairy products, with a total number of 99, of which 1 producer of dairy products within the PDO quality schemes, 7 producers of PGI dairy products, 77 producers of dairy products certified as Traditional Product and 14 producers of products certified as Established Romanian Recipe. This diversity of traditional dairy products indicates an increased concern for the preservation of traditions and the quality of local products.

Figure 6. Distribution of producers of dairy products certified through quality schemes
(Source -map of Romania https://xn--urlaub-in-rumnien-2qb.de/ro/regiuni-romania/
Original: Product logo allocation and caption)

The interest in protecting and promoting authentic Romanian products remains constant, whilst upholding the significance of preserving Romania's cultural values and gastronomic traditions.

CONCLUSIONS

The certification of food products through the PDO, PGI and TSG quality schemes is essential for highlighting the authenticity and superior quality of food products from different geographical areas of the European Union. These schemes attest to the respect for the gastronomic traditions and distinct particularities of each region, contributing to the consolidation of the local culinary identity. Italy, France and Spain are examples of countries with a rich gastronomic diversity, offering certified products that reflect their commitment to protecting and promoting regional traditions.

The sector of dairy products certified by the PDO, PGI and TSG quality schemes stands out for its diversity, authenticity and respect for gastronomic traditions. Italy, France and Spain are recognized as leaders in this industry, offering consumers high-quality, authentic and certified dairy products, reflecting their dedication to excellence and protecting the gastronomic heritage specific to geographic regions.

The evolution of the certification of dairy products in Romania demonstrates an increased interest in protecting and promoting authentic products. Efforts to preserve and promote authentic dairy products remain particularly important for Romania, intending to preserve the country's gastronomic traditions and cultural heritage.

Transylvania is a real epicenter of authentic and quality dairy products. The generous distribution of certified dairy products in this region underlines the firm commitment to the preservation of local gastronomic traditions and the promotion of authentic cultural values. This phenomenon confirms the relevance and gastronomic prestige of Transylvania both domestically and in the international context, contributing to the promotion of the Romanian culinary identity.

The protection and promotion of PDO, PGI, Traditional Product and Established Recipe dairy products represent an important step in the preservation of cultural heritage and in the affirmation of Romanian gastronomic values in an international context.

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PRELIMINARY STUDIES ON OBTAINING A CHEESE MADE EXCLUSIVELY FROM WHEY ENRICHED WITH PUMPKIN POMACE POWDER

Roxana Nicoleta RAȚU[1], Florina STOICA[2], Florin Daniel LIPȘA[1], Andra ŞULER[3], Casiana Agatha PETRESCU[4], Nicoleta STĂNCIUC[5], Iuliana APRODU[5], Oana Emilia CONSTANTIN[5], Gabriela RÂPEANU[5]

[1]"Ion Ionescu de la Brad" Iasi University of Life Sciences, Faculty of Agriculture, Department of Food Technologies, 3 Mihail Sadoveanu Alley, 700489, Iasi, Romania
[2]"Ion Ionescu de la Brad" Iasi University of Life Sciences, Faculty of Agriculture, Department of Pedotechnics, 3 Mihail Sadoveanu Alley, 700489 Iasi, Romania
[3]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[4]"Olga Sturdza" Agricultural Technological High School, 38 Mihail Kogălniceanu, Miroslava, 707305, Iaşi, Romania
[5]"Dunărea de Jos" University of Galati, Faculty of Food Science and Engineering, Department of Food Science, Food Engineering, Biotechnology and Aquaculture, 800201, Galați, Romania

Corresponding author email: roxana.ratu@iuls.ro

Abstract

Pumpkin (Cucurbita maxima) is a popular vegetable widely cultivated and consumed due to its rich content in biological active compounds and essential nutrients. Pumpkin pomace (PP) powder, which is a by-product derived from the pumpkin industry, has garnered considerable attention as a potential useful component for enhancing the overall quality of foods.
The study involved the addition of varying amounts (1 and 2%) of pumpkin powder to whey cheese compositions. This study aims to evaluate the effects of PP powder addition on the physical, chemical, color, microbial and sensory characteristics of whey cheese, alongside its impact on the product's nutritional value. PP powder is a good source of phytochemicals such as carotenoids and polyphenols, with remarkable antioxidant capacity. The results indicate that the incorporation of PP powder resulted in enhanced nutritional and colour characteristics of the cheeses. Furthermore, the incorporation of pumpkin powder resulted in a substantial increase in the levels of phytochemicals and antioxidant activity. The resulting supplemented cheeses offer a unique color profile, appealing to health-conscious consumers seeking innovative dairy products. Developing these products has the potential to facilitate sustainable food production and offer consumers a wider range of food options with improved attributes.

***Key words**: antioxidant activity, cheese, food ingredients, pigments, pumpkin pomace.*

INTRODUCTION

The food industry has become more interested in utilizing agricultural by-products as potential sources of food ingredients due to the changing consumer preferences for natural and clean-label ingredients. Through specific processing of these by-products, such as fruit and vegetable pomaces, they can be utilized to produce food microstructures that possess desirable functional qualities (Moelants et al., 2014).

Pumpkin, scientifically known as *Cucurbita* L., is a squash fruit vegetable that is part of the *Cucurbitaceae* family, sometimes referred to as the gourd family. It is classified into 130 genera and 800 species. Pumpkins are considered to be economically significant species cultivated on a global scale. They are extensively utilized in the food industry for the commercial manufacturing of various products (pumpkin pie, flour, seed oil, seeds as snacks, bread, cookies, desserts, cereals, ice cream, pumpkin butter, soups, etc.) (Kaur et al., 2020).

A wide variety of shapes, sizes, and colors can be found in pumpkins. There has been an increasing interest in pumpkin fruit and pumpkin-derived products by various industries such as agriculture, food processing, pharmaceuticals, and feed. This interest comes from the nutritional and health-promoting properties of the phytochemicals, proteins and oil found in pumpkin seeds, as well as the polysaccharides present in pumpkin fruit (Kuchtová et al., 2016). Pumpkin is commonly consumed through several methods, including fresh consumption, cooking, and storage in frozen or canned forms. Pumpkin is a rich source of β-carotene, dietary fibre, pectin, mineral salts, vitamins, and other health-promoting elements (Kundu et al., 2014).

Large amounts of peels, seeds, and pomace are produced as a result of industrial pumpkin processing into puree, juice, and other products (Kampuse et al., 2018). Presently, these by-products are employed as animal feed (Valdez-Arjona and Ramírez-Mella, 2019), representing a relatively low-value application.

These facts could contribute to the development of pumpkin by-products into a diverse range of food products. The utilization of these by-products has been used to supplement cereal flours in bakery foods, soups, sauces, instant noodles, and spices, also as a natural pigment in pasta, dairy products, beverages, snacks and flour blends (Villamil et al., 2023).

Nowadays natural food colorants have established a significant presence in important food applications. Natural colorants, such as carotenoids, have been effectively utilized in various coloring systems, including bakery foods (solid phase) and beverages (liquid phase). Carotenoids are widely recognized for their vibrant orange, and yellow hues, predominantly found in fruits and vegetables, which play a significant role in imparting appealing flavors to many food and beverage products (Rodriguez-Amaya et al., 2019).

The current study aims to investigate the potential of pumpkin (*Cucurbita* sp.) pomace, which is derived from the extraction of carotenoids from pumpkins, as a bioactive ingredient for cheese production.

This study aimed to evaluate the effects of PP powder on the physicochemical properties, phytochemicals, color, microbial and sensory attributes of cheeses enhanced with PP.

MATERIALS AND METHODS

The raw material was represented by 80 liters of whey obtained after processing milk in order to obtain a semi-paste cheese (Rațu et al., 2023).

The cheese was processed in the Milk Processing Workshop at USV Iași.

As for the whey, qualitative determinations were made to determine the content of dry matter, water, fat content, protein, ash, and pH value.

The AOAC method no. 925.23 was utilised to evaluate the total solids (TS) present in whey. Subsequently, the samples underwent dehydration using a Memmert UFE 700 forced air oven manufactured by Memmert GmbH in Schwabach, Germany. The water content (W) was determined by the disparity, as indicated by the equation W (%) = 100% -TS (%) (Rațu et al., 2021).

The acid-butyrometric Gerber method describe by Dick et al. (2001) was utilised to evaluate the fat percentage of whey.

The protein contents were assessed using the Kjeldahl method, which was implemented on a Velp Scientifica DK 6 digestion unit and UDK 7 distillation system (Velp Scientifica, Usmate, Italy), following the established methodology of the International Dairy Federation (IDF) (Usturoi et al., 2017; Simeanu et al., 2015).

The total mineral content of crude ash was evaluated by incinerating it at a temperature of 550ºC in a Super Therm C311 furnace (SuperTherm SRL, Romania) after burning it on a Bunsen funnel. The incineration process continued until the samples stopped smoking, following the standards outlined in AOAC method no. 945.46.

The pH metre (WTW InoLab, Xylem Analytics GmbH, Weilheim, Germany) was calibrated before measuring the pH using a glass electrode and a temperature probe. The pH was tested using buffer solutions with pH values of 4 and 7 (Rațu et al., 2023).

Pumpkin fruits (Golden Nugget variety), were purchased in November 2023 from a market in Iasi County, Romania, when they had reached full maturity. The fruits underwent immediate processing so the pumpkin juice was extracted (Bosch MES3500, Drachten, Holland), yielding the pumpkin pomace. Subsequently, the pomace was freeze-dried for 50 hours at a temperature of -42 °C and a pressure of 0.10 mBar. This process was carried out using BIOBASE BK-FD10T equipment Jinan, China.

In addition, the freeze-dried pomaces were ground into a fine powder using MC 12 machinery (Stephan, Germany) and stored in glass jars at room temperature and in the dark until analysis. The final powder underwent sterilization using a UV lamp to eliminate contaminants.

ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), gallic acid solution, n-hexane, ethanol, acetone, sodium carbonate, Folin–Ciocalteu reagent, sodium hydroxide, aluminium chloride, sodium carbonate was purchased from Sigma Aldrich (Schnelldorf, Germany).

Extraction of bioactives from PP. The phytochemicals from PP powder were extracted using the ultrasound-assisted extraction approach described by Lima et al., (2019). To extract bioactive compounds, a mixture of 1.0 g of PP powder and 10 mL of n-hexane/acetone solvent mixture (3:1, *v/v*) or 70% ethanol (for total polyphenols and flavonoids) was utilized. The mixture was then subjected to ultrasound treatment for 35 minutes at a temperature of 40°C and a frequency of 37 kHz (Elmasonic S 180 H, Elma, Germany). The obtained crude extract was subsequently subjected to centrifugation for 10 minutes at a speed of 6500 rpm and a temperature of 4°C. After that, the supernatant was examined for total carotenoids, β-carotene, total flavonoids, total polyphenols, and antioxidant activity.

The determination of carotenoids, phenolic compounds and antioxidant activity of PP extract.

Total carotenoid content. A spectrophotometric study was conducted to quantify and ascertain the quantities of total carotenoids and β-carotene in the extract, following the methodology outlined by Nistor et al. (2022), with minor adjustments. To summarise, 0.2 mL of the extract was dissolved in the extraction solvent combination. It was then placed in the UV quartz cuvette and the absorbance was measured at a wavelength of 450 nm for total carotenoids and 470 nm for β-carotene using a UV-VIS spectrophotometer (Analytik Jena - Specord 210 Plus, Germany). The findings were presented as milligrams per 100 grams of dry weight (d.w.).

Contents (mg/100 g d.w.) = (A x Mw x Df)/(m x L x Ma),

where A - Absorbance of the sample; Mw - molecular weight; Df - sample dilution rate; m - Mass/weight of extract; L - length of the optical path of the cuvette (1 cm); Ma - molar absorptivity (2500 L/mol/cm for carotenoids, 2590 L/mol/cm for β-carotene).

Total flavonoid content. The content of total flavonoids of PP extract were determined using the aluminium chloride technique (Horincar et al., 2019). Shortly, 250 μL of the PP extract and 75 μL of 5% sodium nitrite ($NaNO_2$) were combined with 2 mL of distilled water. Then after 5 minutes 150 μL of aluminium chloride ($AlCl_3$) was added to the mixture. 0.5 mL of sodium hydroxide (NaOH) 1 M was added to the mixture after 6 minutes. The mixture was then measured using a UV–VIS spectrophotometer (Analytik Jena - Specord 210 Plus, Germany) at 510 nm. As a standard, a calibration curve for catechin was used and the results were expressed as milligrams of catechin equivalents per 100 grams of dry weight (mg CE/100 g d.w.).

Total polyphenolic content. The Folin-Ciocalteu method was used to measure the PP extract total polyphenolic contents (Horincar et al., 2019). In short, 200 μL of the PP extract, 1 mL of the Folin-Ciocalteau reagent, and 15.8 mL of distilled water were combined. After 10 minutes, 3 mL of Na_2CO_3 20% was added to the mixture. The final mixture was measured at 765 nm using a UV-VIS spectrophotometer (Analytik Jena - Specord 210 Plus, Germany) after 60 minutes of dark storage at room temperature. A standard curve for Gallic acid was utilized, and the results were represented as milligrams of Gallic acid equivalents per 100 grams of dry weight (mg GAE/100 g d.w.).

Antioxidant activity (ABTS). The antioxidant activity was determined using the ABTS radical cation decoloring reaction (Mihalcea et al., 2021). To summarise, 0.20 mL of extracts diluted in a mixture of n-hexane and acetone, along with the supernatants obtained from extracting total carotenoids from PP powder, were combined with 1.98 mL of the ABTS+ solution. The mixture was then left to react for 2 hours in the absence of light. The mixture's absorbance (Af) was quantified at a wavelength of 734 nm (Analytik Jena - Specord 210 Plus, Germany).

The blank absorbance was measured at 734 nm using a 1.98 mL ABTS solution (in ethanol) and 0.20 mL ethanol in the absence of the extract (A0). The results were reported as µmol of Trolox equivalents per gram of dry weight (µmol TE/g d.w.).

Also, the inhibition percentage was calculated.

% Inhibition = (A0- Af)/A0 x 100.

Preparation and characterization of supplemented whey cheese.

The manufacture of whey cheese was carried out in accordance with industrial production technology, which involves the gradual heating of the whey until it reaches a temperature of 90°C. To improve the yield, we added 20 mL of citric acid to the whey at 70°C to lower its pH value to 5.53. Once the whey reached 85-90°C, we added 150 ml of DAIRSAL+, a pure product based on magnesium chloride and calcium chloride, in accordance with Reg. CE No. 231/212, and stopped the heat at the point of flocculation. The precipitate formed (whey cheese) is collected in baskets and left for 7 hours at a temperature of 18-20°C to cool and drain by self-pressing. After cooling, it was packed in plastic casseroles and stored in a refrigeration system at temperatures between 4-6°C. After cooling the whey cheese, we added powder to the PP-fortified product. Two experimental batches were made, batch CPP1, where an addition of 1% was used, and batch CPP2, where an addition of 2% was used. We kept the experimental groups under the same conditions as the control group. Marketers market whey cheese as fresh cheese and advise against storing it for more than 4-5 days.

For chemical analysis, about 100 g of cheese was taken from various parts of the cheese mass. We determined the water and dry matter content in accordance with the Association of Official Analytical Chemists (AOAC, 2005). The fat content was determined according to Van Gulik (IDF, 2008), protein contents were assessed using the Kjeldahl method (AOAC 2003), and ash was obtained by dry ashing at 550°C (AOAC 935.42).

The crude fibre was determined using the methodology outlined by Gavril (Rațu et al., 2024). The part that remained after undergoing digestion with standard sulfuric acid and sodium hydroxide was detected. Essentially, 2.0 g of the substance underwent hydrolysis in 299 mL of 1.25% sulfuric acid, followed by a 30-minute heating process. The mixture underwent vacuum filtration, followed by three rinses with hot distilled water. Subsequently, it was subjected to an additional 30-minute heating process using 200 mL of 1.25% sodium hydroxide. Finally, the combination was subjected to another round of vacuum filtration. After initial neutralisation with hydrochloric acid, the digested sample underwent three rinses using hot distilled water. The residual substance was transferred into a crucible, subjected to a drying process lasting 2 hours at a temperature of 100ºC within an oven, and subsequently chilled in a desiccator prior to being measured in terms of weight. The sample in the crucible was subjected to a temperature of 500ºC for a duration of 5 hours in order to fully eliminate all carbonaceous substances. Subsequently, the crucible holding ash was subjected to desiccation, followed by cooling and subsequent weighing.

% crude fiber = [loss in weight (g) after ignition)/(weight of the original sample (g)] × 100.

Colour analysis. The colour parameters of the samples were evaluated using a MINOLTA Chroma Metre model CR-410 (Konica Minolta, Osaka, Japan) equipped with a CIE Lab scale. The outcomes of the colour measurements were denoted as L*, representing the degree of lightness (where L* = 0 for black and L* = 100 for white), a*, encompassing a spectrum from red to green, and b*, encompassing a spectrum from yellow to blue. After calibrating the device using a white plate, the CIELAB colour parameters were measured three times.

The hue angle-colour appearance (Hue angle = 180 + arctan(b*/a*) for quadrant II (−a*,+b*); Hue angle = arctan (b*/a*) for quadrant I (+a*,+b*) and Chroma-colour intensity [$\sqrt{(a^*)^2 + (b^*)^2}$] were also calculated (Dag et al., 2017).

Sensorial analysis. A group of twenty individuals, ranging in age from 24 to 65, with an equal distribution of 60% women and 40% males, evaluated the sensory attributes of fortified cheese samples. The panel members were provided with information regarding the overarching objective of the study, as well as the requisite protocols for managing personal data. The panel members were instructed to examine a total of 9 descriptors, encompassing appearance, section appearance, odor, aroma, texture, color, taste, aftertaste, and the overall evaluation. The analysis was conducted in compliance with the requirements outlined in ISO 13299 (2016). Faccia et al. (2012) reported that the assessors used a seven-point hedonic scale (1 = extremely low; 7 = extremely high) to award a score for each quality.

Microbiological analyses. Every analytical step involved in counting the microbiological load of whey cheese was carried out in a sterile setting using three duplicates for every lab sample. Using a laboratory blender (Seward, West Sussex, UK) set to run at 250 rpm for five minutes, 10 g of cheese were homogenized with 90 mL of buffered peptone water (Bio-Rad, Marnes-la-Coquette, France) in preparation for microbiological tests. The serial dilutions were created by combining 1 mL of the prior dilution with 9 mL of buffered peptone water in test tubes. The spread and pour plate techniques separated the bacteria, yeast, and molds from the dilutions (Suler et al., 2021a; Najgebauer-Lejko et al., 2022). The non-selective Plate Count Agar (PCA) supplemented with 1 g/L skimmed milk powder and Potato Dextrose Agar (PDA; Scharlau, Barcelona, Spain) along with the selective chromogenic agar Rapid *E. coli* 2 (RE) and Rapid Staph (RS; Bio-Rad, Marnes-la-Coquette, France) were the microbiological media used for plating after inoculation with 1 mL of sample. In compliance with ISO 7218 (2016), total aerobic bacteria were counted following a 72-hour incubation period at 30°C on injected PCA plates. Following five days of incubation at 28ºC on PDA plates, the number of yeast and molds was counted. Following a 24-hour incubation period at 37°C, colonies of *Escherichia coli* and other coliforms were identified on RE media under ISO 16140 (2021). Additionally, all samples were grown on RS medium, which ensures that coagulase-positive staphylococci (*Staphylococcus aureus*) will be found and counted in 24 hours at 37°C.

Following incubation, the automated colony counter Scan 1200 (Interscience, Saint-Nom-la-Bretèche, France) was used to count the microorganisms in the cheese samples. The results were represented as logarithmic colony forming units per gram (log CFU g^{-1}) (Suler et al., 2021b).

Statistical Analysis. The data presented in this study consist of mean values, with a standard deviation of the mean. These values represent the means obtained from triplicate analyses. The Data Analysis Toolkit in Microsoft Excel program was utilized to do statistical data analysis. The quantification of significant differences between samples was conducted using a one-way analysis of variance (ANOVA), following the assessment of normality and equality of variances. A post hoc analysis using Tukey's test was conducted at a significance level of 5% ($p < 0.05$) using Minitab Inc., State College, PA, USA.

RESULTS AND DISCUSSIONS

Table 1 present the results of phytochemical, physicochemical contents and the ABTS radical scavenging activity of the PP extract.

Table 1. Phytochemical and physicochemical analysis of PP powder

Parameters	PP powder
Total carotenoids (mg/100 g d.w.)	31.02±0.15
β-caroten (mg/100 g d.w.)	28.04±0.11
Total flavonoids (mg CE/100 g d.w.)	42.11±0.28
Total polyphenols (mg GAE/100 g d.w.)	109.02± 0.55
ABTS (μmol TE/g d.w.)	1264.20±10.23
Inhibition (ABTS) %	77.53± 0.57
L*	74.89±0.12
a*	7.82±0.08
b*	41.58±0.06

Therefore, the PP extract had a notable carotenoids content of 31.02±0.15 mg/100 g d.w. and a remarkable antioxidant activity of 1264.20±10.23 μmol TE/g d.w., with an inhibition of 77.53 ± 0.57%. In their research, Hussain et al. (2021) examined the peel, pulp, and seeds of *C. maxima* pumpkins, focusing on the identification of diverse nutritional components that contribute to a range of health benefits. Therefore, the authors reported a total carotenoid value of 35.2 ± 0.49 mg/100 g powder, a total polyphenolic content of 134.59 ± 1.24 mg GAE/100 g powder, and a total flavonoid content of 77.11 ± 0.63 mg CE/100 g powder for the pumpkin pulp.

Pinna et al. (2023) reported a total carotenoid value of 1006.00 ± 78.72 μg β-carotene/g d.w. and an antioxidant activity of 1490.77 ± 69.74 μg TE/g d.w. (ABTS) for pomace of Delica vanity variety.

These variations could be caused by the origin material's phytochemical variability and the kind of solvent combination utilized during the extraction process.

The estimated values for L*, a*, and b* were determined to be 74.89, 7.82, and 41.58, respectively, based on the colour parameters. b* parameter that represents blue-to-yellow intensity, indicates a trend towards yellow shades in PP powder due to the high carotenoid content. Pinna et al. (2023) reported the CIELab param ± 1.15 for L*, 10.00 ± 1.00 for a*, and 27.33 ± 0.58 for b*. Based on the colour indices, it was determined that the PP powder was situated within quadrant I (+a*, +b*).

The phytochemical profile and antioxidant activity by the use of the ABTS method of the control and supplemented cheeses are presented in Table 2. Consistent with expectations, there is a notable rise in the levels of carotenoids and antioxidant activity as the concentration of PP powder increases. Thus, the cheese variants exhibited a total carotenoids content with values between 21.22 ± 0.611 mg/100 g d.w and 32.65 ± 0.86 mg/100 g d.w. Regarding the antioxidant activity, there has been an observed rise from 379.82 ± 3.45 μmol TE/g d.w. for CPP1 to 426.38 ± 3.86 μmol TE/g d.w. for CPP2. Extracts from the fruit of Sea buckthorn (*Hippophae rhamnoides* L.) were also tested as colourants for cream cheese (Ghendov-Mosanu et al., 2020).

Eters for pomace of the Delica vanity variety as follows: 29.33

Table 2. Phytochemical characterization of PP-incorporated cheeses

Parameters	Type of cheeses		
	CC	CPP1	CPP2
Total carotenoids (mg/100 g d.w.)	-	21.22±0.61[b]	32.65±0.86[a]
Total flavonoids (mg CE/100 g d.w.)	3.85±1.63[c]	11.02±1.99[b]	20.01±2.14[a]
Total polyphenols (mg GAE/100 g d.w.)	8.52±3.59[c]	24.63±1.96[b]	37.13±2.17[a]
ABTS (μmol TE/g d.w.)	162.29±3.22[c]	379.82±3.45[b]	426.38±3.86[a]
Inhibition (ABTS) %	11.59±0.49[c]	26.84±0.71[b]	39.11±0.67[a]

Means with the same letter in each row are not significantly different (p > 0.05).

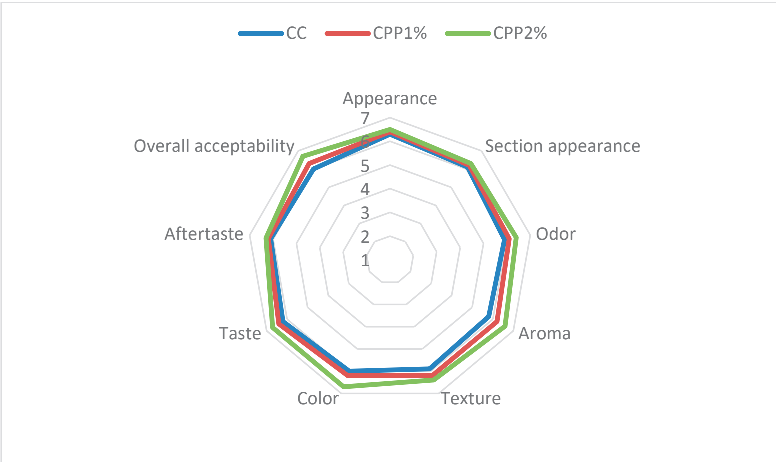

Figure 1. Sensory evaluation scores of controls (CC) and supplemented cheeses (CPP1, CPP2)

Table 3. Physicochemical properties of PP-incorporated cheeses

Parameters	Type of cheeses		
	CC	CPP1	CPP2
Moisture (%)	79.44±0.51[a]	77.62±0.16[b]	75.11±0.15[c]
Total solid (%)	20.56±0.50[c]	22.38±0.16[b]	24.89±0.19[a]
Fat (%)	4.09±0.10[b]	4.41±0.10[a]	4.52±0.11[a]
Protein (%)	9.97±0.10[c]	10.15±0.06[b]	10.97±0.07[a]
Ash (%)	0.98±0.03[c]	1.18±0.03[b]	2.15±0.08[a]
Crude fibre (%)	0.00±0.00[c]	2.42 ±0.11[b]	4.11±0.09[a]

Means with the same letter in each row are not significantly different ($p > 0.05$).

Table 4. Colour parameters of PP-incorporated cheeses

Parameters	Type of cheeses		
	CC	CPP1	CPP2
L*	93.94±0.34[a]	87.82±0.41[b]	86.26±0.35[c]
a*	-1.72±0.13[a]	0.88±0.03[b]	1.32±0.10[c]
b*	10.79±0.42[a]	15.71±0.48[b]	19.61±0.22[c]
Chroma	10.93±0.41[a]	15.73±0.48[b]	19.66±0.21[c]
Hue angle	178.59±0.02[a]	1.51±0.01[b]	1.49±0.01[c]

For each type of cheese, letters indicate a comparison across colour parameters; Means with the same letter in each row are not significantly different ($p > 0.05$).

Table 5. Microbial quality of PP-incorporated cheeses

Parameters	Type of cheeses		
	CC	CPP1	CPP2
TAB	7.51 ± 0.48[a]	7.92 ± 0.12[a]	8.11 ± 0.25[a]
Yeast	0.90 ± 0.11[a]	1.44 ± 0.41[a]	1.55 ± 0.32[a]
Molds	0.46 ± 0.09[a]	1.94 ± 0.23[a]	2.21 ± 0.30[a]
Coliforms	2.48 ± 0.24[a]	2.61 ± 0.17[a]	2.67 ± 0.26[a]
Escherichia coli	Not detected	Not detected	Not detected
Coagulase-positive staphylococci	Not detected	Not detected	Not detected

TAB - total aerobic bacteria count. Means with the same letter in each row are not significantly different ($p > 0.05$).

The primary measured pigments and polyphenols from the extracts of the fruits were carotenoids (8.27 mg/L total carotenoids) and polyphenols (1842.86 mg/100 g d.w., respectively. In terms of sensory evaluation, the addition of Sea buckthorn extract increased with 2.04% the average organoleptic score compared with tartrazine-supplemented cheeses.

The findings displayed in Table 2 support the enhanced nutritional value of cheeses incorporating pumpkin pomace powder, as evidenced by the observed increase in total carotenoids and antioxidant activity.

Chemical composition of control and supplemented cheeses (CPP1, CPP2). The results of chemical composition of analysed samples are presented in Table 4.

The chemical composition data of three types of whey cheese, namely plain (CC), and those enriched with 1% (CPP1) and 2% (CPP2) powder (PP), demonstrates notable nutritional improvements. It is worth mentioning that there is a clear correlation between the concentration of PP and the moisture content, indicating a higher density of the product. Additionally, the total solids, fat, protein, and ash contents gradually increase, suggesting an enhanced nutrient profile. CPP2 has the most elevated concentrations of total solids and protein, suggesting an enhanced nutritional density resulting from the incorporation of PP. In addition, the ash content, which serves as an indicator of mineral content, experiences a substantial increase, especially in CPP2, indicating a higher intake of minerals. Notably, the PP worts had a significantly greater crude fibre content. Specifically, CPP2 contains more than four times the fibre compared to CC, highlighting the potential for enhanced digestive health benefits. The addition of PP to the whey cheese enhances its nutritional qualities, indicating its potential for developing healthier and more nutritious goods.

In another study, the utilization of saffron (*Crocus sativus* L.) as a pigment for newly produced ovine cheese involved the incorporation of a concentrated extract (1000 mg/L) into 2 L of pasteurized ovine milk.

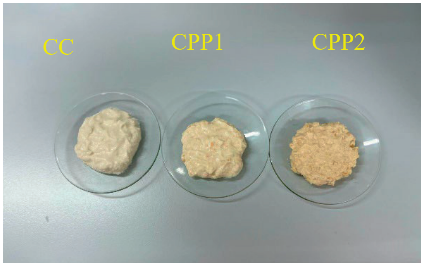

Figure 2. Personal images of the cheese without PP, control (CC); cheese with 1% PP (CPP1); cheese with 2% PP (CPP2)

The incorporation of saffron did not have any significant impact on the levels of moisture, total protein, salt, and lipids. However, these cheese samples exhibited the greatest antioxidant capacity values (up to 25.97% radical scavenging activity). The cheese samples containing the lowest amount of saffron (50 mg/L) exhibited comparable sensory scores to the control cheeses (Aktypis et al., 2018).

Colour evaluation of supplemented cheeses samples. Table 5 displays the CIELAB parameter values for the supplemented cheeses.

The enriched cheeses have a high level of yellowness, which is characteristic of carotenoid compounds, as indicated by the b* values.

Luminosity was influenced by the PP powder concentration, the L* values decrease with powder addition, while a* and b* values increased together with powder concentration. Similar results were found by Durmaz et al. (2020) in ice creams enriched with microalga powder as coloring agent.

The cheeses with higher PP powder concentrations also showed higher Chroma values, which measure the intensity of color. The results mentioned are linked to the visual characteristics of the sample and the detection of carotenoids. The hue angle was positioned in the first quadrant in the color solid for enriched cheeses, indicating the yellowness of all samples.

Microbial Quality. The summary of the microbiological examination for the prevailing samples (CC, CPP1, CPP2) is presented in Table 5, which indicates that the product can be considered safe for eating. Both *Escherichia coli* and coagulase-positive staphylococci (*Staphylococcus aureus*) were not detected in any of the samples. All cheese samples exhibited the presence of coliforms; however, their occurrence did not reach statistical significance when compared to the control sample. The 2% PP fortified cheese sample exhibited the highest viable microbial counts, including total aerobic bacteria, yeast, moulds, and total coliforms, in comparison to the control cheese sample. However, it is important to note that the recorded data falls within the permissible limits as stipulated by European Regulation 2073/2005.

Sensory evaluation of supplemented cheeses.

Consumers preferences for taste, aroma, colour, and overall quality were determined through sensory evaluations.

The sensory qualities and general acceptability of the cheeses, which were supplemented with varying concentrations of PP powder, were evaluated (Figure 2). The attributes evaluated were appearance, section appearance, color, aroma, texture, taste, odor, aftertaste, and overall acceptability. The findings of sensory evaluations are presented in Figure 1. The panellists provided positive evaluations for all evaluated products. The acceptability of the cheeses supplemented with PP powder was assessed based on their taste, aftertaste, and odor. The cheeses with pumpkin powder were evaluated as having an acceptable odor, aroma, and appearance. Furthermore, the cheeses were appreciated for their soft, fine, and crumbly texture. The addition of PP to cheese resulted in a visually appealing yellow hue, which can be attributed to the enrichment of pumpkin pigments.

The results of the sensory assessments point to the 2% PP powder-added cheese as having the highest "general acceptability" value. In a study by Ghendov-Mosanu et al. (2020) the authors prepared a cheese by combining sea buckthorn powder with sunflower oil. The sensory panel results of cheese manufactured with extract were shown to be superior to those of tetrazine-supplemented cheese, mostly attributed to the presence of chlorophylls, carotenoids, and total phenolic content in the extract.

CONCLUSIONS

The research emphasises the possibility of using pumpkin pomace as a sustainable and nutrient-dense food ingredient, which has the ability to enhance the health appeal of conventional dairy products such as whey cheese. This development addresses the increasing consumer need for functional foods that offer beneficial effects on health.

The incorporation of PP powder into the cheeses resulted in an enhancement of both the carotenoid content and antioxidant activity.

Cheese prepared with PP powder showed better results in the sensory panel due to the carotenoids, and total phenolic content of powder as compared to control sample. Sensorial analysis revealed that panelists appreciated the improved color of the enriched cheeses. The results indicated that the incorporation of carotenoids as an colorant in food products not only satisfies consumer preferences but also improves sensory characteristics.

This study presents novel opportunities for the use of by-products from food processing and for the production of innovative dairy products that are enhanced with nutritional value.

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EXPLORING THE POTENTIAL OF GRAPE POMACE POWDER AS A FUNCTIONAL INGREDIENT IN YOGURT

Florina STOICA[1], Roxana Nicoleta RAȚU[2], Irina Gabriela CARA[3], Denis ȚOPA[1], Gerard JITĂREANU[1]

[1]"Ion Ionescu de la Brad" Iasi University of Life Sciences, Faculty of Agriculture, Department of Pedotechnics, 3 Mihail Sadoveanu Alley, 700489, Iasi, Romania
[2]"Ion Ionescu de la Brad" University of Life Sciences Iasi, Faculty of Agriculture, Department of Food Technologies, 3 Mihail Sadoveanu Alley, 700489, Iasi, Romania
[3]"Ion Ionescu de la Brad" Iasi University of Life Sciences, Research Institute for Agriculture and Environment, 3 Mihail Sadoveanu Alley, 700490, Iasi, Romania

Corresponding author email: roxana.ratu@iuls.ro

Abstract

Consumer demand for functional yogurts has been rising recently, particularly for those made by adding plant-based ingredients or their bioactive components. Grape, Vitis vinifera L., is abundant in antioxidant-rich phenolic compounds and dietary fiber. Although grape pomace is an agro-industrial by-product and its handling can lead to socioeconomic and environmental issues, it can be possible to valorise by extracting its bioactive components. The goal of this study was to develop functional yogurt by adding 1 and 2% of grape pomace (YGP1 and YGP2). The impact of fortification was assessed on the enhanced yogurts' physicochemical properties, total phenolic content, antioxidant activity, and sensory acceptance. Grape pomace powder had high total polyphenols contents (20.39 ± 0.23 mg GAE/g d.w.) and antioxidant activity (88.16 ± 0.45%). Values of polyphenols (8.88± 0.06 mg GAE/g d.w.) and antioxidant activity (89.47 ± 0.39%) significantly increased in fortified yogurts compared to control sample. Therefore, it is possible to conclude that grape pomace powder can be used to produce novel, functional yogurt.

Key words*: antioxidant activity, food ingredients, functional yogurt, grape pomace, pigments.*

INTRODUCTION

Food and food selection for a healthy lifestyle have been the main concerns of human society since ancient times. Grapes (*Vitis vinifera* L.). are the most cultivated fruit worldwide, with an estimated production of more than 79 million tons in 2018 (Cardell et al., 2019). About 75% of grape production is transformed into wine production, resulting in residual by-products of 20–30% After grapes are destemming and pressed, a by-product known as grape pomace (GP) is produced (Cheng et al., 2010). The main by-products resulting from winemaking are pomace which is made up of the skin, pulp, seeds and bunches (Balbinoti et al., 2020). Because of the compositional features of GP, including its low pH and high sugar content, disposing of it is expensive and difficult. These traits present a significant environmental risk if left untreated (Ungureanu et al., 2010).

Some studies have been done on using GP for food applications because it is well-recognized that GP is a good source of fiber and antioxidants with considerable nutritional activity. For instance, grape skin flour (GSF) from GP has been used in tomato puree (Lavelli et al., 2014), baked goods (Walker et al., 2014),

while grape seed flour has been added to cereal bars, pancakes, and noodles (Rosales Soto et al., 2012) and bread (Hoye & Ross 2011). Compared to synthetic antioxidants, GP antioxidants, which comprise polyphenol components including anthocyanins, flavanols, catechins, and proanthocyanidins, can be considered fully safe (Rosales Soto et al., 2012). Due to their strong antioxidant activity, these compounds might provide disease-prevention and health-promoting benefits (Choi et al., 2010; Hogan et al., 2010). Because of this, such compounds are now being explored as novel components or food additives that can improve the nutritional value of various food products while also potentially offering a solution to the waste disposal issue (Peng et al., 2010). Anthocyanins are widely utilized natural food colorants that exhibit pH-dependent colour gradients. They find application in various popular food items, including beverages, desserts, ice cream, and dairy goods. Anthocyanins possesses several significant health features, including its anti-cancer activity, which has been observed to exhibit chemopreventive and chemoprotective effects in both *in vivo* and *in vitro* settings across various cancer cell lines. Additionally, anthocyanins has been found to possess antioxidant and anti-inflammatory properties (Khoo et al., 2017).

Due to the presence of active probiotic bacteria, yogurt is already regarded as a nutritious diet; yet, fiber and phenolic antioxidant components are absent from it (Karaaslan et al., 2011). The information that is currently available on the addition of GP to yogurt (Tseng & Zhao 2013) is favourable in terms of the viability of utilizing GP as a novel ingredient.

The objective of this study was to assess the impact of GP on the physicochemical characteristics, phytochemical composition, antioxidant capacity, colour, and sensory aspects of yoghurts fortified with GP. Furthermore, the study sought to ascertain if powders enhanced with phenolic compounds could serve as a sustainable food ingredient in the production of foods that are higher in nutrients.

MATERIALS AND METHODS

A winery (Iaşi University of Life Sciences) supplied the nonfermented GP of the Feteasca Neagra varietal. The Rediu Iași Research Station of the University of Life Sciences contributed 200 liters of cattle milk. The skins underwent mechanical separation, then were dried in an oven for 48 h at 40±2°C, reaching a moisture content of 9.80%. The GP were ground (0.5 mm) using a kitchen grinder. GP was sanitized in an autoclave for 15 minutes at 121 °C before being used to make yogurt.

DPPH (2,2-diphenyl1-picrylhydrazyl), gallic acid, sodium acetate, potassium chloride solution, ethanol, sodium carbonate, methanol, sodium carbonate, Folin-Ciocâlteu reagent, sodium hydroxide, aluminium chloride, were acquired from Sigma Aldrich (Schnelldorf, Germany).

The ultrasound-assisted extraction method was utilized to extract the bioactives from GP powder. Therefore, 1.0 g of GP was mixed with 10 mL of an 80% ethanol solution acidified with citric acid (ratio 7:1, v/v) and then treated with ultrasound for 25 minutes at 35°C and a frequency of 37 kHz. The resulting supernatant was collected and centrifuged for 10 minutes at 6500 rpm at 4°C. Thereafter, the GP extract was used for phytochemicals (anthocyanins, flavonoids, and polyphenols) and antioxidant activity analysis.

A modified pH differential method employed by Lipșa et al. (2024) was utilized to determine the total monomeric anthocyanin content. At 520 nm and 700 nm, the absorbance of diluted extracts using various buffer solutions at pH 1.0 and 4.5 was determined. The findings were exhibited as mg cyanidin-3-glucoside (C3G)/g of dry weight (dw).

The content of total flavonoids in GP extract was measured by the technique described by Dewanto et al. (2002). Shortly, 0.25 mL of the GP extract and 0.075 mL of 5% sodium nitrite were combined with 2 mL of distilled water. The mixture is left to act for 5 minutes, after which 0.15 mL of 10% aluminium chloride is added and left to act again for 6 minutes, then 0.5 mL of 1M sodium hydroxide is added. The absorbance of the resulting mixture was read at a wavelength of 510 nm. The total flavonoid content is expressed as mg catechin equivalents/g sample (mg CE/g d.w.).

The Folin-Ciocâlteu spectrophotometric method described by Dewanto et al. (2002) was used to measure the total polyphenolic content. In a test tube, 200 μL of the GP extract was added, followed by 1 mL of the Folin-Ciocâlteu reagent, and 15.8 mL of distilled water. They were left to stand for 10 minutes, after which 3 mL of 20% sodium carbonate was added and left to stand for one hour at room temperature in the dark. The absorbance was read at a wavelength of 765 nm. The results obtained were expressed as mg gallic acid equivalents/g sample (mg GAE/g d.w.).

The method for determining the antioxidant activity by neutralizing the DPPH radical was carried out according to the protocol described by Castro-Vargas et al. (2010). DPPH stock solution was obtained by mixing 3.8 mg of DPPH with methanol, in a 100 mL volumetric flask. Afterward, 100 µL of the sample to be analyzed (GP extract) was added to a test tube, along with 3.9 mL of DPPH (A sample). For the control sample, 100 µL of methanol was used, and 3.9 mL of DPPH (A control). The absorbance was read after 90 minutes of rest in the dark at a wavelength of 515 nm. The results were represented as µmol of Trolox equivalents (TE)/g d.w. The variation of the antioxidant capacity was studied by determining the inhibition (I%) for each sample to be analyzed, using the following equation: I% = (A control-A sample)/A control x 100.

Collection, sampling, and analysis of unpasteurized milk. 200 litres of milk were removed in clean containers from the farm's storage tank. It was kept in a 4°C for 25 hours. After that, milk was thoroughly homogenized and added to the analytical tests in the laboratory. The AOAC procedures were utilized to determine the physicochemical parameters of milk samples, which included moisture content, total solid, pH, solid non-fat content, protein content, and fat content (Rațu et al., 2024).

Preparation of yogurt enhanced with GP. Full-fat milk was utilized in the technological process; it was not normalized milk. After 30 minutes of pasteurization at 60°C, the milk was chilled to 36°C and then specific lactic bacteria were introduced. Starter culture YF-L812 (commercial product, Chr. HANSEN, Denmark) comprising a blend of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (2:1) was added to milk when the temperature reached 42°C.

The inoculated milk was fermented for around 6.5 hours at 42°C, or until a final pH of 4.8 was reached. At this stage, yogurt and sterile GP powder were combined at a concentration of 60 g/kg and then divided into pots. Analyses were carried out on the samples as soon as they were produced and stored at 4°C. There were two distinct yogurt products made. Each production batch of yogurt was split into three batches: one batch (control, YC) had no GP, while the other two batches included enhanced yogurt (YGP1-1% GP, YGP2-2% GP).

The samples' pH, fat, ash, moisture content, and total protein were measured using the techniques recommended by the Association of Official Analytical Chemists (AOAC) (Usturoi et al., 2017; Tseng & Zhao, 2013).

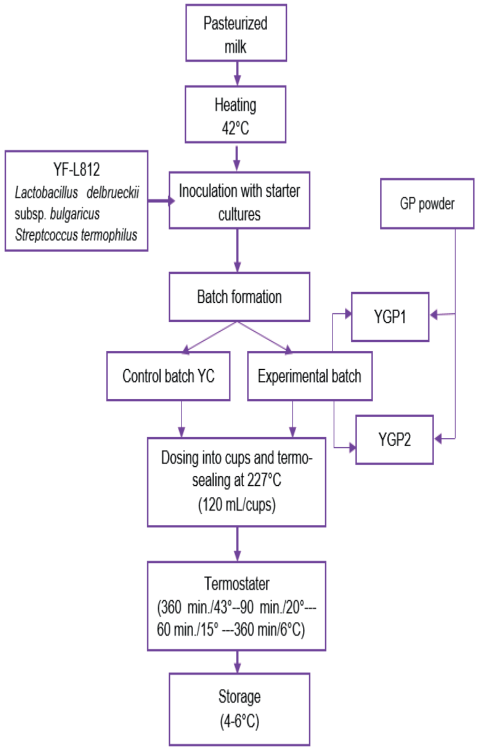

Figure 1. Flow diagram illustrating the processing steps of a functional yoghurt prototype

Colour analysis. The colour of the control and value-added yogurt were examined using the portable colorimeter with illuminator C-MINOLTA ChromaMeter CR-410 (Konica Minolta, Osaka, Japan). The color parameters determined were L*, a* and b*. Three different replicates of each sample were available.

Sensorial analysis. A panel of 16 different panellists conducted the sensory assessment of yogurt samples. A hedonic scale from 1-9 was used to evaluate the sensory attributes, where 1 is the weakest/unpleasant and 9 the strongest/very pleasant. Appearance, colour, aroma, texture, taste, odour, aftertaste, and overall acceptability are the evaluated attributes. Panel members are non-smokers aged 24-40 years and they have studies in the field of the food industry. The samples were arranged in a random configuration. The participants were provided with details regarding the study's overarching objective and the procedures for handling personal data.

Statistical Analysis. Statistical analysis of the data was performed using the data analysis tools package of Microsoft Excel software and using the statistical processing software Minitab 19. Standard deviations were calculated from triplicate experiments.

RESULTS AND DISCUSSIONS

The polyphenolic, flavonoid, and anthocyanin contents in ethanolic extracts from GP extract were measured using spectrophotometric methods (table 1). The total polyphenolic content was 20.39±0.23 mg GAE/g d.w., while the total flavonoid content was 8.28±0.78 mg CE/g d.w. Additionally, a good concentration of total anthocyanin content was observed, measuring 1.56±0.22 mg C3G/g d.w. and also a remarkable antioxidant activity of 24.15±0.12 µmol TE/g d.w. Serea et al. (2021) extracted bioactive compounds from red grapes peel from the Băbească neagră variety using ethanol 96% acidified with 0.1 N HCl on ultrasound assisted extraction and obtained higher anthocyanin values (4.29±0.04 mg C3G/g) similar values for phenolic compounds of 22.28 ± 2.96 mg GAE /g and a smaller antioxidant activity (14.07±1.03 mM Trolox/g). Rockenbach et al., (2011), reported total anthocyanin content values of 1.84-11.22 mg G3G/g d.w, in the extracts of four red grape pomace samples (Bordeaux, Cabernet Sauvignon, Isabel, and Merlot varieties) grown in Brazil. The authors determined also the degree of inhibition of the DPPH free radical (using the ß-carotene/linoleic acid method) of 41.13%, for the extracts obtained from grapes of the Bordeaux variety. Novak et al. (2008) reported a total flavonoid yield of 4.99 ± 0.07 mg CE/g d.w., using ethanol acidified with 1% hydrochloric acid, from freeze-dried grape skin of Alfrocheiro (*Vitis vinifera* L.). González-Centeno et al. (2014) stated that ultrasound treatment for 30 minutes led to the extraction of polyphenols with yields of 5.37 - 31.87 mg GAE /100 g fresh grapes.

These variations could be caused by the origin material's phytochemical variability and the kind of solvent combination utilized during the extraction process.

Table 1. Phytochemical characterizations and colorimetric parameters of the GP powder

Parameters	GP powder
Total anthocyanin content (mg C3G/g d.w.)	1.56±0.22
Total flavonoid content (mg CE/g d.w.)	8.28±0.78
Total polyphenol content (mg GAE/g d.w.)	20.39± 0.23
Antioxidant activity (DPPH, µmol TE/g d.w.)	24.15±0.12
Inhibition %	88.16± 0.45
L*	23.36±0.16
a*	10.37±0.05
b*	3.32±0.02

Based on the colour indices, the GP powder was found in quadrant I (+a*, +b*).

Based on colour characteristics, values for L*, a*, and b* parameters were estimated at 23.36, 10.37, and 3.32. Regarding the parameter a*, which characterizes the tendency towards red, it was seen that GP powder exhibited a high a* value attributed to its elevated concentration of anthocyanins.

The primary chemical quality indices were identified to establish the raw material milk's quality parameters. Table 2 presents the chemical composition values of samples of cow's milk.

The water content of the raw milk was 87.07±0.05% and that of total solids was 12.93±0.07%. Milk's solid components, primarily fat and protein, contribute to its significant economic and nutritional value. The mean fat content was 3.99±0.03%, resulting in a mean solid-non fat content of 8.94±0.06%.

Table 2. Chemical contents values of raw milk

Parameters	Mean
Water (%)	87.07±0.05
Fat (%)	3.99±0.03
Protein (%)	3.37±0.03
Total Solids (%)	12.93±0.07
Solid-non fat (%)	8.94 ±0.06
pH	6.57±0.01

The average value of the protein level was 3.37±0.03%. The findings suggest that all indicators of raw milk quality meet the criteria for assessing the overall quality of the milk.

Phytochemical profile and the DPPH free radical scavenging capacity of control and supplemented yogurt are displayed in Table 3.

The products obtained were analysed to determine their overall phytochemical composition. The inclusion of GP powder in yoghurt products resulted in a dose-dependent increase in phytochemicals (such as anthocyanins, flavonoids, and polyphenols) as well as enhanced free radical scavenging capabilities, as compared to the control group. Thus, the GP-supplemented yogurt variants presented an anthocyanin content with values between 10.22±0.88 mg C3G/100 g d.w. (YGP1) and 21.09±0.97 mg C3G/100 g d.w. (YGP2). As for the antioxidant capacity, it has increased from 24.35±1.17 µmol TE/g d.w. to 35.53±1.19 µmol TE/g d.w. Therefore, the GP powder addition in the composition of the yogurts led to elevated levels of total polyphenolic compounds, total flavonoids, total anthocyanins content, and higher DPPH free radical scavenging capacity. Also, the bioactive components derived from GP had a favorable impact on the antioxidant activity of the enhanced yogurts, resulting in higher values compared to the control samples. By raising the amount of total anthocyanins and antioxidant activity, respectively, in yogurt samples manufactured with GP powder, the results shown in Table 3 validate their additional value. Our results comply with Marchiani et al. (2016) who added grape pomace of Chardonnay, Moscato and Pinot noir types as natural sources of polyphenolic compounds in yogurt formulation products. The yogurt composition with grape skin flour exhibited a significant increase in total polyphenolic contents (+55%) and antioxidant capacity (+80%) compared to the reference sample.

Table 3. The content of phytochemical compounds and the antioxidant activity of added-value yogurt samples

Parameters	**Type of yogurts**		
	YC	YGP1	YGP2
Total anthocyanin content (mg C3G /100 g d.w.)	-	10.22±0.88^{b}	21.09±0.97^{a}
Total flavonoid content (mg CE/g d.w.)	1.28±0.20^{c}	3.15±1.03^{b}	6.23±1.04^{a}
Total polyphenol content (mg GAE/g d.w.)	2.58±0.29^{c}	4.98±0.20^{b}	8.88± 0.16^{a}
Antioxidant activity (µmol TE/g d.w.)	13.45±1.33^{c}	24.35±1.17^{b}	35.53±1.19^{a}

The presence of distinct letters within rows denotes statistically significant variations between the samples ($p < 0.05$).

Chemical composition of control and value-added yogurts (YGP1, YGP2). The results of chemical composition of analysed samples are presented in Table 4.

The analysis of the obtained data indicates differences between the yogurts with de addition of powder obtained from grape pomace for protein and total solid ($p < 0.05$).

The results presented in Table 4 showed that the added-value yogurts with GP powder are characterized by a significantly higher protein content than the control one ($p<0.05$). In contrast, the fats contents of the value-added yogurts are similar to the control. In another study yogurt containing grape skin flour presented significantly lower pH and fat content (−20%) than the control (Marchiani et al., 2016). With the increasing concentration of GP powder, the ash content showed a small increase from 0.86% in the case of the YGP1 sample with an addition of 1% GP to 0.96% in the case of the YGP2 sample with an addition of 2% GP.

Colour evaluation of value-added yogurts samples. Colour is an important sensory characteristic that influences consumer acceptability of foods (Spence, 2015). The yogurts were also examined using CIELAB colorimetric parameters. The results of measurements of colour parameters L*, a*, and b* of the control and GP-supplemented yogurts were presented in table 5. The bioactive chemicals present in the GP powder have a tendency to turn red, as evidenced by the significant rise in the a* value with increasing concentration of the GP powder ($p<0.05$). Additionally, according to the L* and b* values the added-value yogurts show low levels of lightness and yellowness, when the GP powder concentration increases.

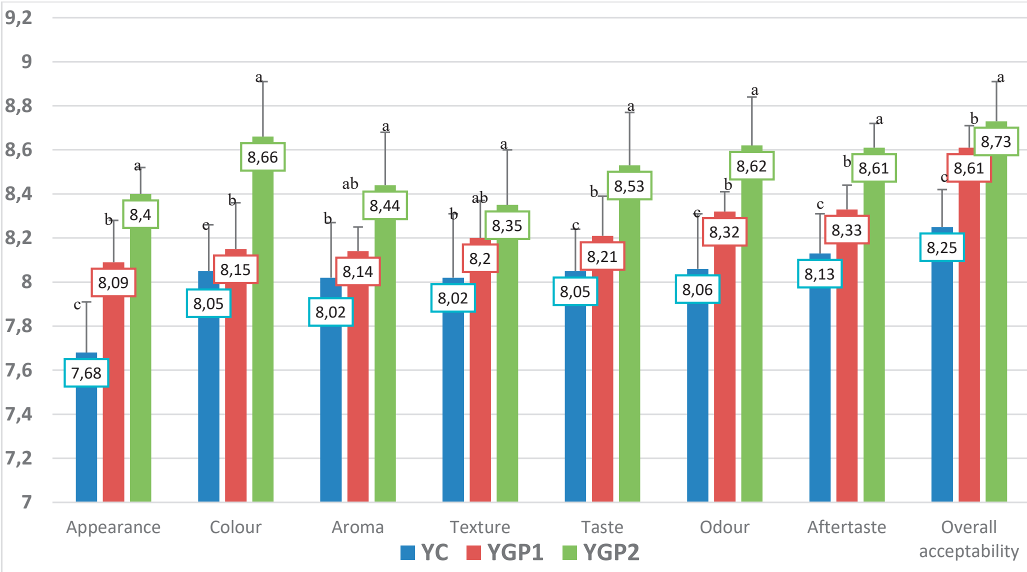

Figure 2. Sensory evaluation values of control and added-value yogurts (YGP1, YGP2) (the presence of distinct letters "a", "b", and "c" in the columns show statistically significant differences ($p < 0.05$))

Table 4. Chemical composition of added-value yogurts samples

Parameters	**Type of yogurts**		
	YC	YGP1	YGP2
Moisture (%)	86.42±0.19^{a}	83.22±0.17^{b}	80.19±0.15^{c}
Total solid (%)	13.58±0.12^{c}	16.78±0.16^{b}	19.81±0.19^{a}
Fat (%)	3.73±0.03^{a}	4.01±0.02^{a}	4.02±0.01^{a}
Protein (%)	3.66±0.08^{a}	4.13±0.05^{ab}	4.21±0.07^{b}
Ash (%)	0.74±0.03^{b}	0.96±0.12^{a}	1.09±0.11^{a}
pH	4.65±0.10^{a}	4.54±0.04^{b}	4.46±0.03^{b}

The presence of distinct letters within rows denotes statistically significant variations between the samples ($p < 0.05$).

Table 5. Colour data of YC, YGP1 and YGP1 yogurts

Parameters	**Type of yogurts**		
	YC	YGP1	YGP2
L*	93.54±0.22^{a}	88.35±0.51^{b}	84.59±0.66^{c}
a*	-2.35±0.13^{a}	4.45±0.33^{b}	8.63±0.54^{c}
b*	16.66±0.14^{a}	9.06±0.14^{b}	5.11±0.24^{c}

Averages with distinct letters indicate a comparison across colour parameters; the use of distinct letters in each row signifies significantly different final results ($p< 0.05$).

L* and b* parameters decreased together with powder concentration, but a* increased in the tested yogurt models. A similar trend was obtained by da Silva et al. (2019) who added blackberry anthocyanin-rich extract to a food system.

The sensory evaluation of the analysed yogurts was conducted using a 9-point hedonic scale. The attributes followed were appearance, colour, aroma, texture, taste, odour, aftertaste, and overall acceptability. The average results acquired from the sensory analysis are presented in Figure 2. As the GP powder concentration increased in the obtained yogurts, the red colour intensified, due to the presence of pigments in the GP powder especially anthocyanins (Figure 3). The YGP2 sample with 2% GP was acceptable and exhibited the highest scores for all nine attributes. The inclusion of GP in yogurts samples increased the red colour, so the panellists noted that the yogurts varied in colour, with YGP2 being the most reddish.

Figure 3. Pictures of the yogurt without GP, control (YC); yogurt with 1% GP (YGP1); yogurt with 2% GP (YGP2) (Own source)

YGP2 yoghurt samples yielded the highest ratings for all eight attributes at 2% GP. The GP- supplemented yogurts samples were praised for having an agreeable aroma, odour, and colour. The yogurts with added GP powder were assessed as having a balanced taste, aroma, and aftertaste. Regarding the general acceptability, it was shown that the addition of GP powder does not substantially influence the basic sensory characteristics (colour, taste, aroma). All of the proposed yogurts were positively evaluated by the panellists. The utilization of jabuticaba (*Myrciaria jaboticaba* (Vell) O. Berg) peel powder as colorant in yogurts was investigated by Freitas-Sá et al., (2018). In the sensory evaluation, yoghurts incorporated with *Jabuticaba* had the highest results in terms of appearance (6.6-6.8), flavour (6.9), and overall liking (6.8) when compared to the other flavoured yoghurts.

The obtained samples have an attractive colour with red shades, in correlation with the added GP powder concentration. Sensory analysis showed that yogurts containing GP have acceptable quality characteristics and may be well-accepted by consumers.

CONCLUSIONS

The global characterization of the grape by-products extract proved that GP extract exhibited higher anthocyanins content with good antioxidant activity. Our results underscore the significance of GP powder as an abundant reservoir of bioactive constituents exhibiting antioxidant properties. Consequently, we propose its integration as a natural constituent in fortified yoghurt.

The GP-supplemented yogurts showed higher levels of total phenolic contents and antioxidant potential compared to the plain yogurt. Sensory study revealed that panellists were pleased with the yogurts samples' increased colour. Overall, the value-added yogurts was found to be acceptable. This trend presents novel prospects for the dairy industry to address the increasing consumer desire for functional foods by developing original, nutritious, and palatable yogurt products. The obtained results certify the quality of natural ingredients with bioactive potential of powders obtained from the skin and seeds of grape berries of the Feteasca neagra variety, for use in the food industry to obtain functional products, promoting the principles of the circular economy.

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A REVIEW ON BIOGENIC AMINES IN CHEESE

Ioana Roxana ȘOIMUȘAN, Oana Andreea MASTAN, Ioan LADOȘI, Adina Lia LONGODOR, Aurelia COROIAN

University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, 3-5 Mănăștur Street, 400372, Cluj-Napoca, Romania

Corresponding author email: aurelia.coroian@usamvcluj.ro

Abstract

Biogenic amines fulfill different roles in the proper functioning of the human body. They result from oxidation processes in food. Biogenic amines are mainly found in fermented products such as wine, fish, meat and cheeses. Their existence in small quantities does not affect people's health, but in a larger volume leads to poisoning. The most common biogenic amine poisonings were found in tyramine and histamine. The main symptoms of poisoning are increased blood pressure and headaches. The concentration of biogenic amines in cheeses is influenced by several factors: temperature, baking time, pH, the culture used, environmental conditions, the origin of the raw material (raw or pasteurized milk and microbiological load. Compliance with hygienic conditions and the correct manufacturing process does not increase the amount of biogenic amines.

Key words: *cheeses, control, health, methods, storage.*

INTRODUCTION

Biogenic amines have roles in blood pressure, synaptic transmission, and cell growth. Foods containing biogenic amines are broken down by the human body via monoamine oxidase and diamine oxidase (Broadley et al., 2010). This type of process can be different for each person, psychological factors, genetic factors, foods rich in biogenic amines or products that do not have beneficial effects on human health (drugs, tobacco and alcohol) called monoamine oxidase inhibitors (EFSA, 2011).

Biogenic amines can appear in fermented products at different stages, such as the hygiene of raw materials, the manufacturing process, the state of fermentation and the time of fermentation. Biogenic amines in food do not disturb human health if they are consumed in low quantities, but their existence in large amounts can cause health problems and poisoning (Doeun et al., 2017). Biogenic amines are formed as a result of more steps: microbial decarboxylation of amino acids with the help of microorganisms, reductive amination, and the last step is transamination of ketones and aldehydes (Wójcik et al., 2024). The main factor with impacts to composition of biogenic amines is the decarboxylase capacity of amino acids, followed by the pH level, free amino acids, water activity, temperature and the proportion of the microbial population. The presence of biogenic amines from cheeses is due to non-starter lactic bacteria *(Enterococcus, Lactobacillus, Lactococcus, Leuconostoc* and *Streptococcus*) and some Gram-negative bacteria (*Pseudomonadaceae* and *Enterobacteriaceae*) (Andic et al., 2010). The most common intoxications were found in histamine and tyramine, the presence of other biogenic amines (putrescine, cadaverine or phenylethylamine) can accentuate the negative effect of histamine (Omer et al., 2021). Therefore, every stage should be done correctly, and in hygienic conditions to reduce any risks that can affect the health of the consumers (Ruiz-Capillas & Herrero, 2019). The contamination process can appear in different stages, such as production, distribution, and consumption. The methods of prevention of biogenic amines can be: using high quality raw material, controlling the temperature, and using good manufacturing practices (Dapkevicius et al., 2000). Biogenic amines can influence food quality, with subsequent impact on people's health, that's why it's important to accord the necessary importance.

MATERIALS AND METHODS

To present a database for this study we conducted a systematic search of scientific publications in the Google Scholar, ScienceDirect PubMed and Scopus databases. In the tables below, the concentrations of the main biogenic amines (histamine, tyramine, putrescine, cadaverine, phenylethylamine) in milk from different animal species (cow, sheep and goat) and various types of cheese are highlighted.

RESULTS AND DISCUSSIONS

Tyramine

Cheese incorporates a large amount of free amino acids as a result of proteolysis, a favorable temperature, pH and water activity, it is an environment that contains the necessary characteristics for optimum biogenic amines development (Benkerroum et al., 2016). In the case of histamine, there are several cases of poisoning, the most frequent being in Ceddar cheese and Swiss cheese (Vale & Gloria, 1998). According to Linares et al. (2013) tyramine poisoning leads to a hypertensive crisis characterized by an increase in blood pressure of ≥180/120 mmHg and severe headaches. 150-750 mg/kg is the allowed concentration for tyramine. Levels greater than 1080 mg/kg are harmful to human health (Shalaby, 1996). EFSA (2011) recommends that the maximum level of tyramine should be between 110-750 mg/kg.
Mohamed et al. (2010) analyzed which are the levels of biogenic amines from cheeses (Cheddar, Ras and Gouda). The results indicated that tyramine recorded the highest tyramine content (93.33%), followed by cadaverine (80%).

Histamine

The main food with the most histamine poisoning is fish, followed by cheese. Cheddar and Swiss cheese contain high amounts of histamine and are responsible for producing more outbreaks (Stratton et al., 1991). Lehane & Olley (2000) state that for sensitive people, an amount of 5-10 mg of ingested histamine is dangerous, a limit of 10 mg of histamine is stated to be tolerated by the human body, a concentration of 100 mg presents a degree of toxicity average, and the 1000 mg level is considered toxic. EFSA (2011) recommends that the maximum level of histamine be between 50-100 mg/kg, 20-28 mg/kg for phenylethylamine, 250-950 mg/kg for the total concentration of biogenic amines allowed for consumption.
Among the three types of analyzed cheeses, the highest concentration of biogenic amines was found in Ras cheese histamine (86.67%), followed by Cheddar cheese (73.33%), and Gouda (53.33%). The mean value of histamine was 12.98 for Cheddar cheese, 17.43 for Ras cheese and 7.65 for Gouda cheese (Mohamed et al., 2010).

Table 1. Biogenic amines from cheeses (mg/kg)

	Histamine	Tyramine	Putrescine	Cadaverine	Reference
Blue cheese	20.3-62.5	63.1-9.9	20.7-61.5	8.30-11.8	EFSA (2011)
Blue pasteurized milk cheese	0-127.02	0-526.63	0-237.56	40-89.4	Fernandez et al. (2007)
Blue raw milk cheese	0-1041.81	0-1051.98	0-875.8	0-756.78	Fernandez et al. (2007)
Brie	30.9-40.0	-	-	-	Ladero et al. (2009)
Camembert	0-40.0	0-4.9	0-82.2	0-266.8	Ladero et al. (2009)
Cheddar	25.4-40.0	-	48	-	Valsamaki et al. (2000)
Curd	1.0	2.0	0.9	0.7	Novella-Rodriguez et al. (2002)
Emmental	23.5-117.5	52.5-64.5	3.9-38.0	98.3	Ladero et al, 2009; Mayer et al. (2010)
Feta cheese	84.6	246	193	82.8	Valsamaki et al. (2000)
Fresh cheese	3.1-37.5	12.5-48.0	5.3-40	10.5-44.7	EFSA (2011)
Gorgonzola	23.7-255.3	13.2-247.6	3.2-31.3	33.7-748.2	Mayer et al. (2010)
Mozzarella	39.7	-	-	-	Ladero et al. (2009)
Parmesan	10.9-40.0	6.4-29.9	1.8-75.9	3.2-15.6	Innocente, N. & D'Agostin (2002)
Pasteurized goat's milk cheese	6.2	10.7	14.4	32.5	Novella-Rodriguez et al. (2004)

Table 2. Biogenic amine composition from different species (mg/kg)

Product type	Type of milk	Cadaverine	Histamine	Phenylethylamine	Putrescine	Reference
Raw milk	cow	-	-	-	-	Novella-Rodriguez et al. (2000)
Cheeses (raw milk)	sheep	123.0	50	-	107.6	Mercogliano et al. (2010)
	sheep	26.7	-	28.5	394.0	Schirone et al. (2011)
	goat	349.6	15.5	9.2	217.7	Pinho et al. (2004)
	goat	88.6	88.3	11.6	191.7	Novella-Rodriguez et al. (2002)
	goat	93.5	116	159	940	Galgano et al. (2001)
	cow	-	-	-	-	Fernandez et al. (2007b)
Cheese (raw milk) Parmigiano	cow	3.2	10.9	-	1.8	Mayer et al. (2010)
	cow	15.4	28.4	9.3	75.7	Innocente & D'Agostin (2002)
Extra hard grana	cow	-	248	-	-	Mayer et al. (2010)
Feta	goat	82.7	84.5	4.8	192	Valsamaki et al. (2000)
Emmental hard	cow	98.2	23.2	-	37	Mayer et al., (2010)
Cheddar	cow	-	25.3	-	4.7	Mayer et al. (2010)
Semi hard	cow	-	-	-	-	Mayer et al. (2010)
Gouda	cow	-	-	-	-	Mayer et al. (2010)
Edam	cow	-	3.1	-	-	Mayer et al. (2010)
Gorgonzola	cow	-	23.6	-	-	Mayer et al. (2010)
Gorgonzola	cow	33.6	255.2	-	3.1	Mayer et al. (2010)

Microorganisms that produce biogenic amines

A greater possibility of the appearance of biogenic amines is found in fermented foods. Biogenic amines can appear from lactic acid bacteria type even if they don't represent a potential for toxicity commonly. Big quantities of biogenic amines were found in streptococci, lactococci, pediococci and enterococci (Özogul & Hamed, 2018). Following genetic studies, it was found that many of these strains harbor genes or operons that encode decarboxylating enzymes or other pathways involved in the biosynthesis of biogenic amines (Marcobal et al., 2012; Wunderlichová et al., 2014). Next, the main non-starter lactic acid species associated with fermented products and involved in the production of biogenic amines are described.

Enterococci

They have impressive ecological adaptability and an ability to grow even under adverse conditions. The enterococci are characterized by their resistance to salt and low pH, the reason why they represent an ideal substrate for the fermentation of traditional cheeses (Foulquié et al., 2006). Analyses made by different sources from fermented products, such as cheeses, fish, meat, human feces and wine showed that the presence of enterococci from these is correlated with the production of biogenic amines (the most representative biogenic amine was tyramine) (Bover et al., 2001; Ladero et al., 2012). The enterococci were also found in artisanal cheeses due to their role in natural microbiota, as well as demonstrated that they can be present in bigger quantities than lactobacilli and lactococci (Suzzi et al., 2000). Starter culture species collected from cheeses represent a high risk of contamination and may co-exist with biogenic amines even though enterococci are generally not found in starter cultures. In dairy products, for example in the milk were detected many strains of *Enterococcus faecalis, E. durans* and *E. faecium* (Giraffa, 2003). Some species of this strain in cheeses have been shown to generate tyramine production (Sarantinopoulos et al., 2001). Rea et al. (2004) analyzed how the production of biogenic amines (tyramine) in Cheddar cheese was affected by the manufacturing and ripening processes of different strains of *E. casseliflavus, E. durans, E. faecium* and *E. faecalis*. The results demonstrated that, after 9 months of ripening, *E. durans* produced the highest concentration of tyramine, and *E. casseliflavus* is the only strain that does not produce tyramine compared to all strains analysed.

Lactobacilli

Lactobacilli are found in various fermented products and are an important category of biogenic amine producers (Spano et al., 2010). *Lactobacillus* species such as *Lactobacillus* *helveticus, Lactobacillus curvatus* or *Lactobacillus buchneri* as well as Gram-negative bacteria have the ability to produce biogenic amines in cheeses (Ladero et al., 2008). At the same time, the presence of histamine was detected in several strains of *L. parabuchneri* isolated from cheeses (Wüthrich et al., 2017). Pachlová et al. (2018) analysed the effect that have *L. curvatus* subsp. *curvatus* and *L. paracasei* on cheeses with these types of strains. Following the study, it was proven that these strains can accumulate tyramine up to 190 mg/kg in dairy products over a maturation period of 90 days.

Streptococci

Although several species of streptococci are important for human health, some of them cause diseases such as endocarditis, bacterial pneumonia, meningitis. In fermented products, such as cheeses, one of the most used streptococci as an initial culture is *S. thermophilus* due to its valuable components, due to the fact that it is present in most stages of their manufacture, due to its baking relationship together with biogenic amines (Delorme, 2008). The presence of tyramine was found in *Streptococcus macedonicus* species from Greek Kasseri cheese (Georgalaki et al., 2000).

Lactococci

The most important lactic bacteria involved in the milk industry, as well as in the manufacture of fermented milk products, are lactococci and lactic species: *Lactococcus* subsp. *lactis, Lc. lactis* subsp. *cremoris, Lc. lactis* subsp. *lactis* biovar *diacetylactis* (Fox et al., 2004). The main putreiscin producing species in dairy products are *E. hirae, E. faecalis* and *Lc. lactis* subsp. *cremoris, Lc. lactis* subsp. *lactis, L. curvatus* and *L. brevis* represents another category of species that can produce putreiscine from agmatine (Ladero et al., 2011; Ladero et al., 2012).

It has been shown that in addition to rotting *Lc. lactis* is capable of producing other types of biogenic amines (2-phenylethylamine and tyramine) which was found in strains of bacteriocinogenic lactococci isolated from raw goat milk (Coton et al., 2011). Two types of strains (*Lc. lactis* subsp *strains*) were detected during the ripening process (90 days) in an assortment of Dutch cheese (Flasarová et al., 2016). Lactococci are a key element for the development of biogenic amines, as the results also demonstrated: the levels of biogenic amines in the control group were very low compared to the control group, where the amounts of biogenic amines obtained were between 500 mg/kg tyramine and 800 mg/kg putrescine (Santos et al., 2003).

The presence of biogeneous amines depending on the period of storage

10 types of commercial Kashar cheese were randomly analyzed, 5 of them were matured and the other 5 were fresh. The values of the main biogenic amines in cheeses (histamine, cadaverine, tyramine, phenylethylamine, etc.), pH and moisture content were evaluated. The experiment lasted 3 weeks, the cheeses were kept at a temperature of 4 ± 1°C. At the end of the study, it was found that matured Kashar cheese has a higher content of biogenic amines compared to fresh Kashar cheese. Regarding the degree of toxicity, toxic levels of histamine were detected in matured cheeses, compared to fresh cheeses that contained no risk of toxicity (Şahin et al., 2019).

The production of biogenic amines was monitored in blue cheese at the beginning of ripening process (12 days) in the following order: γ-irradiated (6, 4 and 2 kGy) and non-irradiated during storage at 5°C. Following irradiation treatment, the cheese's dry weight resulted and biogenic amine content descended from 977 to 430 mg/kg after the irradiation process at 6 kGy, but increased from 1022 to 2311 mg/kg at 90 days in non-irradiated cheeses.

Tyramine is a powerful biogenic amine due to its ability to withstand radiation, it was found that 95% of all non-irradiated cheeses were made up of tyramine. Histamine decreased from 35 mg/kg unirradiated cheese (90 days of processing) to 29 mg/kg after irradiation, then was not detected at all. This procedure is useful for the safety process and detect biogenic amine values in cheeses (Rabie et al., 2011).

The degree of evolution of amino acids and biogenic amines over 4 months of ripening in Feta cheese was investigated. At 60 days of storage, the value of biogenic amines was 330 mg/kg, followed by increasing values to 617 mg/kg at 120 days. During the ripening period, the lowest levels of biogenic amines were identified in phenylethylamine and tryptamine compared to the other predominant amine types, tyramine and putrescine, which recorded values of 69.7% at 60 days and 71.2% at 120 days of storage. During the ripening period, the content of biogenic amines reached 615 mg/kg at 120 days and from 1 to 15 days and from 60 to 120 days the maximum levels were reached. Feta cheese has a high salt content and a pH at low levels, this is the reason why biogenic amines cannot develop under optimal conditions and the decarboxylation process of amino acids cannot take place (Valsamaki et al., 2000). The total content of biogenic amines is 10 to 2000 times higher in ripened cheeses compared to unripened ones (Novella-Rodríguez et al., 2003).

The presence of biogenic amines depending on cultures

Unpasteurized low-sodium Cheddar cheeses were analysed with products containing commercial probiotics (*Lactiplantibacillus* and *Bifidobacterium animalis* ssp. *lactis*) and biogenic amine which produce strains (*Levilactobacillus brevis, L. sacrimbacillus ATCC 362CC; L. sacrimbacillus ATCC 362CC*) to evaluate the content of biogenic amines according to the cultures used over a ripening period of 125 days at different temperatures of 15 or 4°C. The results highlighted the fact that for 125 days maintained at a temperature of 4°C, in the cheese samples inoculated with probiotics the amount of biogenic amines was reduced more compared to those inoculated with biogenic foods. Probiotic cheeses ripened at 15°C had the highest content of biogenic amines (1332 mg/kg). After exceeding the 13-day ripening period, it was observed that the populations of probiotics decreased significantly, and their ability to degrade biogenic amines decreased. To limit the production of biogenic amines in Cheddar cheese, probiotic cultures can be used as an additional option (Gentès et al., 2024).

The impact of different types of starter cultures and ripening periods on biogenic amines (putrescine, tryptamine, phenylethylamine, histamine, spermine and spermine) in cheeses produced from pasteurized sheep's milk were analysed (Renes et al., 2014). The study was based on the analysis of four batches of cheese in duplicate, matured for 7 months. Cheese samples were analyzed by High-performance liquid chromatography (HPLC). Has been demonstrated that tryptamine, phenylethylamine and spermine represent 80% of all biogenic amines studied.

The production of biogenic amines was reduced as a result of the application of starter cultures. The content of biogenic amines was significantly lower ($p < 0.001$) in the batches consisting of autochthonous starter cultures (*Lactococcus lactis* subsp. *cremoris* and *Lactococcus lactis* subsp. *lactis*) also in combination with *Lactobacillus plantaru*.

Butor et al. (2023) set out to investigate the factors responsible for degrading the content of biogenic amines (cadaverine, histamine, putrescine, tyramine, putrescine and phenylethylamine) in foods using *Bacillus subtilis* from Gouda cheeses.

Aerobic and anaerobic conditions combined with different temperatures (30°C, 8°C, 23°C) correlated with medium pH (from 5.0 to 8.0) led to a decrease in biogenic amines.

Cultivation of *Bacillus subtilis* was in an environment consisting of biogenic amines. Cultivation temperature and pH of the medium had a significant effect on the degradation of biogenic amines by *Bacillus subtilis*. At the end of the cultivation period, the content of studied biogenic amines decreased by 60-90%. Due to the positive effects that the strain has it can be used in the food industry to maximize consumer safety.

The presence of biogeneous amines depending on thermal treatments

The levels of biogenic amines were monitored by Novella et al. (2002) in goat cheese under two different conditions: pasteurization or under high hydrostatic pressure. Tyramine was found in both types of cheese, then of histamine, cadaverine and putreiscin. For some of the amines, no changes were observed in the type of treatment applied to the milk (pressurization or pasteurization).

The ripening process can be reduced by using high-pressure treatment, once with their application can change the structure of biogenic amines. The most representative results were observed for tyramine, reaching three times higher levels compared to control samples at a pressure of 50 MPa (MegaPascals). By increasing the high pressure to 400 MPa for a shorter time duration of 5 minutes, the heat treatment mechanism acted differently, resulting in lower tyramine values compared to control cheeses (Novella et al., 2002).

Methods of analysis ion exchange chromatography coupled with tandem detection mass spectrometry

The profile of biogenic amines can be established using chromatography with ion spectrometric detection; therefore 10 biogenic amines were determined from different cheeses. The biogenic amines taken were tyramine, 2-phenylethylamine, spermine, spermidine, trimethylamine, histamine, tryptamine and agmatine. Compared to other existing methods in the literature, an advantage of this method is that the extraction of biogenic amines is determined only with water, without the need for another sample cleaning procedure. At the end of the study, it was illustrated that this method is useful for determining the profile of biogenic amines in cheeses and can also be used for determining food products (Ščavničar et al., 2018).

Salt-assisted liquid-liquid extraction

The next stages of analysis are based on following steps: soluble pre-extraction (using hydrochloric acid), followed by derivatization, salinization and biogenic amine profile analysis by HPLC.

At this time, the extraction time of the hydrochloric acid improved, the reduction from the influence of the average pH on the sample as well as the dose of the reagent. Histamines, dimethylamine and putrescine recorded the most significant levels among the biogenic amines. The method stands out for the simplification of the preparation of the sample and the general improvement of the process, being an agreeable alternative in relation to the existing methods.

They can also be used to analyze the structure of biogenic amines from the food matrix or fish meat (Ramos et al., 2020).

Ultra-High Performance Liquid Chromatography

By using the UHPLC analysis method, the content of biogenic amines in different types of cheeses, namely in 151 samples, was evaluated. Of the cheeses analyzed, only 5% presented total concentrations of biogenic amines higher than 90 mg/100 g, and some samples had values from 150 mg/100 g cheese to 313 mg/100 g cheese. Histamine concentrations were 116 mg/100 g, in a few cases (5%) histamine levels were higher than 17 mg/100 g. The other types of amines in cheeses were detected at different values: tyramine 72%, putrescine 70%, cadaverine 48%, tryptamine 15% with an average concentration of 0.3 mg/100 g (Mayer & Fiechter, 2018). In the case of HPLC-type analysis, each sample is allocated between 20 and 60 minutes, which means a long time, thus UHPLC analysis improves resolution, sensitivity and contributes to decreasing the analysis time for the studied probe. (Loizzo et al., 2013). Mayer et al. (2010), propose a method for the analysis of amines by UHPLC by separating amines in 9 minutes. The results had the following values: 13.5% of the samples had a histamine content above 10 mg/100 g, 22.4% recorded a tyramine content higher than 10 mg/100 g, 8.6% of the samples had a concentration of cadaverine or putrescine greater than 10.2 mg/100 g.

HPLC

A series of cheese samples were analyzed by HPLC analysis methods to determine the content of biogenic amines in them. The raw material from which the cheeses were made had two sources: raw milk or pasteurized milk, at the same time the content of biogenic amines was also evaluated depending on the ripening period. The levels of biogenic amines in cheeses ripened for a short period were lower compared to cheeses ripened for a longer period. At the same time, in terms of milk composition, raw milk recorded a higher composition in biogenic amines compared to pasteurized milk. The most significant result in the composition of biogenic amines was detected in blue cheeses from raw milk. At the same time, among the biogenic amines, tyramine was the most frequently encountered. Through the PCR method, the bacteria that produce tyramine were determined. Following this analysis, there was an affirmative link between the results of the detection of bacteria that produce tyramine by PCR or by the HPLC method.

To reduce the development process of tyramine in cheeses it is recommended to use both methods of analysis (HPLC and PCR) (Fernández et al., 2007).

Kandasamy et al. (2021) obtained a total content of biogenic amines in the analyzed cheeses ranging from 11 to 62 mg/kg. The other biogenic amines had the following values: putrescine (3.45 mg/kg), histamine (13.45 mg/kg), 2-phenylethylamine (with values between 24.20 and 48.02 mg/kg), respectively spermine (11.0-20.45 mg/kg). The highest content of biogenic amines was 62.09 mg/kg and the lowest content was 11.21 mg/kg. Novella et al. (2003), found a similar histamine content with an average value of 18.3 mg/kg.

Sensory changes depending on storage period

In traditional Czech cheese, the influence of storage time and temperature on the development of biogenic amines was monitored (Standarová et al., 2009). Over 7 weeks, the samples were subjected to different temperature treatments: 5°C and 20°C. Biogenic amines were analyzed by the RP-HPLC method. By means of the Czech national standard, analyses related to sensory (color, smell, texture and aroma) and physical and chemical properties were carried out.

The concentrations of biogenic amines are described in order of concentration: cadaverine (120-2400 mg kg^{-1}), tyramine (110-1050 mg kg^{-1}), putrescine (70-760 mg kg^{-1}), histamine (75-41 mg kg^{-1}). The lowest values among biogenic amines were found in spermine, spermidine and tryptamine. After 17 days of storage, the total content of biogenic amines in cheese samples stored at 5°C was 900 mg kg^{-1} in contrast to cheese stored at 20°C Concentrations of biogenic amines had an upward trend during the evolution of the storage period ($P < 0.01$).

In the cheese stored at 5°C, no significant changes were observed in the content of biogenic amines at the end of the storage period (4600 mg kg^{-1}) compared to the cheese stored at 20°C where the concentration of amines tripled. Samples stored at 5°C retain their appropriate sensory characteristics for the duration of safe storage. Storage conditions and the development of biogenic amines are of particular importance in reducing the risk of consuming cheese with toxic concentrations of biogenic amines.

CONCLUSIONS

Safety it's the first requirement who must to be accomplish in food industry and the peoples health. The highest amounts of BA are found in cheeses, the reason why the control should be very rigorous. A greater potential for diseases that can affect the health of consumers is found in cheeses that contain non-uniformly distributed biogenic amines. To minimize the negative effects of biogenic amines, more careful control of the processing steps is recommended: pasteurization, irradiation, hydrostatic pressure, temperature and the starter culture used. It is advisable to respect the limits of biogenic amines in cheeses to avoid the harmful effects they have on human health. Increased attention to the influencing factors of biogenic amines should reduce their occurrence in the dairy category.

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POLYCYCLIC AROMATIC HYDROCARBONS IN CHEESES AS A RESULT OF THE SMOKING PROCESS: A REVIEW

Ioana Roxana ȘOIMUȘAN, Adina Lia LONGODOR, Oana Andreea MASTAN, Daniela LADOȘI, Aurelia COROIAN

University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, 3-5 Mănăștur Street, 400372, Cluj-Napoca, Romania

Corresponding author email: aurelia.coroian@usamvcluj.ro

Abstract

The presence of smoke when smoking cheeses can have negative effects on the health of consumers. Polycyclic Aromatic Hydrocarbons (PAH) result from pyrolysis processes as well as incomplete combustion without organic sources. The factors that influence the concentration of PAH in cheeses are: temperature, moisture content, type of wood used in the smoking process, origin of the raw material (cow's milk, sheep's milk). The main PAH compound that specifies their presence in food products is benzo[a]pyrene. The interest directed at them is due to the carcinogenic and mutagenic effects they have on human well-being.

Key words: *cheese, contamination, pollution, polycyclic aromatic, toxicology.*

INTRODUCTION

Accelerated urbanization and industrialization result in the production of various pollutants such as polycyclic aromatic hydrocarbons (Mojiri et al., 2019). In some European countries it is forbidden to smoke cheeses through smoke, due to the fact that smoking food can contain contaminants that have negative effects on human health. This type of contaminants are polycyclic aromatic hydrocarbons (Guillen et al., 1997). As a result of industrial activity, human actions, the incomplete combustion of organic materials or their pyrolysis process results in the formation of more than 200 chemical substances (Aygun et al., 2005; Ishizaki et al., 2010).

Polycyclic aromatic hydrocarbons (PAHs) are organic compounds containing two or more aromatic rings, made up of carbon and hydrogen atoms, which are pale yellow, white or colorless compounds (Patel et al., 2020). Aromatic rings can be molecularly arranged linearly, angularly or in groups (Abdel-Shafy & Mansour, 2016). According to their molecular mass, PAH can be divided into two categories: low molecular mass (composed of two/three aromatic rings) and high molecular mass (composed of four/more aromatic rings). Contaminations in soil, water and air play an important role because they have carcinogenic and mutagenic effects (Titato & Lancas, 2006). Depending on their molecular mass, the emission of PAHs is of two types: in the form of particles or gas (Lee & Vu, 2010). Regarding the structure of PAHs, they are divided as follows: alternating PAHs (consisting of 6 carbon rings) and non-alternating PAHs (consisting of 6 carbon rings and an additional ring composed of less than 6 carbon atoms), for example fluorine (Gupte et al., 2016). Following the studies carried out, it has been proven that it has a carcinogenic potential for human health, thus it constitutes a global problem.

MATERIALS AND METHODS

To present a database for this study we conducted a systematic search of scientific publications in the Google Scholar, ScienceDirect PubMed and Scopus databases. In the tables below are highlighted concentrations of PAH from different milk products (raw milk, commercial milk, pasteurised milk, smoked cheese), the minimum and maximum values of benzo[a]pyrene (BaP) in cheeses under the influence of different smoking conditions are also presented.

RESULTS AND DISCUSSIONS

Factors influencing PAH

PAH have the following characteristics: low solubility in water, high melting and boiling points, low vapor pressure. All these elements depend on their structure (Lee & Vu, 2010). They are the result of incomplete combustion of organic materials and are produced in various forms, such as in commercial processes or in car workshops (Suchanová et al., 2008).

Thermal methods have a strong influence on the development of polycyclic aromatic hydrocarbons. Thus, Howard & Fazio (1983) suggest that the main factors that influence the hydrocarbons in food are spread over several levels:

1. Heat treatment temperature;
2. The nature of the energy source and
3. Direct or indirect contact with the power source.

Aydinol & Ozcan (2013) mentioned that the smoking process at temperatures higher than 425°C lead to the formation of toxic PAHs, so they recommend that the furnace temperature should not be higher than 425°C.

Another category of factors that influence PAH production are: temperature, oxygen concentration, humidity, type of wood used or type of smoke generation (Guillen & Sopelana 2005; Howard & Fazio, 1980).

THE PRESENCE OF PAH IN CHEESE

Cheese is a food that contains a wide range of varieties, it can be characterized according to the nature of the raw material used (buffalo, cow, sheep, goat), the manufacturing process (the type of curd used, the addition or absence of molds, the ripening time). The smoking process is an owner's option. Smoked cheese exists at a European level in every country, with a variety of cheeses valued for their organoleptic characteristics (Mcllveen & Vallely, 1996).

Hydrocarbon concentrations were analyzed in 7 categories of smoked cheeses (Guillén & Sopelana, 2004). The highest concentrations found are correlated with the hydrocarbons that have the lowest weights (naphthalene, acenaphthylene, fluorene, phenanthrene), as compounds increase, hydrocarbon levels decrease or tend to cease to exist.

In industrial cheese, the PAH concentration was lower (almost 10 times) compared to smoked cheese (Esposito et al., 2015). Suchanova et al. (2008) obtained similar results with different cheese smoking methods, in cow's cheese the most significant level of PAH was obtained. During the smoking of cheeses, accumulations of solid particles can form that have PAHs in their composition and are distributed on the surface of the cheese, a situation encountered in this case was with cow cheese, which had a high concentration of PAH, with values between 75 and 113 μg/kg (Suchanová et al., 2008) in compared to commercial smoked cheese which has a lower content, from 2.3 to 57 μg/kg represented the total values of 12 PAH (Pagliuca et al., 2003). Following the study by Gul et al. (2015) who evaluated the PAH content of smoked and non-smoked industrial cheeses, it was concluded that traditional smoked cheese contains higher amounts of PAH than industrial cheese. This statement is also supported by Pluta et al. (2020), who obtained low levels of PAH in industrial cheeses. Similar PAH results were found in smoked cheeses: naphthalene, acenaphthylene and phenanthrene (Polak et al., 2022), as well as in other studies, the structure with a low molecular mass was found (fluorene, acenaphthene and naphthalene) and high molecular mass compounds (benzo(gly)perylene, benzo(a)pyrene and benz(a)anthracene) (Guillén & Sopelana, 2004). At the same time, the most abundant PAHs are those containing 2, 3 or 4 aromatic rings (Guillén & Sopelana, 2004; Guillén et al., 2007). The PAH analysis of the cheeses from the point of view of the number of aromatic rings was grouped into several categories as follows: 2 or 3 aromatic rings (naphthalene, anthracene, acenaphthene, phenanthrene fluorene), 4 aromatic rings (chrysene, , benz(a)anthracene, fluoranthene), 5 or 6 aromatic rings (dibenz(a,h)anthracene, benzo(b)fluoranthene, benzo[a]pyrene, benzo(k)fluoranthene, benzo(g,h,i)perylene, indeno[1,2,3-cd]pyrene (I[1,2,3-cd]P) (Suchanová et al., 2008).

Sheep milk whey contains the lowest number of hydrocarbons although it contains a higher amount of hydrocarbons compared to other samples. The highest concentration of hydrocarbons was found inside the cheese

(1037.23 μg/kg), in the middle and inside the contents were similar (153.70 and 88.96 μg/kg) (Guillén & Sopelana, 2004). Compounds with a mass greater than 228 (greater than benz(a)anthracene, chrysene or triphenylene) were found in cow, sheep and goat milk samples (Guillén & Sopelana, 2004).

The most contaminated part of the cheese with PAH is found on the surface layers of the cheese, where the amounts are 3 to 6 times more abundant than those of the entire sample. This observation is in agreement with that obtained by Guillen & Sopelana (2004). Removal of 1 to 2 mm of the surface layer reduces PAH concentration by 50-100% (Suchanová et al., 2008).

Table 1. Average PAH level in different dairy products

Product category	The amount of PAH	Reference
Raw milk	5.427 ng/g	Naccari et al. (2011)
Pasteurised milk	6.518 ng/g	Naccari et al. (2011)
UHT milk	5.941 ng/g (Semiskimmed)	Naccari et al. (2011)
	7.752 ng/g (Whole milk)	Naccari et al. (2011)
Commercial milk	0.99 lg/kg	Kishikawa et al. (2003)
Raw milk	1.010 lg/kg (from farm)	Abou-Arab et al. (2014)
	0.36 lg/kg (Commercial)	Abou-Arab et al. (2014)
Smoked cheese	175.43–1037.22 lg/kg	Guillen and Sopelana (2004)
Circassian cheese	19.57 lg/kg (Traditionally smoked)	Gul et al. (2015)
	0.77 lg/kg (Traditional nonsmoked)	Gul et al. (2015)
	6.36 lg/kg (Industrial smoked)	Gul et al. (2015)
	0.49 lg/kg (Industrial nonsmoked)	Gul et al. (2015)
Industrial smoked cheese	10.10 lg/kg	Suchanova et al. (2008)
Smoked cheese	242 lg/kg (smoked with almond shells)	Guillen et al. (2007)
	85 lg/kg (smoked with Dry prickly pear)	Guillen et al. (2007)
Cheese I	10.75 lg/kg	Martorell et al. (2010)
Cheese II	11.75 lg/kg	Martorell et al. (2010)
Cheese III	12.80 lg/kg	Martorell et al. (2010)

High concentrations of polycyclic aromatic hydrocarbons do not always contain a large number of polycyclic aromatic hydrocarbons. For example, the cow's milk cheese sample with a total concentration of 367.53 μg/kg had a content of 28 polycyclic aromatic hydrocarbons, compared to goat's milk cheese which had a total concentration of 194.65 μg/kg and 41 polycyclic aromatic hydrocarbons (Guillén & Sopelana, 2004). The minimum PAH content was detected in non-smoked cheese (1.83 μg/g) (Rawash et al., 2018).

Guillén & Sopelana (2004) used 4 different types of plant material to smoke the cheese: almond skins (*Prunus dulcis*), dry fiber (*Opuntia ficus indica*) and canary pine wood or needles (*Pinus canariensis*). PAH concentrations smoked with almond shells were higher (42.46-739.55 μg/kg) compared to those smoked with dry fiber (29.21-193.11 μg/kg). The presence of very light (naphthalene or acenaphthylene) or heavy (benz(a)anthracene, chrysene, benzopyrene or benzo(gy)perylene) PAH was noted.

Cancerogenic effects

Depending on the PAH compounds, the degree of cancer can be characterized as follows: from moderate to strong (benzo[a]pyrene, dibenz[a,h]anthracene, dibenz[a,j]anthracene, 3-methylcholanthrene and 5-methylchrysene) , relatively weak or inactive (benzo[e]pyrene, benzo[c]phenanthrene, dibenz[a,c]anthracene, fluoranthene and chrysene) (Adeyeye, 2020). The most toxic PAH is Benzo(a)pyrene and in traditionally and industrially ripened cheeses the level of B[a]P was 0.69 and 0.25 μg/kg (Bukowska et al., 2022). The primary element that indicates the occurrence of PAH in food is benzo[a]pyrene, according to animal studies (EFSA, 2008).

PAH are based on 3 types of effect categories: mutagenic, teratogenic and carcinogenic (Jiang et al., 2014). The degree of toxicity increases as the ring structure increases (Hankin et al., 1996). Between the years 1980-2000, they began to study the adverse effects they have on organisms, such as the rat, daphnia (Sun et al., 2021). Since 2000, research on the immunotoxicity, neurotoxicity, genotoxic impact, reproductive and endocrine disrupting effects of PAH has started to be a new topic

(Barron, 2004). PAH exposure is of many types and their effects are correlated with exposure duration, health status, chemical or toxicity (Kim et al., 2013). Among the factors mentioned the most important factor is the duration of exposure (WHO, 1998). On the other hand, the inhalation of PAH and the effects on the cardiovascular system, gastrointestinal system, hematological and musculoskeletal effects are less common. Occupational exposure in an environment with high PAH concentrations in the short term has as symptoms nausea, vomiting, confusion and skin and eye irritation (Goudarzi et al., 2017b). People who have asthma can be affected by short-term exposure to PAH, and people with coronary heart disease are more likely to get thrombosis (Kim et al., 2013). More severe health conditions such as skin, lung or digestive tract cancer occur as a result of repeated exposure to PAH over a longer period of time (Bach et al., 2003; Olsson et al., 2010). Exposure to low doses of PAH causes eye irritation that can result in cataracts (Mumtaz & George, 1995).

Influence of smoke from PAH

In general, smoke conditions can influence PAH levels obtained from smoking and smoked foods (Maga, 1988). One element that reflects the existence of PAH compounds is benzo[a]pyrene contamination demonstrating the existence of a link to smoke generation conditions (Toth & Potthast, 1984). Parameters that influence the production of PAHs during the smoking period are: the type of wood used, the type of smoke generation, the percentage of humidity, the temperature and the oxygen concentration (Howard & Fazio, 1980).

The most used method of preventing the spoilage process in the range of food products is smoking, but nowadays, food smoking is mainly done for the sensory qualities and not for the preservation effect (Bratzler et al., 1969). Smoke performs bacteriostatic and antioxidant roles, imparts flavor and color to high protein foods (Burt, 1988). Cheese can be smoked using two methods: smoking with natural smoke or smoking with liquid smoke. For meat and fish, liquid smoke is used as a preservative and flavoring agent (Hatulla & Luoma, 2001). For example Wendorff et al., (1993) state that phenolic compounds in smoke can prevent mold growth in smoked Cheddar cheese. One way to diversify the flavors is to smoke the cheese. Riha and Wendorff (1993) studied what is the perception of consumers on the choice of the type of cheese and later it was concluded that the most important characteristic of the cheese is the color. In a consumer survey, Hendrick et al. (1960) found that hardwood smoke flavored cheeses were preferred over liquid smoke cheeses.

During the baking of Cheddar cheese under the influence of smoke, the moisture percentage was located between 35.50-37.06. Usually after 3 months of ripening the moisture level increases in smoked cheeses (Rehman et al., 2003).

The results of the sample from a type of processed (industrial) cheese in which a liquid smoke flavor was used, the PAH content found had high values (181 $\mu g\ kg^{-1}$), respectively (9.8 $\mu g\ kg^{-1}$) of the carcinogenic ones (Suchanová et al., 2008). Gilbert & Knowles (1975) state that natural smoking provides a higher degree of preservation than liquid smoking. Benzo[a]pyrene develops during smoking, so as an alternative the method of liquid smoking began to be used to reduce the carcinogenic effect of the compounds. To reduce the risk of PAH formation, it is recommended that the combustion temperature be lower than 300°C (Tulay et al., 2011).

The influence of the type of wood on the PAH content

The elements that generate smoke production are: air supply, combustion temperature, wood moisture (Guillén et al., 2000). PAH content is lower in wet wood compared to dry wood because the temperature at which smoke is produced from wet wood is low (Maga, 1988). Wood moisture has effects on smoke production, for example a low moisture level results in incomplete combustion of the wood, as opposed to a high moisture level that decreases the combustion performance and increases the appearance of smoke, so to reduce the released particles it is recommended a moisture concentration between 15 and 30% (Simoneit et al., 2000).

That is why a low air supply during pyrolysis reduces PAH production (Guillén et al., 2000).

The production of PAHs is influenced by the type of wood used, for example specific resinous (conifers: firs or pines) have positive effects on the development of resin, which is why they can contribute to the decomposition of crisis (Ch), benzo[a]-pyrene (BaP), benzo[a]anthracene (BaA)(Simoneit, 2002) benzo[k]fluoranthene (BkF), ndeno(1,2,3-cd)pyrene (IP), or benzo[k]fluoranthene (BkF) (Simoneit et al., 2000), compared to hardwood smoke which contains reduced amounts of Ch, BaA and BaP (Oros & Simoneit, 2001).

Maga, (1988) proposed the use of hardwoods to reduce the PAH content of smoked products in exchange for the use of resinous lemmas.

The results of the study by Guilleen et al., (2000) illustrate that benzo[a]pyrene, naphthalene, pyrene and phenanthrene were identified. Low molecular weight compounds contain the highest concentrations of PAH. There are differences in the content of PAH and the origin of the raw material, for example beech, oak and cherry shoots contain low amounts of PAHs, in contrast to vine shoots where high concentrations of PAHs are found (due to the high content of methyl derivatives and naphthalene). Benzo[a]pyrene content was high in cheeses smoked with spruce, pine, willow, alder or beech wood (Migdał et al., 2020).

Cherry, beech and oak contain lower amounts of PAHs than vine shoots. Poplar, although a hardwood or softwood, produces higher amounts of PAHs compared to the 4 types of wood previously mentioned (Pallu, 1971). To obtain smoke flavors with low contents of carcinogenic PAH. At temperatures between 270°C and 380°C significant amounts of smoke are produced, and above 500°C the smoke production increases (Migdał et al., 2020).

Among the smoke flavors analyzed, the wood that recorded the highest naphthalene and methyl derivative contents was vine shoots (Guillen et al., 2000).

The analyzed studies demonstrated that one of the most important elements that have an impact on the PAH content is the raw material, namely the wood used in the smoking process. The highest PAH values were detected in cheeses smoked with softwoods such as conifers compared to hardwoods. At the same time, traditional smoked cheeses contain higher amounts of PAHs than industrially smoked cheeses.

Table 2. B[a]P concentration in cheeses

B[a]P content	The method used	Reference
0.011–0.87	Commercially and home-made smoked cheeses	Suchanová et al. (2008)
ND–0.54	Rind of commercial smoked cheeses	Guillén & Sopelana (2004)
<0.11–3.7	Home-made smoked cheeses	Michalski & Germuska (2003)
<0.22–1.65	Effect of different smoking conditions on B[a]P	Michalski & Germuska (2003)
0.04–0.38	Effect of different smoking conditions incl. smoke-flavoured and liquid flavoured cheeses on PAHs	Pagliuca et al. (2003)
ND–0.90	Commercially smoked cheeses	Garcia et al. (1996)
0.15–0.74	Investigation of different smoking conditions on B[a]P	Anastasio et al. (2004)
<0.15–4.1	Investigation of different smoking conditions on PAH	Bosset et al. (1998)

ND*= not detected

CONCLUSIONS

PAH in cheeses present a mutagenic and carcinogenic factor on the health of consumers, which is why their control is necessary to reduce the negative effects they can have. To ensure a high level of preservation of the cheeses, natural smoking is recommended. It is advisable to use hardwoods instead of softwoods to reduce the PAH content in the smoke. One way to reduce the carcinogenic effects of PAH compounds is the liquid fumigation method.

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USING THE BIOPROTECTION CULTURE FOR DRY FERMENTED SALAMI - THE CONTROL MEASURE OF *Listeria monocytogenes* GROWTH

Georgeta ȘTEFAN, Corina Nicoleta PREDESCU

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: durduncorina@yahoo.com

Abstract

The control of Listeria monocytogenes (Lm) growth in dry fermented salami represents an important issue in food safety for the meat processing industry. The dry fermented salami represent ready to eat meat products (RTE) with a long shelf life. According to the Regulation (EC) no.2073/2005, Lm represents a food safety criteria for RTE products . Lm is the pathogen agent of human listeriosis, an emerging global zoonoses. The human listeriosis is one of the most severe food born disease that affects certain risk categories in the human population, mainly transmitted during consumption of contaminated food. The purpose of our study is to evaluate the effect of bioprotection cultures for dry fermented salami to control the Lm growth. The bioprotection cultures contain strains of Pediococcus acidilactici that produces pediocin, a bacteriocin with antimicrobial action against Gram positive bacteria, including Lm. The Lm counts during fermentation-smoking and ripening-drying stages highlights that the Lm number decreased by 3.2 log cfu/g during the 30 days in the batch LII , whereas the reduction in the batch LI (without bioprotection culture - the control batch) was 1,03 log cfu/g.Based on results, the use of bioprotection cultures is a useful measure to control the Lm growth for the dry fermented salami. It represents a preventive measure of human listeriosis during the food consumption.

Key words: *bioprotection culture, dry fermented salami, Lm, Pediococus acidilactici, RTE.*

INTRODUCTION

Listeriosis, zoonotic disease produce by *Listeria monocytogenes* (Lm), recognized a notable growing trend during the last two decades in the socio-economic context development of the human population. The consumption of contaminated food, especially ready-to-eat food, is often identified as the cause. Human listeriosis may affect some of the risk category that includes pregnant women, neonates, immunocompromised patients, and the elderly and it causes pathologies such as meningitis, septicemia, encephalitis and abortions. Lm is responsable of a severe pathologies at these risk categories of human population and it can clinically evolve as meningitis, septicemia, encephalitis and abortions (Vázquez-Boland et al., 2001; Di Pinto et al., 2010; Lopez-Valladares et al., 2018). Several factors, such as the infectious dose, the pathogenic features of Lm ingested with food and susceptibility, health and immunological status of the patients play important roles in relation with clinical presentation of disease (Vázquez-Boland et al., 2001). An important characteristhic of Lm is the capacity to survive inside of infected organism cells , for a long period of time, what makes antimicrobial therapy ineffective and assure the protection against the immune response of host (Caplan et al., 2010). The levels of food contamination as low as 10^2 to 10^4 LMO/g of have been associated with human listeriosis (Farber et al., 1991; McLauchlin, 1991).

According to EFSA and ECDC reports «Listeriosis affected around 2,200 people in 2015, causing 270 deaths – the highest number ever reported in the EU. The proportion of cases in the over 64 age group has steadily increased from 56% in 2008 to 64% in 2015. In addition, during this period, the number of reported cases and their proportion in the over 84s has almost doubled." The European Union One Health 2020 Zoonoses Report shows that listeriosis was the fifth, most frequently reported zoonosis, evolving in humans, at the level of the European Union.

Listeria monocytogenes (Lm) is currently known as one of the transmissible zoonotic agents, mainly through food consumption,

being considered a high-risk microbiological hazard in relation to food safety.
Lm is a ubiquitous bacteria, wide spreads in the environment, being present in water, soil and on various objects. The high resistance of Lm is worth mentioning, being a psychrotrophic microorganism that grows in temperature conditions between 0-45 degrees C. The storage temperature range is between 2-10°C in most ready-to-eat products (RTE), which allows the bacteria to multiply. The growth capacity of Lm during shelf life of RTE, makes this pathogen one of food safety criterion, according to the Regulation (EC) no. 2073/2005 regarding the microbiological criteria for food products. The contamination of food products, frequently incriminated as a source of human infection, is caused by either the use of contaminated animal product and/or contaminated and/or recontaminated through the production environment, on the processing flow, due to deficiencies in good practice and good hygienic practice. There is a wide variety of foodstuffs have been reported as source of infection for humans by consumption (e.g., meat products, soft cheeses, dairy products, smoked fish, and salads) but they were usually refrigerated ready-to-eat products (McLauchlin et al., 1990; Farber et al., 1991; Rocourt, 1996).
Listeria monocytogenes is widespread in the environment and is a potential risk because there is a wide variety of foodstuffs, including the dry fermented sausages, that may be contaminated with LMO (Meloni, 2015). The contamination of foodstuffs recognises various sources, as result of the use of contaminated raw material (i.e., contaminated raw meat for dry feremented products) and/or contamination through the processing environment. The raw meat can be contaminated often, the primary source being the infected animal. Lm could survive and multiply during the fermentation, ripening and drying stages of the dry fermented salami manufacturing. The important resistance of Lm represents the main issue, being a psychrotrophic bacteria and resistant to action of physical and chemical factors. The bacteria can survive and can be grown at a wide range of ph value and a wide range in temperature. The Lm number can be reduced during the manufacturing due the presence of lactic acid, the low value of pH, but an important number of Lm could survive (Farber et al., 1988; Junttila et al., 1989).
The efficiency of bioprotection culture against Lm has been showed by several scientific papers (Korshidian et al., 2021; Aymerich et al., 2019; Saraoui et al., 2018; Bošković et al., 2017 ; Garriga et al., 2015; Winkelströter et al., 2015).
The using of bioprotection culture that contain strains of Pediococcus acidilactici is efficient to inhibit or limit the growth of LMO in dry or semi-dry fermented salami below 26ºC, during the manufacturing stages.
Pediococcus acidilactici produces the pediocin, a bacteriocin with antimicrobial action against Lm. The pediocins are natural bacteriocines produced by several species of *Pediococcus* (i.e., *P. acidilactici*, *P. cellicola*, *P. damnosus*, *P. inopinatus*, *P. pentosaceus*, etc.) (Tomé, et al., 2008; Haakensen et al., 2009). These bacteriocines present an important antimicro-bial action against Gram- positive micro-organisms, especially pathogenic bacteria.
The purpose of our study is to evaluate the effect of bioprotection cultures for dry fermented salami to control the Lm growth during the manufacturing process.

MATERIALS AND METHODS

In order to asses the behaviour of Lm during the manufacturing process of dry fermented salami under action of bioprotection culture6.1×10^7cfu/g(B-LC-20 SafePro™, Chr. Hansen) a trial was contucted to count the Lm during the ripening and drying stages. For this purpose, two experimental lots (LI and LII) of dry fermented salami (below 26ºC) were manufactured, based on the Banatean salami recipe and technology, such as: choper of pork and beef meat, mixing of all ingredients, stuffing the meat mixture, fermentation-smoking (alternatives stages of fermentation and smoking), ripening-drying stages. The duration of Banatean salami production process is 30 days. The manufacturing of dry fermented salami requires microbial fermentation produced by starter culture 5×10^6 cfu/g (BFL-F02®, Chr. Hansen). The starter culture is intended for the dry fermented salami to ensure the characteristics of the meat mixture during the fermentation stage (the acidification process). Both lots contain the starter culture of *Pediococcus pentosaceus* and *Staphylococcus carnosus* (BFL-F02, Chr. Hansen) that is added during the mixing stages, according the technical specification BFL-F02 ver. 4PI-EU-RO 21.08.2012. Each lot has been inoculated with Lm strains directly to the meat recipe, into the bowl chopper. For the artificial inoculation there were used two strains of *Listeria monocytogenes*. The assumed level of contamination was about 1000 cfu/g of sample. The fermentation-smoking stage has been done during 7 days, at 22-24°C in a relative humid air of 85-95%. The ripening and drying stage took place under controlled conditions in temperature (14-15°C) and a humidity of the air raging between 73-75%, until day 30 of the manufacturing process.

LI of Banatean salami with starter culture of *Pediococcus pentosaceus* and *Staphylococcus carnosus* (BFL-F02,Chr. Hansen).

LII of Banatean salami with starter culture of *Pediococcus pentosaceus* and *Staphylococcus carnosus* (5×10^6 ufc/g)) (BFL-F02,Chr. Hansen) and bioprotection culture of *Pediococcusacidolactici*(B-L20SafePro®,Chr. Hansen).

The Lm counts was done during the fermentation-smoking and ripening - drying stages, on 25 g sample on 5 time intervals, such as T0 (day 0), T1 (day 3) , T2 (day 7), T3 (day 14) and T4 (day 30), to counting of Lm on the two lots artificial inoculated. There were tested three inoculated samples (salami stick)/time intervals/lot.

The evaluation of Lm growing has been carried out as a challenge test on the two lots of Banatean salami during the fermentation-smoking and ripening-drying stages. Method of Lm counts is done by the most probable number, using the isolates on Ottaviani & Agosti (incubation the Petri at 37°C, 24 h) and identify 1 to 5 typical colonies using the conventional tests described in the standardized methods ISO 11290-2:2017.

During the fermentation-smoking and ripening-drying stages a complementary test on three samples of Banatean salami (salami stick/each lot/interval of time) was carried out to determine the pH value (measurement by potentiometric method), in five time intervals : T0, T1, T2, T3 and T4.

The results of Lm counts are exprimed as log cfu/g.

RESULTS AND DISCUSSIONS

At the first assessment, the Lm counts registered similar results, with an average of 3.34 log cfu/g for LI and 3.52 log cfu/g for LII. At the end of the fermentation process it is observed an important reduction of Lm number for both batches, 2.64 log cfu/g for LI and 2.04 logcfu/g for LII at T1.The significant decrease carried on for the batch LII, at T2 Lm number being 1.56 log cfu/g. For batch LI, is observed o slightly increase at T2, the Lm number being 3.15 log cfu/g. It can be noted a slight decrease for batch LI, being registered 2.44 log cfu/g at T3. Concerning the batch LII, continued reduction of Lm number, being recorded 1.16 log cfu/g at T3. A significant difference of Lm number is observed between batches at the end of manufacturing production(day 30), i.e for the batch LI being registered 1.89log cfu/g compared to the batch LII where is 0.33 log cfu/g.

Table 1. Results of Lm obtained on LI* of Banatean salami

Sample	day	Log cfu/g	Average of Lm Log cfu/g
1	T0	3.37	3.34±0.03
2		3.35	
3		3.30	
1	T1	2.63	2.64±0.03
2		2.68	
3		2.62	
1	T2	3.18	3.15±0.03
2		3.15	
3		3.12	
1	T3	2.47	2.44±0.03
2		2.40	
3		2.45	
1	T4	1.86	1.89±0.03
2		1.89	
3		1.92	

*LI is the experimental lot of Banatean salami (LI) with the starter culture of *Pediococcus pentosaceus* and *Staphylococcus carnosus* 5×106 ufc/g) (BFL-F02, Chr. Hansen)

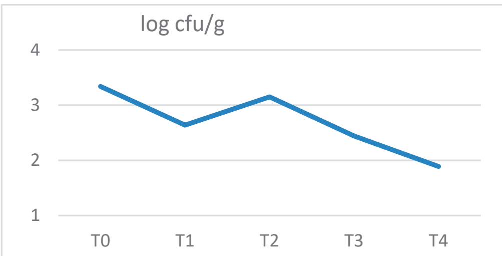

Figure 1. Lm counts in batch LI

Table 2. Results of Lm obtained on LII* of Banatean salami

Sample	day	Log cfu/g	Average of Lm log cfu/g
1	T0	3.57	3.52±0.04
2		3.52	
3		3.48	
1	T1	2.08	2.04±0.03
2		2.04	
3		2.02	
1	T2	1.60	1.56±0.04
2		1.57	
3		1.52	
1	T3	1.20	1.16±0.03
2		1.14	
3		1.16	
1	T4	0.38	0.33±0.04
2		0.30	
3		0.32	

*LII is the experimental lot of Banatean salami with the starter culture *Pediococcus pentosaceus* and *Staphylococcus carnosus* (BFL-F02, Chr. Hansen) and the bioprotection culture *Pediococcus acidolactici* culture (SafePro® B-LC-20, Chr. Hansen Holding)

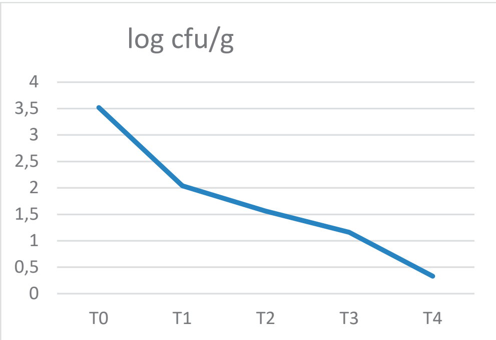

Figure 2. Lm counts in batch LII

Based on the results obtained per each lot of Banatean salami, during fermatation-smoking and ripening-drying stages, the Lm number decreased by 3.2 log cfu/g during the 30 days in the batch LII , whereas the reduction in the batch LI was 1,03 log cfu/g.The assessment of LII on day 30 proves that the bioprotection culture *Pediococcus acidolactici* (SafePro® B-LC-20, Chr. Hansen) and the starter culture *Pediococcus pentosaceus* plus *Staphylococcus carnosus* (BFL-F02, Chr. Hansen) determine an efficient reduction of Lm number. These results are in accordance with observations of Heller-Stahnke (2005): "*… the use of an adjunct culture such as B-LC-20 provides a unique anti-listerial reduction for fermented sausages since it was found that Pediococcus acidilactici is a strong producer of pediocin (which destroys Listeria monocytogenes) at European fermentation temperatures (<26°C) while not being a strong acidifier at 10 this temperature*" (Heller-Stahnke, 2005).

The results of pH evaluation along the fermentation-smoking and ripening-drying stages, expressed as average of triplicate samples, are available in Tabel 3.

Tabel 3. Results of pH along fermentation-smoking and during the ripening and drying stage (expresed as average)

Lot no.	pH	
	LI	LII
T0	5.95±0.02	5.92±0.02
T1	4.78±0.015	4.73±0.03
T2	4.93±0.02	4.74±0.02
T3	4.98±0.01	4.77±0.01
T4	5.0±0.02	4.88±0.02

It is observed an important decrease of pH in both lots, as result of starter culture action, during the fermentation stage. The researches of Foegeding et al. (1992) proved that, as during the dry fermented sausages manufacturing process, the Lm population can be reduce in condition of pH value less than 4.9 at the end of the fermentation process and along the drying stage. The bacteriocin production enhanced the inhibition rate of Lm growth. The pediocin produced by *Pediococcus acidilactici* has an effective activity against Lm growth, during the fermentation stage and during ripening-drying stage. In case of higher pH value, the pediocin will have an antilisterial effect for the remaining Lm. (Foegeding et al., 1992; Nieto-Lazano et al., 2010).

Another study is the one of Korshidian et al. (2021) regarding antibacterial activity of pediocin and pediocin-producing bacteria against *Listeria monocytogenes* in meat products at a wide range of pH. According this study, the pediocin represents an important biopreservatives categorie for meat processing industry. Mehta et al. (2013) showed the importance of pediocines for food industry thanks of their strong activity against food borne pathogenic agents. Furthermore, the pediocin produced by Pediococcus acidilactici, has been considered to be safe (Mehta et al., 2013).

There is a potential risk of Lm contamination of dry fermented salami from various sources (i.e., raw meat, manufacturing processes, etc.) . The use of starter cultures and ensuring the conform condition of ripening and drying can reduce the the potential of LMO growth in this type of sausages (Meloni, 2015) and represents an very useful food safety measure for meat processing industry, considering the ubiquitous character of Lm and the multitude of contamination sources.

The study of of Bungenstock et al. (2020) who identified among the 169 collected isolates, two new bacteriocin-producing isolates which have the potential to contribute to product and consumer safety: *Pediococcus pentosaceus* LMQS 331.3 and *Pediococcus acidilactici* LMQS 154.1 represents an encouragement for the realization of future researches (Bungenstock et al., 2020).

CONCLUSIONS

Based on results of the study, the bioprotection culture is able to inhibit the growth of Lm for dry fermented sausages till the end of ripening-drying stage, compared to a control starter culture alone.

The using of bioprotection cultures in our study proved that the *Pediococcus acidolactici* culture (B-LC-20 SafeProTM, Chr. Hansen) has a major contribution to limit the Lm growth during the manufacturing process and to ensure the microbial safety of dry fermented salami.

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METHODS FOR DETECTING *Bacillus cereus* CONTAMINATION IN DAIRY PRODUCTS - A FOOD SAFETY PERSPECTIVE

Andra Dorina ŞULER[1], Paula POSAN[1], Minodora TUDORACHE[1], Roxana RAȚU[2], Ioan CUSTURĂ[1], Dănuț ENEA[1], Veronica LUNGU[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]"Ion Ionescu de la Brad" Iaşi University of Life Sciences, 3 Mihail Sadoveanu Alley, Iaşi, Romania

Corresponding author email: pauladragut@yahoo.com

Abstract

Every microbial environment starts with an initial contamination from microorganisms present in soil, water, and atmospheric dust. The unique characteristics of each environment ultimately dictate which species or types of associations become dominant. Consequently, microorganisms with high resistance to inhibitory or lethal factors often thrive in food products, on various surfaces, and on machinery and equipment. Bacillus cereus serves as a prime example, widely distributed in nature and considered an opportunistic pathogenic species. Research conducted between 2021 and 2023 focused on several categories of milk powder samples collected from sales networks in Călăraşi County, Ilfov County, and Argeş County. These categories included skimmed milk powder with 1% fat, milk powder with 26% fat, organic milk powder with 26% fat, milk powder for children aged 4-5 years, and of milk powder for infants aged 9-12 months. The analysis of these five products involved two categories of methods: confirmation on MYP agar, blood agar, and real-time PCR testing for the amplification and detection of specific Bacillus cereus DNA. Identification of species within the B. cereus group was conducted using specific tests.

Key words: *Bacillus cereus, microbial niche, milk powder, pathogen.*

INTRODUCTION

A well-balanced diet plays a vital role in the normal growth and development of individuals. Adequate nutrition not only fosters improved health and reduced vulnerability to illnesses but also aids cognitive development and intellectual achievement (Posan et al., 2022). Ensuring the microbiological quality of both raw materials, such as milk sourced from animals raised in suitable microclimates, free from mammary gland inflammatory diseases, under optimal hygienic conditions, and the final product, while considering the intended consumer categories, is of utmost importance. The quality of the finished products depends on the raw materials quality, the ingredients used and the processing technologies (Ianitchi et al., 2023).

Milk holds significant importance as a food product because of its intricate chemical makeup, nutritional value, and excellent digestibility. It stands as a product of immense socio-economic significance, contributing to the overall health and well-being of the population. (Mihai et al., 2022).

To prevent the occurrence of consumer illnesses, all food products need to adhere to bacteriological standards. This entails ensuring that they are free from pathogenic germs and that the levels of saprophytic organisms remain within the maximum permissible limits. (Suler et al., 2021).

There are some bacteria which can contaminate milk through various means. *Bacillus cereus* is one of them. *Bacillus cereus* belongs to the *Bacillus* genus, which falls within the *Bacillaceae* family alongside the *Clostridium* genus. It is characterized as Gram-positive, sporogenous, noncapsulogenous, aerobic, facultative anaerobic, and peritrichous, meaning it has flagella distributed around its cell. It thrives optimally at moderate temperatures (although its spores can withstand high temperatures) and neutral pH levels, with the ability to grow in environments with high moisture content.

In the food processing industry, it serves as a crucial microbiological parameter that must be considered to ensure food safety.

The pathogenicity of *Bacillus cereus* relies on a broad spectrum of extracellular metabolites produced primarily during its logarithmic growth phase and stationary phase. Some of these metabolites are recognized as toxins or virulence factors, identified based on their behaviour in vivo and their epidemiological associations.

Among the main toxins produced by *Bacillus cereus* are diarrheagenic enterotoxin, vomiting toxin, haemolysins, phospholipases, and cytolysins. Both the diarrheagenic enterotoxin and the vomiting toxin are responsible for inducing clinical symptoms associated with two types of gastrointestinal illnesses resembling food poisoning: a syndrome typified by gastrointestinal disturbances, with an incubation period of 8-16 hours (Zhou et al., 2008; Lund et al., 2000; Sastalla et al., 2013).

Foods commonly implicated in these cases include dairy products such as milk, minced meat, portioned meat, vegetables, and cereal preparations containing corn or corn starch. (Alecu & Ţogoe, 2008; Organji et al., 2007)

The number estimated to induce the diarrheal type of food poisoning ranges from approx. 5 to 8 log CFU of vegetative cells or spores of *Bacillus cereus* (Sastalla et al., 2013) and an emetic syndrome, often associated with the consumption of rice and rice-based preparations.

The strains involved in this case develop optimally at temperatures between 35-45°C. The other toxins are virulence factors in wound, eye, or systemic infections produced by *B. cereus*. Diarrheic enterotoxin (ED) is an antigenic, thermolabile protein, composed of several subunits and inactivated by proteolytic enzymes. Emetic toxin (ET) is very resistant to heat (90 minutes at 126°C) and to proteolytic enzymes. *B cereus* strains usually produce one, very rarely, both types of exemplified toxins.

The objective of this study was to assess the prevalence of *Bacillus cereus* in various types of milk powder available in the Romanian market. Subsequently, the isolated strains were examined to determine their classification within the group.

MATERIALS AND METHODS

Research conducted between 2021 and 2023 focused on several categories of milk powder samples collected from sales networks in Călăraşi County, Ilfov County, and Argeș County. These categories included skimmed milk powder with 1% fat, milk powder with 26% fat, organic milk powder with 26% fat, milk powder for children aged 4-5 years, and of milk powder for infants aged 9-12 months.

The varieties of milk powder were purchased from stores across Romania, transported under optimal conditions, and subjected to examination before the expiration date. In total, 100 samples were examined. This number included 63 samples of milk powder with 26% fat (32 in 2021 and 3 in 2022), 10 samples of skimmed milk powder with 1% fat (3 in 2021 and 7 in 2022), 10 samples of organic milk powder with 26% fat (6 in 2021 and 4 in 2022), 10 samples of milk powder for infants aged 9-12 months (3 in 2021 and 7 in 2022), and 10 samples of milk powder for children aged 4-5 years (3 in 2021 and 4 in 2022).

The 5 varieties were analysed using 2 categories of methods: confirmation on MYP agar, blood agar, and real-time PCR testing for amplification and detection of *Bacillus cereus*-specific DNA. Identification of species belonging to the *B. cereus* group was performed through specific tests.

The laboratory protocol consisted of the following steps:

- Sample collection was conducted under aseptic conditions; a quantity of 10 g was placed into sterile plastic bags containing 90 ml of diluent. The samples were homogenized for 10 minutes using a STOMACHER 80 and serial dilutions were subsequently performed (ISO, 2004, 2017);
- The samples were inoculated into tubes containing tryptic soy broth, and the tubes were then incubated for 48 hours at 30°C. Subsequently, they were checked for dense growth, typical of *B. cereus*;
- Passages were performed onto Petri dishes by streaking onto the surface of MYP isolation media (mannitol with egg yolk emulsion and polymyxin B) and blood agar, followed by incubation for 24-48 hours at 30°C;

- Subcultures were then conducted on blood agar from colonies that produced lecithinase to confirm *B. cereus*;
- Confirmation of *B. cereus* was achieved by checking for the presence of lecithinase and the absence of mannitol fermentation on MYP medium. *B. cereus* colonies appeared large, with a dark pink colour on a violet background, surrounded by a zone of egg yolk precipitation. On blood agar, *B. cereus* colonies appeared large, surrounded by a distinct halo of haemolyses, which did not intensify upon refrigeration (specific to *B. cereus*) (Alecu & Țogoe, 2008);
- The enumeration of *Bacillus cereus* (total number of vegetative cells and spores) in the examined products was conducted according to the colony counting technique outlined in ISO 7932:2004 (ISO 7932:2004; Ehling-Schulzet al., 2015).

From each MYP agar plate, 5 lecithinase-positive colonies were selected and transferred onto nutrient agar, from which microscopic preparations were made. *B. cereus* appears as a large, Gram-positive bacillus with rounded ends, arranged in longer or shorter chains, and with ellipsoidal endospores located centrally or sub-terminally, which usually do not deform the vegetative cell.

Following the suspension culture, the following confirmation tests were conducted (Table 1).

Table 1. Confirmation tests for *Bacillus cereus* (ISO 7932:2004)

Confirmation tests	Results
Gram stain	Gram-positive bacillus
Lecithinase (MYP) production	negative
Mannitol fermentation on agar (MYP)	negative
Reduction of nitrates in nitrites	positive
Production of acetyl-methyl-carbinol	positive
Tyrosine decomposition	positive
Growth in the presence of 0.001% lysozyme	positive

These fundamental characteristics are shared with other members of the *B. cereus* group, including the rhizoid strains of *B. cereus* var. *mycoides*, pathogenic bacteria of the crystalliferous insect, *B. thuringiensis*, and those pathogenic to mammals, *B. anthracis*.

Subsequently, the varieties of infant milk powder were subjected to ensure food safety and real-time PCR verification using the *Bacillus cereus* Taqman PCR Detection Kits. The tests were performed according to the manufacturer's instructions.

The enumeration of *Bacillus cereus* (the total number of vegetative cells and spores) in the examined products was conducted according to the colony counting technique outlined in ISO 7932:2004 (ISO, 2004)

For the differentiation of members of the *B. cereus* group, the isolates were then subjected to the following laboratory protocol:

1. Mobility test

Inoculation into BC medium for mobility testing by stabbing with a straight inoculating needle (3 mm suspension of 24-hour culture), incubation for 18-24 hours at 30°C (if bacterial growth diffuses throughout the culture medium, the bacteria are mobile; if growth occurs only along the inoculation line, the bacteria are non-motile). Alternatively, inoculation of a bacterial suspension onto the surface of an agar slope, incubation for 6-8 hours at 30°C, and mobility testing by examining between slide and cover slip (most strains of *B. cereus* and *B. thuringiensis* are mobile, possessing peritrichous flagella. *B. anthracis* and almost all strains of *B. cereus* var. *mycoides* are non-motile).

2. Rhizoid growth

Inoculation onto nutrient agar plates, incubation for 48-72 hours at 30°C. We observed ropelike growth characterized by the production of colonies resembling root structures that can extend several centimetres from the point of inoculation.

B. cereus typically forms rough colonies in a galaxy-like pattern, which should not be confused with the ropelike colonies typical of *B. cereus var. mycoides*. Most strains of this variety are non-motile.

3. Haemolytic activity test

Inoculation of a 24-hour bacterial culture suspension onto a sheep blood agar plate and a tryptic soy agar plate, incubation for 24 hours at 35°C (*B. cereus* cultures typically exhibit strong haemolytic activity, producing a zone of complete beta haemolysis measuring 2-4 mm around the bacterial colony; most strains of

B. thuringiensis and *B. cereus* var. *mycoides* also exhibit beta haemolysis, while those of *B. anthracis* are usually non-haemolytic after a 24-hour incubation).

4. Test for protein toxin crystals

Inoculation of a 24-hour bacterial suspension onto nutrient agar, followed by incubation at 30°C for 24 hours, and then allowing it to stand at room temperature for 2-3 days. Preparation of smears and covering with methanol (for 30 seconds), removal, drying over a flame, and staining with 0.5% basic fuchsin. After gently heating over a flame until vapors appear and allowing to stand for 2 minutes, the process is repeated. After 30 seconds, the dye is removed and the slide is rinsed. After drying (without wiping), examination under immersion microscopy for the presence of endospores and dark tetragonal crystals (diamond-shaped). The crystals are usually smaller than the spores. These crystals are abundant in a 3-4 day old culture of *B. thuringiensis* but may not be detected by staining techniques until after sporangium lysis. Therefore, if free spores cannot be observed, cultures are kept at room temperature for several days and re-examined. Typically, protein toxin crystals are produced by *B. thuringiensis* (ISO, 2004).

RESULTS AND DISCUSSIONS

Various types of powdered milk (a product primarily intended for sensitive consumer categories such as infants and young children) are known to be contaminated with *B. cereus*, particularly its spores. (Organji et al., 2015; ISO, 2007)

The obtained results are presented in Table 2 and Figure 1.

Table 2. The prevalence of *Bacillus cereus* species in the analyzed samples

Sample type	No. of analysed samples	Positive reactions	%	Negative reactions	%
Milk powder 26% G	63	5	7.93	58	92
Skimmed milk powder 1%G	10	2	20	8	80
Organic milk powder 26% G	10	1	10	9	100
Milk powder for children 9-12 months	10	-	-	10	100
Milk powder for children 4-5 years old	7	-	-	7	100
Total	**100**	**8**	**8**	**92**	**92**

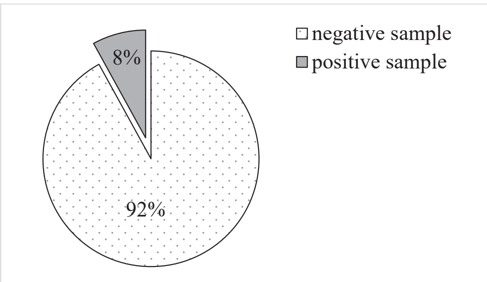

Figure 1. Incidence of Bacillus cereus in the analysed samples

Among the examined products, *Bacillus cereus* was most frequently detected in powdered milk with 26% fat, with a prevalence rate of 7.93%. The bacterial count in these positive samples varied from 1.0 to approximately 3.0 log CFU g-1. For Skimmed Milk Powder with 1% fat, the count of *Bacillus cereus* ranged from 1.0 to about 2.0 log CFU g-1, while for Bio Milk Powder with 26% fat, it was 1.0 log CFU g-1 (Table 3). None of the samples intended for infants and young children tested positive for *Bacillus cereus*.

Table 3. Inventory of *Bacillus cereus* in positive samples

Sample type	*Bacillus cereus* isolates	*Bacillus cereus* count (log CFU g-1)
Milk powder, 26% fat	5	1-3
Skimmed milk powder, 1% fat	2	1-2
Organic milk powder, 26% fat	1	1

The identification of species from the *Bacillus cereus* group in the period 2021-2023, for the Milk powder with 26% fat, Skimmed milk powder with 1% fat, and Organic milk powder with 26% fat category is shown in Tables 4-6.

Table 4. Identification of species from the *Bacillus cereus* group in the period 2021-2023, for the category Milk powder with 26% fat

Year	Number of samples	*Bacillus cereus* group	Number of isolated strains	%
2021	32	*Bacillus cereus* *Bacillus cereus* var. *mycoides*	2 -	6.25 -
2022	31	*Bacillus cereus* *Bacillus cereus* var. *mycoides*	2 1	6,45 3,22
2023	35	*Bacillus cereus* *Bacillus cereus* var. *mycoides*	2 1	5.71 2.85

In 2021, for the Powdered Milk with 26% fat category, 2 strains of *Bacillus cereus* were identified, which represents 6.25% of the total of 32 analysed samples. In 2022, for the Powdered Milk with 26% fat category, 2 strains of *Bacillus cereus* were identified, which represents 6.45% of the total of 31 samples analysed, and one strain of *Bacillus cereus* var. *mycoides* which represents 3.22% of the total of 31 analysed samples. In 2023, 35 samples were analysed, in which 2 samples with *Bacillus cereus* and 1 sample with *Bacillus cereus* var. *mycoides* were identified.

Table 5. Identification of species from the *Bacillus cereus* group in the period 2021-2023, for the category Skimmed milk powder with 1% fat

Year	Number of samples	*Bacillus cereus* group	Number of isolated strains	%
2021	3	*Bacillus cereus* *Bacillus cereus* var. *mycoides*	1 -	33.3 -
2022	7	*Bacillus cereus* *Bacillus cereus* var. *mycoides*	1 -	14.28 -
2023	7	*Bacillus cereus* *Bacillus cereus* var. *mycoides*	1 -	14.28 -

In 2021, for the Skimmed Milk Powder with 1% fat category, a single strain of *Bacillus cereus* was isolated, which represents 33.3% of the total samples analysed.

In 2022, for the Skimmed milk powder with 1% fat category, a single strain of *Bacillus cereus* was isolated, which represents 14.28% of the total of 7 samples. The same situation occurred in 2023.

Table 6. Identification of species from the *Bacillus cereus* group in the period 2021-2023, for the category Organic milk powder with 26% fat

Year	Number of samples	*Bacillus cereus* group	Number of isolated strains	%
2021	6	*Bacillus cereus* *Bacillus cereus* var. *mycoides*	1 -	16.66 -
2022	4	*Bacillus cereus* *Bacillus cereus* var. *mycoides*	- -	- -
2023	6	*Bacillus cereus* *Bacillus cereus* var. *mycoides*	1 -	16.66 -

Both in 2021 and 2023, for the Bio Powdered Milk with 26% fat category, a single strain of *Bacillus cereus* was isolated, which represents 16.66% of the total of 6 analysed samples, in 2022 no strains of *B. cereus* were detected.

The incidence of species from the *Bacillus cereus* group from the total strains identified in the samples analysed is shown in Table 7 and Figure 2.

Out of the 131 samples analyzed across all categories of milk powder, 11 samples were found to contain *Bacillus cereus*, and 2 samples contained *Bacillus cereus* var. *mycoides*. Thus, a total of 13 samples, or 9.91% of the samples, were considered contaminated.

Table 7. Frequency of species from the *Bacillus cereus* group in all the analysed samples

Species	Number of samples	Number of isolated strains	%
Bacillus cereus	131	11	8.39
Bacillus cereus var. *mycoides*		2	1.52
Total		**13**	**9.91**

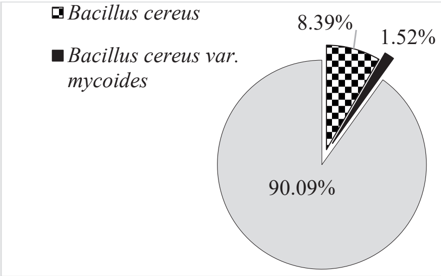

Figure 2. Incidence of species belonging to the *Bacillus cereus* group across all examined samples

CONCLUSIONS

The results of the performed analyses indicate a high level of hygiene and food safety in the processes of obtaining, handling and processing milk powder.

The prevalence of *B. cereus* in powdered milk marketed in Romania demonstrated that there are no significant differences depending on the product, and the contamination level of all analysed products did not exceed 3.0 log. CFU g^{-1}.

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THE USE OF SALMON AND TUNA BY-PRODUCTS IN FISH CRACKERS MANUFACTURE

Ionela Florentina TOMA (ENACHE), Gratziela Victoria BAHACIU, Daniela IANIȚCHI, Nela DRAGOMIR, Iuliana Ștefania BOLOLOI, Carmen Georgeta NICOLAE

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: toma.ionela1998@gmail.com

Abstract

Fish by-products have a great potential for use, both for the food industry and for other industries. Salmon and tuna are valuable species for the food industry, especially those sold as fillets or steaks, but the by-products have a low economic value or are turned into waste. The aim of this study is to obtain new foods by salmon and tuna by-products in order to integrate them into human consumption. Five types of biscuits were created with salmon and tuna byproducts added as salmon oil and fish meat. The products were made in the Bakery Pilot Station of the University of Agronomic Sciences and Veterinary Medicine of Bucharest. After creating the recipes and obtaining the products, the degree of consumer acceptance was established by performing tastings and completing evaluation tests. The evaluation criteria were: taste, aroma, color, general appearance and consistency (five-point hedonic scale). The results showed that the biscuits with salmon meat and dehydrated onion were the most appreciated by consumers.

Key words*: acceptance, consumers, fish, new food products, sensory evaluation.*

INTRODUCTION

The processing of fish by-products is a current topic within the food industry. The conversion of by-products into alternative products or their utilization in various industrial applications has the potential to resolve issues and could emerge as a sustainable solution for the industry (Toma (Enache) et al., 2023).

Fish has been and continues to be widely used as food. As consumption is increasing while fish stocks are decreasing, there is a need to enhance processing efficiency. Fish production may involve several technological processes, depending on the type of products being produced (Idzere et al., 2020).

Beyond the technical and technological advancements in transforming fish by-products into useful products, consideration must be given to social, environmental, political, and economic parameters to understand why and how fish by-product conversion technologies are applicable. This is especially important in the current context where the fisheries sector faces various challenges, such as declining wild fish stocks in favor of aquaculture, increased imports in a global market, and shifting consumer behaviors. These parameters affect and complicate the definition of a strategy for creating and developing fish by-products, as they are directly dependent on the processed raw material (Penven et al., 2013).

In the fishing and aquaculture sector, 35% of the global harvest is lost or wasted each year. Therefore, the utilization of marine by-products must be mandatory to ensure global food security and to meet the increasing demand for fish products (Kaanane & Mkadem, 2020).

Fish is a protein-rich food that readily deteriorates after capture due to bacterial activity, enzymatic action, and chemical oxidation of fats (More & Likhar, 2020; Nicolae & Bololoi, 2023).

Fish processing involves fishing, collection, and transportation, stunning, bleeding, chilling, cutting, packaging, and also the recycling of by-products. The cutting technological stage involves a set of operations aimed at dividing the whole fish into smaller pieces (fish fillets, steaks, loins etc.) (Nicolae, 2020). Various techniques and machinery have been introduced to advance and automate cutting operations to increase processing efficiency and reduce fish by-product quantities (Wenbo et al., 2022).

The need for nutritious, safe, and healthy food because of a growing population, coupled with the pledge to preserve biodiversity and other resources, poses a significant challenge for food industry.

Fish and fish by-products are a natural source of polyunsaturated omega-3 fatty acids, which are highly important in the food and pharmaceutical industries. Conventional fish oil extraction processes include cold pressing, wet reduction, or enzymatic extraction, but the method of supercritical fluid extraction with carbon dioxide under moderate conditions can also be used. Utilizing fish by-products by recovering the oil is particularly interesting when the oil is rich in triglycerides and has a high content of polyunsaturated omega-3 fatty acids (Rubino-Rodrighez et al., 2012).

As food product diversification progresses, various types of foods have been created, including fish biscuits. One variety of this product is represented by biscuits produced with sturgeon *Acipenser sturio* (Linnaeus, 1758) fillet protein concentrate. In this case, the sturgeon fillet protein concentrate was used in biscuit production by replacing low-gluten wheat flour with 5%, 7%, and 10% to ensure biscuit quality and acceptability (Abraha el al., 2018). Another variant of fish biscuits is represented by salt biscuits supplemented with 5% fish protein concentrate, made from Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) by-products. In this case, the fish protein concentrate made from tilapia by-products was used to increase water retention capacity (Ibrahim, 2009). Another variation involved the use of four different recipes containing Nilem carp *Osteochilus vittatus* (Valenciennes, 1842) egg protein concentrate to produce biscuits with better sensory characteristics and higher protein content. (Muslihudin et al., 2021).

Diversifying food sources is essential for ensuring consistent access and sustainability in food supply. Managing and controlling the technological processes in fish processing, inventory, and product quality through traceability leads to increased consumer confidence in food safety, enhanced operational efficiency for all partners in the supply chain, and facilitates potential profit growth for companies in the food industry (Nicolae et al., 2017). Enhancing food production and availability are vital steps toward achieving significant nutritional and economic advancements. It is important to produce a wider variety of foods rich in essential micronutrients, ensuring they're available in ample quantities and accessible to people.

MATERIALS AND METHODS

This scientific study represents a starting point for the creation and development of new technological schemes for producing fish crackers. The proposed and realized crackers were of 5 types: P1 - Crackers with salmon oil, P2 - Crackers with salmon oil and salmon meat, P3 - Crackers with salmon oil and tuna meat, P4 - Crackers with salmon oil, salmon meat, and dehydrated onion, P5 - Crackers with salmon oil, tuna meat, and dehydrated onion (Figure 1).

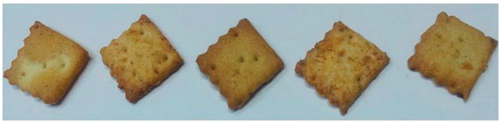

Figure 1. Types of fish crackers: P1 - Crackers with salmon oil P2 - Crackers with salmon oil and salmon meat P3 - Crackers with salmon oil and tuna meat P4 - Crackers with salmon oil, salmon meat, and dehydrated onion P5 - Crackers with salmon oil, tuna meat, and dehydrated onion (own source)

The ingredients used included flour, water, sugar, salt, granulated garlic, dry yeast, and ground white pepper. As innovative ingredients used in the biscuit recipes, fish by-products were employed, namely oil of salmon *Salmo salar* (Linnaeus, 1758) (obtained from fish bellies resulting from salmon filleting) and salmon meat (a by-product from salmon filleting), or tuna *Thunnus thynnus* (Linnaeus, 1758) (a by-product from portioning tuna into steaks). These by-products used in making the biscuits have low economic value if marketed as such. Their utilization in these innovative food products aims to increase economic value and obtain products with novel nutritional and sensory characteristics.

1. Obtaining raw materials from fish by-products

For the production of fish crackers, it was necessary to obtain innovative raw materials: salmon oil, grilled salmon meat, grilled tuna meat.

Salmon oil was obtained through the method of fish oil extraction by wet pressing. This involved qualitatively and quantitatively receiving the salmon by-products, followed by mechanical cleaning and washing. The hygienized by-products underwent mechanical extraction, where the flesh and subcutaneous fat of the salmon were separated from the skin, fins, and other elements that could hinder or impede the fat extraction process. To increase the contact surface of the product with hot air, a grinding step of the fish by-products was necessary. The obtained minced mixture was then heat-treated at a temperature of 95°C, maintaining it for 35 minutes from the moment this temperature was reached at the core of the product (Glowacz et al., 2016). Once the heat treatment was completed, a partial cooling of the obtained product was carried out until it reached 45°C. After reaching the recommended temperature, the product was pressed at a pressure of 25,000 kPa until optimal extraction of the fluid from the product in process was achieved. The resulting fluid was then allowed to settle for one hour, after which the fatty part was centrifuged (FAO, 1986). The obtained oil was cooled to refrigeration temperature, packaged, and stored until use (Figure 2).

Qualitative and quantitative reception of salmon by-products
↓
Cleaning (removal of mechanical impurities)
↓
Laundering
↓
Mechanical extraction
↓
Shredding
↓
Heat treatment at 95°C
↓
Cooling to 45°C
↓
Rent 25,000 kPa
↓
Decanting 1h
↓
Centrifuge
↓
Cooling to 0-4°C
↓
Packaging
↓
Storage

Figure 2. Technological scheme for obtaining salmon oil

The grilled salmon meat was obtained from processing salmon by-products with low-fat content. The meat was extracted mechanically from the salmon skeletons resulting from filleting. First, a qualitative and quantitative reception of the fish by-products was performed, followed by cleaning the by-products by removing any contaminating mechanical impurities and washing the entire mass of the product to chemically remove possible microbiological contaminants. The hygienized by-products were then subjected to mechanical extraction of the meat from the skeletons. The obtained meat was minced to increase its contact surface with the hot air in the oven and was heat-treated at 180°C until it reached proper dehydration, approximately 40% humidity. The product was then cooled to refrigeration temperature, packaged, and stored until use (Figure 3).

Qualitative and quantitative reception of salmon by-products
↓
Cleaning (removal of mechanical impurities)
↓
Laundering
↓
Mechanical extraction
↓
Shredding
↓
Heat treatment at 180°C
↓
Cooling to 0-4°C
↓
Packaging
↓
Storage

Figure 3. Technological scheme for obtaining grilled salmon meat

The grilled tuna meat was obtained by processing tuna by-products resulting from processing tuna into steaks. The processing procedure was similar to that of salmon, with the exception of the fat content concentration of the meat (Figure 4).

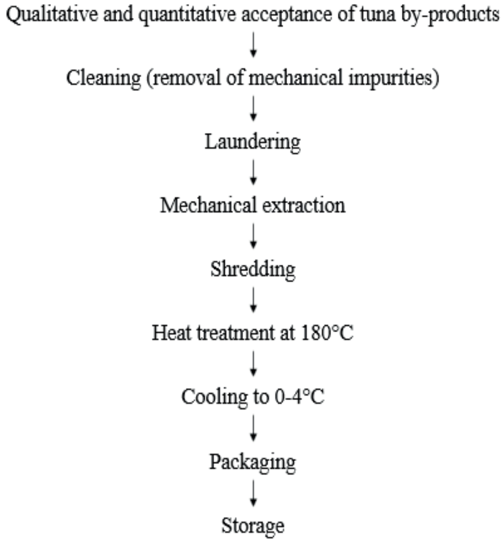

Figure 4. Technological scheme for obtaining grilled tuna meat

2. Consumer acceptance

The sensory evaluation of the fish crackers (with salmon oil and salmon or tuna meat) aimed to determine the sensory impact of including salmon and tuna by-products in the crackers.

The sensory properties of the crackers: external appearance, cross-section appearance, shape, color, taste, aroma, texture, recipe originality, were evaluated using a 5-point hedonic scale. The freshly made products were assessed by 101 participants of different ages, genders, and random social classes. In addition to rating the sensory properties, participants also expressed their decision to purchase or not the products under analysis.

The evaluation sheets were designed in accordance with the acceptability needs of these products in the market.

3. Determining nutritional value

To assess the nutritional and energy contribution of the crackers, as well as their integrity, it was necessary to establish the basic chemical composition, including the content of water, proteins, lipids, and minerals.

The nutritional content was determined utilizing the Softfedima program, a nutritional development tool available at http://softfedima.ro/. This tool facilitates the creation of nutrition facts panels, data sheets, and ingredient statements for various food products. Its formulas can be customized to account for moisture and/or fat content, and the resulting information can be easily printed or saved as a PDF document.

RESULTS AND DISCUSSIONS

1. Developing recipes and products

To streamline the utilization of salmon and tuna by-products, five fish biscuit recipes were created and studied: P1 - Crackers with salmon oil, P2 - Crackers with salmon oil and salmon meat, P3 - Crackers with salmon oil and tuna meat, P4 - Crackers with salmon oil, salmon meat, and dehydrated onion, P5 - Crackers with salmon oil, tuna meat, and dehydrated onion.

Innovation was based on the following considerations:

- Using by-products that could otherwise be lost or turned into waste.
- Using low-value by-products to increase their economic value.
- Innovating and diversifying fish-based products.

The ingredients used in the recipes were purchased from retail or wholesale food stores. After purchase, the fish by-products were processed to obtain the ingredients subsequently used in the biscuit composition.

Crackers are food products with low water content, where wheat flour is the main raw material. They must be crispy, tender, and tasty to be accepted by consumers. The addition of salmon oil, salmon meat, tuna meat, and atypical spices (dehydrated onion - in certain recipes) represents the innovative part of these new recipes.

The ingredient proportion for the salmon oil crackers (P1) was: 57.42% flour, 28.71% water, 8.83% salmon oil, 1.77% sugar, 1.24% salt, 0.88% granulated garlic, 0.62% dry yeast, 0.53% ground white pepper.

To obtain the P1 crackers, flour was placed in a bowl, a well was made in the center, and dry yeast, oil, lukewarm water, and sugar were added. The mixture was kneaded until it started to bind slightly, then salt, granulated garlic, and white pepper were added, and kneading continued until a homogeneous dough was obtained. The dough was covered and left to rise for approximately 1 hour. After rising, the dough was kneaded, rolled out, perforated, and portioned. The formed biscuits were then placed in a preheated oven at 180°C and baked for 10-12 minutes (until fully cooked). After baking, the biscuits were allowed to cool to room temperature, then packaged and stored until consumed (Figure 5).

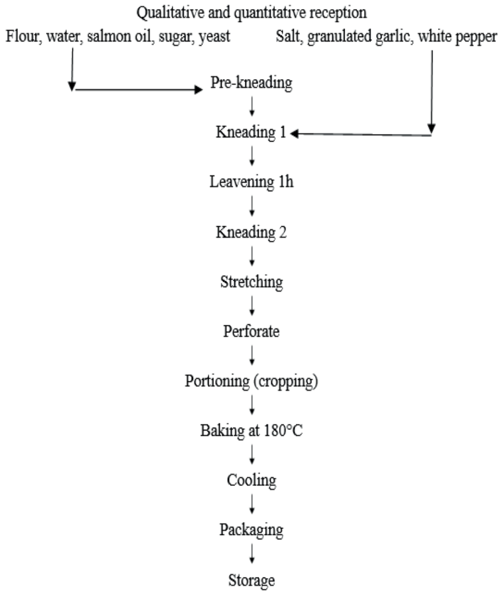

Figure 5. Technological scheme for obtaining crackers with salmon oil - P1

For the salmon oil and grilled salmon meat crackers (P2), the proportion of ingredients was as follows: 47.85% flour, 23.93% water, 16.67% grilled salmon meat, 7.36% salmon oil, 1.47% sugar, 1.03% salt, 0.73% granulated garlic, 0.52% dry yeast, 0.44% ground white pepper.

The technology for obtaining the P2 crackers was similar to that of the salmon oil crackers until after rising. During the second kneading, the grilled salmon meat was added. The dough was then rolled out, perforated, and portioned, and subsequently baked at 180°C for 10-12 minutes. After baking, the biscuits were cooled to room temperature, packaged, and stored (Figure 6).

For the salmon oil and grilled tuna meat crackers (P3), the proportion of ingredients was as follows: 47.85% flour, 23.93% water, 16.67% grilled tuna meat, 7.36% salmon oil, 1.47% sugar, 1.03% salt, 0.73% granulated garlic, 0.52% dry yeast, 0.44% ground white pepper.

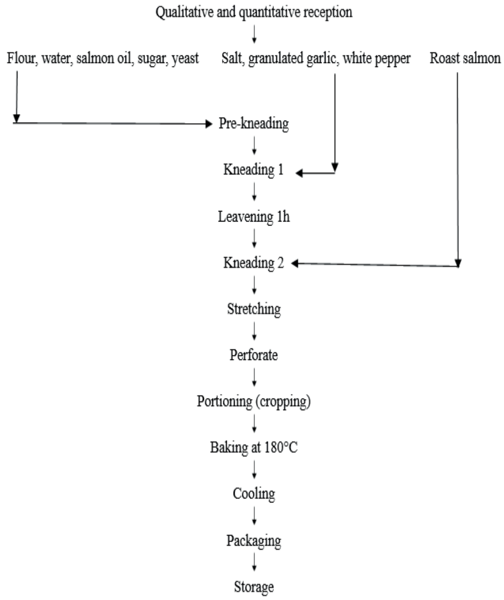

Figure 6. Technological scheme for obtaining crackers with salmon oil and salmon meat - P2

The technology for obtaining the P3 crackers was similar to that of the salmon oil and grilled salmon meat crackers. The difference lied in the type of meat added during the second kneading (Figure 7).

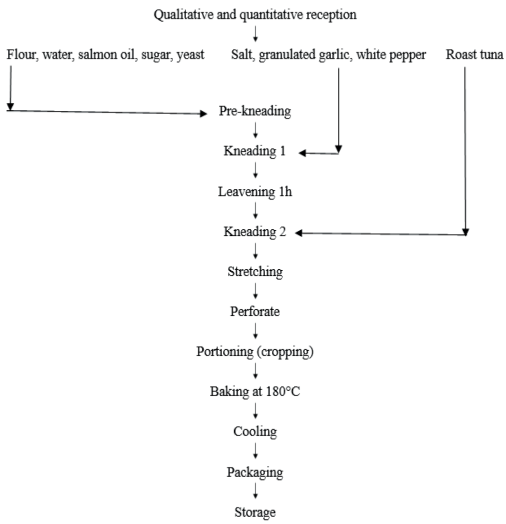

Figure 7. Technological scheme for obtaining crackers with salmon oil and tuna meat - P3

For the salmon oil, grilled salmon meat, and dehydrated onion crackers (P4), the proportion of ingredients was as follows: 45.94% flour, 22.97% water, 16% grilled salmon meat, 7.06% salmon oil, 4% dehydrated onion, 1.42% sugar, 0.99% salt, 0.7% granulated garlic, 0.5% dry yeast, 0.42% ground white pepper.

In terms of execution, the technology for obtaining the P4 crackers was similar to that of the salmon oil and grilled salmon meat crackers. The difference lied in adding dehydrated onion during the second kneading (Figure 8).

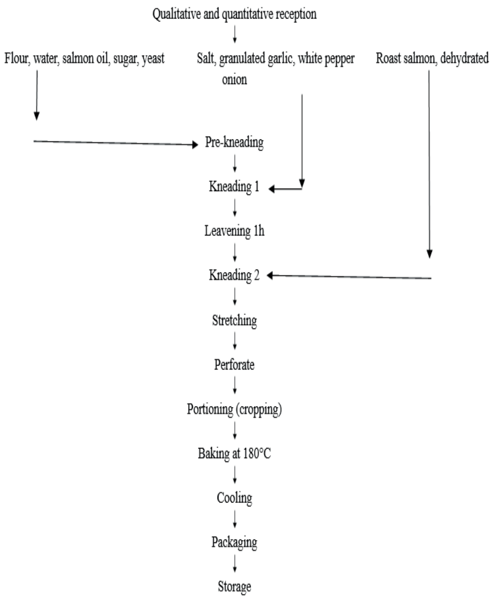

Figure 8. Technological scheme for obtaining crackers with salmon oil, salmon meat, and dehydrated onion - P4

For the salmon oil, grilled salmon meat, and dehydrated onion crackers (P5), the proportion of ingredients was as follows: 45.94% flour, 22.97% water, 16% grilled tuna meat, 7.06% salmon oil, 4.00% dehydrated onion, 1.42% sugar, 0.99% salt, 0.70% granulated garlic, 0.50% dry yeast, 0.42% ground white pepper.

The technology for obtaining the P5 crackers was similar to that of the salmon oil, grilled salmon meat, and dehydrated onion crackers, with the difference lying in the type of meat added during the second kneading (Figure 9).

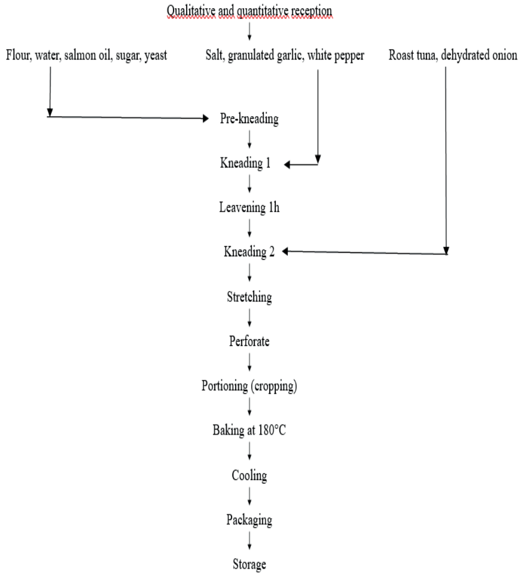

Figure 9. Technological scheme for obtaining crackers with salmon oil, tuna meat, and dehydrated onion - P5

2. Consumer acceptance

In the sensory evaluation study, all products were considered suitable for consumption and received positive evaluations. The majority of the evaluators expressed their willingness to purchase (100% for the salmon oil, grilled salmon meat, and dehydrated onion crackers - P4, 93.07% for the salmon oil crackers - P1, and the salmon oil and grilled salmon meat crackers - P2, 91.09% for the salmon oil and grilled tuna meat crackers - P3, and the salmon oil, grilled tuna meat, and dehydrated onion crackers - P5), supporting the proposal of these crackers for the food industry market.

Sample P1 (salmon oil crackers) was characterized by evaluators as having a pleasant appearance both externally and in cross-section, with the shape being relatively appropriate, and the characteristic color of the biscuits (Figure 10). The taste, aroma, and texture were what set these biscuits apart from regular ones. The taste of the fish oil crackers received a lower score. The aroma was evaluated similarly to the taste. The texture was deemed appropriate by the evaluators, being rated quite similarly across all five types of crackers.

The originality of the recipe received a high score due to its creative and innovative nature. Participants in the sensory evaluation considered sample P1 suitable for commercialization.

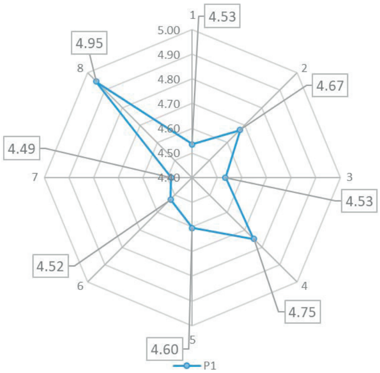

Figure 10. Sensory analysis of crackers with salmon oil (P1)

Sample 2 (salmon oil and grilled salmon meat crackers) was evaluated as having a pleasant appearance both externally and in cross-section, with the shape being relatively appropriate, and the characteristic color of the biscuits (Figure 11). The taste, aroma, and texture of this product were rated higher than in the previous case, being improved by the use of meat and fat from the same fish species. Like in the previous case, the product was considered original, and the purchase decision was favorable.

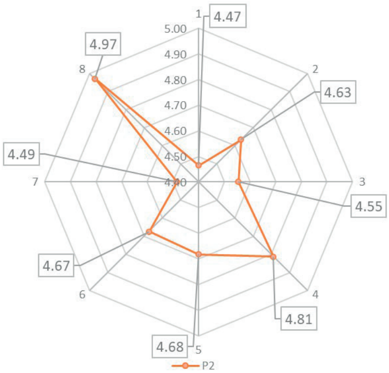

Figure 11. Sensory analysis of crackers with salmon oil and salmon meat (P2)

Sample 3 (crackers with salmon oil and tuna meat) was characterized as having a pleasant appearance both externally and in cross-section, with the shape being relatively appropriate. However, the color was noted to be more grayish compared to the previously described biscuits (Figure 12). The taste of the crackers was considered pleasant but less so than the previous ones. The aroma was appreciated as pleasant, and the texture was noted to be similar to sample P2. The product was deemed original, and the purchase decision was favorable.

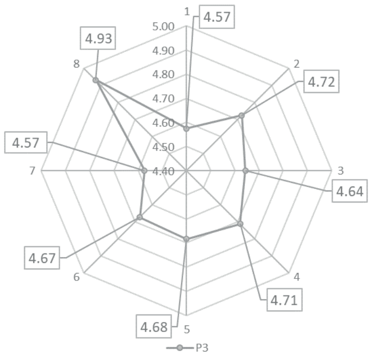

Figure 12. Sensory analysis of crackers with salmon oil and tuna meat (P3)

Sample 4 (crackers with salmon oil, salmon meat, and dehydrated onion) was evaluated as the best product among those analyzed (Figure 13). The product was characterized as having a pleasant appearance both externally and in cross-section, with a suitable shape, and a much more pleasant color than the previously analyzed samples. The taste was considered the most complex, being pleasant and unique. The aroma was deemed very pleasant, with the onion aroma complementing the intense salmon scent. The texture of the biscuits was also highly appreciated. As before, the product was valued as authentic, leading to a positive purchase decision.

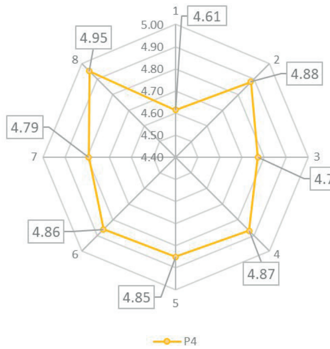

Figure 13. Sensory analysis of crackers with salmon oil, salmon meat, and dehydrated onion (P4)

Sample 5 (crackers with salmon oil, tuna meat, and dehydrated onion) was evaluated as the second-best in terms of quality (Figure 14). The external and cross-sectional appearance were considered very good, with a suitable shape. The color was more grayish than in sample 4 but more pleasant than in the other cases. The taste and aroma were considered of lower quality than sample 4 but higher than the other samples analyzed. The texture was deemed appropriate. As in previous cases, the product was considered original, with a favorable purchase decision.

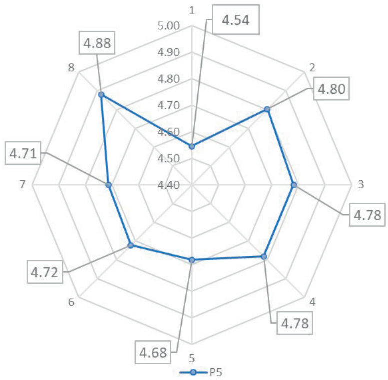

Figure 14. Sensory analysis of crackers with salmon oil, tuna meat, and dehydrated onion (P5)

Following the sensory analysis, it was found that all newly created products were accepted by consumers. Upon comparing all sensory analyses, it was found that the crackers with salmon oil, salmon meat, and dehydrated onion were considered the most relevant variant of fish crackers (Figure 15). They received an overall score of 4.82 out of a maximum of 5 points. In second place, with a score close to that of the crackers with salmon oil, salmon meat, and dehydrated onion, were the crackers with salmon oil, tuna meat, and dehydrated onion, which obtained an average score of 4.74. Crackers with salmon oil and tuna meat obtained an average score of 4.69 and were considered a middle-quality recipe. The second-to-last recipe in terms of quality was that of the crackers with salmon oil and salmon meat, with an average score of 4.66. Crackers with salmon oil proved to be the weakest in terms of quality, obtaining an average score of 4.63.

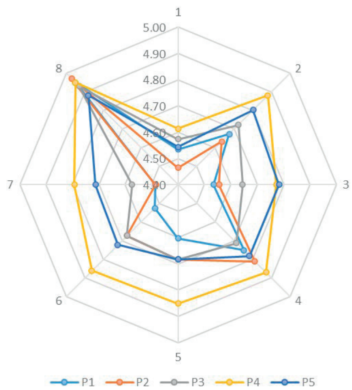

Figure 15. Comparative analysis of the five types of crackers from a sensory perspective

From the perspective of external appearance, crackers with salmon oil, salmon meat, and dehydrated onion obtained the highest average score (4.61), being considered the most appealing crackers. This result was followed by crackers with salmon oil and tuna meat (4.57), crackers with salmon oil, tuna meat, and dehydrated onion (4.54), crackers with salmon oil (4.53), and the weakest sample was that of crackers with salmon oil and salmon meat (4.47).
In terms of appearance in section, the best results were obtained by crackers with salmon oil, salmon meat, and dehydrated onion (4.88), followed by crackers with salmon oil, tuna meat, and dehydrated onion (4.8), crackers with salmon oil and tuna meat (4.72), crackers with

salmon oil (4.67), and crackers with salmon oil and salmon meat (4.63).
The most appreciated shape was that of crackers with salmon oil, tuna meat, and dehydrated onion, with an average score of 4.78, followed by crackers with salmon oil, salmon meat, and dehydrated onion (4.77), crackers with salmon oil and tuna meat (4.64), crackers with salmon oil and salmon meat (4.55), and crackers with salmon oil (4.53).
The most preferred color was that of crackers with salmon oil, salmon meat, and dehydrated onion (4.87), followed by crackers with salmon oil and salmon meat (4.81), crackers with salmon oil, tuna meat, and dehydrated onion (4.78), bisc crackers uits with salmon oil (4.75), and crackers with salmon oil and tuna meat (4.71).
In terms of taste, the most appreciated crackers were those with salmon oil, salmon meat, and dehydrated onion (4.85), followed by crackers with salmon oil and salmon meat, crackers with salmon oil and tuna meat, and crackers with salmon oil, tuna meat, and dehydrated onion (4.68), and crackers with salmon oil (4.60).
The most appreciated aroma was that of crackers with salmon oil, salmon meat, and dehydrated onion (4.86), followed by crackers with salmon oil, tuna meat, and dehydrated onion (4.72), crackers with salmon oil and salmon meat, and crackers with salmon oil and tuna meat (4.67), and crackers with salmon oil (4.52).
In terms of texture, the most appreciated crackers were those with salmon oil, salmon meat, and dehydrated onion (4.79), followed by crackers with salmon oil, tuna meat, and dehydrated onion (4.71), crackers with salmon oil and tuna meat (4.57), and crackers with salmon oil and salmon meat (4.49).
Regarding the originality of the recipe, crackers with salmon oil and salmon meat (4.97) were considered the most appropriate, followed by crackers with salmon oil, salmon meat, and dehydrated onion, and crackers with salmon oil (4.95), crackers with salmon oil and tuna meat (4.93), and crackers with salmon oil, tuna meat, and dehydrated onion (4.88).
In terms of expressing the buying decision, crackers with salmon oil, salmon meat, and dehydrated onion had a percentage of 100%, crackers with salmon oil, and crackers with salmon oil and salmon meat had a percentage of 93.07%, and crackers with salmon oil and tuna meat, and crackers with salmon oil, tuna meat, and dehydrated onion had a percentage of 91.09%.
Taking into account the rankings formed, but also the small differences between scores, it is considered that all products were suitable for consumption, but the most appreciated recipe was that of crackers with salmon oil, salmon meat, and dehydrated onion.
Analyzing Figure 15, it was concluded that crackers with salmon oil, salmon meat, and dehydrated onion (P4) were considered better than the other biscuit samples. Comparing the results obtained in the study with the results obtained by crackers with Nilem carp fish egg protein concentrate (Muslihudin et al., 2021), it is found that the crackers with salmon and tuna were appreciated as having more pleasant sensory characteristics.

3. Determining storage period based on packaging

The shelf life of fish crackers is crucial from both a food safety and economic perspective. Therefore, the duration during which the crackers maintained their consumption characteristics was determined. The newly created products were packaged and stored for 5 months at a temperature of 20-25°C.
All products underwent sensory analysis to determine their shelf life. Three types of packaging were used: paper, high-density polyethylene, and aluminum foil. All analyzed products exhibited similar sensory behavior.
The paper packaging proved to be inadequate and aesthetically unpleasing because the paper absorbed oil from the product. Additionally, its air permeability led to the oxidation of fats in the crackers.
Polyethylene packaging proved to be the most suitable packaging for biscuits as it did not absorb grease and was impermeable. Two packaging variants were tested: under normal atmosphere and under modified atmosphere (70% N2, 30% CO2).
The aluminum foil packaging was not suitable as it interacted with the biscuits, altering their color. Additionally, it was found to be not resistant to prolonged handling.
The polyethylene packaging was considered the most suitable option. The products retained

their sensory characteristics for 2 months under normal atmosphere and for 5 months under modified atmosphere.

4. Nutritional values

Nutritional values represent the ability of foods to provide the body with the nutrients it needs. They are an indicator of the quality of food products. In the case of fish crackers, according to the recipe and technological scheme, food ingredients with their own nutritional values have been introduced.

The basic chemical elements of salmon oil crackers (P1) have been determined (Table 1). Therefore, P1 crackers have a content of 6.8% protein, 10.2% lipids, 46.7% carbohydrates, 1.6% dietary fiber, and 1.3% mineral salts. After determining the basic chemical composition of the salmon oil biscuits, their energy value was calculated, which is 1299.7 kJ/309 kcal.

Table 1. Nutrition declaration of crackers with salmon oil (P1)

Nutritional values per 100 g	
Energy(calories)	1299.7 kJ
	309 kcal
Fat	10.2 g
- of which saturates	1.9 g
Carbohydrate	46.7 g
- of which sugars	1.9 g
Fiber	1.6 g
Protein	6.8 g
Salt	1.3 g

Following the determination of the basic chemical elements of salmon oil and salmon meat crackers (P2), it was found that they contain 10.1% protein, 9.7% lipids, 38.9% carbohydrates, 1.3% dietary fiber, and 1.1% mineral salts (Table 2). The energy value of the salmon oil and salmon meat biscuits was calculated to be 1202.3 kJ/285.9 kcal.

Table 2. Nutrition declaration of crackers with salmon oil and salmon meat (P2)

Nutritional values per 100 g	
Energy(calories)	1202.3 kJ
	285.9 kcal
Fat	9.7 g
- of which saturates	1.5 g
Carbohydrate	38.9 g
- of which sugars	1.5 g
Fiber	1.3 g
Protein	10.1 g
Salt	1.1 g

The crackers with salmon oil and tuna meat (P3) were analyzed for their content of basic chemical elements, resulting in the following composition: 11.6% protein, 9.8% lipids, 38.9% carbohydrates, 1.3% dietary fiber, and 1.1% mineral salts. Their energy value was calculated to be 1231.5 kJ/292.8 kcal (Table 3).

Following the determination of the basic chemical elements of crackers with salmon oil, salmon meat, and dehydrated onion (P4), it was observed that they contain 10.3% protein, 9.4% lipids, 39.7% carbohydrates, 1.3% dietary fiber, and 1% mineral salts. The energy value of crackers with salmon oil, salmon meat, and dehydrated onion was calculated to be 1208.2 kJ/287.2 kcal (Table 4).

Table 3. Nutrition declaration of crackers with salmon oil and tuna meat (P3)

Nutritional values per 100 g	
Energy(calories)	1231.5 kJ
	292.8 kcal
Fat	9.8 g
- of which saturates	1.5 g
Carbohydrate	38.9 g
- of which sugars	1.5 g
Fiber	1.3 g
Protein	11.6 g
Salt	1.1 g

Table 4. Nutrition declaration of crackers with salmon oil, salmon meat, and dehydrated onion (P4)

Nutritional values per 100 g	
Energy(calories)	1208.2 kJ
	287.2 kcal
Fat	9.4 g
- of which saturates	1.5 g
Carbohydrate	39.7 g
- of which sugars	3.4 g
Fiber	1.3 g
Protein	10.3 g
Salt	1 g

The analysis for determining the basic chemical elements of crackers with salmon oil, tuna meat, and dehydrated onion (P5) revealed that they contain 11.7% protein, 9.5% lipids, 39.7% carbohydrates, 1.3% dietary fiber, and 1% salt, with an energy value of 1235.7 kJ/293.7 kcal (Table 5).

Table 5. Nutrition declaration of crackers with salmon oil, tuna meat, and dehydrated onion (P5)

Nutritional values per 100 g	
Energy(calories)	1235.7 kJ
	293.7 kcal
Fat	9.5 g
- of which saturates	1.5 g
Carbohydrate	39.7 g
- of which sugars	3.4 g
Fiber	1.3 g
Protein	11.7 g
Salt	1 g

Comparative analysis of the five biscuit samples from a nutritional perspective (Table 6) revealed that the most energy-rich biscuits were those with salmon oil, followed by biscuits with salmon oil, tuna meat, and dehydrated onion, biscuits with salmon oil and tuna meat, and biscuits with salmon oil, salmon meat, and dehydrated onion. The least energy-rich were biscuits with salmon oil and salmon meat.

In terms of protein content, the richest in protein were biscuits with salmon oil, tuna meat, and dehydrated onion, followed by biscuits with salmon oil and tuna meat, biscuits with salmon oil, salmon meat, and dehydrated onion, and biscuits with salmon oil and salmon meat, with the least protein being in biscuits with salmon oil.

Regarding lipid content, the richest in lipids were biscuits with salmon oil, followed by biscuits with salmon oil and tuna meat, biscuits with salmon oil and salmon meat, biscuits with salmon oil, tuna meat, and dehydrated onion, with the least lipids being in biscuits with salmon oil, salmon meat, and dehydrated onion.

In terms of carbohydrate content, the richest in carbohydrates were biscuits with salmon oil, followed by biscuits with salmon oil, salmon meat, and dehydrated onion, and biscuits with salmon oil, tuna meat, and dehydrated onion, with the least carbohydrates being in biscuits with salmon oil and salmon meat and biscuits with salmon oil and tuna meat.

Regarding dietary fiber content, the richest in fiber were biscuits with salmon oil, while the others were equally poor in dietary fiber.

In terms of mineral salt content, the richest in salts were biscuits with salmon oil, followed by biscuits with salmon oil and tuna meat, and biscuits with salmon oil and salmon meat, while the poorest in salts were biscuits with salmon oil, tuna meat, and dehydrated onion, and biscuits with salmon oil, salmon meat, and dehydrated onion.

Table 6. Comparative analysis of crackers from a nutritional perspective

Specification	Nutritional values per 100 g				
	P1	P2	P3	P4	P5
Energy(calories)	1299.7 kJ	1202.3 kJ	1231.5 kJ	1208.2 kJ	1235.7 kJ
	309 kcal	285.9 kcal	292.8 kcal	287.2 kcal	293.7 kcal
Fat	10.2 g	9.7 g	9.8 g	9.4 g	9.5 g
- of which saturates	1.9 g	1.5 g	1.5 g	1.5 g	1.5 g
Carbohydrate	46.7 g	38.9 g	38.9 g	39.7 g	39.7 g
- of which sugars	1.9 g	1.5 g	1.5 g	3.4 g	3.4 g
Fiber	1.6 g	1.3 g	1.3 g	1.3 g	1.3 g
Protein	6.8 g	10.1 g	11.6 g	10.3 g	11.7 g
Salt	1.3 g	1.1 g	1.1 g	1 g	1 g

Following the nutritional analysis of biscuits with added salmon and tuna, it was found that the resulting biscuits were improved, as they had recipes without additives and were rich in nutrients. Compared to crackers with salmon and tuna by-products, crackers made from sturgeon fillet protein concentrate (Abraha et al., 2018) had a higher percentage of protein (14.63-19.52%) and fat (16.20-16.50%). Additionally, salted biscuits with tilapia protein concentrate (Ibrahim, 2009) had higher protein content (12.50 ± 0.07%) and fat (22.65 ± 0.19%) compared to crackers with salmon oil and salmon or tuna meat. Analyzing the newly created biscuits and comparing them with those in the specialized literature, it was found that the obtained recipes had lower nutritional values.

CONCLUSIONS

Fish by-products are a very good source of food ingredients.

The five newly created types of fish biscuits have been accepted by consumers and can be introduced into the food chain.

Biscuits with salmon oil, salmon meat, and dehydrated onion were the most highly appreciated by consumers, excelling in external appearance, cross-section appearance, color, taste, aroma, and texture, as well as in the expression of purchase decisions.

Biscuits with only salmon oil were rated the lowest, with low scores in all criteria except external appearance, cross-section appearance, color, and product originality.

Packaging in polyethylene packaging proved to be the optimal option for product protection. Packaging in a modified atmosphere extended the shelf life from 2 months to 5 months due to the inability of microorganisms to develop and the prevention of fat oxidation in the product due to the lack of oxygen.

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ANALYSIS OF THE MAIN CAUSES THAT MAY CONTRIBUTE TO THE DECLINE OF SOME ECONOMIC VALUE FISH STOCKS IN THE DANUBE RIVER

Kety BALACI[1], Maria Desimira STROE[2], Angelica DOBRE[2, 3], Monica MARIN[1], Dana POPA[1], Carmen Georgeta NICOLAE[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]Institute of Research and Development for Aquatic Ecology, Fishing and Aquaculture Galati, 54 Portului Street, 800211, Galati, Romania
[3]"Dunarea de Jos" University of Galati, 47 Domneasca Street, 800008, Galati, Romania

Corresponding author email: kety_popa@yahoo.com.au

Abstract

The paper presents the main causes that can contribute to the decrease of fish stocks with economic value in the Danube River, based on an analysis of both the recorded fishing catches and the quantities of fish retained following the control actions carried out by the authorities with the right of inspection and control with the aim of combating fish poaching, as well as the normative regulatory acts in the field of fishing. A broader perspective involves analysing the environmental factors and their role in the decline of economically valuable fish species in the Danube. From the analysed data, it was found that following the specific actions organized and carried out by the competent authorities, significant quantities of fish with economic value, originating from illegal fishing, were confiscated, thus resulting in the fact that both the regulations that sanction acts of poaching and the capacity of the responsible authorities are insufficient.

***Key words**: administrative capacity, environmental factors, fisheries management, illegal fishing, IUU.*

INTRODUCTION

The Danube River Basin is the second largest hydrographic basin in Europe, covering an area of 801,463 km^2. It hosts valuable ecosystems from ecological, economic, historical, and social perspectives, accommodating 79 million people from 19 countries with a wide range of cultures, languages, and historical backgrounds. This is why Padło et al. (2021), calls it "*a symbol of international cooperation and reconciliation between Eastern and Western Europe*". Furthermore, it harbours a rich variety of economically significant fish species.

However, human-induced pressures pose a key challenge for the management of the river (Hein et al., 2019). Severe pollution from agriculture, industry, and municipalities (Winiwarter et al, 2013), navigation, and invasive species (Wong et al., 2007), overfishing, affect water supply for communities, irrigation, hydro energy generation, and industry, as well as opportunities for transportation, tourism, and fishing. IUU fishing and climate change (Kalikoski et al., 2018) also represent major threats to the Danube River, its fish stocks, and have serious implications for the sustainability of commercial fishing and the river ecosystem as a whole.

Combatting IUU fishing has become a crucial means to achieve sustainable fisheries management globally (Samy-Kamal, 2022), a high priority on the international fisheries management agenda (Le Gallic & Cox, 2006; Samy-Kamal, 2022). The European Court of Auditors (2022) considers illegal, unreported, and unregulated fishing to be one of the most serious threats to marine ecosystems, negatively impacting both fish stocks and marine ecosystems as a whole. The European Parliament acknowledges the significant impact this phenomenon has on the environment, the economy (EPRS, 2022), food security (Swartz et al., 2010; FAO, 2018), defining it as a disruption to the fish product market and a disadvantage to responsible fishermen.

Climate change poses another serious threat to fish stocks in the Danube River, as well as to the capacity to manage water resources in the Danube River Basin.
In this work, we aim to investigate and analyse the main factors contributing to this deterioration of fish resources in the Danube, with a focus on the interconnection between illegal, unreported, and unregulated (IUU) fishing and environmental factors, particularly water level and temperature.
Firstly, we will assess the impact of illegal, unreported, and unregulated (IUU) fishing on fish stocks in the Danube. We will analyse the practices and methods to combat IUU fishing and their impact on fish stock health and river biodiversity.
Secondly, we will focus on the environmental factors influencing the dynamics and health of the Danube River ecosystem, with an emphasis on water level and temperature. We will examine the impact of climate change and anthropogenic modifications on the hydrological and thermal regime of the Danube and their consequences for fish resources.

MATERIALS AND METHODS

The analysis of the evolution of environmental factors in the lower sector of the Danube River is based on the daily data collected from 2020 to 2023 by the Autonomous Administration of the Lower Danube.
In analysing the actions taken by authorities to combat IUU fishing, data on reported catches, confiscated quantities, and illegal fishing were utilized from the database of the National Agency for Fisheries and Aquaculture. Additionally, a comparative analysis of the evolution of reported catches and total allowable catches from 2020 to 2023 was conducted, highlighting possible reasons underlying the downward trend in catches, with a focus on IUU fishing. To conduct this analysis, the joint orders approving the regulatory measures for fishing effort and fishing quotas allocated for each year, species, and zones were consulted (Order no. 45/539/2023 approving the regulatory measures for fishing effort and fishing quotas allocated for 2023, by species and zones; Order no. 42/558/2022 approving the regulatory measures for fishing effort and quotas allocated for 2022, by species and zones; Order no. 99/814/2021 approving the regulatory measures for fishing effort and quotas allocated for 2021, by species and zones; Order no. 124/1.159/2020 approving the regulatory measures for fishing effort and quotas allocated for 2020, by species and zones).
Data on reported catches by species were extracted from the National Agency for Fisheries and Aquaculture website. Statistical data processing was carried out using the Excel program from the Office 365 software package.

RESULTS AND DISCUSSIONS

Environmental factors analysis

The phenomenon of global temperature rises in recent years, with values ranging from 1.8 to 4.0°C, leads to changes in meteorological patterns and hydrodynamics, alterations in water levels and stratification, and even acidification of water bodies (Bradley et al., 2015; Madeira et al., 2016).

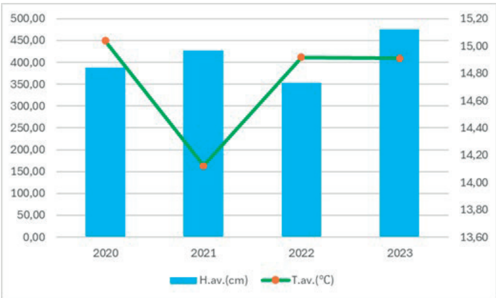

Figure 1. Evolution of environmental factor in the Romanian sector of the Danube River between 2020-2023

Variations in water level and temperature can significantly affect the habitat and life cycle of fish species, influencing their distribution, reproduction, and growth (Simionov, 2020). The relationship between the water level and temperature of the Danube River is inversely proportional during the analysed period (Figure 1). There is a significant fluctuation in the water level during the analysed period. In 2023, the water level (474.93 ± 52.23 cm) is the highest recorded compared to other years, while the minimum value was recorded in 2022 (353.42 ± 52.23 cm), suggesting a period of drought or reduced hydrological regime.

Water temperature shows a smaller variation compared to the water level, but it can have a significant impact on the metabolism and biological activity of fish. It is observed that in 2020, the water temperature (15.04 ± 0.42°C) is higher, which may influence fish reproduction and growth behaviour. In contrast, in 2021, the water temperature decreases (14.12 ± 0.42°C).

Illegal, Unreported and Unregulated Fishing

Illegal, unreported and unregulated (IUU) fishing is a broad term that encompasses various types of fishing activities undermining sustainable fisheries management. It emerged from the recognition that certain fishing activities, while not strictly qualifying as "illegal," also pose a threat to marine ecosystems and fisheries conducted in accordance with sustainable management rules (EPRS, 2022).

IUU fishing undermines conservation and sustainable management efforts for fishery resources, contributing to the overexploitation of fish species, degradation of aquatic habitats, and even affecting communities reliant on them (Petrossian, 2015).

From an economic standpoint, IUU fishing also has a direct impact on countries with developing economies, which together comprise approximately 79% of the world's countries (Petrossian, 2015). According to estimates, the trade associated with IUU fishing deprives developing countries of $9 billion annually, with $1 billion lost by African countries (Black, 2007).

A study conducted by the Marine Resources Assessment Group (MRAG, 2005) estimated that, as a result of IUU fishing, total losses in Guinea, Liberia, Mozambique, Kenya, Seychelles, Sierra Leone, Angola, Namibia, Somalia, and Papua New Guinea amounted to $372 million or 19% of the total catch value. Similarly, in the Asia Pacific Region, IUU catches represent approximately $5,8 billion annually, with total losses ranging from 3,5 to 8,1 million tons, or about eight to 16% of the total reported catch annually (Palma, 2010). Indonesia alone, one of the world's major fishing nations, loses approximately $4 billion annually in profits due to illegal fishing, while the economic loss to the Philippines is estimated at around $894 million annually (Palma, 2010).

In Romania, although there is no clear statistical data regarding IUU fishing, especially since our country's accession to the EU, efforts are being made by authorities to combat these activities. According to ANPA data, during the analysed period, a series of inspection and control actions were undertaken to monitor commercialization, processing, transportation, commercial fishing, recreational fishing, and aquaculture activities in the Danube River Basin, aiming to quantify the extent and consequences of this phenomenon on aquatic resources and to protect fish stocks.

The total number of inspection and control actions varied by year, with the highest number recorded in 2021 (14,803) and the lowest in 2023 (13,047). These actions resulted in the imposition of a significant number of administrative sanctions, as well as warnings to those involved in fishing and the commercialization of aquatic products (Figure 2). The number of criminal complaints and the preparation of criminal files indicate a continuous effort by authorities to enforce the law and sanction those involved in illegal activities.

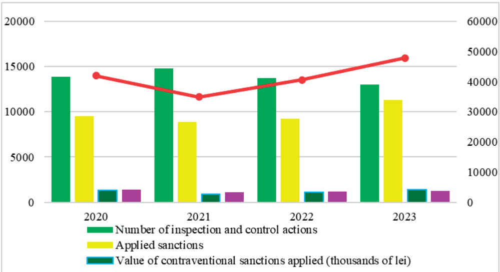

Figure 2. Variation of control and inspection results between 2020-2023

Regarding confiscations of aquatic products and their by-products, we observe a variety of fish species, including sturgeon, native and Asian cyprinids, predatory fish, and other species of fresh, frozen, or processed fish. The quantities confiscated and the values of sanctions applied have fluctuated over the years, with significant values recorded in 2022 and 2023. The distribution by species of the confiscated quantities is presented in Figure 3.

The presence of sturgeons among the confiscated species is certainly concerning and signals a serious issue in the management of fish resources in the Danube River basin. Sturgeons are valuable fish species, both in terms of nutrition and economics (Raposo et al., 2023), and they are vulnerable according to the IUCN Red List.

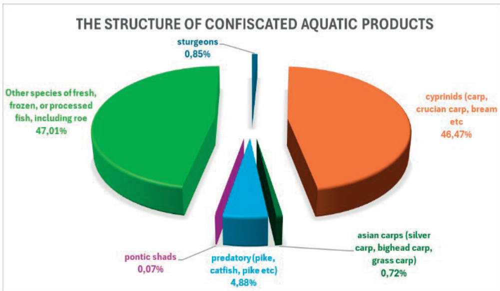

Figure 3. Confiscated aquatic products distribution

Fishing for sturgeons has become subject to strict regulations in many countries due to the dramatic decline in their populations (WWF Romania, 2018).

Article 64(b) of Emergency Ordinance No. 23/2008 on fishing and aquaculture and Article 3(a) of Joint Order No. 85/662/2021 on measures for the recovery and conservation of sturgeon populations in natural fish habitats explicitly prohibits sturgeon fishing for an indefinite period, acknowledging the importance of protecting these species. However, the presence of sturgeons among the confiscated species (Figure 3) suggests that there are still illegal and unregulated activities targeting these vulnerable populations.

The illegal fishing of sturgeons poses a serious threat to the conservation and recovery of these fish populations, endangering not only these species but the entire ecosystem of the Danube River. Sturgeons are known for their essential ecological role in maintaining the health of the aquatic ecosystem and for their contribution to biological diversity (Patriche, 2001).

Table 1. Total confiscated fishing tools and gears between 2020-2023

Specification	**Year 2020**	**Year 2021**	**Year 2022**	**Year 2023**	**Total**
Total confiscated fishing gear (pieces), including:	**20069**	**14926**	**15569**	**15459**	**66023**
Monofilament fishing nets (meters)	16346	10653	11421	9345	47765
Meshes	2162	1665	1618	1897	7342
Traps, cages	762	1748	1322	720	4552
Unknown/unspecified tools	711	789	1142	3398	6040
Recreational fishing gear	88	71	66	99	324
Confiscated vessels	**186**	**152**	**179**	**156**	**673**
Confiscated boat engines	**95**	**75**	**74**	**99**	**343**
Other means of transport confiscated	**23**	**23**	**30**	**31**	**107**

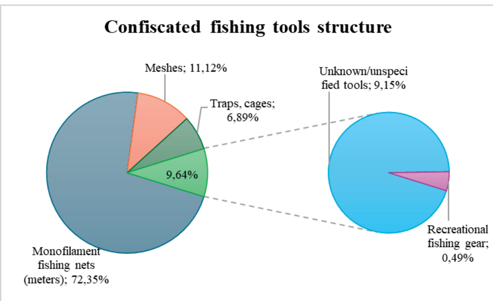

Figure 4. The structure of confiscated fishing tools

The confiscation of fishing gear and other tools used in illegal fishing activities is a crucial aspect in the fight against illegal, unreported, and unregulated (IUU) fishing and in protecting fish stocks in the Danube River Basin. Data on the confiscation of these tools during the period 2020-2023 (Table 1) shows a continuous effort by authorities to combat illegal activities and protect fish resources.

In total, 66,023 fishing gear items were confiscated during the analysed period (Figure 4). This includes a variety of gear such as monofilament nets, seine nets, traps, and cages, as well as unknown, artisanal, or nonspecific gear and recreational fishing gear. Additionally, 673 vessels and 107 other means of transport were seized.

Analysis of TAC vs. IUU

During the period 2020 - 2023, approximately 1,515.31 tons of economically valuable fish (carp, pikeperch, pike, catfish) were caught.

From this quantity, 43% was carp, 29% was catfish, 15% was pikeperch, and 13% was pike, highlighting the dominant nature of peaceful species, especially carp, in the catches made.

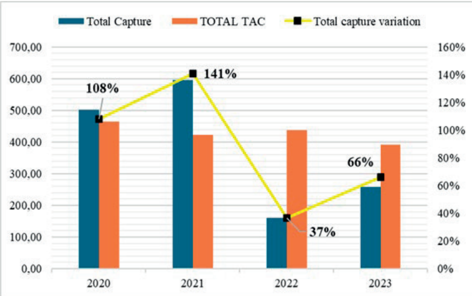

Figure 5. Evolution of important species captures and TAC

Analysing Figure 5, significant variations in reported catch values can be observed, indicating inconsistency in fishing activity, underreporting of catches, or even declines in fish stocks, which require further investigation to determine the cause.

Two opposing periods are highlighted: 2020-2021 records captures above the allowable limit with percentages of achieving the TAC at 108% and 141%, respectively, and the period 2022-2023 with reported catch decreases to 37% in 2022 and 66% in 2023.

In both cases, the situation is concerning. Percentages of TAC achievement exceeding 100% lead to overfishing. This may indicate the presence of illegal or unregulated fishing, where fishermen exceed legal limits to obtain more fish than allocated to them.

In cases where the percentages of TAC achievement are less than 100%, this may indicate either inefficient fishing management or underreporting of catches. Thus, low percentages of TAC achievement can also be an indication of IUU fishing, where a portion of the catch is taken but not reported to avoid regulation.

Extrapolating the results by species (Figure 6), the same pattern of exceeding limits in 2020 and 2021 is evident for three out of four analysed species (carp, pike, catfish), followed by sharp declines in reported catches, while for pikeperch, the percentage remains below unity throughout the period.

In addition to the factors mentioned so far, it is not negligible to consider the hypothesis that variation in climatic factors could be one of the reasons behind these concerning figures, given the variable meteorological and hydrological conditions recorded in recent years.

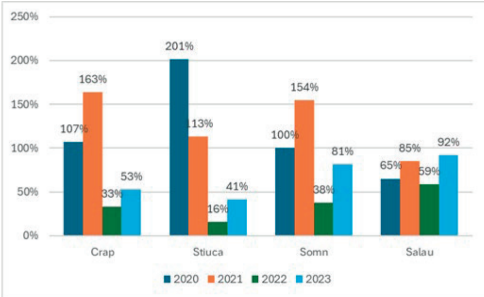

Figure 6. The evolution of Total Allowable Capture (TAC) achievement percentage for the most valuable species in the Danube River

Furthermore, considering the upward trend in actions taken by authorities (Figure 2), it can be said that one of the most important reasons for the decline in catches is their underreporting, either due to illegal fishing or other reasons, such as avoiding regulations or tax evasion. This suggests that the actual fish catch figures could be much higher than those officially reported.

CONCLUSIONS

The Danube River Basin is one of the most valuable water resources in Europe, with vast surface area and significant ecological, economic, historical, and social importance. It is a key region, home to millions of people from 19 countries, with significant cultural and linguistic diversity.

However, the anthropogenic pressures on the Danube River are significant and concerning. Pollution from agriculture, industry, and municipalities, intense navigation, and invasive species are just a few of the major threats facing this valuable ecosystem.

Illegal, unreported, and unregulated (IUU) fishing is a serious problem, undermining efforts for the conservation and sustainable management of fishery resources. The analysed data show that there are still illegal activities targeting vulnerable species, such as sturgeons, despite strict regulations prohibiting their fishing.

Efforts to combat IUU fishing must be intensified and consolidated, and control, monitoring, and sanction measures must be firmly implemented to protect fish stocks and the river ecosystem as a whole.

Additionally, climate change poses a serious threat, impacting water resources and fish habitats. Large variations in water level and temperature can significantly influence fish distribution and behaviour, with consequences for fishing and biodiversity.

In light of these findings, it is evident that an integrated and cooperative approach is necessary for the protection and sustainable management of fishery resources in the Danube River Basin. This involves concerted efforts at the local, national, and international levels, improving regulations and their enforcement, as well as promoting increased awareness and community engagement for the conservation of this highly valuable ecosystem.

By integrating these aspects, this study aims to provide a comprehensive understanding of the factors contributing to the decline of economically valuable fish stocks in the Danube and to identify efficient strategies and solutions for the conservation and sustainable management of these vital resources.

It is essential to address these complex challenges through an interdisciplinary approach and through the active involvement of authorities, local communities, and stakeholders in the decision-making process and implementation of measures for the protection and conservation of the Danube River ecosystem.

RECOMMENDATIONS

- ***Monitoring and control***: Implementing efficient monitoring systems for fishing activities, using modern technologies such as satellites and drones, can help detect and prevent IUU fishing.
- ***Legislative improvements***: Adopting and enforcing stricter regulations and harsher penalties for illegal fishing can deter such practices.
- ***Education and awareness***: Informing local communities and fishermen about the negative impact of IUU fishing and the importance of environmental conservation can encourage their involvement in sustainable practices.

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EFFECT OF WET-AGING WITH VITAMIN C ON QUALITY BIOMARKERS OF *Biceps femoris* MUSCLES COLLECTED FROM SUSTAINABLE MANAGEMENT OF *Cervus elaphuS* L. POPULATION FROM NORTHERN EASTERN CARPATHIANS, ROMANIA

Paul Corneliu BOIȘTEANU, Elena-Iuliana FLOCEA, Marius Mihai CIOBANU

"Ion Ionescu de la Brad" Iasi University of Life Sciences,
3 Mihail Sadoveanu Alley, 700490, Iasi, Romania

Corresponding author email: marius.ciobanu@iuls.ro

Abstract

*This work aims to investigate Wet-Aging with Vitamin C on chemical properties of Biceps femoris muscles collected from sustainable management of Cervus elaphus L. population from Northern Eastern Carpathians, Romania. 2% Alkaline Vitamin C Powder per 0.520 kg muscle sample, 4% Alkaline Vitamin C Powder per 0.504 sample kg, and 6% Alkaline Vitamin C Powder per 0.496 kg sample were used. Samples were protected from light and kept at 2°C for 10 days in order not to accelerate oxidative stress of the muscle samples. The influence of Vitamin C used in the wet-aging method was tested by performing quality biomarkers analyses. Data distribution was evaluated using SPSS Statistics 26.0 software. Non-parametric Independent Samples Kruskal-Wallis test was performed to analyze how the percentage of protein, water, collagen, and fat varied with the concentration of vitamin C in muscle samples. The results suggest that there are significant differences for quality biomarkers (***p < 0.001) in the percentage of fat, water, protein, and collagen in particular, between the percentages of 4.00 % and 6.00% Vitamin C introduced. Bonferroni correction was applied to counteract errors for multiple assays and to reduce the chance of an erroneous conclusion.*

Key words*: game meat, quality component, vitamin C.*

INTRODUCTION

In terms of nutritional value and organoleptic properties, game meat meets the demanding expectations of specialists (McMillin & Hoffman, 2009; Boișteanu et al., 2024).

Game meat is a rich source of bioactive compounds (Ciobanu et al., 2023). Conjugated linoleic acid (CLA) is found, as well as carnosine and anserine. It has been shown, that carnosine and its analogs, anserine and N-acetyl carnosine can significantly reduce infarct volume and improve neuronal function (Czarniecka-Skubina et al., 2022; Min et al., 2008). The quality of meat from species resulting from game management is influenced, according to the literature (Wiklund et al., 2010; Węglarz, 2010) by seasonal differences and physiological factors of the species. The color can be attributed to physical activities, stress, or dietary changes caused by the season (Neethling et al., 2017; Frunză et al., 2023). Differences in color between species are due to differences in myoglobin (Mb) content, proportion of muscle fiber type, and intramuscular fat content. Meat with a higher myoglobin (Mb) content also has a higher concentration of Iron, which in turn promotes the oxidation process (Farouk et al., 2007; Neethling et al., 2017). Intramuscular fat contributes to the sensory attributes of meat. The gender of the animal influences fat deposition, with an important impact on the flavor of the meat. Female game species assimilate protein differently and tend to accumulate more fat over time than males, which have a higher fat percentage at any chronological age (Ciobanu et al., 2022a).

Preservation processes in the case of meat from the exploitation of game are carefully chosen to sustain post-mortem quality for a longer time (Postolache et al., 2015). Maturation processes are consistent with improved tenderness and flavour Wet-aging, respectively Dry-aging is the most widely used preservation method in the meat industry (Ha et al., 2019). They influence juiciness, and flavors and ultimately

result in the desired palatability (Anchidin et al., 2023; Ciobanu et al., 2022b).
Velotto et al. demonstrated in 2015 the effects of two methods, Wet-aging and Dry-aging often used in the meat industry. They stated that both methods at 7 days post-mortem resulted in similar palatability. In contrast, sensory tests revealed differences, with the Wet-aging method compared to the Dry-aging method having a higher percentage of consumers as preference. A study of increased interest was that of Yu et al., 2023 who brought to our attention that Wet-Aging compared to Dry-aging resulted in improved water-holding capacity.

MATERIALS AND METHODS

The study included the game mammal species (*Cervus elaphus* L.) which was harvested in accordance with the Romanian National Legislation on Hunting, Hunting and Wildlife Protection (Hunting and Game Fund Protection Law no. 407/2006, 2010) during the hunting season 2022-2023 (winter), to control the population density in the area of hunting ground no. 24 in Frasin, Suceava, Romania. The available food during the cold season is limited, so supplementary food in concentrated (cereals, seeds) and succulent (beetroot, potatoes, and carrots) forms was administered. The samples taken for determination of chemical and physical analyses were m. *Biceps femoris* (duplicate samples/musk). According to the health inspection in the first 24 h postmortem, samples were collected, identified according to gender and age class, sealed in sterile bags, and transported to the laboratory under refrigerated conditions (0-5°C) (Regulation (EC) no. 853/2004 of the European Parliament and of the Council, 2004).
After the samples arrived in the laboratory, 100% natural alkaline Vitamin C powder (bioroots) was used: 100% sodium L-ascorbate. To avoid any conflict of interest for these products, the brand name of the manufacturer has been kept anonymous. We chose to use them in the study to investigate a scenario similar to a real case. According to Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives, for Ascorbic Acid (E 300) no dose limits are being quantum satis*. The experimental set-up has been designed by the Regulation. 2% Vit C at 0.520 kg sample, 4 Vit C at 0.504 kg Vit C, and 6% Vit C at 0.496 kg were used. Samples were refrigerated and separated from light so as not to accelerate the oxidative stress of muscle samples.
At 48 h post-mortem *Biceps femoris* samples were obtained, weighed, and vacuum packed in vacuum-embossed bags, 2 layers, inner layer 60 μm polyethylene suitable for food contact, outer layer 15 μm polyamide, UV filter. Samples were vacuumed with ATM Machinery, having a 630W chamber, refrigerated at 2°C, and separated from light for 10 days.
For biomarker quality analysis of the experimental samples after 10 days of Wet- Aging the samples were ground and homogenized using an electric grinder. Fat (%), water (%), protein (%), and collagen (%) contents were evaluated using the Omega Bruins Food-Check Near Infrared (NIR) spectrophotometer with infrared light beams (Bruins Instruments GmbH, Puchheim, Germany).
All statistical analyses were performed using the SPSS v.26 software package (SPSS Inc., Chicago, IL, USA). The non-parametric Independent Samples Kruskal-Wallis test was performed to analyse how the percentage of protein, water, collagen, and fat varied with Vitamin C concentration in muscle samples.

RESULTS AND DISCUSSIONS

According to the results presented in Table 1, application of the Independent Samples Kruskal-Wallis test to evaluate differences in the percentage of fat (%) of experimental Biceps femoris muscle samples according to the percentage of vitamin C added using the wet maturation method shows that there are significant differences (***p<.001) between the groups tested.

Table 1. Independent-Samples Kruskal-Wallis Test Summary Fat % with Vitamin C

Total N	**20**
Test Statistic	16.731[a]
Degree Of Freedom	3
Asymptotic Sig. (2-sided test)	<.001

[a]The test statistic is adjusted for ties.

As can be seen in Table 2, there are also significant differences (***p<.001) in water content between the groups tested. During the wet-aging process, Vitamin C can form bonds with water molecules, thus contributing to the maintenance of optimal moisture levels in muscle samples subjected to the Wet-Aging method. Vitamin C can form electrostatic interactions with water molecules via partial electric charges in its molecular structure. These interactions may contribute to the stability and cohesion of the system, helping to maintain moisture in muscle (Morrissey et al., 1998).

Table 2. Independent-Samples Kruskal-Wallis Test Summary Water % and Vitamin C

Total N	20
Test Statistic	16.722[a]
Degree Of Freedom	3
Asymptotic Sig. (2-sided test)	<.001

[a]The test statistic is adjusted for ties.

Vitamin C is known for its antioxidant properties. During the wet ripening process, vitamin C can protect proteins from oxidative stress in muscle caused by free radicals or other oxidative processes (Morrissey et al., 1998).

According to the data presented in Table 3, there are significant differences in the protein content of the analyzed samples with a high level of significance (***p<0.002).

Table 3. Independent-Samples Kruskal-Wallis Test Summary Protein % and Vitamin C

Total N	20
Test Statistic	14.531[a]
Degree Of Freedom	3
Asymptotic Sig. (2-sided test)	.002

[a]The test statistic is adjusted for ties.

These results suggest that Vitamin C in different concentrations (2%, 4%, 6%) had a significant impact on the protein level in the analyzed muscle samples. We can affirm the impact of Vitamin C and how it can protect biomolecules, such as proteins, against oxidative damage.

The results presented in Table 4 indicate that the percentage of vitamin C used in the wet aging process can influence the beffe content of experimental *Biceps femoris* muscle samples. This finding may be of importance in understanding the effects of wet aging and the role of Vitamin C in maintaining the structural integrity of collagen following the experimental process.

Table 4. Independent-Samples Kruskal-Wallis Test Summary Beffe % and Vitamin C

Total N	20
Test Statistic	15.016[a]
Degree Of Freedom	3
Asymptotic Sig. (2-sided test)	.002

a. The test statistic is adjusted for ties.

In the graphical configuration shown in Figure 1 one can see the differences between the level of quality biomarkers (%) as a function of the percentage of Vitamin C (Control Sample (0), 2%, 4%, 6%). In the case of the Fat level, as the percentage of Vitamin C increases, the level of Vitamin C also increases. In the case of water, protein, and collagen the process is reversed, increasing the percentage of Vitamin C up to 6% decreases the amount of water in the test samples significantly.

From Table 5, one can see the significant differences between .00%-4.00% Vitamin C and 0.00%-6.00% Vitamin C at the fat level. The percentage of 6.00% Vitamin C significantly increased the amount of fat.

Table 5. Pairwise Comparisons of Vitamin C-Fat%

Sample1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.[a]
.00%-2.00%	-4.900	3.703	-1.323	.186	1.000
.00%-4.00%	-8.900	3.703	-2.403	.016	.098
.00%-6.00%	-14.600	3.703	-3.942	<.001	.000
2.00%-4.00%	-4.000	3.703	-1.080	.280	1.000
2.00%-6.00%	-9.700	3.703	-2.619	.009	.053
4.00%-6.00%	-5.700	3.703	-1.539	.124	.743

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .050. [a]Significance values have been adjusted by the Bonferroni correction for multiple tests.

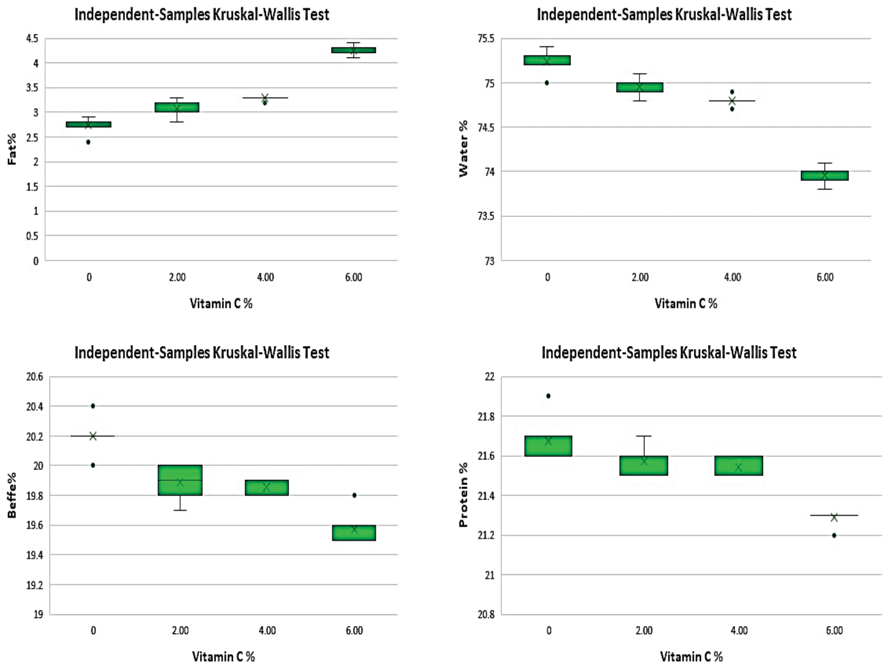

Figure 1. Level of quality biomarkers (Fat %), Water (%), Beffe (%), and Protein (%) as a function of the percentage of added Vitamin C (Control Sample (0), 2%, 4%, 6%)

Table 6. Pairwise Comparisons of Vitamin C-Water%

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig[a]
6.00%-4.00%	5.600	3.718	1.506	.132	.792
6.00%-2.00%	9.800	3.718	2.636	.008	.050
6.00%-.00%	14.600	3.718	3.927	<.001	.001
4.00%-2.00%	4.200	3.718	1.130	.259	1.000
4.00%-.00%	9.000	3.718	2.421	.015	.093
2.00%-.00%	4.800	3.718	1.291	.197	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .050. [a]Significance values have been adjusted by the Bonferroni correction for multiple tests.

In the case of Water level, according to Table 6, the most significant differences are observed between 00%-4.00% Vitamin C and .00%-6.00% Vitamin C. The Water level decreases with increasing percentage of Vitamin C added to the Wet-aging method.

Table 7. Pairwise Comparisons of Vitamin C-Protein%

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig[a]
6.00%-4.00%	7.200	3.643	1.976	.048	.289
6.00%-2.00%	9.200	3.643	2.525	.012	.069
6.00%-.00%	13.600	3.643	3.733	<.001	.001
4.00%-2.00%	2.000	3.643	.549	.583	1.000
4.00%-.00%	6.400	3.643	1.757	.079	.474
2.00%-.00%	4.400	3.643	1.208	.227	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .050. [a]Significance values have been adjusted by the Bonferroni correction for multiple tests.

Protein level as a function of Vitamin C percentage shown in Table 7 varies significantly in the experimental samples between 6.00%-2.00% Vitamin C and .00%-6.00% Vitamin C. Similar case according to Table 8 and at collagen level, significant differences between .00%-6.00% Vitamin C.

Table 8. Pairwise Comparisons of Vitamin C-Collagen (%)

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.[a]
6.00%-4.00%	6.400	3.699	1.730	.084	.502
6.00%-2.00%	7.300	3.699	1.973	.048	.291
6.00%-.00%	14.300	3.699	3.866	<.001	.001
4.00%-2.00%	.900	3.699	.243	.808	1.000
4.00%-.00%	7.900	3.699	2.136	.033	.196
2.00%-.00%	7.000	3.699	1.892	.058	.351

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .050. [a]Significance values have been adjusted by the Bonferroni correction for multiple tests.

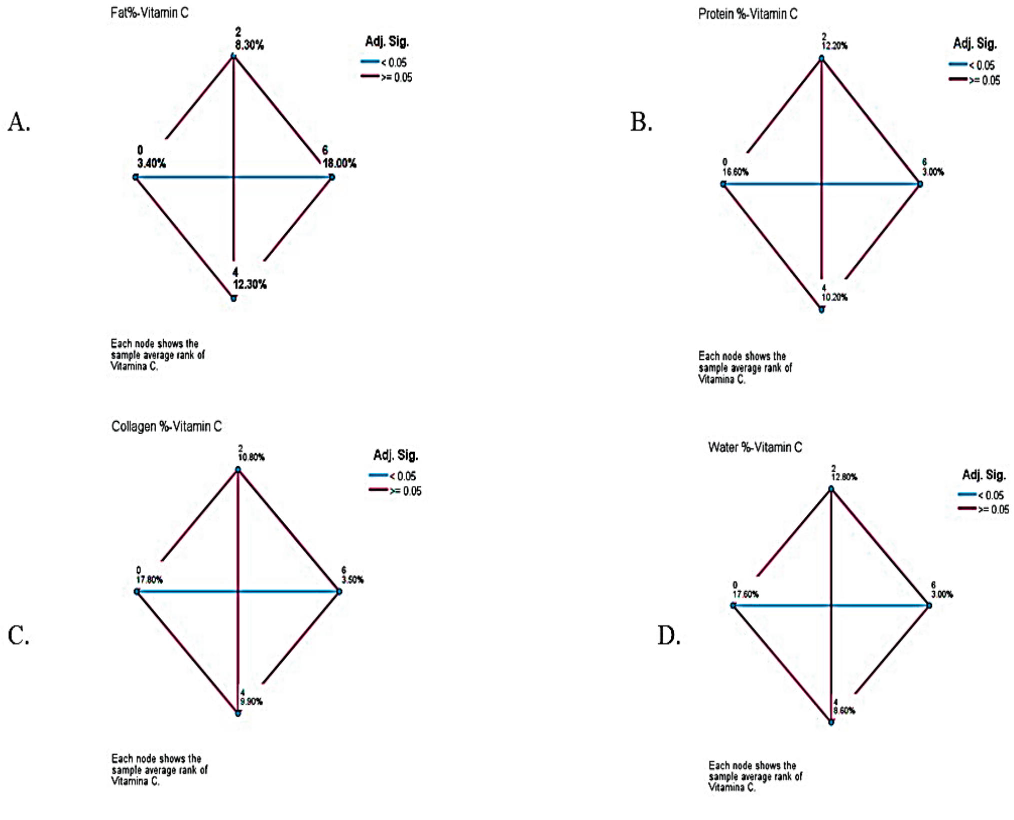

Figure 2. Mean Rank Distribution of the percentages of 0%, 2%, 4%, and 6% Vitamin C in the muscle samples tested to control the level of A (Fat), B (Protein), C (Collagen), and D (Water)

The mean rank of Vitamin C percentages is a statistical measure that indicates the average position of a Vitamin C concentration under study in the whole data set. According to Figure 2, Vitamin C is the variable of interest, which in this study refers to the Vitamin C concentration in the muscle samples. According to the distribution of the Mean Rank

Distribution of the percentages of 0%, 2%, 4%, 6% Vitamin C in the muscle samples tested for controlling the level of A (Fat %), B (Protein %), C (Collagen) and D (Water), for all the parameters tested significant differences are observed between the control sample (0.00% Vitamin C) and the sample with 6.00% Vitamin C.

CONCLUSIONS

The presented results significantly conclude the differences in biomarkers of quality, and the presence of different percentages of Vitamin C in the Wet-Aging method for *Biceps fermoris* muscle samples from *Cervus elaphus* L. The results suggest that there are significant differences for fat, water, protein, and collagen in the percentage (***p < 0.001) of in particular, between the percentages of 4.00 % and 6.00% Vitamin C introduced. The Wet-Aging method with Vitamin C is an efficient and sustainable technique for improving the quality of raw material. However, the final quality of the meat depends on other factors such as biological factors of the species and processing conditions.

In future studies, we aim to test the antioxidant activity of vitamin C in the targeted muscle samples.

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EFFECT OF LIGHT INTENSITY ON GROWTH PERFORMANCE, PHYSIOLOGICAL STATE AND TISSUE COMPOSITION OF *Polyodon spathula* (Walbaum, 1792) JUVENILES

Marian Tiberiu COADĂ[1, 2], Elena SÎRBU[4], Neculai PATRICHE[4], Victor CRISTEA[2, 3], Mirela CREȚU[1, 3, 4], Alina ANTACHE[1, 5], Săndița PLĂCINTĂ[1], Veta NISTOR[4], Magdalena TENCIU[4]

[1]"Dunărea de Jos" University of Galați, Faculty of Food Science and Engineering, 47 Domnească Street, 800008, Galați, Romania
[2]"Dunărea de Jos" University of Galați, Cross-Border Faculty, 800008, Galați, Romania
[3]"Dunarea de Jos" University of Galați, Faculty of Food Science and Engineering, Romanian Center for Modelling Recirculating Aquaculture Systems, 800008, Galați, Romania
[4]Institute for Research and Development in Aquatic Ecology, Fishing and Aquaculture, 54 Portului Street, 800211, Galati, Romania
[5]Alexandru Ioan Cuza University, Faculty of Biology, Department of Biology, 700506, Iasi, Romania

Corresponding author emails: sirbu.elena@asas-icdeapa.ro, magdalena.tenciu@asas-icdeapa.ro

Abstract

This study aimed to investigate the effect of light intensity on the growth performance, hematological profile, and biochemical composition of muscle tissue of paddlefish juveniles, reared in a recirculating aquaculture system (RAS). Two experimental variants were created: V1- where the rearing units have green color, and the average of the light intensity was 105 lx (mean fish weight was 21.65 ± 0.6 g), and V2- where the rearing units have white color, and the average of the light intensity was 30 lx (mean fish weight was 22.15 ± 0.8 g). Fish were fed at an intensity of 2.5% BW for 48 days. Growth of juvenile paddlefish under 30 lx was significantly better than that of paddlefish under 105 lx (p < 0.05), as indicated by final weight (1158.20 ± 21.90 g), specific growth rate (2.18% ± 0.07%/day) and feed conversion coefficient (0.80 ± 0.28 g/g). In conclusion, the paddlefish juveniles can adapt to various light intensities and grow favorably under low light conditions in a recirculating aquaculture system.

Key words: *growth performance, hematology, light intensity, paddlefish, recirculating aquaculture systems (RAS).*

INTRODUCTION

Growing fish outside their natural habitat can negatively impact their appetite for food, health, welfare, and overall growth if environmental conditions differ from the optimal conditions for the farmed species. The physiology of fish is affected by light intensity, time of exposure to light, and color of the rearing environment (Coadă, 2012).

Artificial environments such as rearing systems that are very different from natural fish habitats can negatively affect fish feeding activity, fish health, welfare, and growth, especially if conditions are stressful for the fish (De Silva & Anderson, 1994; Jobling, 1994; Brännäs et al., 2001). Some possible environmental factors influencing the performance of cultured fish include tank color and light intensity.

Some studies point to the fact that light is the result of a combination of different external and ecological factors, including color spectrum (quality), intensity (quantity), and day-night period (Downing, 2002).

The exposure of juvenile beluga to red ambient light negatively affects growth performance, compared to exposure to blue light (Bani et al., 2009). The color of the growth unit and the environmental conditions in a given genre can influence it (Barcellos et al., 2009). Choosing suitable colors for growth units can be a beneficial practice in aquaculture, especially during the larval stage. Most teleost fish are visual foragers during this developmental phase. This visual behavior can lead to a decrease in feed assimilation, subsequently reducing feed conversion efficiency and increasing mortality. The appropriate color of rearing units allows for the creation of contrast between the feed and tank walls, thereby enhancing feed intake assimilation by larvae and juvenile fish (Martin-Robichaud, 1998; Tamazouzt et al., 2000; Rotllant et al., 2003).

Popular color shades for growing units are as follows: white, yellow, green, dark/dark blue, red, and black. In the case of juvenile carp, better growth was obtained in the blue tank, but the results were insignificant, compared to the other rearing units. Another study carried out on juvenile carp (100-160 g) showed that there were no significant differences between batches of juvenile carp reared in white, black, and green tanks. Several studies have highlighted that marine fish in the larval stages show a preference for darker rearing units (Papoutsoglou et al., 2000; Naas et al., 1996).

Studies on Nile tilapia maintained in a white-rearing medium have reported color-induced metabolic effects, resulting in higher respiration rates compared to black, blue, green, yellow, and red-rearing conditions (Fanta, 1995).

The literature demonstrates that fish can adapt to different colors of the rearing environment but may change their integument color, a characteristic that could have detrimental effects on marketing (Vissio et al., 2020).

Since the introduction of American paddlefish into the Soviet Union in the 1970s, paddlefish aquaculture expanded to several European and Asian countries, with a wild population now established in the Danube River / Black Sea watershed (Naiel et al., 2021).

Some studies of paddlefish farming in Europe indicate that paddlefish aquaculture will continue to expand, although it is challenged by the suitability of the species for high-intensity production (Jarić et al., 2018). In the past, there has been significant research into paddlefish farming in Russia, Moldova, and Romania, after which farming began in commercial farms for meat production (Vedrasco et al., 2001).

Although *Polyodon spathula* (Walbaum, 1792) is a valuable fish species, there were no studies regarding its growth and adaptation to light intensity. In this context, this study aims to investigate the effects of light intensity on the growth and stress response of this species. Furthermore, our study seeks to provide knowledge on rearing this species in recirculating aquaculture.

MATERIALS AND METHODS

Fish and Experimental Design

The biological material used in the experiment was represented by juvenile *Polyodon spathula* (Walbaum,1792), provided by SCDP Nucet, Dâmbovița. A number of 78 fish aged four months, with an average individual weight of 20.9±5.5 grams, were distributed in four rearing units (two white and two green, respectively. The population of the breeding units was followed after weighing and biometric measurements to obtain an equal density and a batch with a normal distribution.

a) The growing module contains four Ewos-type units measuring 1.4 × 1.4 × 0.6 m. Each rearing unit's water column, basin shape, and water volume meets the technological requirements specific to the species *Polyodon spathula*.

b) Water quality conditioning units aim to reduce water consumption by improving water quality, keeping it within the optimum range of the main physicochemical parameters: dissolved oxygen, ammonia nitrogen concentration, solid particle concentration, pH, and carbon dioxide. Dissolved oxygen (DO) requirements of the culture species were insured by an oxygenation aeration unit, represented by a Hagen compressor with a capacity flow rate of 1.5 m^3/h. At an intermediate period of three days, the physicochemical parameters remained within the optimal range by replacing 15-20% of the water volume.

c) Throughout the experimental period, juvenile paddlefish were fed Classic Extra 1P pelleted, with a protein concentration of 45% and lipids of 12%. The total amount of feed daily was administrated manually in six equal rations and represented 2.5% of overall fish biomass.

Monitoring water quality in terms of physicochemical parameters

Monitoring of water quality was established during the whole experiment period through periodic determinations for ammonia nitrogen compounds and daily for temperature, pH, and dissolved oxygen. The monitoring of these relevant water quality parameters was made using the Hanna HI 98,186 (HANNA Instruments, Cluj-Napoca, Romania) for temperature and dissolved oxygen, while pH was measured with the pH meter WTW, 340 (Sigma-Aldrich, Darmstadt, Germany). For determination of the concentrations of nitrogen compounds, we use the Spectroquant NOVA 400 portable spectrophotometer (Merck general laboratory equipment, Enschede, Netherlands), with compatible kits from Merck (Merck laboratory equipment, Enschede, Netherlands).

The experiment evaluated the effect of growth unit color and light intensity on growth performance, biochemical composition of meat, and physiological response, for 48 days. Brightness was adjusted using translucent polycarbonate plates (6 mm). Light exposure of the growth units is based on natural photoperiodicity. Thus, the white units covered with white transparent polycarbonate light intensity have been observed with an average daily value of 105 lx and a maximum of 350 lx. In the green growing units covered with green transparent polycarbonate, the average daily value was around 30 lx, with a maximum of 80 lx. The measuring of light intensity has been measured with the Lxmeter TESTO 545 (Testo AG, Lenzkirch, Germany).

Growth performance assessment

Biotechnology indicators are essential to obtain information on the resulting technological performance and the efficiency of the breeding system used. The principal biotechnological parameters used to determine the growth performance of *Polyodon spathula* in the present study are described by Sîrbu et al. (2022).

Blood sampling and screening procedures

Examination of the blood profile of fish can provide insights into the physiological condition and health status of fish in aquaculture. Therefore, hematology, combined with other routine diagnostic methods, could prove valuable information in identifying and evaluating conditions that cause stress or diseases affecting production performance. At the end of the experimental period, while ensuring the integrity of the technological aspect of the experiment, approximately 2.5 ml of biological blood samples were collected from ten fish/rearing units. This sampling represented 50% of the fish population in each rearing unit and was conducted by manipulating juvenile paddlefish under strict biosecurity conditions. The blood sampling of fish was collected by caudal vein puncture along the lateral line. The hematological parameters analyzed were red blood cell count - RBCc ($x10^6$ cells/µL), hemoglobin - Hb (g/dL), and hematocrit – PVC (%). Blood analysis was performed by a method used in fish hematology described by Sîrbu et al. (2022).

The relative and absolute number of leukocytes has been performed to evaluate modifications under the influence of different light intensities in the leukocyte profile and are described by Dima et al. (2022). For the determination of total serum protein (g/dL) and glucose (mg/dL) VetTest® Chemistry Analyzer and IDEXX VetTest kits (IDEXX Laboratories, Inc., Westbrook, ME, USA) were used. To minimize the impact of diet on the results of biochemical and hematological indices, the fish were not fed on the day preceding the sampling.

Methods for determining the biochemical constituents of fish meat

The biochemical analyses of body composition (for each experimental variant, considered for the average weight of the individuals) were performed at the end of the experiment. Tissue biochemical determinations of juvenile paddlefish reared in a recirculating system were used to estimate feed retention capacity. Diet utilization and the influence of growth system environmental conditions were assessed by analysis of the main biochemical parameters: crude proteins, lipids, and ash. Proteins were determined with Gerhardt equipment by using the Kjeldahl method, fats were determined by Soxhlet solvent extraction method (petroleum ether) with Raypa extraction equipment (C. Gerhardt GmbH & Co. KG, Königswinter, Germany), the dry matter was determined by heating at a temperature of 105±2ºC using Sterilizer Esac (Systec GmbH & Co. KG, Linden, Deutschland) and ash was evaluated by calcification at temperatures of 550±20°C, in a Nabertherm furnace (Nabertherm GmbH, Bremen, Germany).

Methods of statistical data processing

Statistical analyses of the biotechnology, hematological, and biochemical indicators obtained have been processed statistically in Microsoft Office Excel 2019 and SPSS 26.0 (SPSS Inc., Chicago, IL). Statistical differences between variables were tested using a *t*-test. All the results are expressed as means ± standard deviation (SD). Results with a $p<0.05$ were considered significant.

RESULTS AND DISCUSSIONS

Several research investigations have shown that different colors of the rearing environment can influence the growth performance and health of fish (Coadă, 2012).

Assessment of dynamic water quality parameters

Temperature. The species *Polyodon spathula* tolerates a relatively large temperature range of 0-35°C, allowing it to distribute to other continents. During the experiment, the temperature variation in the two experimental variants was within the optimal tolerance range of sturgeon growth, 20.9÷25.8°C with an average of 22.8±1.76°C in the growth units of the white variant (V1), and in the green variant (V2) temperature range of 20.6÷25.2°C at an average value of 22.6±1.8°C (Figure 1), without any statistical differences ($p>0.05$) between the two experimental variants (Figure 1).

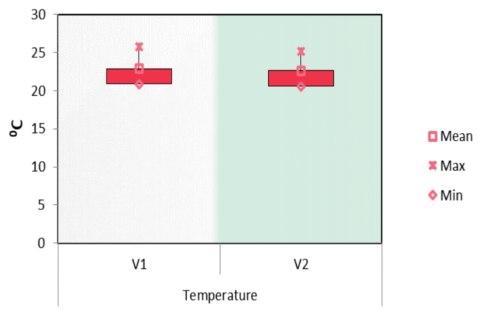

Figure 1. Temperature variation values for the two experimental variants

Dissolved oxygen. The values of DO are illustrated in Figure 2. During the analyzed period, DO values ranged from 9 to 11.87 mg/L, with an average of 10.6±1.32 mg/L in the white variant (V1). In the green variant (V2), DO values fluctuated between 8.9 and 13.9 mg/L, with an average of 11.3±2.28 mg/L. (Figure 2). No statistical differences ($p>0.05$) were recorded between the V1 and V2 variants.

The pH was kept within optimal limits for sturgeon growth, ranging from pH 8.03 (in the white variant) to pH 8.9 (in the green variant), without any statistical differences (p>0.05) between the experimental variants (Figure 3).

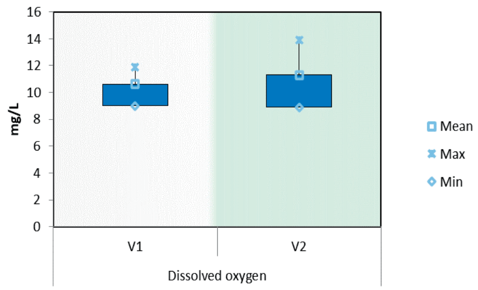

Figure 2. Dissolved oxygen variation values for the two experimental variants

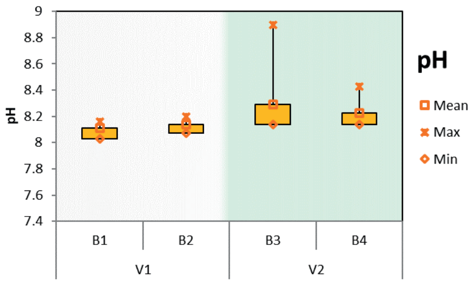

Figure 3. pH variation values for the two experimental variants

Regarding the ammonium values, the statistical analysis showed significantly higher concentrations (p>0.05) in the B4 basin (0.17 mg/L), while no significant differences (p>0.05) were recorded in the concentration registered in B1 (0.07 mg/L), B2 (0.05 mg/L) and B3 respectively (0.11 mg/L) (Figure 4).

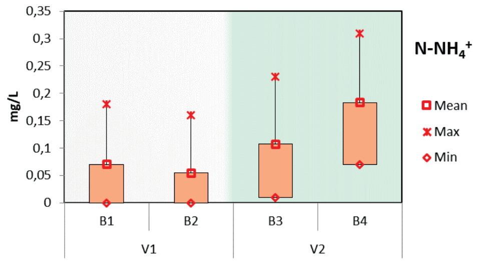

Figure 4. Ammonium ion variation values for the two experimental variants

Across the two experimental variants, the average nitrate concentration was significantly higher ($p<0.05$) in variant V2 (36.35±6.9 mg/L), compared to variant V1 (where an average value of 26.75±5.64 mg/L was recorded) (Figure 5).

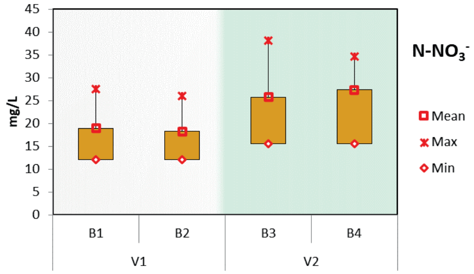

Figure 5. Nitrate variation values for the two experimental variants

During the experimental period, nitrite showed a similar growth trend as nitrate. A significantly higher valuer ($p<0.05$) was recorded in the case of green growth units (variant V2), (with an average value of 0.08 mg/L in B3 and 0.17 mg/L in B4), while in the white growth units (B1 and B2), significant ($p<0.05$) lower values were recorded (an average value of 0.04 mg/L and 0.034 mg/L) (Figure 6).

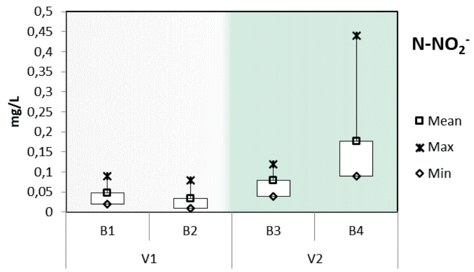

Figure 6. Nitrite variation values for the two experimental variants

Assessment of biotechnology indicators

The rearing of *Polyodon spathula* in a recirculating aquaculture system under the action of light intensity aims to obtain superior biotechnological indicators. These can be used to assess physiological conditions and growth performance. The growth rate of juvenile paddlefish in the white variant was hindered by high light intensity (V1). As a consequence, the mean individual mass, recorded at the end of the experiment, was smaller in the white-rearing units (B1 47.3 g/fish respectively B2 37.3 g/fish) compared to that registered in the green-rearing units (B3 63.2 g/fish respectively B4 61 g/fish) (Table 1).

Regarding the technological plasticity of the species, the influence of growth unit color and light intensity on the final biomass obtained in both experimental variants can be observed. As a result, in the green variant (V2), the juvenile paddlefish increased from initial biomass of 1.05 kg/m^3 to 2.95 kg/m^3, and in the white variant (V1), it grew from 1.08 kg/m^3 to 2.05 kg/m^3.

To identify any significant differences in total growth gain within and between experimental variants, the Pairwise Comparisons Test was applied (Table 2).

In this study, light intensity played a significant role ($p<0.05$) in the growth of juvenile paddlefish. The results obtained in the present study showed that light intensity significantly affected the growth of paddlefish, and the highest performance resulted in the 30lx light intensity green variant - V2. Differences in morphology and behavior, such as the presence of rostrum and the digestive and filtering mechanism of paddlefish compared to sturgeon, mean that the response to foraging is different, which also complicates nutritional issues therefore, adequate light intensity could improve feeding efficiency.

Table 1. Performance indicators show the evolution of growth gain in both experimental variants

Indicators	Experimental Phase	Variant V1 B1	B2	Mean	Variant V2 Mean	B3	B4
Total biomass [g]	Initial	429.6	436.5	433.05	421.4	421.9	420.8
	Final	897.04	746.7	821.87	1179.1	1200.06	1158.2
Total biomass [kg/m^3]	Initial	1.07	1.09	1.08	1.1	1.06	1.05
	Final	2.24	1.87	2.05	2.95	3.0	2.9
Biomass gain [g]		467.44	310.2	388.82	757.8	778.16	737.4
Fish number	Initial	20	20	20	19	19	19
	Final	19	20	19.5	19	19	19
Survival [%]		95	100	97.5	100	100	100
Mean weight [g/fish]	Initial	21.48	21.83	21.65	22.16	22.18	22.15
	Final	47.2	37.3	42.3	62.1	63.2	61.0
Experimental time		48	48	48	48	48	48

Table 2. The estimation of the marginal population means using the Pairwise Comparisons test

Pairwise Comparisons

Dependent Variable: Final_weight

Variant	**(I) Unit**	**(J) Units**	**Mean Difference (I-J)**	**Std. Error**	**Sig.**[d]	**95% Confidence Interval for Difference**[d] **Lower Bound**	**Upper Bound**
V1	B1f	B2f	9.878[a,b]	8.806	1.000	-14.013	33.768
		B3f	-19.457[a,b]	9.041	.208	-43.986	5.071
		B4f	-13.745[a,b]	8.918	.766	-37.940	10.450
	B2f	B1f	-9.878[a,b]	8.806	1.000	-33.768	14.013
		B3f	-29.335[a,b,*]	8.930	.009	-53.563	-5.107
		B4f	-23.623[a,b]	8.806	.054	-47.513	.268
V2	B3f	B1f	19.457[a,b]	9.041	.208	-5.071	43.986
		B2f	29.335[a,b,*]	8.930	.009	5.107	53.563
		B4f	5.712[a,b]	9.041	1.000	-18.816	30.241
	B4f	B1f	13.745[a,b]	8.918	.766	-10.450	37.940
		B2f	23.623[a,b]	8.806	.054	-.268	47.513
		B3f	-5.712[a,b]	9.041	1.000	-30.241	18.816

Based on estimated marginal means
*The mean difference is significant at the 0.05 level.
[a]An estimate of the modified population marginal mean (I).
[b]An estimate of the modified population marginal mean (J).
[d]Adjustment for multiple comparisons: Bonferroni.

The results of our experiment are similar to those of the previous study, from which it has found that low light intensity (V2 - with a daily mean luminous intensity of 30 lx) induces an increase in body weight in juvenile paddlefish. The two different intensities of luminosity used in the experiment significantly influenced growth performance parameters (FCR, SGR), a result confirmed by some previous studies in other species (Karakatsouli et al., 2007; Karakatsouli et al., 2008).

Based on the values obtained for SGR and FCR, it is suggested that the daily light intensity of 30 lx (V2) was favorable for food conversion and retention.

In the present study, the SGR (2.18 g%/day in green variant) and FCR (0.78 g/g) of juvenile paddlefish have been significantly influenced by different light intensities. The results obtained on the effect of light intensity on the growth performance of juvenile paddlefish are also similar to other studies that reported that blue and green light can facilitate the growth of *Verasper moseri* (Yamanome et al., 2009), *Scophthalmus maximus* (Wu et al., 2021), *Macrobrachium rosenbergii* (Wei et al., 2021), *Penaeus vannamei* (Fei et al., 2020a; Fei et al., 2020b) and *Strongylocentrotus intermedius* (Yang et al., 2020). Also, larvae of Atlantic cod (*Gadus morhua*) are exposed to increased somatic growth when exposed to blue or green light spectra (Sierra-Flores et al., 2016). The green-light spectrum is beneficial for barfin flounder (*Verasper moseri*) to achieve higher growth rates, while the red-light spectrum harms its growth. Optimal light intensities vary among different fish species.

Figure 7 demonstrates the inversely proportional correlation relationship between SGR and FCR evolution.

Light is a vital environmental factor that influences the growth and survival of teleosts. The survival rates obtained in this study indicated that the lower 30 lx light intensity was safe for juvenile paddlefish. The light might affect fish feeding and other issues, such as improved appetite, increased ratio consumption, and high food conversion efficiency, which influence the early stages of fish and have commonly been reported to be responsible for faster teleost growth under continuous light conditions (Villamizaret et al., 2011). Light influences the development of aquatic animals from early life stages to adult reproductive stages.

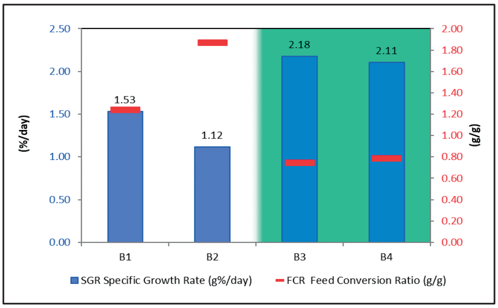

Figure 7. Relationship between FCR (feed conversion rate) and SGR (specific growth gain)

Investigation of the possible influence of light intensity on the blood profile of *Polyodon spathula* (Walbaum, 1792) juveniles

To assess the effect of light intensity on the hematological profile of juvenile paddlefish, the variation in the values of hematological indices, erythrocyte constants, and different types of leukocytes at the end of the experimental period in the two variants have been analyzed. To investigate the influence of light intensity on the physiological condition of juvenile paddlefish reared in a recirculating system, hematological indicators such as hematocrit (PVC, %), hemoglobin concentration (Hb, g/dL), and red blood cell count, RBCc ($\times10^6$ cells/μL) were assessed. Furthermore, erythrocyte constants derived as mean corpuscular volume MCV (fL), mean corpuscular hemoglobin MCH (pg), and mean corpuscular hemoglobin concentration MCHC (g/dL) have been calculated.

The results of the hematological indices are presented in Table 3. At the end of the experimental period, RBCc, PVC, and Hb showed insignificant differences (p>0.05), the values of the white variant (V1) being similar to the values of the green variant (V2).

The skin of juvenile paddlefish in the white rearing units (V1 - variant with a daily average luminous intensity of 105 lx) underwent a slight depigmentation compared to the green variant (V2 - with a daily mean luminous intensity of 30 lx) and acquired a darker shade of skin, a phenomenon also observed in juvenile carp. Indeed, some studies have reported that skin coloration in teleost fish is under the multiparametric control of both external and internal (physical, nutritional, genetic, and neurohormonal) factors (Papoutsoglou et al., 2000; Karakatsouli al., 2010).

Table 3. Hematological indices of paddlefish in the two experimental variants.

Hematological parameters	**White color (V1)**	**Green color (V2)**	***p-value***
RBCc ($\times10^6$ cells/μL)	0.98±0.06	0.96±0.06	0.73
PVC (%)	34.54±1.56	32.09±1.54	0.27
Hb (g/dL)	6.53±0.24	6.35±0.22	0.58
MCV (fL)	356.60±18.99	351.70±31.80	0.89
MCH (pg)	68.46±4.73	68.90±4.89	0.94
MCHC (g/dL)	19.34±1.29	20.04±0.84	0.65

RBCc - red blood cell count, PVC – hematocrit, Hb – hemoglobin, MCV - mean corpuscular volume, MCH - mean corpuscular hemoglobin, MCHC - mean corpuscular hemoglobin concentration. Results are presented as duplicate means ± standard error.

Hematological indices have been measured to evaluate general health status and nutritional and environmental conditions affecting the fish (Hoseinifar et al., 2011).

Circulating blood erythrocyte numbers of juvenile paddlefish were kept constant in both variants. In the experimental green variant V2 the mean was $0.96\pm0.06\times10^6$cells/μL, while in variant V1 the mean was $0.98\pm0.06\times10^6$ cells/μL. In the present experiment, the obtained values were lower than those obtained in adult paddlefish reared in open systems, where the range is between 1.04-1.36×10^6/μL (Bucur et al., 2009). Circulating blood hemoglobin and hematocrit of juvenile paddlefish showed no significant difference between V1 (105 lx - mean daily light intensity) and V2 (30 lx - mean daily light intensity). This indicator aligns with values reported by other studies conducted for the species *Polyodon spathula*, where hemoglobin levels fell within the range of 6.2-9.6 g/dL (Bucur et al., 2009; Coadă et al., 2012). Therefore, a possible explanation for the observed increase of hematocrit could be the heightened erythropoiesis in response to the potentially stressful impact of light (Valenzuela et al., 2006). However, this situation was not found in our experiment, with the number of

erythrocytes, hemoglobin, and hematocrit recorded at the end of the experiment being similar.
According to our results, high (V1 – white variant with a daily average luminous intensity of 105 lx) and low (V2 – green with a daily mean luminous intensity of 30 lx) light intensity had no significant effect on these parameters. Similar results have been previously reported for *Huso huso* (Musavi et al., 2022) and other species (Adeoye et al., 2016; Zamini et al., 2014).
The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) showed no significant differences (p>0.05), the values of the high light intensity variant (V1) being approximately similar to those of the low light intensity variant (V2). Standard reference intervals of blood parameters are relevant for assessing and observing fish welfare. In the past, numerous normal reference ranges for hematological indices of farmed and wild fish have been established (Bocioc et al., 2015; Fazio et al., 2019).

Assessment of leukocyte reactions
The analysis of blood smears from quantitative and qualitative aspects can offer valuable information to study innate/native immunity. To determine the influence of different light intensities on the hematological profile, the leukocyte profile of juvenile paddlefish subjects from the two experimental variants was also studied, which included the determination of a relative number of leukocytes and the absolute number of leukocytes (Figures 8 and 9).
Lymphocytes (%). Concerning small lymphocytes between experimental white and green variants, no significant differences (p>0.05) were observed, but the highest value was recorded in the green variant (71%). The relative number of large lymphocytes was not significantly different between the experimental variants (p>0.05), however, compared to the value obtained between variants there was a 6% increase in the white variant (V1).
Monocytes (%) identified at the end of the experiment showed no significant difference (p>0.05) in the relative number in both experimental variants.
Neutrophil granulocytes (%) from leukogram showed that there was an insignificant increase (p>0.05) in the white experimental variant (14%) compared to the green experimental variant (6%). Basophilic and eosinophilic granulocytes were absent.

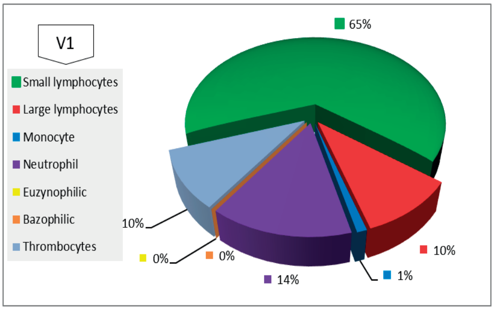

Figure 8. Variation in the relative number of leukocytes in the white variant - V1

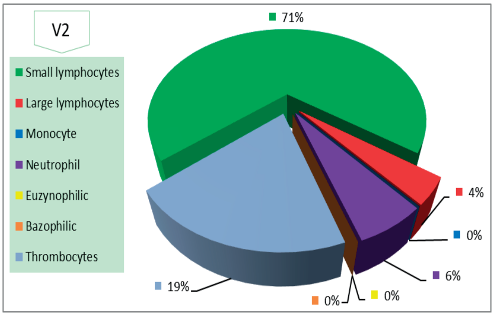

Figure 9. Variation in the relative number of leukocytes in the green variant – V2

The *relative number of thrombocytes* showed an insignificant increase (p>0.05) in the 9% green variant compared to the white variant.
Analysis of blood smears from a quantitative and qualitative aspect can provide the necessary information to study innate/native immunity. The modifications of the various types of cells that form the leukocytic complex are presented in Figures 10 and 11.

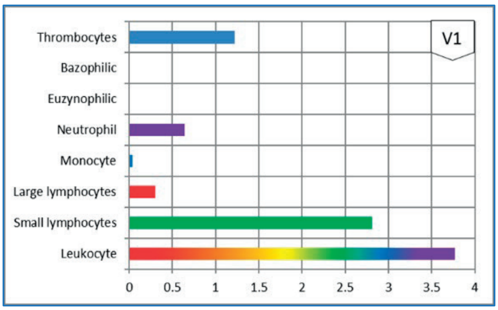

Figure 10. The variation of the absolute number of leukocytes in the white variant (V1)

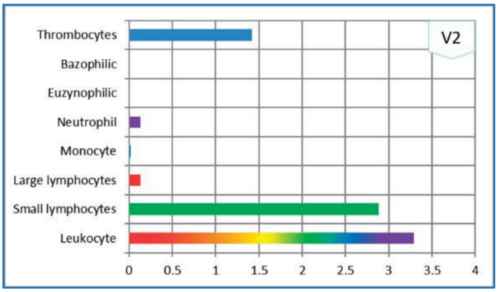

Figure 11. The variation of the absolute number of leukocytes in the green variant (V2)

At the end of the experimental period, no significant differences were observed between the experimental variants on the leukocyte complex (p>0.05). The absolute number of small lymphocytes was dominant in the total leukocytes and showed approximately the same tendency as in the number of leukocytes.

The absolute number of neutrophils recorded at the end of the experiment showed slightly higher values in the experimental variant with light intensity of 105 lx (V1) compared to V2 (light intensity of 30 lx). For the absolute number of platelets, a tendency of reduction was observed, but it was not statistically (p>0.05) in the 105 lx light intensity variant (V1).

The present study found that juvenile paddlefish exposed to 30 lx showed relatively more leukocyte counts, probably suggesting enhanced immunity with low light intensity in this species. At the same time, leukocytes are also known to play an essential role in innate immunity, and their number could be considered an indicator of fish health status (Alcorn et al., 2003). The relative number of leukocytes registered in our study is sensitively equal under the conditions of its growth influenced by a light intensity of 105 and 30 lx, a similar observation was reported by other authors at a light intensity between 7-600 lx (Ruchin et al., 2006). Basophilic and eosinophilic granulocytes were absent, and this finding was also observed by other authors (Buchtíková et al., 2011).

The most numerous cells of the total granulocyte leukocytes are the neutrophils, which play a role in antimicrobial defense due to their specific properties. At the end of the experiment, the number of neutrophilic granulocytes has the same tendency to increase as the other leukocytes (lymphocytes, monocytes). Thus, the relative number of neutrophils increases by 14% in the white light variant, compared to 6% in the green variant. In contrast, platelets showed an insignificant increase of 19% in the green variant compared to the white variant (10%). In the study on the effect of photoperiod on the growth of juvenile Siberian sturgeon (*Acipenser baerii*), hematological indices were reported to be within normal limits under optimal photoperiod conditions. In contrast, pronounced neutrophilia and leukopenia were observed for this species. After statistical evaluation of the absolute number of leukocytes (lymphocytes, monocytes) at the end of the experiment, no statistically significant differences were obtained for the two colors of light, white and green. This implies that the juvenile paddlefish's body was adapted and remained unaffected by light intensity.

Since most previous studies examining these effects of light have tended to investigate the influence of single colors of light or white light (full spectrum) on fish growth and immunity, there is presently limited information available on the effects of light color variation on fish physiological ecology (Xueweijie et al., 2022).

Investigation of serum biochemical parameters. At the end of the experimental period, the results of serum biochemical parameters indicate normal blood glucose and total protein levels in the sturgeon. In both experimental variants, glycemia recorded an average value between 84.03±1.08 mg/dL in the white variant (V1) and 81.19±1.22 mg/dL in the green variant (V2), with no significant differences (p>0.05) between the experimental variants (Figure 12).

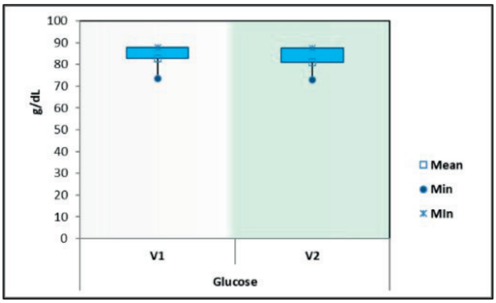

Figure 12. Analytical values of glucose concentration in the serum blood of juvenile paddlefish were determined at the end of the experimental period

In the case of the total proteins, the results showed no significant differences (p>0.05) between the experimental variants, with an average of 3.92±0.09 mg/dL in the white variant (V1) and 3.88±0.10 mg/dL in the green variant (V2) (Figure 13).

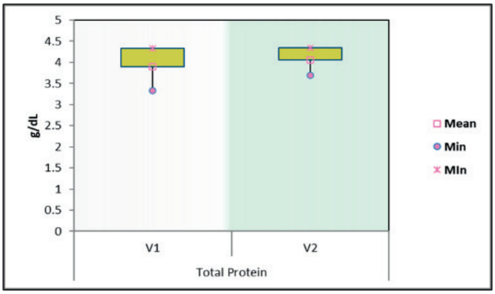

Figure 13. Analytical values of total protein in the serum blood of juvenile paddlefish were determined at the end of the experimental period

Determination of serum glucose in fish is the most efficient and rapid method of assessing stress. A high-value marker for stress is the serum glucose level, while the serum total protein level is primarily a synthetic indicator of stress, the state of nutrition of the body. There are significant influences on serum biochemical concentrations by the following factors: age, gender, environmental conditions, and nutritional diet (Banan et al., 2013). In our study, serum glucose and serum total protein showed no significant differences between the variants with different light intensities, the values obtained being within the normal range for these parameters. Usually, blood contains 40-90 mg of glucose per 100 mL of blood. According to some authors, blood glucose in some freshwater fish species ranges from 25-200 mg/dL. Normal blood contains 3.5- 5.5 mg of total protein per 100 ml of blood (Misaila et al., 2009). Usually, animal husbandry, including fish farming, involves a high density of animals in a limited space. The results obtained in the present experiment were observed at the species of *Acipenser stellatus*, and the blood normally contains 30-75 mg/dL (Patriche et al., 2011).
According to some studies, there are reports that light conditions may influence blood glucose and lactate levels, Baekelandt et al., 2019 reported that pike (*Sander lucioperca*) exposed to red light had higher blood glucose levels compared to fish exposed to white light. Concerning total protein and glucose levels, there were no significant differences between the different light intensities in terms of serum parameters of swordfish. The results for total protein are similar to that of tilapia (Wang et al., 2023) and red porgy, with no significant differences in total protein and globulin levels between different photoperiod groups (Biswas et al., 2006).

Biochemical composition analysis of the muscle tissue of juvenile Polyodon spathula (Walbaum, 1792)

The results recorded in the present study related to the effect of light intensity on the meat biochemical composition of juvenile paddlefish, reared in a recirculating aquaculture system have been compared to those reported by other authors. Biochemical analyses of muscle tissue were performed at the end of the experimental period.
The results obtained from determining the biochemical composition of the muscle tissue of juvenile paddlefish are illustrated in Figures 14 and 15.

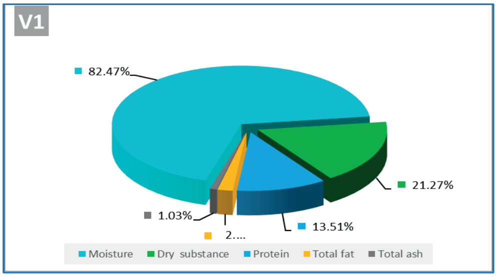

Figure 14. The biochemical composition of the muscle tissue of juvenile paddlefish in the white variant at the end of the experimental period

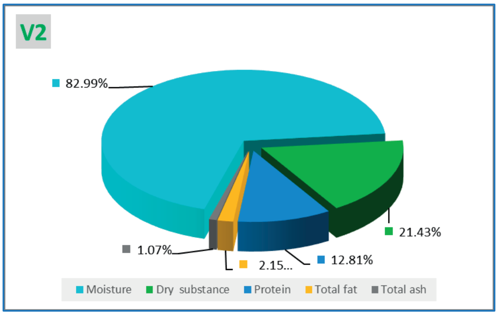

Figure 15.The biochemical composition of the muscle tissue of juvenile paddlefish in the green variant at the end of the experimental period

The muscle tissue biochemical composition analysis revealed no statistically significant differences ($p > 0.05$) in proteins, lipids, ash, and moisture levels between juvenile paddlefish reared in high and low light-intensity conditions. The evaluation of the composition of the muscle tissue of the juvenile paddlefish showed no significant differences between proteins, lipids, ash, and moisture of paddlefish reared in high and low light intensity. These results indicate a lower percentage of protein compared to other sturgeon species, where the protein content of the oarfish muscle tissue is 14% (Bucur et al., 2009) or even 16-19% (Simeanu et al., 2012). Regarding the fat content in the muscle tissue of juvenile paddlefish, low values were recorded in both experimental variants. Based on these data, the species *Polyodon spathula* is placed in the category of fish with a relatively low lipid content (2.45-3.96%), which is confirmed by other authors (Simeanu et al., 2012). Also, in the study on proximate chemical composition analysis of sturgeon *Polyodon spathula* meat aged two and three summers, it was observed that between the two ages, there was no significant difference in the chemical composition of the fillets (Simeanu et al., 2022). Sturgeon as a meat product is a relatively new, albeit burgeoning field, with a protein and fat profile similar to salmonids (Gao et al., 2020; Pelic et al., 2019).

CONCLUSIONS

Light intensity significantly influenced the growth of juvenile paddlefish in RAS. Fish exposed to light at 30 lx showed the highest growth performance of all treatment groups. This information provides a starting point for the rearing of paddlefish in RAS. In this study, in which we examined the responses of paddlefish juveniles to different light intensities to exposure, it was concluded that low light intensity (green color) simulated growth performance compared to high light intensity (white color), which can simultaneously improve growth and not affected of the immune response of the species without stressing paddlefish juveniles. Our results indicate the paddlefish juveniles can adapt to various light intensities and grow favorably under low light conditions in a recirculating aquaculture system. Furthermore, we recommend conducting additional research to enhance the lighting regime to augment both fish production and welfare. Investigating optimal light conditions, such as intensity, duration, and color spectrum, can provide valuable insights into how these factors influence the physiological well-being and growth of fish. This research could contribute to refining aquaculture practices, ensuring not only increased productivity but also the overall health and quality of the fish population. Additionally, a thorough examination of behavioral responses to different lighting conditions can aid in designing environments that promote the natural behaviors of the fish, ultimately contributing to improved welfare and sustainable aquaculture practices.

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RESEARCH STUDY OF MAMMAL SPECIES OF HUNTING INTEREST IN FOUR HUNTING FUNDS IN TELEORMAN AND PRAHOVA COUNTIES

Luiza-Georgeta CRĂCIUNICĂ, Cristina Ileana COVALIU-MIERLĂ

National University of Science and Technology Politehnica Bucharest,
Faculty of Biotechnical Systems Engineering, 313 Splaiul Independentei Street,
District 6, Zip Code 060042, Bucharest, Romania

Corresponding author email: cristina_covaliu@yahoo.com

Abstract

The need to ensure biodiversity conservation is seen as a measure to adapt to climate change and at the same time to protect vulnerable species by preserving and restoring ecosystems. For this purpose, in this article, was study the vulnerability of three species of mammals of hunting interest (deer, rabbit and wild boar) and their adaptation to the effects of climate change in two hunting funds in Teleorman County (hunting fund 1 Flămânda and hunting fund 62 Islaz) respectively Prahova county (hunting fund 43 Lapoș and hunting fund 11 Gherghița).

***Key words**: biodiversity conservation, mammal species.*

INTRODUCTION

Natural habitats represented by forests and farmland management opportunities provide benefits and variety, offering hunters seeking opportunities to pursue both large game such as deer, wild boar, and small game such as pheasant and rabbit. In addition, these landscapes offer wildlife enthusiasts and lovers the chance to observe and photograph animals in their natural habitat.

While agriculture can alter wildlife habitats, agroecosystems (agricultural ecosystems) can also contribute to biodiversity conservation by providing recreational activities such as hunting and the aesthetic pleasure of observing wildlife in agricultural environments. Thus, agroecosystems serve as providers of various biodiversity and wildlife services and benefits to society, providing individuals with both non-use and use values by providing these goods and services.

The existence of diversified and abundant natural resources in Romania has led to an increase in cases of damage caused by wild animals both on private and public property. Consequently, the implementation of a consistent policy to prevent, combat and compensate losses has become imperative (Ionescu-Lupeanu, 2023).

The sustainable use of wildlife, whether for our direct (productive) or indirect (non-productive) purposes, is an undeniable aspect of societies. However, there are policies that advocate the end of productive exploitation. Wildlife, considered renewable, retains this quality only when the ecosystems remain unaltered and undegraded. Biodiversity conservation requires stopping practices that harm wildlife and biodiversity, regardless of the method of their exploitation (Knoche & Lupi, 2007). The trend in this sector is similar to other sectors involving the use of natural resources: demand is increasing, but natural resources remain in decline due to various pressures and threats from various human activities.

Understanding the influence of climate change on the quality and connectivity of natural habitats is essential for the effective conservation of a wild species and the management of the country's population (Zhenhua et al., 2024).

In Teleorman County, environmental disturbances have negative repercussions on wildlife, leading to disturbances among game species and diminishing their natural food sources, including supplementary feeding in colder seasons.

Meanwhile, in Prahova County, there has been a slight increase in poaching incidents over the last five seasons. Given the potential of the region for natural resources and rural development, it is essential to impose strict measures on future poaching, especially as regards poaching through trapping. In general, tourism has minimal impact on the area, including the influence on hunting activities. In this article, the vulnerability of three species of mammals of hunting interest (deer, rabbit and wild boar) and their adaptation to the effects of climate change were studied in two hunting grounds in Teleorman county (hunting grounds 1 Flămânda and hunting grounds 62 Islaz) and Prahova county (hunting fund 43 Lapoș and hunting fund 11 Gherghița).

MATERIALS AND METHODS

Analysis and comparison of optimal and evaluated populations of mammal species of hunting interest in four hunting grounds in Teleorman County, respectively Prahova County, during 15 May 2022 and 14 May 2023.
As species of hunting mammals representative for Teleorman and Prahova counties, was carry out a comparative study of wild boar, deer and rabbit species in two hunting funds (FC) in Teleorman County (hunting fund 1 Flămânda and hunting fund 62 Islaz) respectively in Prahova county (hunting fund 43 Lapoș and hunting fund 11 Gherghița).
The primary indigenous wildlife species thriving in the hunting areas FC 1 Flămânda and FC 62 Islaz, listed in order of prevalence, include:
Rabbit: Serving as the primary game species under management, rabbits flourish across the entire hunting grounds except for pastures and areas near water bodies like swamps.
Deer: Ranking as the second most significant game species in terms of management, deer inhabit approximately 20% of the hunting grounds, enjoying favorable living conditions.
Wild boar: Occasional sightings of wild boars occur, particularly during autumn seasons, influenced by the types of agricultural crops cultivated in the vicinity.
Hunting fauna presentation on FC 43 Lapoș and FC 11 Gherghița.
The relationship between fauna and vegetation must be considered bidirectionally. Forests provide optimal habitats, and the forested area significantly contributes to the credibility of a hunting ground. Cervids, such as deer, with higher year-round food requirements, can cause considerable damage, particularly in areas with increasing population density, if food sources are not adequately managed. To address this, the cultivation of crops like maize, beet, or barley is recommended, especially where undergrowth is well established, to enhance food availability.
Supplementary feeding is administered as follows:
Deer: Hay or alfalfa feeders are provided from November onwards.
Wild boar: Concentrated feed is placed at feeding points between November and February.
Salt is administered throughout the year, with 10% in the first quarter, 45% in the second quarter, 40% in the third quarter, and 15% in the fourth quarter.
In these two hunting areas, studies have focused on mammalian species of hunting interest, including the European hare (*Lepus europaeus*), wild boar (*Sus scrofa*), and various deer species (*Capreolus capreolus*) (Figures 1, 2, 3).

Figure 1. Rabbit species (*Lepus europaeus*)
https://ro.wikipedia.org

Figure 2. Wild boar species (*Sus scrofa*)
https://ro.wikipedia.org

Figure 3. Deer species (*Capreolus capreolus*)
https://ro.wikipedia.org

For each species, we recognized the importance of determining how to establish optimal herds aligned with creditworthiness categories. The creditworthiness or biogenic capacity of a hunting reserve is defined by various factors, including ecological, geomorphological, edaphic, climatic, and biotic elements (Daneti, 1968) which influences for positive or negative the lives of animals living on that land (Almasan et al., 1966) (Tables 1, 2, 3).

Table 1. Optimal herds corresponding to the creditworthiness categories, for rabbits (Almasan et al., 1965; Order 393/2002)

Name	Creditworthiness category			
	I	II	III	IV
Points awarded by creditworthiness category	91-112	61-90	33-60	8-32
Adequate optimal herds pcs/100 ha productive land	25-15	10-14.9	5-9.9	1-4.9

Table 2. Optimal herds corresponding to creditworthiness categories, for deer (Almasan et al., 1965; Order 393/2002)

Name	Creditworthiness category			
	I	II	III	IV
Points awarded by creditworthiness category	111-140	76-110	42-75	6-41
Adequate optimal herds; pcs/100 ha productive land	9-11	7-8.9	5-6.9	0.5-4.9

Table 3. Optimal herds corresponding to creditworthiness categories, for wild boar (Almasan et al., 1965; Order 393/2002)

Name	Creditworthiness category			
	I	II	III	IV
Points awarded by creditworthiness category	81-100	55-80	30-54	5-29
Adequate optimal herds; pcs/100 ha productive land	0.7-0.8	0.5-0.6	0.3-0.4	0.05-0.2

Knowing it helps to establish the productivity of hunting funds, to take the most appropriate management measures, to achieve optimal productivity in the shortest time. In order to find the main causes or factors positively or negatively influencing the existence of game species, it is imperative to investigate the fluctuation of actual game populations and harvests. Such data must express as accurately as possible the situation on the ground, i.e. be based on highly accurate game assessment or inventory methods. The field assessment was carried out according to the provisions of the Order of the Ministry of Environment, Waters and Forests nr. 2847/2022 on the approval of the Instructions on the assessment of the numbers of certain species of hunting fauna admitted to hunting and for regulating the way of establishing their harvest quotas.

RESULTS AND DISCUSSIONS

Deer (*Capreolus capreolus* L.)

The evaluation of the deer herds was done through direct observations, in two stages which, corroborated, in order to provide an image as close as possible to the reality on the ground, both in terms of herd size and its structure by age classes, sexes and health status.
The direct, visual evaluation during the mating period (running) was organized in July and August (first decade). During this period, very easy observations were made, which provide essential data on deer herds. It was easy to observe the age categories in males, an assessment could be made on the annual increase in young exemplars under one year old, respectively it was possible to easily establish the sex: ration ratio of the deer population.
In each of the four hunting grounds, there were identified the places suitable for running where, as the case may be, one or more observation points were installed, from where the rafters in that area could be observed and heard well. The observations were held for 2-3 consecutive days, at all established points, simultaneously, early in the morning (before light) and in the afternoon, until dark. The second stage of evaluation of deer herds was based on visual observations made on flocks during the winter period (January-February).

Wild boar (*Sus scrofa* L.)

The evaluation of wild boar herds was carried out in January - February by visual observations at feeding points and by reading traces left on the substrate (earth, snow). The visual assessment procedure consisted of direct observations. The observations were made simultaneously, on the same day, at all feeding points within each hunting fund and were made at least two times during February.

Hare (*Lepus europaeus*)

The herd size assessment in hares was carried out on the basis of direct visual observations on sample areas or sample strips. The observations were made in late winter - early spring (February - March), recommended in January when the specimens are relatively grouped for mating, preferably on sunny, windless days, in the evening and morning hours, when rabbits are more active. The evaluation of the herds was carried out both in cultivated agricultural land and in the forests of the plain area.

The calculation of the herds by categories of land was made using the formula:

$$Ef.c. = (S.c.: S.p.) \times n \quad (1)$$

Ef.c = the population calculated on a given category of land, in exemplars;

S.c. = the total area of the land category in the hunting fund, in ha;

S.p. = area of sample area travelled from the same category of terrain, in ha;

n = the number of specimens found on the sample area travelled, in the same category of terrain.

When centralizing the data, the results obtained by categories of land were totaled, excluding from the calculation the non-productive areas for rabbits of the hunting fund, finally establishing the total herd on its entire productive area.

Using the methods for assessing mammalian species of hunting interest, from the analysis of the above summary data, it follows that:

- for the deer species, the largest evaluated herds are in the hunting fund FC 43 Lapoș from Prahova county (218 pcs) as Table 4, which has Creditworthiness category I, which means that the area is populated, probably because the food was enough and there are not anthropogenic activities in the area;
- from the point of view of animal husbandry, the area is not so active, no negatively activity influencing the silence of the hunt; for the wild boar species, the largest evaluated herds are in the hunting fund FC 43 Lapoș from Prahova county (69 pcs) as Table 5, which has Creditworthiness category II, which means that the area had no significant negative influences of the anthropogenic activities in the area (e.g. chemical fertilizer from agriculture);
- the rabbit species, the largest assessed herds are in the hunting fund FC 1 Flămânda from Teleorman county (775 pcs) as it is observed in Table 6, which has Creditworthiness category I, meaning that that the area is overpopulated, probably because the enough fauna and the non- existence of the human activities;
- in addition to the agricultural works that are carried out on large areas, unevenly and over a long period of time, the vulnerability of the three studied species of mammals of hunting interest is due to poaching with greyhounds or dogs, with a noose, with a beacon or with hunting weapons and the burning of stubble after the harvesting of grasslands or the chaotic grazing in areas cultivated with perennials;
- the lands within the FC 1 Flămânda and FC 62 Islaz hunting funds are public property (forest, water surface, pasture, and part of the agricultural land) and private property of the citizens of the communes within the radius of which the hunting fund is surrounded;
- as a form of agricultural land exploitation in the past, the associative-cooperative form prevailed as well as that of organizing the exploitation of these lands in large agricultural farms;
- the territory of the FC 11 Gherghița hunting fund is quiet from the point of view of industrial activity. Consequently, the pollution phenomenon does not affect the way the studied area and, implicitly, the existing hunting species;
- the phenomenon of poaching has seen a reduced magnitude in recent seasons, no cases of poaching with weapons have been discovered. In 2022, a case of poaching with greyhounds was discovered;
- tourism has no significant influence on the area in general and game in particular;
- the territory of the hunting fund 43 Lapoș is a quiet one from the point of view of industrial

activity. The pollution phenomenon does not, therefore, affect in any way the studied area and, implicitly, the existing hunting species;
- from the point of view of animal husbandry, the area is active, a significant number of domestic animals being grazed annually. In total, about 2000 sheep, goats and cows, belonging to 12 sheepfolds, graze within the hunting fund, the influence being felt negatively, especially under the aspect of the silence of the hunt;
- reanalyzing the criteria for ranking of hunting funds;
- elaboration of a hunting management plan at national level;
- active involvement of the administrator of the national hunting fund in the evaluation of the populations of hunting interest (Gheta et al., 2022);
- limitation of the extraction quota;
- the popularization of the impact that this drastic decrease, of the wild boar herd, has on the environment (Cocor et al., 2022).

Of all the three species of mammals of hunting interest evaluated, it is observed that the rabbit species with creditworthiness category I has large herds in all the hunting funds evaluated, which means that human activities from the have not influenced on this species.

Table 4. Centralizer of evaluated deer species and harvest quotas

Crt. no	Name Hunting Fund	Hunting Fund Manager	Optimal Effectives (pcs)	Effectives evaluated (pcs)		Harvest quotas for hunting season 2022/2023 (pcs)		Proposed harvest quotas for hunting season 2023/2024 (pcs)	
				2022	2023	Approved	Accomplished	According to the formula	By Manager
1	FC 1 Flămânda	AJVPS TELEORMAN	60	57	57	4	4	4	3
2	FC 62 Islaz	AJVPS TELEORMAN	80	73	74	5	5	5	5
3	FC 11 Gherghița	AVPS CODRII VLASIEI	74	180	177	55	55	69	65
4	FC 43 Lapoș	AVPS MUFLONUL	80	223	218	35	20	69	35

Table 5. Centralizer of evaluated wild boar species and harvest quotas

Crt. no	Name Hunting Fund	Hunting Fund Manager	Optimal Effectives (pcs)	Effectives evaluated (pcs)		Harvest quotas for hunting season 2022/2023 (pcs)		Proposed harvest quotas for hunting season 2023/2024 (pcs)	
				2022	2023	Approved	Accomplished	According to the formula	By Manager
1	FC 1 Flămânda	AJVPS TELEORMAN	5	2	2	2	2	0	2
2	FC 62 Islaz	AJVPS TELEORMAN	15	6	6	6	6	0	6
3	FC 11 Gherghița	AVPS CODRII VLASIEI	25	26	40	11	10	20	20
4	FC 43 Lapoș	AVPS MUFLONUL	20	62	69	40	32	30	45

Table 6. Centralizer of rabbit species evaluated and harvest quotas

Crt. No.	Name Hunting Fund	Hunting Fund Manager	Optimal Effectives (pcs)	Effectives evaluated (pcs)		Harvest quotas for hunting season 2022/2023 (pcs)		Proposed harvest quotas for hunting season 2023/2024 (pcs)	
				2022	2023	Approved	Accomplished	According to the formula	By Manager
1	FC 1 Flămânda	AJVPS TELEORMAN	850	775	775	30	30	58,125	35
2	FC 62 Islaz	AJVPS TELEORMAN	750	685	685	30	30	51,375	35
3	FC 11 Gherghița	AVPS CODRII VLASIEI	500	548	546	40	40	175	60
4	FC 43 Lapoș	AVPS MUFLONUL	100	134	145	15	13	40	16

CONCLUSIONS

For each wild species, it is imperative to effectively identify optimums for all creditworthiness categories. The quality of a hunting ground, or its biogenic capacity, encompasses ecological, geomorphological, edaphic, climatic and biotic factors that influence the well-being and life cycles of resident animals. Understanding these factors is crucial to stabilizing gameland productivity, implementing appropriate management strategies, and effectively maximizing productivity. In order to identify the primary causes or factors that have a positive or negative impact on game species, a thorough investigation of herd fluctuations and actual game harvests is essential. This research must be based on precise methods of game assessment or inventory, aligned with the criteria for classifying hunting grounds in Romania for species such as: pheasant, rabbit, deer, wild boar, etc.

From these analyses, we considered it necessary to draw up sustainable game management action measures for these mammal species of hunting interest on the four hunting funds in Teleorman and Prahova counties, in the context of climate change:

- revision of existing populations through "refreshing the blood" actions;
- increasing the nutritional potential of hunting resources. this will be done by: creating the network of hunting units, buildings and installations, creating natural conditions for game feeding, complementary game feeding, combating game pests.

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IDENTIFICATION OF POTENTIAL EFFECTS OF MINERAL AGGREGATES EXTRACTION ACTIVITY FOLLOWING AN ADEQUATE IMPACT ASSESSMENT ON PROTECTED BIRD SPECIES IN TWO NATURA 2000 SITES FROM TELEORMAN AND PRAHOVA COUNTIES

Luiza-Georgeta CRĂCIUNICĂ, Cristina Ileana COVALIU-MIERLĂ

National University of Science and Technology Politehnica Bucharest,
Faculty of Biotechnical Systems Engineering, 313 Splaiul Independentei Street,
District 6, Zip code 060042, Bucharest, Romania

Corresponding author email: cristina_covaliu@yahoo.com

Abstract

By its very nature, the extraction of mineral aggregates invariably has an impact, often negative, on the ecosystem where it occurs. In essence, it requires that any extraction of mineral aggregates likely to have a significant effect on protected bird species be subject to an appropriate assessment detailing this impact on them and how will align with the conservation objectives of the two Natura 2000 sites. In this article two Natura 2000 sites from Teleorman and Prahova counties (ROSPA0024 OLT-DANUBE confluence and ROSPA0152 IALOMIȚA corridor) where study considering the impact evaluation of mineral aggregates extraction on the protected bird species.

***Key words**: environmental impact, mineral aggregates extraction.*

INTRODUCTION

Preserving biodiversity is a prevalent global challenge, and Europe's natural environment encompasses numerous habitats and ecosystems. Romania is proactive in nature conservation and biodiversity preservation byestablishing a national network of protected areas based on scientific identification.

Compared to 2016 when some areas of the country were declared Natura 2000 sites (http://www.mmediu.ro), a negative influence on bird species of community interest having various causes was observed.

Some of the causes that may negatively influence the protected bird species monitored in Natura 2000 sites are thefollowing (Guide to Extraction of Non-Energy Minerals and Natura 2000 Executive summary, European Union, 2019):

- intensity of mineral aggregate extraction activity on a relatively small area, observing a cumulative impact of this activity;
- lack of exact knowledge of the species of birds protected by hunters, which can be easily confused with unprotected ones, which leads to a decrease in their numbers;
- climate change which has led to landslides, very high temperatures, heavy rainfall in the spring months leading to flooding of protected bird species habitats;
- the magnitude of the industrialization phenomenon which, through the emissions produced, causes significant damage;
- the chemichal fertilisers used in agriculture has led to major imbalances in the food chains and pyramids of terrestrial and aquatic ecosystems where protected bird species have their natural habitat;
- the - excessive utilisation of chemical fertilizers has a negativ impact to both soil and groundwater (Lingke et al., 2020; Ausubel & Waggoner, 2008; Awuah-Offei & Adekpedjou, 2011; Fischer-Kowalski et al., 2011; Bringezu & Bleischwitz, 2009; Krausmann et al., 2009; Popa et al., 2012).

Like all other forms of land use, the non-energy mineral extraction industry must operate within the framework of European environmental legislation, which includes the Birds and Habitats Directives (Strateanu et al., 2009).

The European Birds and Habitats Directives are cornerstones of EU biodiversity policy and enable Member States to work together to protect and ensure the survival of Europe's most threatened and vulnerablespecies and habitat types. Article 6 of the Habitats Directive sets out the procedure to be followed for authorizing plans and projects likely to have a significant effect on a Natura 2000 site (Guide to Extraction of Non-Energy Minerals and Natura 2000 Executive summary, European Union, 2019).

The two main objectives of European directives are:

- protect the species itself throughout the EU (through species protection provisions);
- conserve certain rare and threatened habitat types or the central habitats of certain rare and threatened species to ensure their uninterrupted survival (through site protection provisions, which led to the creation of the Natura 2000 network).

Birds in Natura 2000 sites: ROSPA0024 OLT-DANUBE confluence and ROSPA0152 IALOMIȚA corridor are monitored because they are considered species of Community interest, species that are endangered, vulnerable, rare, endemic on the territory of the European Union. We chose for the study these birds of community interest, because following field monitoring, a decrease in population numbers was observed due to the activity of extracting mineral aggregates.

The quality of surface water is a parameter which needs continuous and carefully monitoring, because the chemical, physical and biological process from water mass are dynamic, are inside in cycling compartments of nutrients and are most vulnerable compartments to nitrites pollution from agricultural and husbandry fields. The continuous monitoring of surface water is a necessity because their quality influences the quality of fresh and underground water.

MATERIALS AND METHODS

Two Natura 2000 sites were chosen as study areas, from Teleorman and Prahova counties, in which ten protected bird species were evaluated considering the impact of the mineral aggregates extraction activity on them.

We chose the monitoring method depending on each species, namely the linear transect method of 1 km and 5 km, a method that complies with the StandardGuide for monitoringbird species of community interest in Romania, within the project "Completing the level of knowledge ofbiodiversity by implementing the monitoring system of the conservation status of bird species of community interest in Romania and reporting based on Article 12 of the Birds Directive 2009/147/EC", financed by the Large Infrastructure Operational Program 2014-2020, guide approved by the Order of the Ministry of Environment, Waters and Forests nr. 1358 of 6 August 2021.

The method involved the numerical evaluation of the population numbers of a number of 10 birds, and for the species of limicole waterfowl, the monitoring was done during the nesting period April 15-June 15, the numerical evaluation being made in the colony and at a distance based on vocalizations and observations of bird colonies. Linear transects in these species of limicole birds were not restricted only to the waterfront, but also the areas of swamps, flooded agricultural lands were followed.

Identification of the geographical studied area in Teleorman and Prahova counties of the mineral aggregates extraction activity in Natura 2000 sites.

The Natura 2000 network is a European ecological network of protected natural areas comprising special protection areas for birds (SPA) established in accordance with the provisions of Directive 79/409/EEC on the conservation of wild birds (Birds Directive).

At Teleorman county level, was identified 1 Natura 2000 site where the extraction of mineral aggregates takes place, namely: ROSPA0024 OLT-DANUBCONFLUENCE - an area of 14672 ha (Figure 1).

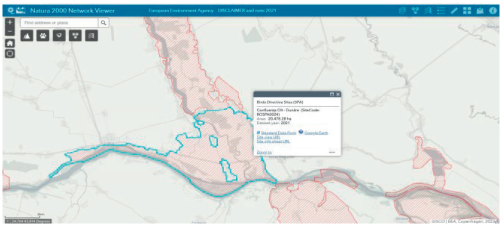

Figure 1. Location of the site ROSPA0024 OLT-DANUBE CONFLUENCE (http://ananp.gov.ro/ariile-naturale-protejate-ale-romaniei/)

In this site, bird species were monitored: *Cygnus cygnus* (winter swan) (Figure 2), *Phalacrocorax pygmaeus* (lesser cormorant) (Figure 3), *Falco vespertinus* (evening bream) (Figure 4), *Alcedo atthis* (kingfisher) (Figure 5), *Anas crecca* (dwarf duck) (Figure 6).

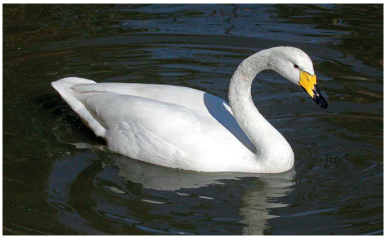

Figure 2. Species *Cygnus cygnus* (winter swan) (https://ro.wikipedia.org)

Figure 3. Species *Phalacrocorax pygmaeus* (little cormorant) (https://ro.wikipedia.org)

Figure 4. Species *Falco vespertinus* (evening bream) (https://ro.wikipedia.org)

Figure 5. Species *Alcedo atthis* (kingfisher) (https://ro.wikipedia.org)

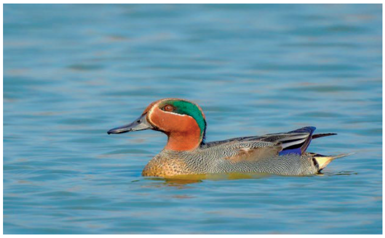

Figure 6. Species *Anas crecca* (dwarf duck) (https://ro.wikipedia.org)

At Prahova county level, one Natura 2000 site was identified where the mineral aggregates extraction activity takes place, namely: ROSPA0152 IALOMIȚA CORRIDOR - area of 25307.90 ha (Figure 7).

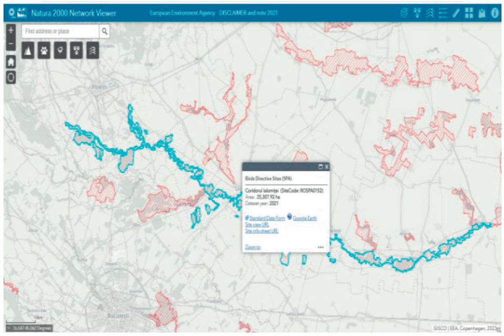

Figure 7. Location of the site ROSPA0152 IALOMIȚA CORRIDOR (http://ananp.gov.ro/ariile-naturale-protejate-ale-romaniei/)

In this site, bird species were monitored: *Alcedo atthis* (kingfisher) (Figure 8), *Ixobrychus minutus* (dwarf heron) (Figure 9), *Nycticorax nycticorax* (night heron) (Figure 10), *Falco vespertinus* (evening heron) (Figure 11), *Egretta garzetta* (lesser egret) (Figure 12).

Figure 8. Species *Alcedo atthis* (kingfisher) (https://ro.wikipedia.org)

Figure 9. *Ixobrychus minutus* (dwarf heron) (https://ro.wikipedia.org)

Figure 10. Species *Nycticorax nyctocorax* (Night heron) (https://ro.wikipedia.org)

Figure 11. Species *Falco vespertinus* (evening bream) (https://ro.wikipedia.org)

Figure 12. Species *Egretta garzetta* (little egret) (https://ro.wikipedia.org)

Adequate assessment of the impact of mineral aggregates extraction activity on protected bird species in two Natura 2000 sites in Teleorman and Prahova counties was done. The type and degree of environmental impacts vary significantly from site to site depending on various factors. They must therefore be determined on a case-by-case basis.

In order to estimate the potential impact on the two sites Natura 2000, ROSPA0024 OLT-DANUBE CONFLUENCE and ROSPA0152 IALOMIȚA CORRIDOR an impact assessment matrix was developed.

The value of the impact generated by the activity of extracting mineral aggregates on species was consider by the consequences and probability depending on the degree of damage and the possibility of occurrence.

The calculation formula used was:

Impact = probability x consequence (1)

The probability categories are defined according to the Table 1. The consequences were calculated according to the Table 2.

Table 1. Probability categories (Ozunu & Anghel, 2007)

Probability	**Value**	**Observations**
Inevitable	5	The effect will definitely happen
Very likely	4	The effect will manifest frequently
Probably	3	The effect will occur with reduced frequency
Improbable	2	The effect will manifest occasionally
Very unlikely	1	The effect will occur accidentally

Description of consequences are defined according to the Table 2.

Table 2. Description of consequences (Ozunu & Anghel, 2007)

Degree of impairment	**Value**	**Description**
Disastrous	5	Reduction of local populations by81%-100%
Very important	4	Reduction of local populations by 61%-80%
Important	3	Reduction of local populations by 41%-60%
Moderate	2	Reduction of local populations by 21%-40%
Insignificant	1	Reduction of local populations by 0%-20%

The impact matrix was calculated according to the probability of occurrence of the impact and the maximum for useable consequences.

Impact levels of mineral aggregate extraction activity are defined according to the Table 3.

Table 3. Impact levels of mineral aggregate extraction activity (Ozunu & Anghel, 2007)

Value	Impact level
15-25	Significant negative
5-15	Moderately negative
1-5	Negative insignificant

RESULTS AND DISCUSSIONS

Using the bird monitoring method, in order to interpret the matrix of the impact determined by the extraction of mineral aggregates activity on bird species of community interest from two Natura 2000 site ROSPA0024 OLT-DANUBE CONFLUENCE and ROSPA0152 IALOMIȚA CORRIDOR, we used the methodology described below (Ozunu & Anghel, 2007).
The matrix below shows the level of impact of mineral aggregate extraction activity on bird species of community interest from the site ROSPA0024 OLT-DANUBE CONFLUENCE and is defined according to the Table 4. The impact shall be deemed to be moderately negative.

Table 4. Matrix of consequences of the mineral aggregates extraction activity on bird species of community interest from the site ROSPA0024 OLT-DANUBE CONFLUENCE

Consequence	*Cygnus cygnus*	*Phalacrocorax pygmaeus*	*Falco vespertinus*	*Alcedoa tthis*	*Anas crecca*
5					
4					
3					
2	x				
1		x	x	x	x

From the analysis of the information contained in the table above, of the five bird species studied, it appears that for a single bird species, namely *Cygnus cygnus*, the consequence being considered of a moderate level, signifies a reduction of the local population 21-40%, for the rest of the bird species the consequence being considered of an insignificant level signifies a reduction of the local population 0-20%.
The matrix below shows probability of occurrence of negative effects of mineral aggregates extraction activity on bird species of community interest from site ROSPA0024 OLT-DANUBE CONFLUENCE and is defined according to the Table 5. The impact shall be deemed to be moderately negative.

Table 5. Matrix of probability of occurrence of negative effects of mineral aggregates extraction activity on bird species of community interest from site ROSPA0024 OLT-DANUBE CONFLUENCE

Probability	*Cygnus cygnus*	*Phalacrocorax pygmaeus*	*Falco vespertinus*	*Alcedo atthis*	*Anas crecca*
5					
4					
3					
2	x				
1		x	x	x	x

From the analysis of the information contained in the table above, of the five bird species studied, it appears that for only one bird species, namely *Cygnus cygnus*, the impact of the mineral aggregate extraction activity will occasionally manifest, and for the rest of the bird species the impact of the mineral aggregate extraction activity will occur accidentally.
The impact of mineral aggregate extraction activity on the bird species is considered insignificantly negative as evidenced by the analysis of the information contained in Table 6, it appears that for the studied bird species from the site ROSPA0024 OLT-DANUBE CONFLUENCE.

Table 6. Matrix of the impact determined by the extraction of mineral aggregates activity on bird species of community interest from the site ROSPA0024 OLT-DANUBE CONFLUENCE

Impact	*Cygnus cygnus*	*Phalacrocora xpygmaeus*	*Falco vespertinus*	*Alcedo atthis*	*Anas crecca*
15-25					
5-15					
1-5	4	1	1	1	1

In order to estimate the potential impact on the site ROSPA0152 IALOMIȚA CORRIDOR, an impact assessment matrix was developed.
The matrix below shows consequences of the implementation of mineral aggregates extraction activity on bird species of community interest from the site ROSPA0152 IALOMIȚA CORRIDOR CONFLUENCE and is defined according to the Table 7.

Table 7. Matrix of consequences of the implementation of mineral aggregates extraction activity on bird species of community interest from the site ROSPA0152 IALOMIȚA CORRIDOR

Consequence	*Alcedo atthis*	*Ixobrychus minutus*	*Nycticorax nycticorax*	*Falco vespertinus*	*Egretta garzetta*
5					
4					
3		x	x	x	x
2	x				
1					

From the analysis of the information contained in the table above, of the five bird species studied, it appears that for a single bird species, namely *Alcedo atthis*, the consequence being considered of a moderate level, signifies a reduction of the local population 21-40%, for the rest of the bird species the consequence being considered of an important level signifies a reduction of the local population 41-60%.

The matrix below shows probability of occurrence of negative effects of mineral aggregates extraction activity on bird species of community interest from the site ROSPA0152 IALOMIȚA CORRIDOR CONFLUENCE and is defined according to the Table 8.

Table 8. Matrix of probability of occurrence of negative effects of mineral aggregates extraction activity on bird species of community interest from the site ROSPA0152 IALOMIȚA CORRIDOR

Probability	*Alcedo atthis*	*Ixobrychus minutus*	*Nycticorax nycticorax*	*Falco vespertinus*	*Egretta garzetta*
5					
4					
3		x	x	x	x
2	x				
1					

From the analysis of the information contained in the table above, of the five bird species studied, it appears that for only one bird species, namely *Alcedo atthis*, the impact of the mineral aggregate extraction activity will occasionally manifest, and for the rest of the bird species the impact of the mineral aggregate extraction activity will occur with reduced frequency.

The impact of mineral aggregate extraction activity on the bird species is considered moderately negative as evidenced by the analysis of the information contained in Table 9, it appears that for the studied bird species from the site ROSPA0152 IALOMIȚA CORRIDOR**.**

Table 9. Matrix of the impact determined by the mineral aggregates extraction activity on bird species of community interest from the site ROSPA0152 IALOMIȚA CORRIDOR

Impact	*Alcedo atthis*	*Ixobrychus minutus*	*Nycticorax nycticorax*	*Falco vespertinus*	*Egretta garzetta*
15-25					
5-15		9	9	9	9
1-5	4				

The analysis of the level of impact of mineral aggregates extraction activity on bird species of Community interest from the site ROSPA0024 OLT-DANUBE CONFLUENCE took into account the consequences and probability of negative effects taking into account the particularities of the area, the technical characteristics of the project, the degree of reversibility of the effects produced and the observations made in the field. The result is defined as the level of impact, the impact being considered an insignificant negative.

The analysis of the level of impact of mineral aggregates extraction activity on bird species of Community interest from the site ROSPA0152 IALOMIȚA CORRIDOR took into account the consequences and probability of negative effects taking into account the particularities of the area, the technical characteristics of the project, the degree of reversibility of the effects produced and the observations made in the field. The outcome is defined as the level of impact, the impact being considered moderately negative.

Compared to the data contained in the Natura 2000 Standard Form prepared in 2016 (http://www.mmediu.ro) for the bird species from the site ROSPA0024 OLT-DANUBE CONFLUENCE: *Cygnus cygnus* (winter swan) - 5 ex., *Phalacrocorax pygmaeus* (lesser cormorant) - 450 ex., *Falco vespertinus* (evening bream) - 25 ex., *Alcedo atthis* (kingfisher) - 6 ex., *Anas crecca* (dwarf duck) - 1 ex. and for the bird species from the site ROSPA0152 IALOMIȚA CORRIDOR: *Alcedo atthis* (kingfisher) - 30 ex., *Ixobrychus minutus* (dwarf heron) - 15 ex., *Nycticorax nycticorax* (night heron) - 60 ex., *Falco vespertinus* (evening heron) - 300 ex., *Egretta garzetta* (lesser egret)- 50 ex., it was found that the population decreased by a small percentage of up to 10% of the total bird populations of Community interest at site level, due to the extraction of mineral aggregates from these Natura 2000 sites.

The monitoring of threatened species revealed a change in the number, composition and distribution of wild bird species analyzed that may indicate changes in ecological processes, especially in the capacity to sustain sustainable populations of essential species.

The need to ensure biodiversity conservation is seen as a measure to adapt to climate change and at the same time protect vulnerable species by preserving and restoring ecosystems.

From the study it is found that the bird species *Falco vespertinus* (Evening heron) is a vulnerable species that requires the adoption of conservation measures, both active and long-term restrictive measures.

CONCLUSIONS

From the number of specimens of bird species analyzed and included in the Standard Form of the two Natura 2000 sites established in 2016, as well as from the analysis of the information contained in the impact matrices, it appears that the extraction of mineral aggregates in the two studied Natura 2000 sites may have an insignificant negative impact in the OLT-DANUBE CONFLUENCE ROSPA0024 site and a moderate negative impact in the Natura 2000 site ROSPA0152 IALOMIȚA CORRIDOR.

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ADVANCING THE SUSTAINABILITY OF AQUACULTURE THROUGH ECOSYSTEM SERVICES MONETIZATION

Angelica DOBRE[1, 2], Lorena DEDIU[1], Desimira Maria STROE[2], Mirela CREȚU[1, 2], Floricel Maricel DIMA[2]

[1]"Dunarea de Jos" University of Galati, 47 Domneasca Street, 800008, Galati, Romania
[2]Institute of Research and Development for Aquatic Ecology, Fishing and Aquaculture Galati, 54 Portului Street, 800211, Galati, Romania

Corresponding author email: angelica.dobre85@yahoo.com

Abstract

Most of the human interaction with nature affects ecosystems, influencing human life quality. The aquaculture industry plays a pivotal role in global food security, but its growth poses multifaceted challenges to environmental conservation, urging the implementation of sustainable measures to safeguard aquatic ecosystems. In this context, Payments for Ecosystem Services (PES) have emerged as a pioneering approach to incentivize and ensure sustainable practices within the aquaculture sector. This study emphasizes the fundamental role of PES, it highlights its substantial impact in ameliorating environmental repercussions, conserving biodiversity, and enhancing water quality within aquacultural zones. The study underscores the importance of collaborative efforts among stakeholders and advocates for effective monitoring systems to reinforce the efficacy of PES initiatives in fostering sustainable aquaculture practices. By centering on Romania's aquacultural context, this paper provides crucial insights into the implementation of PES within diverse socio-economic and environmental landscapes, contributing pertinent perspectives to the overarching discourse on sustainable aquaculture practices worldwide for a safe future.

***Key words**: aquaculture, biodiversity conservation, economic incentives, environmental services, sustainable practices.*

INTRODUCTION

The relationship between humans and nature is a reciprocal cycle, where human actions impact ecosystems, and ecosystems, in turn, affect human life quality. Human decisions shape today's ecosystems, influencing aspects like land use, water management, and fisheries (Liu et al., 2007; Chapin et al., 2010).
The aquaculture industry is a vital component of the global economy, crucial for ensuring food security amid population growth (FAO, 2018; Turkowski, 2021). Recognizing the need for sustainable practices in aquaculture, the concept of Payments for Ecosystem Services (PES) has gained attention as an innovative approach. PES aims to harmonize socioeconomic development with ecological conservation, providing positive incentives for enhanced ecosystem services (Anderson et al., 2015; Chen et al., 2021; Blandon et al., 2016).
This paper delves into the significance of PES in the aquaculture industry, examining research and case studies showing its impact on sustainable practices and environmental conservation. Additionally, we address challenges and opportunities in implementing PES, stressing the importance of stakeholder collaboration for achieving sustainability goals.

MATERIALS AND METHODS

This study relies on the comprehensive collection and analysis of information gathered from reputable scientific platforms, including ResearchGate, Science Direct, and Google Scholar. The sources encompass research studies, reports from national and international organizations, and pertinent academic publications addressing topics related to aquaculture, conservation of aquatic ecosystems, and the application of Payments for Ecosystem Services (PES).
The research was conducted utilizing key search terms such as "aquaculture", "incentives", "environmental services", "ecosystems services", "PES", "Payments for Ecosystem Services", and specific services/functions (e.g., provisioning, regulating, supporting, cultural, filtration,

carbon, nutrient remediation, carbon trading, etc.).
To establish a global perspective on fish production trends, data from FishStatJ - Software for Fishery and Aquaculture Statistical Time Series, the Food and Agriculture Organization (FAO) on global aquaculture production, the European Commission Database, and the Statistics National Institute database were employed.
Microsoft Excel was used for the organization and analysis of gathered information and the ArcMap program from the ArcGIS 10.7.1 package was used for creating the map.

RESULTS AND DISCUSSIONS

The global aquaculture production has experienced an upward trend from 2010 to 2021 (Figure 1). These figures highlight the economic and nutritional importance of both fishing and aquaculture sectors, especially in China and, more broadly, in Asian countries, which have been leading in terms of production since 2011 (FAO, 2022).

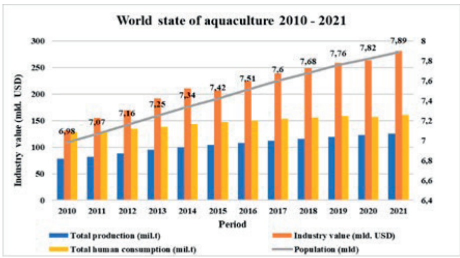

Figure 1 Global Production and Consumption trends of Aquatic Products from 2010 to 2020 (source: FishStatJ)[1]

Thus, the total aquaculture production has seen a steady increase from year to year, in the period 2010-2021, starting from 78 million tons in 2010 and reaching 126.03 million tons in 2021. This growth is strongly correlated with the expansion of the global population which increased from 6.98 mld. in 2010 to 7.89 mld. in 2021 (r^2=0.99). On average, production has experienced an annual increase of 1.84%. The smallest advancement during this period was recorded in 2020 compared to 2019 (2.42%±1.84%), a situation understandable given the global circumstances affected by the COVID-19 pandemic. The most significant increase was recorded in 2012 compared to the production in 2011 (8.04%±1.84%).
Similarly, the value of the aquaculture industry has consistently increased, being directly influenced by the increase in fish and other aquatic products consumption, registering an average growth rate of 5.47% in the period 2010-2021. The year 2011 marked the highest increase in value (18.06%±5.47%), while in 2015 the industry faced a decrease of 2% compared to the previous year.

The current state of aquaculture in Romania

Aquaculture in Romania primarily operates in two distinct directions: one involves extensive or semi-intensive cultivation of cyprinids in polyculture, utilizing natural basins such as ponds, reservoirs, and lakes, while the other focuses on the intensive cultivation of salmonids. Additionally, the freshwater aquaculture industry has diversified and expanded to include other species such as sturgeon, African catfish, tilapia, and perch (www.anpa.ro).

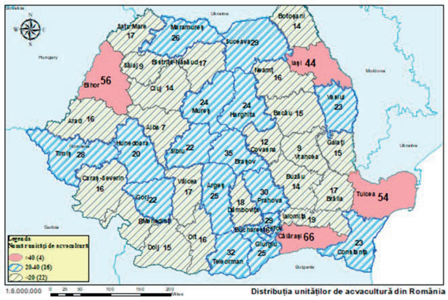

Figure 2 The distribution of aquaculture units with valid licenses across counties in Romania as of the year 2023 (Source: original map)

According to the Aquaculture Units Register (update by National Agency for Fishery and Aquaculture), in 2023, Romania had 944 aquaculture farms with valid licenses (Figure 2), occupying an area of 69,766.98 ha, out of which 168 nurseries, 690 farms, and 86 mixed.
Counting approximately 267 farms covering an area of 189.19 ha, trout farming represents approximately 28% of the total aquaculture farms in Romania, while pond cyprinids aquaculture and other freshwater species - 72%

[1] The parameter "Total human consumption" takes into consideration production values, supply values, exports, imports and non-food use products

- span over an area of 69,577.79 ha, with a total of 677 farms.
In the global context, Romania demonstrates a high potential for the development of the aquaculture sector, supported by a hydrographic network spanning over 843,710 ha (Pila et al., 2023), climatic and geographic diversity, providing opportunities for the cultivation of various fish and crustacean species in different regions. Furthermore, the country's long history in fishing and aquaculture contributes to a solid foundation of knowledge and expertise in the field.
Comparing with international trend, in Romania, from 2010 to 2021, aquaculture experienced relatively slow evolution both in terms of production (with an average annual evolution rate of 3.08%) and industry value (Figure 3). At the same time, it is important to note that there were significant fluctuations and certain periods experienced production declines, such as the year 2020, which was influenced by the COVID-19 pandemic.

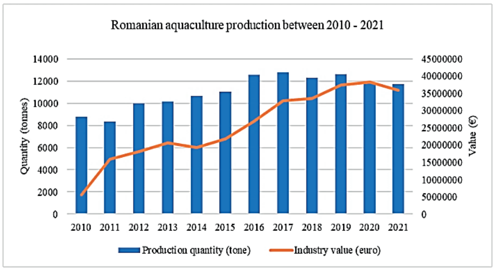

Figure 3 Evolution of Romanian aquaculture production and industry value between 2010-2021
(Source: FishStatJ)

The average annual growth rate of the sector value[2] during this period was approximately 20.10%, indicating a consistent increase, from €5,605,148.85 in 2010 to €35,860,002.26 in 2021. This growth could be attributed to rising prices in the global market and increased demand for fish products. An important microeconomic theory states that the intersection of supply and demand in the market determines both the price and the equilibrium quantity for the respective product (Marshall, 2009). This suggests that this increase in market prices of fish meat may stimulate production to meet the increased demand.
This observation suggests that when market prices for fish products are rising, aquaculturists are often encouraged to increase their production to satisfy demand. With the increase in prices, an opportunity may arise for producers to earn higher profits, motivating them to invest in technology to amplify production. Thus, the relationship between market prices and quantity produced reflects the interaction between supply and demand in a dynamic economic environment.
In this general context, the traditional producers, operating in large pond farms, are in disadvantage in comparison with those operating intensive farms, given the lower unitary production imposed by low technologic intervention capacity. The adaptation to dynamic economic environment of these farms is slower and therefore they are more likely to lose market share in the competition with small farms that integrate intensive technology. In order to be competitive and sustainable large pond fish farms have to adopt ecological aquaculture principles (Costa-Pierce, 2002) which, beside the technical issues of ecosystem design, incorporates socio-cultural aspects, targeting the development of human community while decreasing aquaculture’s inherent risks and environmental impacts, especially those on biodiversity (Bosma & Verdegem, 2011). Therefore, by switching the focus toward developing ecosystem services, large traditional farms could also improve their economical sustainability.
Fluctuations and unexpected events, such as the COVID-19 pandemic, have demonstrated that the aquaculture in Romania faces challenges in competitiveness compared to other regions, emphasizing the sector’s fragility and the need for continuous adaptation to changing market conditions and the environment.
The combined insights highlight the potential for sustainable aquaculture development in Romania, with adequate legal framework and financial support. A strategic investment in aquaculture infrastructure, technology, and education could further drive the growth of the industry, creating economic opportunities while promoting environmental sustainability. This

[2] This parameter contains aquaculture production statistics by country or territory, species item, FAO Major Fishing Area and culture environment

approach aligns with global trends towards sustainable food production.

Ecosystems services

Considering that markets, global trade, and consumer preferences influence the growth of the sector (Subasinghe, 2009), in direct proportion to the expansion of the aquaculture industry, concerns have arisen about its impact on the environment and aquatic ecosystems (Froehlich et al., 2018; Poore & Nemecek, 2018; Weitzman, 2019) and the health of resident species (van Senten et al., 2018).

Uncontrolled expansion of aquaculture has led to issues such as water pollution, degradation of natural habitats, and a decline in biodiversity in affected regions (Troell et al., 2014; Henriksson et al., 2021). In recent years, significant progress has been made in developing approaches that consider ecological sustainability in aquaculture (Brugère et al., 2019; Alleway, 2019).

Some authors (Overton, 2023; Alleway et al., 2023) argued that aquaculture, when done correctly and in appropriate locations, using best practices, can provide ecosystem benefits, from habitat provision to improved water quality and biological control, thereby reducing the risk of negative environmental impacts. Innovative strategies are needed to meet the United Nations Sustainable Development Goals in a timely manner and in the context of a growing human population. These strategies must allow to produce sustainable biological resources with minimal environmental impact and ensure healthy food, sustainable energy, and harmless materials, contributing to biodiversity conservation (Duarte et al., 2022).

Already used at a global scale, a form of sustainable aquaculture practice is represented by integrated multitrophic systems (IMTA) From an environmental standpoint, this method aims to maintain water quality and carbon sequestration, stock enhancement and biodiversity conservation (Zhou et al., 2022; Arcade et.al., 2023).

Moreover, IMTA help reduce inorganic sulfur in sediments, distribution of dissolved inorganic selenium, and nutrient cycling; distribution and seasonal variation of picoplankton and contributes to the concept of a circular economy through nutrient recycling (Fang et al., 2015). Therefore, through this practice, aquaculture becomes a crucial provider of environmental services to society.

Romania has a great potential for IMTA development and also from the perspective of aquatic ecosystem services this direction is also promoted in National Multi-Annual Strategic Plan for Aquaculture 2021-2030 (www.anpa.ro).

The transformation of conventional aquaculture into sustainable practices, such as IMTA, promotes economic viability and environmental protection. Private and financial benefits from multitrophic aquaculture platforms include sales of produce and services, energy savings, and improved productivity. Environmental benefits encompass mitigate global warming and enhanced water quality (Zugravu et al., 2016).

It has been observed that the initial emphasis on ecosystem services as an educational concept, designed to stimulate public interest in biodiversity conservation, has gradually shifted towards a growing focus on transforming ecosystem services into tradable commodities in potential markets (Kaiser et al., 2023).

Moreover, it was suggested (Galappaththi & Berkes, 2014; Bottema et al., 2019) that promoting aquaculture through economic incentives is not only necessary but also crucial for attaining farm management that considers environmental factors extending beyond the farm boundaries, a concept referred to as "beyond-farm management". Ecosystem services encompass the transfer of materials, energy, and information from natural resources to the services rendered by built and human capital, with the goal of enhancing human well-being (Costanza et al., 1997).

In the Millennium Ecosystem Assessment (MEA) Report, 2005, ecosystem services are presented as the benefits people obtain from ecosystems. TEEB, 2010 classified them into provisioning, regulating, supporting, and cultural services (Figure 4).

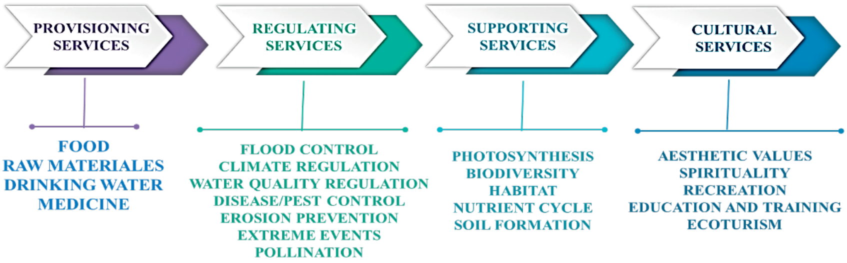

Figure 4. Classification of Ecosystem Services (according to TEEB, 2010)

Cole & Moksnes (2016) consider that one crucial aspect for policymakers who must make difficult decisions about allocating scarce financial resources for environmental protection is assessing the value of the benefits provided by nature in monetary terms.

As methods for the monetary evaluation of ecosystem services have advanced, there has been increased interest in developing market-based instruments that generate conservation incentives. Among these instruments, Payments for Ecosystem Services (PES) stand out (WWF Romania, 2016).

Payments for Ecosystems Services

Payments for Ecosystem Services (PES) are becoming increasingly widespread as a method of ecosystem management through the use of economic incentives. The economic approach within PES aims to incorporate ecosystem services into a market model, placing particular emphasis on efficiency (Farley & Costanza, 2010).

A simplified definition of the concept, as provided by Engel et al (2008), presents PES as a management tool where economic incentives are provided to participants in exchange for environmental services. Wunder (2005) further expands on Engel's (2008) explanation and defines the concept of Payments for Ecosystem Services (PES) as a voluntary transaction in which different users of environmental services "pay" a provider of ecosystem services under the condition that the flow of benefits remains constant. This description highlights an approach focused on market and customer requirements, preferences, and needs. Such an approach takes into account customer feedback and adapts products, services, or strategies to meet market demands and trends, aiming to maximize customer satisfaction and success in the competitive environment.

On the other hand, Muradian et al. (2010) focus on the actors involved in PES: institutions and intermediaries. They consider these actors to play a key role in correcting market failures and addressing issues.

Researchers believe that these types of incentives could yield results where traditional management approaches have failed (Kazakova, 2007; Engel et al., 2008; Blandon et al., 2016).

Unfortunately, there are a limited number of studies evaluating the effectiveness of these payments in aquaculture or other industrial sectors that provide environmental services. Even fewer studies focus on the impact on cultural services (tourism, recreation, spiritual and aesthetic values of the ecosystem), with the main focus being on provisioning, regulating, and supporting services.

Nevertheless, these incentives represent an opportunity to align the aquaculture industry with the United Nations' goals for human prosperity and environmental sustainability. Even though the ecosystem can provide a multitude of ecosystem services, the compensatory payment scheme should target only those production services that can realistically be exploited by landowners.

Payments for Ecosystem Services meet the following conditions: they are voluntary, there is a well-defined environmental service, there is at least one buyer, there is at least one provider, and they relate to the provisioning services that the ecosystem offers (Fripp, 2014; Silva-Muller, 2022).

In contrast to other types of incentives, such as eco-certification, in the case of PES, contracts include conditions that impose restrictions on land and/or resource use or establish environmental outcomes for a predefined number of land units (Wunder, 2005; Pagiola et al., 2008).

These specific requirements have significantly reshaped the approach to aquaculture practices and the conservation of aquatic ecosystems, as we will further explore in detail.

1. The impact of Payments for Ecosystem Services (PES) on aquaculture practices and the conservation of aquatic ecosystems and biodiversity.

The implementation of Payments for Ecosystem Services (PES) has had a significant impact on aquaculture practices and the conservation of aquatic ecosystems. PES programs have incentivized aquaculturists to adopt innovative techniques and strategies to reduce their negative environmental impact. These include more efficient use of water resources, reduced use of chemicals, and improved habitat conditions for wild species (Chen et al., 2021).

The idea of biodiversity conservation and the protection of wild species through PES implementation is sustained also by Martinez-Harms & Balvanera (2012). As a result, PES initiatives have supported conservation efforts for vulnerable species and contributed to maintaining ecological balance in aquatic ecosystems.

In a recent study, Duarte et al. (2022) present that the benefits of algae in the context of sustainability, extending beyond aquaculture and impacting a diverse range of industries, are highlighted. These benefits include ensuring food security, promoting population health, providing clean and affordable energy, contributing to the fight against climate change through long-term carbon sequestration (Sondak et al., 2017, Duarte et al., 2017), with potential for industrial innovation and future development, responsible production system implementation, and generating significant positive environmental effects, with additional societal benefits (Hasselström et al., 2018).

Furthermore, algae cultivation has helped alleviate poverty through the implementation and monitoring of innovative techniques, as seen in communities in northern Brazil (Freddi & Aguilar-Manjarrez, 2003; Rebours et al., 2014). Additionally, PES has led to improved knowledge among farmers about algae aquaculture and technologies, making algae a significant source of income.

2. The contribution of PES (Payments for Ecosystem Services) to improving water quality and reducing pollution.

The implementation of Payments for Ecosystem Services (PES) has led to improvements in water quality and a reduction in pollution in aquaculture areas (Barbier, 2007). This aspect is reinforced by other similar studies (Senff et al., 2018). Moreover, by adopting responsible practices such as efficient waste management and the use of more effective water filtration technologies and discontinuing the use of poisons and toxic substances to eliminate unwanted species, aquaculturists have reduced the impact of pollution on the aquatic environment.

In a study conducted in shrimp farms, (Hukom et al., 2020) where farms receiving financial incentives were compared with those which did not benefit from these incentives, was demonstrated that incentives beneficiaries have improved quality water parameters (dissolved oxygen, ammonia, salinity and temperature), reduced nutrient discharge, enhanced technical efficiency and higher production levels.

Shrimp farming has gained significant attention in recent years, intensifying in many countries (Primavera, 1997). This has been the main cause of the disappearance of mangrove habitats and, consequently, the reduction of ecosystem services provided by them. Therefore, sustainable shrimp farming practices are continuously sought after (Gunawardena & Rowan, 2005).

The bivalve industry has also experienced significant growth, considering the role it plays in supporting ecosystems, creating habitats (van den Burg et al., 2022), carbon sequestration (Han, 2017), nutrient removal, and water quality improvement, thereby preventing eutrophication (Troell et al. 1999; Marinho-Soriano et al. 2011; Gentry et al., 2020).

While some methods of aquaculture may harm the aquatic environment or interfere with vital services, others have the potential to provide notable environmental advantages. As a result, it is crucial for the former to support the endeavors of the latter. Thus, the implementation of Payment for Ecosystem Services (PES) becomes imperative in this context.

Debates and Challenges in Implementing PES in Aquaculture

While the concept of Payments for Ecosystem Services (PES) holds significant potential for promoting sustainable aquaculture and conserving aquatic ecosystems, there are examples of flawed implementations of these programs (Vatn, 2010). Often, these incentives are used to address an immediate problem rather than focusing on prevention (Sone et al., 2019). Such inadequate implementations can result in limited impact on aquaculture practices or the surrounding environment, raising questions about the effectiveness of these programs.

They assume that farmers and, in general, providers of ecosystem services are paid for a certain behavior. This implies, on the one hand, that the proposed goal is difficult to achieve, and on the other hand, that human greed knows no bounds. In situations where payments are made for behavior that should be considered normal, such as environmental protection (Gneezy et al., 2011; Kruijssen et al., 2022), the implementation of PES could lead to a diminishing of certain moral values (Bowles, 2016).

Additionally, Vatn (2010) argues that the distinction between incentives and compensation is important in terms of the relationships built between the involved agents, based on the idea of "reciprocal exchange" rather than goodwill, influencing the level of control and reciprocity within contracts.

Certainly, one might contemplate the longevity of the "beneficial" effects associated with the implementation of ecosystem service payments, especially considering the prospect of ceasing such payments. A crucial aspect to ponder is whether the positive impacts observed during the period of payments would endure and remain perceptible if ecosystem monitoring were continued post the cessation of payments. (WWF Romania, 2014*)*

1) Accurate Assessment of Ecosystem Services

It is not difficult to identify the services provided by an aquaculture farm. What presents a challenge is quantifying these services (van den Burg et al., 2022). We are not discussing provisioning services, which can be evaluated at market prices. The issue lies with the evaluation of regulating, cultural, and supporting services, where exact accounting values cannot be established.

Furthermore, researchers are concerned that intrinsic and socio-cultural aspects of ecosystems may transform into interchangeable values, thereby reducing ecosystem complexity and integrity (Dextre et al., 2022; Cole and Moksnes, 2016). The process of economizing nature through labeling is considered unethical (McCauley, 2006; Peterson et al., 2010; Munda, 2004).

Additionally, it has been observed that some small and medium-sized farms may face financial difficulties in implementing the necessary changes to qualify for PES, without necessarily leading to a collective effort to improve ecosystem functionality, as seen in Lombok, Indonesia (Senff et al., 2018). Chen et al. (2021) emphasize the need for an accurate determination of the environmental services that will be the subject of the transaction and the effects of aquaculture species on the environmental service involved in the scheme to establish precise ways of mitigation/ prevention.

2) The Role of Collaboration Among Stakeholders

Government institutions play a significant role in the outcomes of PES implementation in aquaculture. Many PES schemes have failed due to the lack of adequate or incomplete governmental support (Wunder, 2007; Senff, 2018; Silva-Muller, 2022). Policies regarding the implementation of payments and methods for evaluating ecosystem services can be vague and inconsistent, as seen in certain regions of Russia where these incentives are active (Yakovlev & Mikhaylov, 2020).

Studies by Chen et al. (2020) highlight that the success of PES implementation in aquaculture largely depends on close collaboration between government authorities, the aquaculture industry, environmental organizations, and local communities. Therefore, effective

collaboration ensures better-coordinated planning and implementation of PES programs, addressing specific challenges and ensuring long-term sustainability (Chen, 2021).
One of the conditions of PES is the existence of a provider. This implies that there is a right of ownership over the environmental service to be the subject of the transaction (Vatn, 2010).

3) Undermining Compensation Policies
Gordon et al. (2015) highlight the potential risk of "greenwashing," where aquaculturists may adopt sustainable practices only to receive financial rewards without a genuine commitment to environmental conservation. This underscores the importance of continuous monitoring and evaluation of the impact of PES programs.
Alternatively, there is the possibility that the value of payments is too low, given that they depend on the market value of the service provided, such as the price of carbon sequestration. Consequently, owners of aquaculture farms may not be willing to provide environmental services, potentially violating one of the defining rules of PES (van de Burg, 2022).
This raises the need for funding from not only public sources but also private organizations, companies, the public, and non-profit organizations, among others. Such an example is represented by the emergence and development of AIPs (aquaculture improvement projects). AIPs are partnerships between private actors in the aquaculture industry aimed at enhancing sustainability in the aquaculture sector that involve engaging and empowering value chain actors to collectively address sustainability issues (https://sustainablefish.org/). AIPs reflect a broader trend of using market-based approaches to promote sustainable and responsible food production (Bottema, 2019).
Furthermore, Vatn (2010) and Kosoy & Corbera (2010) raise concerns regarding how payments are delivered to the providers of environmental services within compensation transactions. Payment for ecosystem services can manifest as incentives tied to the level of provision or as rewards for positive actions, with the distinction resembling the cost-sharing dynamics found in sales-compensation relationships.

CONCLUSIONS

The synthesis concludes by affirming that PES holds promise in promoting sustainable aquaculture and conserving aquatic ecosystems, provided that challenges are addressed and recommendations implemented.
The importance of continuous research, collaboration, and awareness is highlighted to ensure the long-term success of PES in the aquaculture industry.
In summary, the analysis underscores the intricate relationship between aquaculture, ecosystem services, and the potential of PES to foster sustainability in the industry, both globally and in the specific context of Romania.
From the analyzed data regarding aquaculture in Romania, it can be observed that our country has significant opportunities for the expansion and consolidation of this sector in a sustainable manner. Considering the generous natural resources, as well as the extensive experience in fishing and aquaculture, the country has all the prerequisites to develop a strong and competitive ecological aquaculture industry. However, to achieve this, an integrated approach is necessary to promote sustainable practices, biodiversity conservation, and water quality maintenance. Additionally, the involvement and collaboration of all stakeholders are essential, as well as the implementation of efficient policies, regulations, and economic support to ensure that sustainable aquaculture development is carried out responsibly and in line with the country's environmental and social objectives.

RECOMMENDATIONS

To improve the implementation of Payments for Ecosystem Services (PES) in aquaculture, considering the specific context of Romania, the following concrete suggestions can be proposed:
- Develop a Clear and Incentive Legal Framework: Draft and implement a specific legal framework for PES in aquaculture,

providing clarity and substantial incentives for producers adopting sustainable practices.

- Establish a National Fund for Aquaculture PES: Create a national fund exclusively dedicated to PES in aquaculture, funded by budgetary resources and contributions from the private sector, to financially support projects and initiatives for conserving aquatic ecosystems.
- Implement an Efficient Monitoring System: Develop and implement an efficient monitoring system to assess the impact of PES in aquaculture. This system should provide clear performance data and contribute to the continuous optimization of programs.
- Introduce an Educational and Awareness Program: Launch a national education and awareness program for communities, farmers, and consumers to promote the importance of PES in aquaculture and the benefits it brings to ecosystems.
- Enhance Collaboration Among Various Stakeholders: Facilitate collaboration between governmental bodies, non-governmental organizations, the private sector, local communities, and researchers. This collaboration can consolidate resources and expertise to ensure the effective implementation of PES.
- Tailor PES to the Diversity of Romanian Aquaculture: Customize PES programs to account for the diversity of aquatic systems and species cultivated in Romania. Specific approaches can be developed for freshwater and marine farms, as well as different fish and crustacean species.
- Involve Local Communities in Decision-Making: Ensure the active participation of local communities in the decision-making process regarding PES projects. Consultations and involvement of locals can contribute to identifying the most suitable solutions for conserving aquatic ecosystems.
- Promote Examples Through Successful Case Studies: Create and promote successful case studies of aquaculture farms in Romania that have successfully implemented PES programs. These examples can serve as inspirational models for other producers and communities.
- Financial Accessibility for Small and Medium-Sized Farmers: Establish specific financial facilities for small and medium-sized farmers wishing to participate in PES programs. This may include grants, preferential loans, or financial insurance schemes.
- Integrate PES into Regional Development Strategies: Integrate PES into regional development strategies to ensure these programs align with overall economic development and environmental conservation goals in respective regions.
- Engage Key Stakeholders in Aquaculture: Actively involve farmer associations, research organizations, and other key stakeholders in the aquaculture sector in developing and promoting PES initiatives, ensuring they are tailored to the realities and needs of local industries.

Implementing these proposals could contribute to the efficiency and adaptation of PES programs in Romanian aquaculture, ensuring they align with the country's specific context and sustainability objectives.

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THE INFLUENCE OF THE FOOD TYPE ON THE DEVELOPMENT OF PIKE-PERCH (*Sander lucioperca*, Linneaus -1758) IN THE POST-EMBRYONIC PERIOD

Gheorghe DOBROTĂ, Nicoleta-Georgeta DOBROTĂ, Nino MARICA, Silvia RADU

Research and Development Station for Fisheries Nucet, Nucet, 549 Principală Street, Nucet, Dâmbovița County, Romania

Corresponding author email: dobrota19dng@yahoo.com

Abstract

The culture of the pike-perch(Sander lucioperca, L.-1758), in intensive aquaculture, is difficult to achieve due to its character as an active predator in the water mass. In recent years, significant quantities of pike-perch are requested by consumers, being more and more numerous those who prefer this species. Choosing the most appropriate food category is very important, because it can make the difference between the success or failure of development in the post-embryonic development stage. The experimental works for the development of the pike-perchtook place in three variants, with different food being administered, as follows: variant 1 - zooplankton (Daphnia sp.) was administered, variant 2 - prussian carp larvae (Carrassius gibelio) were administered and variant 3 - granulated feed was administered.The growth was carried out in "Evos" fiberglass tanks. The results obtained were very good in variant 2. Good results were also obtained in the other two variants (variant 1 and variant 3). The decision of the way of post-embryonic development remains at the farmer's choice..

Key words*: food, post-embryonic development, Sander lucioperca, survival, weight.*

INTRODUCTION

The culture of pike-perch (*Sander lucioperca*, L.-1758) is currently carried out in extensive or semi-intensive systems as a complementary species. Through the intensive growth of this species, it has been demonstrated that it can consume additional food such as granulated fodder (Bódis et al., 2007; Ebrahimi & Vahabzadeh, 2014; Hubenova et al., 2015; Javid & Falahatkar, 2021).

Results regarding the feeding of pike-perch fingerlings with additional feed have been made since the "90s" by Zakęś & Demska-Zakęś, (1996); Zakęś (1999) și Horváth et. al. (2013).

A group of researchers from Hungary(Molnar et. al., 2004) carried out an ample experiment, through which they evaluated the effect of the pike-perch fry accommodation to feed. They also tested the effects of stocking density on behavior and feeding. The method of feeding (live food or granulated feed) proved to have a significant effect on feed consumption and implicitly on the increase in growth. Fish feed with live food had the fastest growth rate (Gardeur et. al., 2007; Hamza et. al., 2007; El Kertaoui et. al., 2019). On the contrary, according to those presented by other researchers, it was shown that stocking density did not significantly influence individual growth. But at higher densities, the specific consumption was better. Thus, both the stocking density and the type of feed influence the feeding behavior (Rasouli et al., 2014).

The objective of this experiment was to establish a regime of supplementary feeding as suitable as possible for the larvae of the pike-perch, so as to find an effective correlation between the growth rate and the type of food administered.

MATERIALS AND METHODS

The experimental works were carried out for three consecutive years, at the Research and Development Station for Fisheries Nucet.The variable factor in this experiment was the type of food administered.

The experiments were carried out in "Evos" type fiberglass tanks, with a capacity of 1000 liters (Figure 1). The shape of the tanks is parallelepiped, have rounded corners and they are supplied with water from the outside by

free fall.The evacuation is carried out through a pipe centrally located at the bottom of thetank, thus obtaining a circular current in the tank.
The average water level in the experimental tanks was approximately 0.55 cm. The technological water flow was correlated with the temperature, being 8-14 l/minute/tank.

Figure 1. Evostype fiberglass tanks (original photo)

To maintain proper hygiene, the fiberglass tanks are washed and disinfected with $Ca(ClO)_2$, then filled with water and stocked. The vertical water level initially rises to 0.3-0.35 m. With the development of the fish, the water level rises to 0.55-0.6 m on the 20th day of post-embryonic period.
The experimental variants were carried out in triplicate, the variable being the type of food administered. Since the pike-perch is an active predatory species in which cannibalism is easily manifested and the predatory instinct is quickly triggered (Dobrotă et al., 2022), it was decided to stock 1000 exemplars/tank (Figure 2). At the same time, the quality of food is very important for the development of fish during the post-embryonic period, in the first year of growth or later and is specific to the species and the applied technology (Dobrotă et. al., 2022).
The realization of the experiments was carried out in stages, as follows (Table 1):

- ↳ to obtain conclusive results, three experimental variants were established, in triplicate, with different feeding for each variant;
- ↳ a number of 3 tanks were used for each experimental variant;
- ↳ the experiments took place in three consecutive seasons;
- ↳ granulated fodder and live food were used for feeding;
- ↳ feed was administered "ad-libitum" and its consumption was monitored as well as the environmental conditions of the technological water;
- ↳ theperiodof post-embryonic development was 41 days.

The quantity and method of administration of food

During the period of post-embryonic development in the pike-perch, feeding was done as follows:

- ■ in variants 1 and 2, live food was distributed (*zooplankton and prussian carp larvae*), and in variant 3 granulated feed (*Aqua Garant Start*);
- ■ for all experimental variants, during the first 10 days, a mixture of boiled egg yolk (50%) was administered with an extruded granulated feed (*Aqua Garant Start*, 50%), intended specifically for feeding fish at this stage of development. The mixture was distributed 5 times a day between 8^{00} - 20^{00} hours;
- ■ when the pike-perch fry reached the age of 17 days, they switched to the specific feeding for each experimental variant, as follows:
  - ⟶ variant 1 – administration of live food (*Daphnia* sp.) from cultures from SCDP Nucet;
  - ⟶ variant 2 – administration of fish larvae (*Carasus auratus*);
  - ⟶ variant 3 – feeding with Aqua Garant Start granulated feed 0.4 mm.
- ■feeding for variants 1 and 2 was carried out twice a day, at 8^{00} and 15^{00}, and the amount depending on how it is consumed by the fish material;
- ■ for variant 3, the administration method was chosen with the help of automatic belt feeders, between 8^{30}-20^{30} hours (Figure 3);
- ■ the feed administered increased quantitatively with the increase in weight of the fish;
- ■ at approximately 37 days, the fish showed the phenomenon of cannibalism, so they were transferred to the rearing ponds in the first summer.

Table 1.Stocking formula of "Evos" tanks with 7-8 day old larvae

No.	The experimental variant	Tank volume (l)	Ex./tank	No. tanks	Total ex./ variant	Food type	No. years
1	Variant 1 (V1)	1000	1000	3	3000	zooplankton	3
2	Variant 2 (V2)	1000	1000	3	3000	fish larvae	3
3	Variant 3 (V3)	1000	1000	3	3000	feeding with fodder	3

Figure 2. Stocking with 7-8 day old larvae (original photo)

Table 2. Hydrochemical parameters recorded (average values) during the three years of study from the water source and experimental tanks

No. crt.	The chemical parameter		U.M.	Parameter values		
				Optimum according to Order MMGA no. 161/2006	Water source	Experimental modules (Evostype)
					Average of the three years	
1	pH		pH units	7-7.8	7.2	7.4
2	Calcium(Ca^{2+})		mg/l	90-120	34.4	33.6
3	Magnesium(Mg^{2+})		mg/l	10-40	14.1	15.56
4	Ca^{2+}/ Mg^{2+}		mg/l	5	2.43	2.15
5	Total hardness		(^0D)	12	8.06	8.28
6	Alkalinity		mg /l	200-400	176.9	189.1
7	Organic substance		mg $KMnO_4$/l	20-60	16.8	20.56
8	Disolved oxygen		mg/l	5-12	4.25	5.2
9	Nitrates(NO^-_3)		mg/l	2.5-4	0.062	0.156
10	Nitrites(NO^-_2)		mg/l	0.03	0.0012	0.0024
11	Phosphates(PO^{3-}_4)		mg/l	0.05-1.5	0.042	0.086
12	Chloride	Cl^-	mg/l	40	9.54	10.6
		NaCl	mg/l	30	15.78	17.53
13	Ammonia (NH^+_3)		mg/l	lack	lack	lack
14	Ammonium (NH^+_4)		mg/l	0.5-1	0.14	0.54

RESULTS AND DISCUSSIONS

Monitoring the environmental indicators of technological water from the source and the Evostype experimental tanks

The quality of the growth environment is of particular importance because it can guarantee the success in the post-embryonic development period. During the development period of the pike-perch fry in the "Evos" tanks (with different types of food), water samples were taken 2 times a week to monitor the hydrochemical indicators. Dissolved oxygen and water temperature were recorded daily. The dissolved oxygen value was over 5.5 mg/l (Figure 4). In order to maintain oxygen above this value, measures have been initiated, such as: cleaning the tanks of uneaten food and fish excrement, constant water flow, etc.

The main hydrochemical parameters of the water from the supply source and from the experimental basins ("Evos" type tanks), were within optimal limits for fish farming water, according to the Ordinance MMGA no. 161/2006 (Table 2).

Figure 3.Automatic belt feeders (original photo)

Figure 4. Monitoring dissolved oxygen and water temperature (original photo)

Figure 5. The phenomenon of cannibalism (original photo)

Figure 6. Biometric measurements at the end the postembryonic period (original photo)

Evaluation of the growth performance of pike-perch fry

The growth and development of the pike-perch during the post-embryonic development period was 41 days (586-668 degree days), ending with the appearance of the accentuated phenomenon of cannibalism (Figure 5).

The experimental works in the post-embryonic period were carried out under similar technological conditions in all variants and in all years of the study, the only variable element being the food administered.

The main biotechnological indicators monitored in the growth and development experiments during the growth period, in an intensive system, in the pike-perch (*Sander lucioperca*, L. 1758) (Table 3 and Figure 6) were: average weight (W); survival rate (%); feed conversion ratio (FCR); real growth rate (Sr); daily growth rate (GR) (Olaniyi et. al., 2013).

Average mass of fish material (W) - g/ex

→ the best value of the average mass obtained after 41 days was in variant 2, tank T5 in the first year of study of 4.154 g/ex, and the lowest was obtained in variant 3, tank T9, from the third year of study of 1.878 g/ex;

→ in the first year of study, the best value was in variant 2 of 4.103 g/ex, the lowest in variant 3 of 1.927 g/ex and of 2.545 g/ex in variant 2;

→ in the second year of study, the best value was in variant 2 of 4.08 g/ex, the lowest in variant 3 of 1.961 g/ex and 2.479 g/ex in variant 2;

→ in the third year the best value was in variant 2 of 4.094 g/ex, the lowest in variant 3 of 1.962 g/ex and of 2.477 g/ex in variant 2;

→ we can conclude that, the best value obtained in all the years of study was in variant 2, variant 1 ranked second and variant 3 had the lowest value (Figure 7).

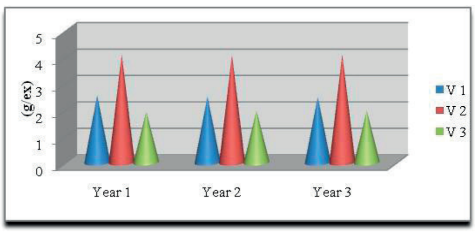

Figure 7. Average mass variation

Table 3. The main biotechnological indicators obtained at the end of each experimental period (average values/experimental variants/years)

The year	Variant	Tank	SV med. (%)	W medium (g)	LT Average (mm)	Sr (g)	GR (g/day)	FCR (g feed/g biomass gain)	SGR (%/day)
1	V1	T1+T2+T3	61.8	2,545	65	1571.1	37.41	1.97	16.37
	V2	T4+T5+T6	65.2	4,103	75	2673.4	63.65	2.18	17.59
	V3	T7+T8+T9	46.9	1,927	59	902	21.48	1.67	14.95
2	V1	T1+T2+T3	60.8	2,479	65	1505.5	35.85	1.93	16.26
	V2	T4+T5+T6	65	4.08	77	2652.2	63.15	2.18	17.58
	V3	T7+T8+T9	47.3	1,961	60	925.4	22.03	1.68	15.17
3	V1	T1+T2+T3	61.4	2,477	64	1520.3	36.2	2,3	16.36
	V2	T4+T5+T6	65	4,094	76	2658.5	63.3	1.97	17.55
	V3	T7+T8+T9	46.8	1,962	60	916	21.81	1.83	15.01

Note: V1– zooplankton feeding; V2 – feeding with fish larvae (prussian carp); V3 – feeding with granulated fodder

Survival rate (%)

→ the best value of the survival rate obtained after 41 days was in variant 2 T4 tank, in the second year of study of 66.8%, and the lowest was obtained in variant 3, T9 tank, in the third year of study by 45.6%;

→ in the first year of study, the best value was recorded in variant 2 of 65.2%, the lowest in variant 3 of 46.9% and of 61.8% in variant 1;

→ in the second year of study, the best value was recorded in variant 2 of 65.0%, the lowest in variant 3 of 47.3% and of 60.8% in variant 1;

→ in the third year of study, the best value was recorded in variant 2 of 65.0%, the lowest in variant 3 of 46.8% and of 61.4% in variant 1;

→ we can conclude that the best value obtained in all the years of study was in variant 2, variant 1 was in 2nd place and variant 3 recorded the lowest value (Figure 8).

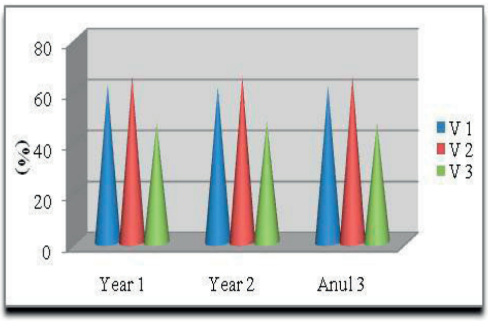

Figure 8. Variation in survival rate

When pike-perch consumes food different from the specific one, it shows a state of stress. With the increase in age and weight, it acquires the morphological and behavioral characteristics of adults, begins to feed actively in the water mass and has the instinct to catch organisms that move in front of it, they trigger its attack instinct. Also, the swimming of his peers can trigger this instinct, which he attacks, causing injury or even death. In the variants where live food was administered (prussian carp larvae or zooplankton), a lower rate of cannibalism was observed.

Feed conversion ratio (FCR)

→ the best value of the conversion coefficient was in variant 3, the T9 tank in the second year of study, of 1.63, and the weakest was in variant 1, the T2 tank, in the second year of study, of 2.4;

→ in the first year of study, the best value was in variant 3 of 1.67, the lowest value being in variant 2 of 2.18 and 1.97 in variant 1;

→ in the second year of study, the best value was in variant 3 of 1.68, the lowest value being in variant 2 of 2.18 and 1.5 in variant 1;

→ in the third year of study, the best value was in variant 3 of 1.83, the lowest value being 2.3 in variant 1 and 1.97 in variant 2;

→ we can conclude that, the best value obtained in all the years of the study was in variant 3, on the 2nd place was variant 1 and the lowest value was variant 2. The very good quality of the granulated feed that was administered in variant 3, and a higher conversion coefficient was obtained than the other experimental variants. Thus, we can explain the superiority of the conversion coefficient in variant 3 compared to variants 1 and 2 by the fact that live food has a high water content (Figure 9).

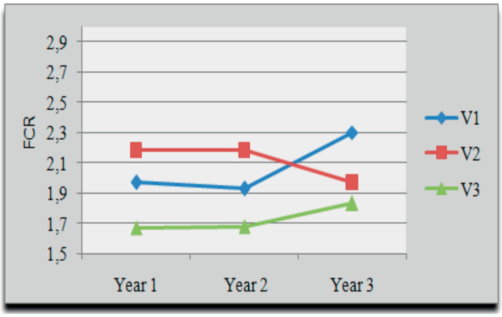

Figure 9.Variation in feed conversion ratio (FCR)

Real growth rate (Sr)

Real growth rate (Sr) registered different values in the three variants as follows: in variant 1 it was 1505-1571 g; in variant 2 of 2652-2673 g and in variant 3 of 902-925 g (Figure 10).

Close values were recorded within the experimental variants. The administration of food specific to the pike-perch was a determining factor in achieving a higher growth rate.

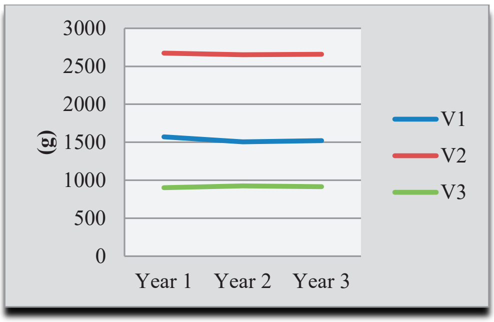

Figure 10.Variation in real growth rate (Sr)

Daily growth rate (GR)

Daily growth rate (GR) was different for each variant, with values of 35.85-37.41 g/day in variant 1, with 63.15-63.65 g/day in variant 2 and 1.48-22.03 g/day in variant 3 (Figure 11). Close values were recorded within the experimental variants.

Parameters that influenced the daily growth rate were: the percentage of survival obtained at the end of the post-embryonic period, the total amount of biomass obtained and the individual mass.

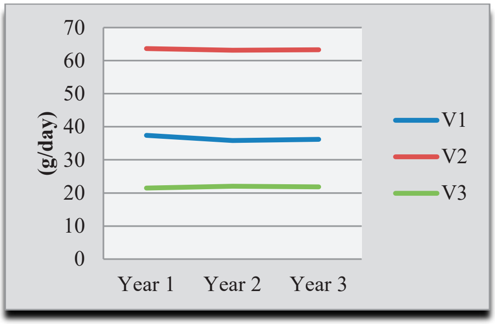

Figure 11.Variation of daily growth rate (GR)

The Pearson coefficient had positive values during the entire period of the experiments, resulting in the fact that the biological material developed homogeneously, this being due to the abundance of food in all experimental variants. The positive correlation between the length of the fish and their mass also shows the homogeneous development within the experimental variants (Table 4).

Table 4. The average correlation between length-weight within the experimental variants

Year	Experimental variant	Correlation	Pearson coefficient
I	1	Positive	0.785
	2	Positive	0.799
	3	Positive	0.582
II	1	Positive	0.996
	2	Positive	0.990
	3	Positive	0.988
III	1	Positive	0.882
	2	Positive	0.998
	3	Positive	0.758

The coefficient of variability demonstrates that the variable body mass comes from the type of food administered, which caused the development of variable batches of fish (Table 5).

It is noted, superior results obtained in variants 2 and 3 where live food was administered in terms of the percentage of survival and average mass, compared to variant 3 where the feed consisted of granulated fodder.

Table 5. Minimum, maximum, average values, standard deviation and coefficient of variability of body mass, between experimental variants

Year	Variant	No. ex. stocked	No. ex. harvested	Mass (g)			CV (%)
				M min.	M max.	M mean ± SD	
I	1	3000	1853	2.021	2.988	2.546 ± 0.21	13.01 ± 0.015
	2	3000	1957	3.707	4.586	4.102 ± 0.22	19.99 ± 0.018
	3	3000	1406	1.349	2.202	1.926 ± 0.15	12.18 ± 0.012
II	1	3000	1825	2.068	2.981	2.478 ± 0.23	10.29 ± 0.03
	2	3000	1950	3.604	4.488	4.081 ± 0.25	15.95 ± 0.022
	3	3000	1418	1.507	2.315	1.962 ± 0.27	10.58 ± 0.016
III	1	3000	1844	2.088	2.993	2.478 ± 0.18	14.63 ± 0.015
	2	3000	1948	3.716	4.585	4.095 ± 0.22	19.25 ± 0.018
	3	3000	1405	1.409	2.582	1.963 ± 0.19	10.48 ± 0.016

CONCLUSIONS

The average weight of the pike-perch species, obtained at the end of the post-embryonic development period, had significantly different values between the three experimental variants. Very good values were recorded in variant 2, good values in variant 1 and slightly appreciable values in variant 3.

The most obvious difference was determined by the survival rate, which recorded significantly higher values in the variants where live food was administered compared to the variant where granulated feed was administered.

The specific growth rate recorded similar values. Biotechnological indicators such as: real growth rate [Sr], survival rate (Sv), daily growth rate [GR]and feed conversionratio (FCR) were differentiated from which it follows that the availability of pike-perch specific food favors growth and decreases the manifestation of cannibalism.

The quality and type of food administered have a positive or negative effect on the growth and development of *Sander lucioperca*, during the post-embryonic development period.

Since pike-perch is a predatory species, even if it has good environmental conditions and very good food, after the age of 36 days it starts to become predatory. Organisms in motion that are near him trigger his attack instinct and he no longer differentiates between his peers and other fish, thus manifesting the phenomenon of cannibalism.

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RESEARCH ON THE EVOLUTION OF MORPHOLOGICAL INDICES IN THE *Cyprinus carpio*- SPECIES DURING THE COLD SEASON IN THE CONTEXT OF CLIMATE CHANGE

Nicoleta-Georgeta DOBROTĂ, Gheorghe DOBROTĂ, Silvia RADU, Mioara COSTACHE, Daniela RADU, Nino MARICA

Research and Development Station for Fisheries Nucet, loc. Nucet, 549 Principală Street, Dâmbovița County, Romania

Corresponding author email: dobrota19dng@yahoo.com

Abstract

Due to the increasingly warm winters of the last 20 years, the wintering of cultured carp is achieved with increasing numerical and weight losses. Water temperatures during the winter period reach 10-12°C, temperatures that intensify the metabolism and set the fish material in motion. Because these temperatures are for short periods, no natural food grows in the water. The paper presents the results of the research carried out in the cold seasons of 2020-2021, 2021-2022, and 2022-2023 on cultured carp aged two summers, from the Research and Development Station for Fisheries Nucet. Wintering was done in earthen ponds, in two variants: variant V1 - without feed distribution and variant V2 - with feed distribution. In view of this study, somatic measurements were carried out, both in autumn and in spring, to assess the change in the values of the condition coefficient, survival percentages, as well as the decrease in weight and number, during the cold season period, in the context of climate change.

Key words: *carp, global climate, morphological indices, temperature.*

INTRODUCTION

Aquaculture continues to significantly expand its production, making it the fastest-growing food production sector globally. Freshwater ecosystems are strongly threatened by climate change. In the context of climate change, phenotypic plasticity takes on particular relevance, as this process allows organisms to cope with the unpredictability of environmental stressors over time (Chang et al., 2021; Costache et al., 2021; Popa et al., 2022). The common carp *Cyprinus carpio* (Linnaeus, 1758) is one of the most widely-distributed freshwater fishes in the world (Cocan & Mireșan, 2018; Anin et al., 2021). In the technological process of carp farming, in fish farms that use earth ponds, for the cold period of the year, the fish is stored at a high density, in special wintering earth ponds. The temperature of technological water influences: the oxygen content of the water, the amount of oxygen assimilated by aquatic organisms, the availability of food, including metabolic activity (Dobrotă, 2022). In these situations, the growth rate of fish is influenced in direct proportion to water temperature, which in turn is correlated with latitude (Hokanson, 1977). Temperature plays a critical role in the growth and development of aquatic animals (Ngoan, 2018). Fish, being poikilothermic, may particularly be sensitive to temperature variations resulting from climate change (Sae-Lim et al., 2017; Adhikari et al., 2018). Metabolism and physiology, as well as feeding behavior and growth performance of most finfish species are likely to be affected (Marcogliese, 2008; Lemasson et al., 2018; Anin et al., 2023).

During the winter period, when the fish does not feed, the organism consumes its own reserves to cover the energy needs. First of all, the hydrocarbons are exhausted, but they are consumed quickly, then the fatty substances follow, and only after they are exhausted, the proteins are broken down. The intense and long-term consumption of protein substances leads to the death of the body. It was found that the accumulation of adequate fat reserves in the carp's body ensures its good wintering, influencing both weight loss and survival (Gheracopol, 1971; Dobrotă, 2008).The dynamics in the fat content of fish is especially under the influence of quantity and quality of food (Oprea & Georgescu, 2000). As the water temperature drops below 10º C, the cultured carp's behavior changes becoming less less active, and when the temperature value approaches 1º C they are inactive (hibernate) and a series of changes in metabolism occur. The purpose of the research was to highlight the correlation between environmental factors and fish food consumption during the cold season and implicitly their increase or decrease in weight. The method of capitalizing the additional feed administered to the fish and the amount of feed consumed was followed depending on the oscillating value of the temperature and concentrations of dissolved oxygen in the water.

The condition factor or Fulton coefficient is an index studied by specialists as being very valuable for the indications it can give them in terms of how carp overcome the winter period (Latiu et al., 2022; Savin et al., 2022; Thangaraji et al., 2023).

MATERIALS AND METHODS

During the periods October 2020-March 2021, October 2021-March 2022 and October 2022-March 2023, the experimental works were carried out at the Research and Development Station for Fisheries Nucet (RDSF Nucet). The experimental base is located in the main bed of the Ilfov stream, downstream of the Ilfoveni reservoir. The experimental ponds (B1, B2, B3, B4, B5 and B6) with the surface of 1000 m^2/pond and the depth between 1.5-2.4 m, were used for the wintering of the experimental biological material. The stocking of two-summer old carp for wintering was realized between October 10 and 25. The average weight of the biological material was between 557-621 g/ex, being stocked in ponds with an area of 1000 m^2, in monoculture. During the warm season, wintering ponds were left to dry and disinfected with lime chloride, more intensively in wet areas. The water supply was made from a common supply channel, through supply monks, where was installed a metal screen with a 4 mm mesh, to prevent the entry of wild fish species. Since the supply channel was common, we can state that, in all 6 experimental ponds, the physico-chemical characteristics of the water were identical.

Wintering of carps was done in earthen ponds, equipped with water supply and drainage facilities, in two variants, in triplicate, as follows:

- Variant V1, without feed distribution, in ponds B1, B2 and B3, with a stocking density of 1000 kg/pond (10000 kg/ha);
- Variant V2, with feed distribution (Aller Classic - 3 mm) was administered in ponds B4, B5 and B6, with a stocking density of 1000 kg/pond (10000 kg/ha).

At the end of each winter season, between March 16 and April 9, the biological material was fished, and the duration of the winter period was 135 ± 2 days.

During the experimental period, the data on the environmental parameters, water quality, food consumption were recorded and interpreted, at the beginning and at the end of the experimental period, biometric measurements were made to determine the morphological indices studied. In order to establish the physico-chemical parameters of water, water samples were collected at intervals of 6-7 days, and their analysis was carried out in the hydrochemistry laboratory of RDSF Nucet, according to the methodology in the specialized literature.

The results obtained in the three years of study, during the winter season, were statistically processed using: Excell (Office 2021), Windows xp and descriptive statistics.

RESULTS AND DISCUSSIONS

During the whole period of the experiments, the hydrochemical parameters of the technological water were monitored and the fresh water supply system of the wintering ponds, as well as the duration of covering the ponds with ice, were monitored. The analysis and interpretation of the results of the water samples was carried out in accordance with the "Regulation on the classification of surface water quality in order to establish the ecological status of water bodies", correlated with the data from the specialized literature for waters used for fish farming (Order no. 161, 2006) (Table 1).

The main factor of importance on the behavior of the carp during the winter period is the water temperature, which led us to follow its evolution. Air and water temperatures were recorded during the three experimental years in the cold season (2020-2021, 2021-2022 and 2022-2023). Positive air temperatures were recorded in January and February (low quantitative precipitation). The month of March was colder during the night, with temperatures lower than normal for the period, and during the day with precipitation in the form of snow.

In the three experimental winter seasons, the water temperature recorded average values in the range of 2.0 - 9.6°C, and air temperatures of up to 10.5°C were recorded during the day (Table 2). For short periods of time, a transparent ice was formed, with no mortality of fish material recorded.

This highlights the need for a continuous supply of well-oxygenated water to the wintering ponds.

Table 1. The main parameters of technological water recorded for the winter period 2020-2023 (average values)

No. crt.	The chemical parameter		UM	Parameter values		
				Source	Experimental ponds	Optimum according to quality standards
				Average of the years 2020-2023		
1.	pH		pH units	7.2	7.6	7-7.8
2.	Total hardness		(^{0}D)	13.8	14.6	12
3.	Calcium (Ca^{2+})		mg/l	41.6	40.4	90-120
4.	Magnesium (Mg^{2+})		mg/l	38.92	38.76	10-40
5.	Ca^{2+} / Mg^{2+}		mg/l	1.06	1.04	5
6.	Alkalinity		mg/l	154.8	172.8	200-400
7.	Organic substance		mg $KMnO_4$/l	13.84	22.64	20-60
8.	Oxygen		mg/l	3.50	5.72	5-10
9.	Nitrites (NO_2^-)		mg/l	0.0012	0.0024	0.03
10.	Nitrates (NO_3^-)		mg/l	0.026	0.098	2.5-4
11.	Phosphates (PO_4^{3-})		mg/l	0.016	0.056	0.05-1.5
12.	Ammonia (NH_3^+)		mg/l	lack	lack	lack
13.	Ammonium (NH_4^+)		mg/l	0.008	0.056	0.5-1
14.	Chloride	Cl^-	mg/l	10.60	12.37	30
		Na Cl	mg/l	17.53	20.46	20

Table 2. Average water and air temperatures for the winter period 2020-2023

Month and year	Water temperature			T med. air, ˚C
	T min., ˚C	T max., ˚C	T med., ˚C	
2020-2021 Season				
December 2020	1.8	3.8	2.3	-1.9
January 2021	1.9	3.4	2.0	1.3
February 2021	2.5	5.0	3.6	4.1
March 2021	3.5	13.0	8.7	8.5
2021-2022 Season				
December 2021	2.0	5.0	3.8	5.1
January 2022	1.5	2.5	2.3	0.8
February 2022	2.5	6.5	4.1	5.8
March 2022	5.0	12.5	9.6	10.3
2022-2023 Season				
December 2022	3.5	5.0	4.2	2.4
January 2023	2.0	5.0	3.5	3.1
February 2023	3.0	7.5	4.7	4.4
March 2023	5.0	10.5	7.7	10.5

In variant V2 (with feed distribution), in the three seasons during which the experiments were carried out, food was administered in amount of 1% of the total weight of the stocked biological material, when the water temperature exceeded 10°C, and 0.5% when the temperature was between 6-10°C. No feed was administered below temperature of 6°C. The food was administered at fixed points with the easy possibility of controlling its consumption.

When unconsumed feed was found, no more food was given in the next period until it was consumed. The amount of feed administered in the 2020-2021 winter season was 310 kg, in the 2021-2022 season it was 280 kg, and in the 2022-2023 season it was 320 kg. The feeding was carried out with an extruded granulated feed (Figure 1) - *Aller classic* 3 mm which has the following content: fish meal (15 %), soybean meal (25%), blood meal (10 %), corn (25%), wheat (20%), fish oil (2.5%) and vegetable oils (2.5%). The biochemical characteristics of the feed are as follows: NFE 43.5%, protein 30.0%, ash 7.0%, fat 7.0%, cellulose 5.0%, active urease 0.3%, and as minerals P and N. The feed also contains vitamins: D3, A and E.

Figure 1. Granulated feed *Aller classic* 3 mm

Figure 2. Biological material at stocking in autumn 2020

In the 2020-2021 winter season, the winter stocking were made between 26-30 October 2020, with carp aged two summers (Figure 2), with the following average weights: in the V1 variant 602 g/ex, and in V2 variant 600 g/ex. In the autumn, the stocking of the wintering ponds was carried out respecting the established density (10000 kg/ha), so in both variants, the number of exemplars per pond was found in the range 1623-1704. During the period 04.05-04.09.2021, the spring fishery was conducted with the following results (Table 3, Figure 3):

- the average harvested quantities were: in the V1 variant - 889 kg, and in the V2 variant - 1050 kg;
- the average weights were: in the V1 variant - 573 g/ex, and in the V2 variant - 641 g/ex.;
- survival had lower values in the V1 variant - 89.7% compared to the V2 variant - 98.2%.

Figure 3. Biological material in the spring of 2021

Figure 4. Biological material at stockingin autumn 2021

Figure 5. Biological material in the spring of 2022

In the 2021-2022 winter season, the winter stocking were made between 21-27 October 2021, with carp aged two summers (Figure 4), with the following average weights: in the V1 variant 607 g/ex, and in V2 variant 584 g/ex. In the autumn, the stocking of the wintering ponds was carried out respecting the established density (10000 kg/ha), so in both variants, the number of exemplars per pond was found in the interval 1610 - 1795. In the period 04.04-10.04.2021, the spring fishery was conducted with the following results (Table 4, Figure 5):

- the average harvested quantities were: in the V1 variant-870 kg, and in the V2 variant-1059 kg;
- the average weights were: in the V1 variant-585 g/ex, and in the V2 variant-630 g/ex.;
- survival had lower values in the V1 variant-90.3% variant compared to the V2 variant-98.2% .

In the 2022-2023 winter season, the winter stocking were made between 24-28 October 2022, with carp aged two summers (Figure 6), with the following average weights: in the V1 variant 611 g/ex, and in V2 variant 604 g/ex.

Table 3.The results obtained for the two experimental variants in the winter season 2020-2021

Recorded parameters		Variant V1 (without feed distribution)			VariantV2 (with feed distribution)			V1	V2
Pond		B1	B2	B3	B4	B5	B6	Average	Average
Stocking in the fall	Quantity (kg)	1000	1000	1000	1000	1000	1000	1000	1000
	W average (g/ex)	592	602	613	587	597	616	602	600
	No. specimens	1689	1661	1631	1704	1675	1623	1661	1667
	Ex/ha	16892	16611	16313	17036	16750	16234	16605	16673
	Kg/ha	10000	10000	10000	10000	10000	10000	10000	10000
	Fulton coefficient	3.73	3.62	3.58	3.61	3.59	3.57	3.64	3.59
Spring fishing	Sv (%)	89.8	89.6	89.8	98.1	97.9	98.6	89.7	98.2
	Quantity (kg)	856	859	847	1045	1046	1058	854	1050
	W average (g/ex)	564	577	578	625	638	661	573	641
	No. specimens	1517	1488	1465	1671	1640	1601	1490	1637
	Ex/ha	15169	14884	14649	16712	16399	16006	14901	16372
	Kg/ha	8555	8588	8467	10445	10462	10580	8537	10496
	Fulton coefficient	2.82	2.8	2.78	3.46	3.45	3.46	2.8	3.46

Table 4. The results obtained for the two experimental variants in the 2021-2022 winter season

Recorded parameters		V1 Variant (without feed distribution)			V2Variant (with feed distribution)			V1	V2
Pond		B1	B2	B3	B4	B5	B6	Average	Average
Stocking in the fall	Quantity (kg)	1000	1000	1000	1000	1000	1000	1000	1000
	W average (g/ex)	588	621	612	557	592	603	607	584
	No. specimens	1701	1610	1634	1795	1689	1658	1648	1714
	Ex/ha	17007	16103	16340	17953	16892	16584	16483	17143
	Kg/ha	10000	10000	10000	10000	10000	10000	10000	10000
	Fulton coefficient	3.61	3.59	3.62	3.58	3.6	3.6	3.61	3.59
Spring fishing	Sv (%)	91.4	89.3	90.2	97.8	98.5	98.3	90.3	98.2
	Quantity (kg)	881	866	864	1059	1058	1061	870	1059
	W average (g/ex)	567	602	586	603	636	651	585	630
	No. specimens	1554	1438	1474	1756	1664	1630	1489	1683
	Ex/ha	15544	14380	14739	17558	16639	16302	14888	16833
	Kg/ha	8814	8657	8637	10588	10582	10612	8702	10594
	Fulton coefficient	2.91	2.87	3.01	3.51	3.59	3.53	2.93	3.54

In the autumn, the stocking of the wintering ponds was carried out respecting the established density (10000 kg/ha), so in both variants, the number of specimens per pond was included in the interval 1608 - 1686. In the period 04.03-04.07.2023, the spring fishery was conducted with the following results (Table 5, Figure 7):

- the average harvested quantities were: in the V1 variant - 855 kg, and in the V2 variant - 1049 kg;
- the average weights were: in the V1 variant-580 g/ex, and in the V2 variant - 644 g/ex.;
- survival had lower values in the V1variant - 90.1% compared to the V2 variant - 98.4%.

Figure 6. Biological material at stocking in autumn 2022

Figure 7. Biological material in the spring of 2023

Table 5. The results obtained for the two experimental variants in the 2022-2023 winter season

Recorded parameters		V1 Variant (without feed distribution)			V2 Variant (with feed distribution)			V1	V2
Pond		B1	B2	B3	B4	B5	B6	Average	Average
Stocking in the fall	Quantity (kg)	1000	1000	1000	1000	1000	1000	1000	1000
	W average (g/ex)	611	601	622	593	608	612	611	604
	No. specimens	1637	1664	1608	1686	1645	1634	1636	1655
	Ex/ha	16367	16639	16077	16863	16447	16340	16361	16550
	Kg/ha	10000	10000	10000	10000	10000	10000	10000	10000
	Fulton coefficient	3.59	3.64	3.68	3.67	3.57	3.65	3.64	3.63
Spring fishing	Sv (%)	90.2	90.6	89.4	98.1	98.4	98.6	90.1	98.4
	Quantity (kg)	853	861	851	1052	1047	1047	855	1049
	W average (g/ex)	578	571	592	636	647	650	580	644
	No. specimens	1476	1507	1437	1654	1618	1611	1474	1628
	Ex/ha	14763	15075	14373	16543	16184	16111	14737	16279
	Kg/ha	8533	8608	8509	10521	10471	10472	8550	10488
	Fulton coefficient	2.83	2.96	3.02	3.61	3.49	3.55	2.94	3.55

The weight losses and the increase in growth (g) per season and experimental variants are presented in Figure 8.

Figure 8. Weight loss and growth factor over years and experimental variants

In the season 2020-2021, the experimental variant V1 (without feed distribution), decreases in the average individual weight were recorded in all three experimental ponds. The physiological losses for each pond were: B1- 25 g/ex; B2- 25 g/ex; B3- 35 g/ex. In the experimental variant V2 (with feed distribution) increases in the average individual weight were recorded in all three experimental ponds. The increases recorded for each pond were: B4- 38 g/ex; B5- 41 g/ex; B6- 45 g/ex.

In the season 2021-2022, the experimental variant V1 (without feed distribution), decreases in the average individual weight were recorded in all three experimental ponds. The physiological losses for each pond were: B1- 21 g/ex; B2- 19 g/ex; B3- 26 g/ex. In the experimental variant V2 (with feed distribution) increases in the average individual weight were recorded in all three experimental ponds. The increases recorded for each pond were: B4- 46 g/ex; B5- 44 g/ex; B6- 48 g/ex.

In the season 2022-2023, the experimental variant V1 (without feed distribution), decreases in the average individual weight were recorded in all three experimental ponds. Physiological losses for each pond were: B1- 33 g/ex; B2- 30 g/ex; B3- 30 g/ex. In the experimental variant V2 (with feed distribution) increases in the average individual weight were recorded in all three experimental ponds. The increases recorded for each pond were: B4- 43 g/ex; B5- 39 g/ex; B6- 38 g/ex.

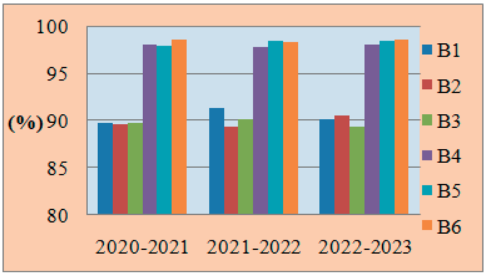

Figure 9. The percentage of survival variation in the three years of study in experimental ponds

In the three years of the study, on experimental variants, the survival rate (Sv %) were between 89.3% and 98.6% (Figure 9).
In the 2020-2021 season, in the V1 variant (without feed distribution) the survival percentage was in the range of 89.6-89.8%. The lowest percentage was recorded in pond B2, where Sv=89.6%, and in the other two ponds (B1 and B3) identical survivals of 89.8% were recorded. In the V2 variant (with feed distribution) the survival recorded values between 97.9-98.6%, the highest survival being recorded in the B6 pond of 98.6%, the lowest being recorded in the B5 pond of 97.9% and in pond B4 a survival rate of 98.1% was recorded.
In the 2021-2022 season, in the V1 variant (without feed distribution) the survival percentage was in the range of 89.3-91.4%. The lowest percentage was recorded in pond B2, where Sv=89.3%, the highest was recorded in pond B1, where Sv=91.4%, and in pond B3 Sv=90.2% was recorded. In the V2 variant (with feed distribution) the survival recorded values between 97.8-98.5%, the highest survival being recorded in the B5 pond of 98.5%, the lowest being recorded in the B4 pond of 97.8% and in pond B6 a survival rate of 98.3% was recorded.
In the 2022-2023 season, in the V1 variant (without feed distribution) the survival percentage was in the range of 89.4-90.6%. The lowest percentage was recorded in pond B3, where Sv=89.4%, the highest was recorded in pond B2, where Sv=90.6%, and in pond B1 Sv= 90.2% was recorded. In the V2 variant (with feed distribution) the survival recorded values between 98.1-98.6%, the highest survival being registered in the B6 pond of 98.6%, the lowest being recorded in the B4 pond of 98.1% and in pond B5 a survival rate of 98.4% was recorded.
In all the years of study, at stocking, in both experimental variants, the Fulton coefficient had close values, being in the range of 3.57 - 3.73.

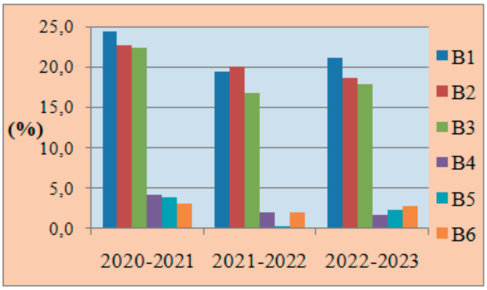

Figure 10. Reduction of the Fulton coefficient in percent (%) during the cold season by years and experimental ponds

The reduction of Fulton coefficient by years and experimental variants was between 16.9% and 24.4% for V1, respectively 0.3% to 4.2% for V2 (Figure 10).
In the 2020-2021 season, for spring fishing in the V1 variant (without feed distribution), a sharp decrease in this coefficient was observed, the lowest value being recorded in the B3 pond of 22.3%, the highest value being recorded in the B1 pond of 24.4% and in the B2 pond the value of 22.7% was recorded. In the V2 variant (with feed distribution) a slow decrease of this coefficient was observed, its lowest value being recorded in the B6 pond of 3.1%, the highest value in the B4 pond of 4.2%, and in the B5 pond there was a decrease in the Fulton coefficient of 3.9%.
In the 2021-2022 season, for spring fishing in the V1 variant (without feed distribution), a sharp decrease in this coefficient was observed, the lowest value being recorded in the B3 pond of 16.9%, the highest value being recorded in the B2 pond of 20.1% and in the B1 pond the value of 19.4% was recorded. In the V2 variant (with feed distribution), a slow decrease of this coefficient was observed, its lowest value being recorded in the B5 pond of 0.3%, the highest

value in the B4 pond of 2.0%, and in the B6 pond there was a decrease in the Fulton coefficient of 1.9%;

In the 2022-2023 season in spring fishing in the V1 variant (without feed distribution), a sharp decrease in this coefficient was observed, the lowest value being recorded in the B3 pond of 17.9%, the highest value being recorded in the B1 pond of 21.2% and in the B2 pond the value of 18.7% was recorded. In the V2 variant (with feed distribution) a slow decrease of this coefficient was observed, its lowest value being recorded in the B4 pond of 1.6%, the highest value in the B6 pond of 2.7%, and in the B5 pond there was a decrease in the Fulton coefficient of 2.2%.

CONCLUSIONS

The survival rate of the two summers carp, during the cold season, in the V2 variant was clearly superior to the V1 variant, in all ponds in all three years of the study, as follows: in the V1 variant it fell within the range of 89.3-91, 4%, compared to the V2 variant where it fell within the range of 97.8-98.6%. Thus, it was concluded that if the carp of two summers is fed in the cold season, the numerical losses are reduced.

From the point of view of weight loss, in the V1 variant it was found that they had values that fell within the range of 19-35 g/ex. In the case of variant V2, an increase in the average weight is found in all the years of the study in all ponds, with values between 38-48 g/ex.

In the case of the Fulton coefficient, in the V1 variant, a sharp decrease was found, ranging from 16.9% to 24.4%, compared to the V2 variant, where it had a slight decrease, ranging from 0.3% to 4.2%.

In conclusion, as much as the temperature allows, feeding the carp during the cold season reduces the numerical losses and causes an increase in the individual weight, obtaining a vigorous stocking material in the spring. Numerical and quantitative losses are minimal.

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NEW DATA FOR HELMINTH FAUNA OF BUFONIDAE (AMPHIBIA) IN THE REPUBLIC OF MOLDOVA

Elena GHERASIM, Dumitru ERHAN

State University of Moldova, Institute of Zoology, 1 Academiei Street, MD-2028, Chișinău, Republic of Moldova

Corresponding author email: gherasimlenuta@gmail.com

Abstract

The paper presents data on the identification of the helminth fauna structure of Bufo bufo Linnaeus, 1758 and Bufotes viridis Laurenti, 1768 species, ecaudata amphibian from Bufonidae families, and the determination of its role as bioindicators and as vectors for parasitic agents specific to animals. As result of helminthological investigations during 2013-2023 years, in Bufonidae species 19 helminths species was established. The helminthological research carried out on the species Bufo bufo highlighted the presence of 19 species of helminths, of which the predominant parasitic agents are trematodes which make up 47.4%, nematodes with 31.5%, acanthocephals with 15.8% and monogeneans with 5.3%. In Bufotes viridis species, the presence of 18 species of helminths was established, of which helminth species from the trematode class represent the predominant group with 44.4%, nematodes with 38.9%, acanthocephales with 11.1% and monogeneans with 5.6%. The helminthological researches were carried out depending on the age of the host, the type of habitat and the area. The bioindicator significance of the established parasite species was presented for an ecological assessment of the studied area.

***Key words**: Bufo bufo, Bufotes viridis, helminth fauna, Republic of Moldova.*

INTRODUCTION

Anurans are very important in biocenoses. They are predators for a complex of aquatic and semi-aquatic invertebrate species (rarely of vertebrates). In addition, they are prey for vertebrate predators of a higher trophic level, represented by intermediate, intercalary (mesocercar), additional, paratenic (metacercar) and final hosts for helminthes of different taxa (Sessions & Ruth, 1990; Thiemann & Wassersug, 2000; González & Hamann, 2007; Chikhlyaev & Ruchin, 2014; Chikhlyaev et al., 2016a; Chikhlyaev et al., 2016b, Chikhlyaev et al., 2018a, Chikhlyaev et al., 2018b; Gherasim, 2020).

Habitat conditions significantly influence the formation of a helminth fauna in amphibians. They form the features of its biology and ecology, i.e. lifestyle and breadth of the dietary spectrum (Ruchin & Fayzulin, 2019a; Ruchin & Fayzulin, 2019b).

The diversity of the parasitic fauna specific to the amphibian species *Bufo bufo* and *Bufo viridis* from the Bufonidae family is a component of both terrestrial and aquatic biotopes (only for the reproduction period) and forms certain specific ecological interrelations. The parasitic factor is one of the main biotic factors, which determine the numerical effectively of the hosts, and through their numerical regulation, the structure and functioning of the ecosystem as a whole is influenced.

In the context of assessing the state of ecosystems and recognize the sources of spread of parasitosis in domestic animals, wild and humans, for the first time in the Republic of Moldova a complex batraco-helminthological study of the *Bufo bufo* and *Bufo viridis* amphibian species from the Bufonidae family was performed, as well as identifying their role as hosts, as vectors, but also their role in the formation and maintenance of foci of parasitic agents.

MATERIALS AND METHODS

The study area includes natural and anthropogenic, aquatic and terrestrial ecosystems specific to the *Bufo bufo* and *Bufo viridis* amphibian species in the Central, Northern and Southern area of the Republic of Moldova. The collection of the material was carried during the active period of their annual life cycle (March-October) and of all age categories: embryos, larvae, juveniles, subadult and adult individuals of toads. Helminthological investigations were carried out over a period of 10 years (2013-2023).

The determination of amphibian species, both of adult forms, embryonic and larval stages, was carried by classical deductive methods, which refer to parameters, morphometric indices and body coloration (Bannikov et al., 1971 Bannikov et al., 1977; Arnold & Burton, 1986).

The helminthological analysis of biological samples was performed according to the standard method proposed by Skrjabin, which involves the examination of all the internal organs of the animal (Skrjabin, 1928). Helminthological research of the parenchymal organs was performed with the help of compressors, and the digestive tract - by successive washes. The collection, fixing, determination and processing of the helminthological material was carried after the methods proposed by various authors (Gashev et al., 2006; Kuzmin, 2012; Petrocenko, 1956; Ryzhikov et al., 1980; Serghiev, 2001; Sudarikov, 1964). The determination of the helminthological material was performed after standard methods (Ryzhikov et al., 1980).

To quantify the contamination characteristic by helminthes, the Intensity indice was calculated (*II, exemplars*) – the minimum and maximum number of parasites of a species and the extensivity of invasion (*EI, %*) – the percentage of host contamination by a species of parasite.

Laboratory helminthological investigations of biological samples of *Bufo bufo* and *Bufo viridis* to the presence of helminths or helminthic elements (eggs, larvae), allowed to obtain data of special value in order to determine the importance of amphibians in the formation and maintenance of outbreaks of common parasitic organisms in wild animals, pets and human.

RESULTS AND DISCUSSIONS

Given the fact that toads, as well as the other species of amphibians on the territory of our country, are amphibians species which live in two types of characteristic habitats: terrestrial and aquatic during the entire annual and vital life cycle, they are represent some of the main vectors of parasitic agents from the aquatic life environment to the terrestrial one, and vice versa. One of the main factors that essentially influence the activity of toads is represented by environmental factors, which are directly reflected on the structure of their helminthic fauna.

For the complex characteristic of the helminthic fauna of toads, throughout the country the multiannual phenology of these ecaudate amphibian species was evaluated. (Table 1).

According to the data obtained, it was established that the phenological phases of the annual life cycle of toads depends primarily on air and soil temperature, but atmospheric precipitation and relative air humidity, the distribution and distance of the location of breeding habitats from the habitats of nutrition, rest and hibernation have a complementary, but no less important role in the formation of intra- and interrelationships ecological specio-specific of toads with their living environments, with biological diversity, which on the whole characterize the diversity of their specific helminth fauna.

Table 1. Phenology of toads (*Bufo bufo, Bufo viridis*) in the conditions of the Republic of Moldova

No	Species	Coming out of hibernation	Moving to breeding sites	Ontogenesis: Embryonic development	Ontogenesis: Larval development		Moving from summer places	Initiation of the hibernation phase
				Egg	Larvae	Achieving metamorphosis and exiting juveniles on land		
		t^0C	t^0C	Duration / days	Duration / days	Duration / days	t^0C	t^0C
1.	*Bufotes (Bufo) viridis*	+12.7	+15.2	9-10	41-43	41-45	+11.2	+7.0
2.	*Bufo bufo*	+7.1	+8.9	9-15	39-42	42-43	+11.2	+6.8

The helminthological research carried to amphibian species from the Bufonidae family revealed the presence of 22 helminth species, of which 19 species of helminths in *Bufo bufo* and 18 species of helminths in *Bufo viridis* where established. From a taxonomic point of view, the parasitic agents detected in toads fall into three Phylum (*Platyhelminthes, Nematoda, Acanthocephala*), four classes (*Trematoda, Monogenea, Secernentea, Palaeacanthocephala*), ten orders (*Plagiorchiida, Echinostomida, Diplostomida, Spirurida Ascaridida, Strongylida, Rhabditida, Echinorhynchida, Polymorphida, Polystomatida*), 17 families (*Omphalometridae, Haematoloechidae, Gorgoderidae, Lecithodendriidae, Cyathocotylidae, Pleurogenidae, Diplodiscidae, Diplostomatidae, Strigeidae, Macroderoididae, Cosmocercidae, Spirocercidae, Molineidae, Rhabdiasidae, Echinorhynchidae, Centrorhynchidae, Polystomatidae*) and 21 genera (*Opisthioglyphe, Haematoloechus, Gorgoderina, Pleurogenes, Pleurogenoides, Prosotocus, Diplodiscus, Strigea, Haplometra, Holostephanus, Tylodelphys, Cosmocerca, Oswalcocruzia, Spirocerca, Ascarops, Agamospirura, Rhabdias, Acanthocephalus, Pseudoacanthocephalus, Sphaerirostris, Polystoma*).

In the evaluation of the scientific data obtained and reflected in Table 2, we can mention that each host species of toads from the Bufonidae family is characterized by a specific helminthic fauna structure for which the main helminthological indices are variable, and the extensivity of invasion (*E.I* - %) - oscillates from 1.1% of cases (*Opisthioglyphe ranae* species in *Bufo bufo* host species) to - 75.0% of cases (*Oswaldocruzia filiformis* in *Bufo viridis* host species). At the same time, the intensivity of invasion (I.I - ex.) of helminths in a host is quite fluctuating, it can vary from – 1 ex. (*Diplodiscus subclavatus* in *Bufo bufo* host species, *Pleurogenes claviger* in *Bufo viridis* host species) up to - 630 ex. in a single host specimen (*Ascarops strongylina* in *Bufo bufo* host species) or 145 ex. in *Bufo viridis* host species (Table 2).

In the Common European Toad (*Bufo bufo*) of the 19 detected species of helminths, the predominant group is represented by trematodes (n = 9) which constitute 47.49% of cases, nematodes with 31.6% of cases (n = 6), acanthocephals with 15.8% of cases (n = 3) and monogeneans (n = 1) with 5.3% of cases (Figure 1).

In the European Green Toad (*Bufo viridis*) of the 18 species of detected helminths, the predominant group is also represented by trematodes (n = 8) which constitute 44.4% of cases, nematodes - 38.9% of cases (n = 7), acanthocephals - 11.1% of cases (n = 2) and monogeneans (n = 1) with 5.6% of cases (Figure 1).

Table 2. The degree of invasion of toads with parasitic agents

No.	Host / Invasion	*Bufo bufo*		*Bufo viridis*	
		EI - %	II – ex.	EI - %	II – ex.
Trematoda					
1.	*Opisthioglyphe rane*	1.1	3	2.27	1-6
2.	*Haematoloechus variegatus*	4.9	1-2	2.27	1-2
3.	*Gorgoderina viteliloba*	12.3	1-4	-	-
4.	*Pleurogenes claviger*	42.6	1-15	2.27	1
5.	*Pleurogenoides medians*	9.55	1-21	4.55	1-13
6.	*Prosotocus confusus*	40.2	1-33	32.8	1-64
7.	*Diplodiscus subclavatus*	1.20	1	-	-
8.	*Strigea sphaerula,* mtc.	-	-	2.27	1-4
9.	*Haplometra cylindracea*	-	-	44.5	1-29
10.	*Holostephanus volgensis,* mtc.	42.3	1-6	-	-
11.	*Tylodelphis excavata,* mtc.	11.3	55	27.7	1-89
Secernentea					
12.	*Cosmocerca ornata*	64.3	1-8	20.00	1-6
13.	*Oswaldocruzia filiformis*	21.4	1-3	75.00	1-9
14.	*Oswaldocruzia duboisi*	28.6	1-5	21.4	1-3
15.	*Spirocerca lupi,* larva	-	-	73.2	1-95
16.	*Ascarops strongylina,* larva	22.5	1-630	12.3	1-145
17.	*Agamospirura sp.,* larva	21.2	8-32	42.3	1-32
18.	*Rhabdias bufonis*	70.0	5-48	59.09	1-18
Palaeacanthocephala					
19.	*Acanthocephalus ranae*	21.4	1-3	10.0	3
20.	*Pseudoacanthocephalus bufonis*	11.9	2-8	26.3	1-11
21.	*Sphaerirostris teres,* larva	1.96	1-3	-	-
Monogenea					
22.	*Polystoma integerrimum*	12.3	1-2	20.0	1-3

Thus, due to the above, the degree of infestation with helminths of ecaudate amphibians from the Bufonidae family, from the territory of our country, is quite high, being recorded in the *Bufo viridis* species in 64.5% of cases, and in the *Bufo bufo* species in 57, 3% of cases.

We can explain this insignificant difference in the degree of helminth infestation of toads as a result of their interaction with the living environments they are inhabit.

Bufo viridis is a species of synanthropic amphibian that lives near humans (orchards, vegetable gardens, vineyards, agricultural land with well-drained soil, parks, etc.), avoiding wooded areas. While *Bufo bufo* is not a synanthropic species, on the contrary it populates the wooded, forested areas, lowland areas, as far as possible from the human factor (Cozari & Gherasim, 2021).

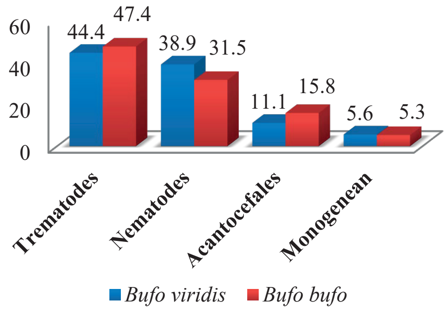

Figure 1. Indices of extensivity of taxonomic classes of helminths in toads

Each of these areas is characterized by a varied diversity of fauna, which directly influences their parasite structure.

In the study of the helminth fauna in amphibians, one of the main factors influencing the diversity of the parasitic fauna of a host is its age, which is accompanied by certain morpho-physiological, ethological and ecological changes, which also leads to a change in the probability of infection (Gherasim & Erhan, 2024).

The biological and ecological peculiarities of toads are very closely related to their age, and in this case the way of life is the same that determines the diversity of the parasitic fauna. Thus, toads are often infected with monogenea and up to two years of age, despite the fact that this parasite are directly develops.

This situation is explained by the fact that the toads infection takes place during reproductive migrations through streams, the environment where monogenean larvae are also found. Then, the extensivity and intensivity of the invasion increases steadily, synchronized, until the toads reach the age of 5 years, after which these indicators approximately at the same level remain.

The reason that the young forms of the toads are infected with this species of monogeneans is due to the fact that the juveniles spend part of the time on the surface of the water in the aquatic pools, the area that represents a real reservoir, where the probability of meeting the larval stages of the parasite, it is higher than in adult amphibians, which may have more contact with the terrestrial habitat, or may go to a greater depth of water. In many cases, the increase of parasite numbers is simply due to the large amount of food ingested by the host (Gherasim & Erhan, 2024).

For a complex assessment of the degree of helminth infestation of *Bufo bufo* and *Bufo viridis* species depending on age, specimens from four ontogenetic periods were evaluated helminthologically: embryos (ponta), larvae, juveniles and adults.

In ponta and larvae of toads, throughout the country, they were not invasive stability elements, but in their juveniles the presence of three species of trematodes (*Opisthiogliphe ranae, Haplometra cyclindracea, Strigea sphaerula*) was established, which constitutes 13.6% of total helminth species and a species of nematode (*Cosmocerca ornata*) which constitutes 4.5% of the cases. Thus, toad juveniles were infected with parasitic agents in 18.2% of cases, while in the adult forms all helminth species represented in table no. two were stable (Table 2, Figure 2).

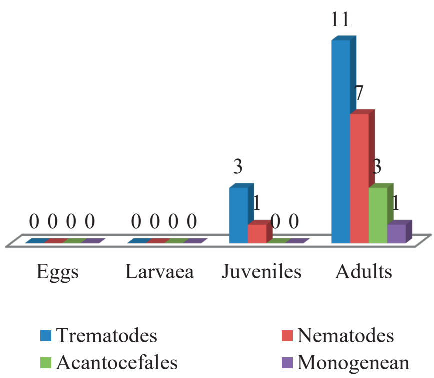

Figure 2. The structure of the helminthic fauna in toads depending on the age host

The helminthological analysis of the data allowed us to establish that one and the same helminth species can be common to toads from different ontogenetic periods, thus 18.2% of the total helminth species detected are specific to both juvenile and adult forms. The infestation both of juveniles and adult forms of amphibians from the Bufonidae family with one and the same species of helminths is explained by the fact that the young forms (juveniles) of these species, after metamorphosis process, immediately leave the water bodies in which they were developed and inhabits areas specific to adult forms.

In order to determine the diversity of the helminthic fauna in toads depending on their main phenological phases, the amphibians were investigated on the entire annual life cycle during the spring-summer-autumn seasonal periods. Thus, during the course of the helminthological investigations in the spring season, it was established that the toads when they come out of the hibernation phase are characterized by a poor structure of their helminthic fauna, but also a prevalence of invasion of 8.5% of cases. Along with the increase in the environmental temperature, during their movement to the summer places (breeding pools), the infestation toads was recorded in 9.2% of cases. Later, after completing the reproductive process, the toads dont form couples, thus leaving the breeding pools, their nutritio are intensify and the their parasite structure at the end of the summer season is recorded with a prevalence of invasion in 71.0% of cases. Therefore, toads are the species of amphibians that form aggregations, and during the breeding period they usually accumulate many parasitic agents at the same time, because during this short period the probability of contact between the parasite and the host considerably increases.

In the autumn period, when the environmental factors dont represent the ecological optimum for toads, they are leave the summer sites and are characterized by the highest degree of infestation with helminths, which constitute 92.5% of cases. Gradually, with the establishment of the environmental conditions with low temperatures, towards the initiation of the hibernation phase, the prevalence of the invasion takes on lower values, being recorded in 62.0% of cases (Figure 3).

According to the data obtained, it was established that the variation of ecological factors is only a very important aspect not only for the vital activity of toads, but also for their helminthic fauna.

The high degree of helminth infestation of toads during the period of leaving summer sites is the result of the presence of obligate intermediate hosts in the development of life cycle for certain helminth species.

Although during the autumn season, when the toads initiate the hibernation phase, their infestation was established in 62.0% of cases, in the spring when they come out of the hibernation phase, the structure of the helminthic fauna is much reduced. This situation does not allow us to conclude that the reduction of the vital functions of the amphibian body under the influence of low temperatures during the winter season significantly reduces the structure of their helminthic fauna.

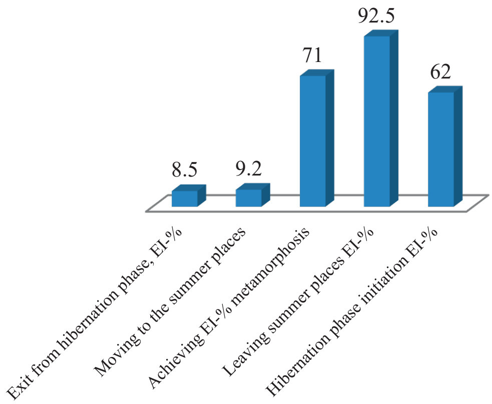

Figure 3. Degree of helminth infestation of toads depending on their phenological phases

At the same time, toads are also characterized by an infestation with different species of helminths, thus delimiting the two habitats of the species. With the post-reproductive migrations, the toads back to the summer sites and they have the capacity to lose certain parasitic agents. The toads returning from the breeding pools to the summer sites carry with them the aquatic parasitic fauna, but, moving up to the hibernation areas, they gradually lose it.

The annual monitoring and forecast of the parasitological situation of toads, makes it possible to assess the risks of invasion of a certain effective of animals, but also taking into account the number of susceptible animals. The forecast for the Republic of Moldova is of a general nature, because the biology of helminths for the different species presents significant differences depending on their

distribution area and their biological cycle.
Although the Republic of Moldova is a country with a small area of 33,846 km^2, the climatic conditions in the Center, North and South areas are different, and the forecasts have their own characteristics regarding the population and diversity structure of the intermediate hosts (for biohelminths), as well as the living environment conditions in a certain area.
According to the evaluation of the helminthological data depending on the area, the structure of the helminthic fauna in toads demonstrates that one and the same host species, analyzed from different points of its range, has a qualitatively and quantitatively different helminthic fauna (Figure 4).

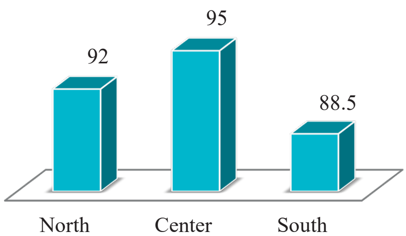

Figure 4. The structure of the helminthic fauna in toads depending on the area

So, these divergences regarding the structure of the helminthic fauna and the degree of helminth infestation of amphibians depending on the area, are not only to the trophic factor, but also to the zonal preference of amphibians and their adaptability to abiotic factors.
In order to evaluate the degree of helminth infestation of toads in the aspect of mono- and polyinvasions during their annual and life cycle, it was established that both in the *Bufo bufo* species (Figure 5) and in the *Bufo viridis* species the infestation in the aspect of monoinvasion was established (Figure 6).

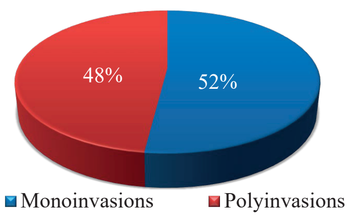

Figure 5. The structure of the helminth fauna in the *Bufo bufo* species in aspects of mono- and polyinvasion

The diversity of the helminthic fauna in amphibians depending on the biological cycle of the parasitic agents allowed us to realize their evolutionary characteristic.

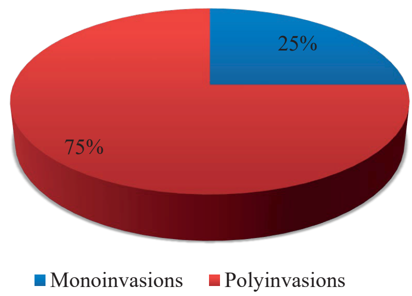

Figure 6. The structure of the helminth fauna in the*Bufo viridis* species in aspects of mono- and polyinvasion

Thus, of the 22 species of helminths detected in amphibians, 22.7% (n = 5 species) develop according to the monoxen model (*Cosmocerca ornata, Oswaldocruzia filiformis, Oswaldocruzia duboisi, Rhabdias bufonis, Polystoma integerrimum*), 18.2% of the species (n = 4 species) develop according to the dixen model (*Diplodiscus subclavatus, Agamospirura* sp., *Ascarops strongylina, Acanthocephalus ranae*), 54.5% of the species (n = 12 species) develop according to the trixen model (*Gorgoderina viteliloba, Haematoloechus variegatus, Pleurogenes claviger, Pleurogenoides medians, Prosotocus confusus, Haplometra cylindracea, Opisthioglyphe rane, Tylodelphis excavata, Holostephanus volgensis, Spirocerca lupi, Pseudoacanthocephalus bufonis, Sphaerirostris teres*, larva) and 4.5% of the species (n = 1 species) develop according to the tetraxen model (*Strigea sphaerula*, mtc.) (Figure 7).

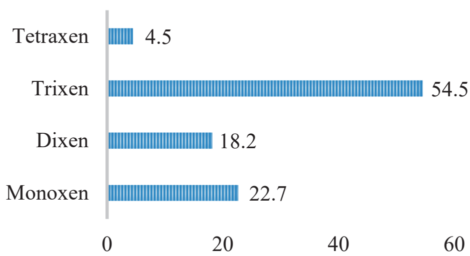

Figure 7. Evolutionary characteristics of the parasitic agents detected in toads

When evaluating the helminthological data obtained, it was established that adult forms of parasitic agents are predominate in the structure of the helminthic fauna of *Bufo bufo* species, so that when the host species is infected with trematodes, 22.2% are metacercariae, and 77.8% are adult forms of parasitic agents, when the host species are infected with nematodes 33.3% are the larval stages, and 77.7% are the adult forms, when the host species are infested with acanthocephals, it was established that the adult and larval forms are in a 1:1 report, both adult and larval forms they each constitute 50.0%, and the monogeneans are represented by a single species, and this is an adult form (100.0%) (Figure 8).

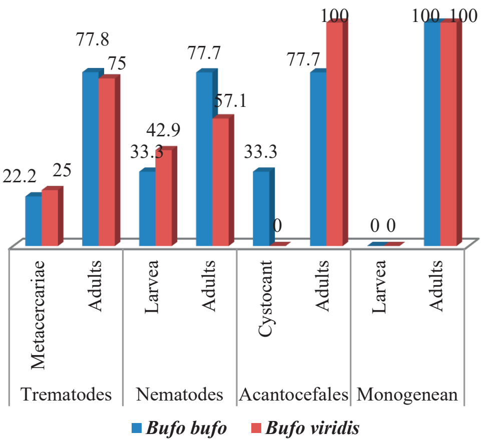

Figure 8. Helminthological indices of the *Bufo bufo* and *Bufo viridis* species depending on the ontogenesis of the parasitic agents

The helminthological analysis of the obtained data revealed that in the structure of the helminthic fauna of the Bufo viridis species the adult forms of the helminths predominate, so that when the host species are infected with trematodes, 25.0% are metacercariae, and 75.0% are the adult forms, when the infestation of the host species with nematodes, 42.9% are the larval stages, and 57.1% are the adult forms, when the host species was infested with acanthocephals, it was established that the helminth specimens are as adult forms and represent 100.0%, and the monogeneans are represented by only one species, and this is an adult form (100.0%) (Figure 8). Therefore, according to the evaluation of the structure of helminthic fauna in amphibians from the Bufonidae family depending on the ontogenetic phases of the their helminths detected in the host species and establishing their role as vectors for various helminths species common other vertebrates animals, it was found that both the *Bufo bufo* species and *Bufo viridis* species are an increased interest in the vectorization of parasitic agents common to fish in 9.1% of cases (*Tylodelphis excavata*, mtc., *Holostephanus volgensis*, mtc.), birds in 22.7% of cases (*Ascarops strongylina*, larva, *Tylodelphis excavata*, mtc.), mammals in 9.1% of wild cases: wild boars, insectivorous rodents (*Ascarops strongylina*, larva), carnivorous mammals (dog, fox, wolf), occasionally domestic mammals: goats, horses, bulls, pigs (*Spirocerca lupi*, larva) and human in 4.5% of cases (*Pseudoacanthocephalus bufonis*) (Figure 9).

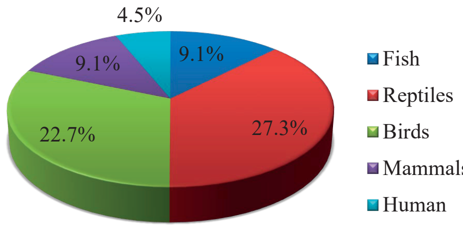

Figure 9. The degree of vectorization of parasitic agents by toads to various groups of animals

Although in toads the larval forms of parasitic agents don't have been determined as predominant forms, though the *Bufo bufo* and *Bufo viridis* species have a very important role in the vectoring of parasitic agents common to domestic animals, wild, pets and humans.

The increased degree of vectorization of the parasitic agents by toads to vertebrate animals is due to the trophic relations in the ecosystem (prey-predator), but also the possibility of simultaneous infestation of a single host with more species of parasitic agents.

In *Bufo bufo* species, their infestation with until to three species of helminths simultaneously was determined. According to the assessments, it was found that amphibians in 52.4% of cases were infested with a single species of helminths, in 37.6% of cases - with 2 species of helminths and 10.3% of cases - with 3 species of helminths (Figure 10).

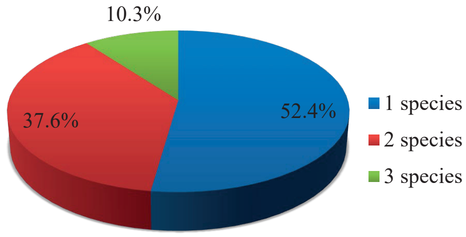

Figure 10. The degree of co-infection of the *Bufo bufo* species

In the *Bufo viridis* species, their infestation with until to four species of helminths simultaneously was established. According to the assessments, it was found that the amphibians in 25.0% of cases were infested with only one species of helminths, in 35.0% of cases - with 2 species of helminths, 35.0% of cases - with 3 species of helminths and 5.0% of cases - with 4 species of helminths (Figure 11).
It is known that amphibians have a very important role in the functioning of ecosystems as consumers and in regulating the population of invertebrates in an ecosystem.

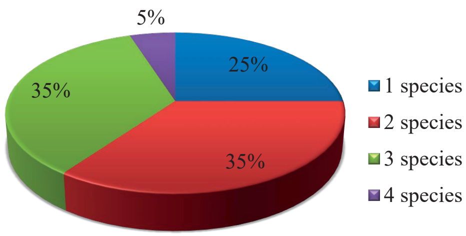

Figure 11. The degree of co-infection of the *Bufo viridis* species

At the same time, according to our data obtained on the territory of our country, the role of toads as definitive hosts was determined in 63.6% of cases (*Cosmocerca ornata, Oswaldocruzia filiformis, Oswaldocruzia duboisi, Rhabdias bufonis, Polystoma integerrimum, Diplodiscus subclavatus, Acanthocephalus ranae, Gorgoderina viteliloba, Haematoloechus variegatus, Pleurogenes claviger, Pleurogenoides medians, Prosotocus confusus, Haplometra cylindracea, Opisthioglyphe rane*), intermediate in 22.7% of cases (*Agamospirura* sp., *Tylodelphis excavata, Holostephanus volgensis, Spirocerca lupi, Strigea sphaerula*) and as paratenic hosts in 13.7% of cases (*Ascarops strongylina, Pseudoacanthocephalus bufonis, Sphaerirostris teres*) for a large diversity of helminth species common to fish, reptiles, birds, mammals and humans (Figure 12).

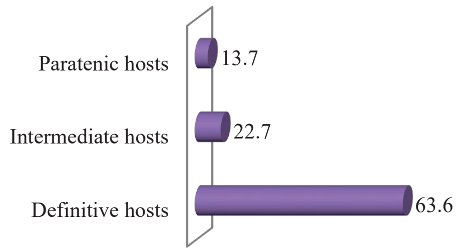

Figure 12. The role of toads as hosts of parasitic agents

Along with determining the role of toads as hosts for a large diversity of helminth species that are specific to fish, reptiles, birds, mammals and humans, at the same time, the data obtained also show their importance as bioindicators of ecosystems populated by these toads.
The distribution and the appearance dynamics of parasites in a certain environment, time and in different hosts, and the factors that are found during the host's relationship with the parasite at the individual level, or at the population level represent a complex study with a deep approach to various biological, ecological and helminthological aspects both to the host organisms and for the parasites.
The presence of obligatory stages of finding parasites in the external environment (natural) creates additional some impediments because the parasite must adapt to two different living environments - inside the host organism and outside it. Therefore, in addition to spreading in the environment, the parasitic agents must have the ability to spread over time and survive the direct influence of adverse climatic conditions while waiting for their improvement, or finding a new host. This activity is achieved by including in the life cycle of the parasite either an inactive or latent stage with a number of

protective adaptations, or an intermediate host, or even both. Both the latent stage and the intermediate host enable to the parasite, to some extent, to avoid the dangers associated with fluctuations conditions in environmental and to prolong its existence over time.

Any biological cycle of a parasitic agent is highly dependent on the obligate hosts involved in its realization, and the differences tend to relate to the processes of transmission and synchronization of the life cycles of the parasite and the host, which ensure the survival of the next generation.

Therefore, the monitoring of the helminth fauna in the ecaudate amphibians of *Bufo bufo* and *Bufo viridis* from the family Bufonidae, in various biotopes, depending on the intrinsic and extrinsic factors, it has of particular bioecological, medical and veterinary importance, and the data obtained have a particular contribution to the prevention of transmission parasitic agents to humans and animals involved in the biological cycles of parasites agents with a zoonotic and epizootic role.

So, according to the batraco-helminthological research carried on toads, we can conclude that the helminthic fauna of these host organisms, with the amphibious mode of life, which populate the most diverse living environments and with the highest degree of anthropization, are of particular importance not only theoretical, but also practical in science, because these species of amphibians actively participate in the formation and maintenance of foci of parasitic agents common to fish, birds, mammals (domestic, wild) and the human.

CONCLUSIONS

The study of the helminthic fauna in the ecaudate amphibians of *Bufo bufo* and *Bufo viridis* in the Republic of Moldova revealed the presence of 22 species of helminths, of which 19 species of helminths in *Bufo bufo* and 18 species of helminths in *Bufo viridis*. Taxonomic the parasitic agents detected in toads fall into three phylum, four classes, 10 orders, 17 families and 21 genera.

In this study, a complex ecological analysis of toads was carried out in which the structure of the parasite communities and the degree of invasion in relation to the phenology of the hosts was determined. These results allowed us to conclude that the variation of ecological factors during an annual cycle of toads represents a very important aspect on the formation of helminth fauna, but the divergent degree of invasion recorded in different phenological phases is the result of the presence of obligatory intermediate hosts in the biological cycle of the specific agents parasites.

At the same time, toads are also characterized by a certain infestation with different species of helminths, thus delimiting those two habitats of the species, terrestrial and aquatic (only during the reproduction period). Along with the post-reproductive migrations, towards the summer sites, the toads carry the aquatic parasitic fauna, but, moving towards the summery areas, they gradually lose it. Monitoring and annual predict of the parasitological situation of toads, makes it possible to assess the risks of invasion of a certain number of hosts.

It was determined that the biological and ecological peculiarities of the toads are very closely related to their age criterion, which determines the diversity of their parasitic fauna as well as the formation of the relationship in the parasite-host system from the juvenile stage.

The role of toads in the vectorization, formation and maintenance of foci of parasitic agents common to fish, reptiles, birds, mammals and human was determined.

ACKNOWLEDGEMENTS

This research work was carried out with the support of framework of the postdoctoral project “Helminthic fauna of amphibians (Amphibia), their importance as vectors in the formation and maintenance of parasitic zoonoses” no. 23.00208.7007.05/PDII and the project ”Evaluation of the structure and functioning of animal world and aquatic ecosystems under the influence of biotic and abiotic factors in the context of ensuring ecological security and the well-being of the population no. 010701

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A HISTOLOGICAL STUDY OF THE ZEISSL MEMBRANE FOUND IN THE DIGESTIVE TRACT OF THE DANUBE SALMON *Hucho hucho* (Linnaeus, 1758)

Andrada IHUȚ[1], Paul UIUIU[1], Camelia RĂDUCU[1], Daniel COCAN[1], Radu CONSTANTINESCU[1], Alexandru SAVA[1] , Tudor PAPUC[1], Vioara MIREȘAN[1], Camelia MUNTEANU[2], Viorel MICLĂUȘ[3], Vasile RUS[3]

[1]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Animal Science and Biotechnologies, 3-5 Mănăştur Street, 400372, Cluj-Napoca, Romania
[2]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Agriculture Sciences, 3-5 Mănăştur Street, 400372, Cluj-Napoca, Romania
[3]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Veterinary Medicine, 3-5 Mănăştur Street, 400372, Cluj-Napoca, Romania

Corresponding author email: camelia.raducu@usamvcluj.ro

Abstract

The digestive system of fish is different from one species to another, depending on the type of food ingested. Danube salmon Hucho hucho (Linnaeus, 1758) is a critically endangered species of the Salmonidae family with the threat of extinction looming large, therefore conservation efforts are urgently needed. This study presents a detailed microscopic analysis of the Zeissl membrane in Danube salmon, through histological means. The biological material analyzed consisted of six fish raised in a specialized breeding farm for restocking purposes. Samples were collected from the gastrointestinal tract and processed using histological paraffin embedding. The Zeissl membrane was present in all studied segments, but its structure and development varied. It comprised the stratum compactum (SC) and stratum granulosum (SG). The SC formed a continuous membrane with unequal thickness in most of the studied segments, except for the cardia area of the stomach, where it appeared discontinuously, and the duodenum, where it only appeared between the apertures of the pyloric caeca. Aside from its role in limiting gastrointestinal distension, it may have other implications for the morphophysiology of the Danube salmon digestive tract.

Key words*: stratum compactum, stratum granulosum, Zeissl membrane.*

INTRODUCTION

Danube salmon *Hucho hucho* (Linnaeus, 1758) is a critically endangered species of the Salmonidae family with the threat of extinction looming large (Holcík et al., 1988; Ihuț et al., 2014). Conservation efforts are being made to protect this species both in its natural habitat (in situ) (Zitek et al., 2004, 2008; Schmutz et al., 2002; Ratschan, 2014) and outside of it (ex-situ) (Holcík, 1995; Holzer, 2011; Stráňai, 2012). To adopt conservation measures for any species, it is important to understand its biological characteristics (Holcík et al., 1988; Ihuț et al., 2014; 2017; 2020), which may help to improve the species' conservation efforts.

One important biological characteristic is the particularities of the digestive apparatus in fish (Moisa et al., 2022). This is different from one species to another, being also dependent on the type of food ingested. Danube salmon can ingest up to 50% of its body weight in a single feeding session since it is known as a voracious predator (Šubjak, 2013). The digestive system of fish is divided into four segments, as stated by Harder (1975) and Teresa & Maciej (2019). The headgut, which includes the oral cavity and pharynx, is responsible for capturing food. The foregut, consisting of the esophagus and stomach, is where food digestion begins. The midgut, located between the pyloric caeca and the rectum, is responsible for continuing digestion and nutrient absorption. Finally, the hindgut is the rectum. From the foregut to the hindgut, the digestive wall consists of four layers (mucosa, submucosa, muscularis, and serosa).

According to histological literature, there is a unique membrane within the inner mucous layer (Adlersberg et al., 1955; Vitanov et al., 1995; Bacha & Bacha, 2006; Sapundzhiev et al., 2017), which is not present in all species, and its presence could distinguish the gut wall across species. It is present in carnivorous species and has an important role in limiting stomach distension and preventing perforation when consuming large amounts of food, including bones (Zahariev, 2011). Lim (1922) identified in the gastric mucosa of the cat a thick condensation in the inner part of the mucosa as Zeissl membrane and Oppel's stratum compactum. Adlersberg et al. (1955) and Gherman (1993) describe the Zeissl membrane as a dense connective tissue compressed at the base of the tubules that form the subglandular layer. The membrane is not elastic and separates the muscle fibers within the inter-glandular tissue from muscularis mucosae (Lim, 1922). Some authors referred to this structure only as lamina subglandularis (Vitanov et al., 1995; Bacha & Bacha, 2006), composed of two different layers: the stratum compactum (SC) and the stratum granulosum (SG) (Bacha & Bacha, 2006).

In the SG, there are cells with granular cytoplasm, which some authors consider immune cells (Khojasteh et al., 2009). The Zeissl membrane has been observed in several mammalian species, including cats, tigers, rats, and horses (Lim, 1922; Trautman & Fiebiger, 1957; Bacha & Bacha, 2006; Zahariev et al., 2010; Sapundzhiev et al., 2017). In these cases, it was present in the stomach and appears to be disposed of between the bottom of the gastric glands and muscularis mucosae (Singh & Kannabathula, 2018). In carnivores, it can extend into the intestine (Bacha & Bacha, 2006). Regarding fish, the Zeissl membrane has been reported in Rainbow trout, *Oncorhynchus mykiss* (Khojasteh et al., 2009; Yonkova et al., 2017), Chinook salmon, *Oncorhynchus tshawytscha* (Greene, 1912), Atlantic salmon, *Salmo salar* (Gulland, 1898), Northern pike, *Esox lucius* (Bucke, 1971; Sadeghinezhad et al., 2015), Brown trout, *Salmo trutta* (Burnstock, 1959), Common dentex, *Dentex dentex* (Carrassón et al., 2006), Mosquitofish, *Gambusia affinis* (Cengiz & Unlu, 2006) and Asian knifefish, *Notopterus notopterus* (Khadse & Gadhikar, 2017).

Since there is no information in the literature regarding the presence of the Zeissl membrane in Danube salmon, this study aimed to verify the structure and development of this layer in various segments of the digestive tract microscopically.

MATERIALS AND METHODS

All procedures involving animals were conducted following Romanian (Law 43/2014) and European legislation (EU Directive 63/2010). The Ethics Committee of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca (No. 117/2018) approved the study. The six specimens of Danube salmon used in this study were raised in captivity in a trout farm from Transylvania, Romania, to create a breeding stock for repopulating the region's mountain and submontane rivers. For microscopy assessment, several transversal fragments in the form of slices with a thickness of 4 mm for each were collected from the following regions: the foregut (the cardia and pyloric region of the stomach), the midgut (duodenum, pyloric caeca, and the posterior portion of the medium intestine), and the hindgut (anterior portion of the rectum). The samples were fixed by immersion in Stive solution for 24 hours, dehydrated in ethanol alcohol, clarified with n-Butanol, and finally embedded in paraffin. Thin sections of 5 µm were taken and stained with Goldner’s trichrome method. The histological slides were examined under an optical microscope (Olympus BX41) and images were captured using a digital camera (Olympus E330). The images were further processed using Adobe Photoshop CS 2.0.

RESULTS AND DISCUSSIONS

The microscopic analyses proved the presence of the Zeissl membrane in Danube salmon, in all studied segments with some differences between components.

In the histology structure of the cardiac stomach, the Zeissl membrane appears to be composed of both SC and SG. The SC was discontinuous and uneven in thickness over the entire circumference. The SC is present on about 30% of the stomach circumference in the cardiac region. The SG was better represented than the SC, being present on about 80% of the circumference. In certain regions, the thickness of the SC is roughly half that of the lamina propria. Meanwhile, in areas where the SC is present, SG is about double in thickness, reaching up to the muscularis mucosae. It is worth noting that in these regions, the muscle cells from the muscularis mucosae seem to be tightly compacted with a mostly longitudinal alignment (Figure 1).

The SG exhibits a more pronounced thickness in regions where the SC is not present. It is located between the lamina propria at the bottom of the glands and the muscularis mucosae. The ratio of thickness between lamina propria and SG is approximately equal. In these regions, the muscularis mucosae are composed of either smaller or larger fascicles, spaced apart one from another (Figure 2).

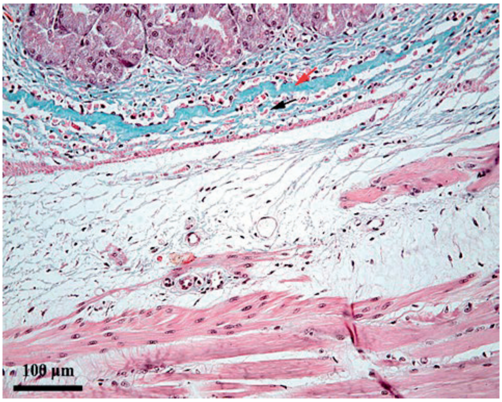

Figure 1. Stomach cardia area, Goldner's trichrome stain; red arrow – *stratum compactum*; black arrow – *stratum granulosum*

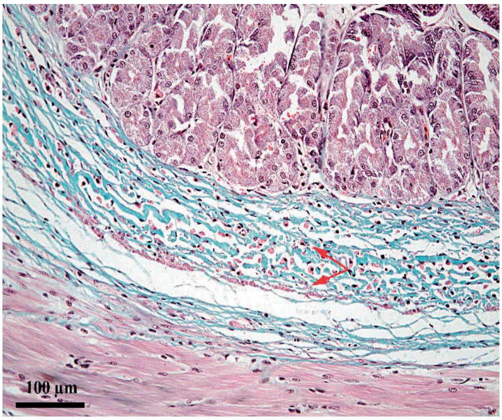

Figure 2. Stomach cardia area, Goldner's trichrome stain; red arrow – *stratum granulosum*

The presence of the Zeissl membrane in the pyloric stomach was continuous. The SC was noticeably thicker and uneven in thickness throughout its entire circumference. The SG was well developed in areas where the SC is thicker. In these areas, the fibril component of the SG appears to be quite compact to the muscularis mucosa, giving the appearance of doubling, and the cells from the SG occupy the space between the two lamellas (Figure 3). In the areas where the SC is thinner, the SG is approximately four times thicker. The granular cells are numerously represented in the areoles delimited by the fibrillar component in this layer (Figure 4).

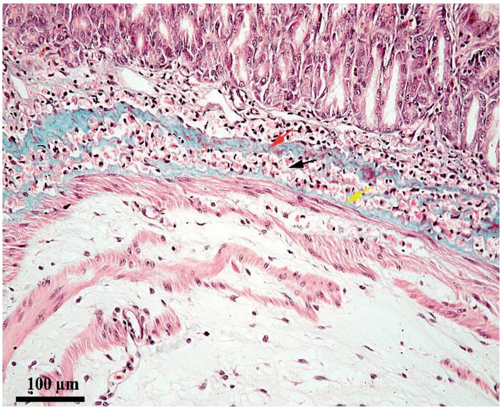

Figure 3. Stomach pyloric area – double layered aspect of the *stratum compactum*, Goldner's trichrome stain; red arrow – *stratum compactum*; black arrow – *stratum granulosum*; yellow arrow – double layered aspect of the *stratum compactum*

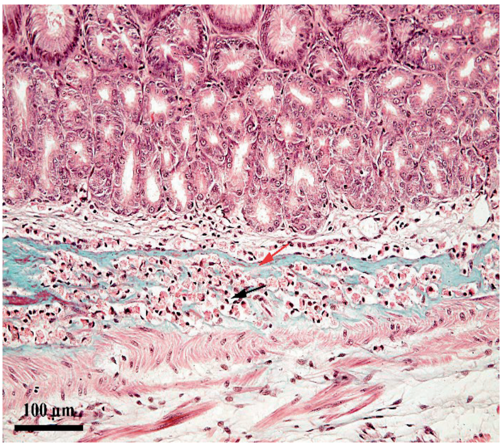

Figure 4. Stomach pyloric area, Goldner's trichrome stain; red arrow – *stratum compactum*; black arrow – *stratum granulosum*

In the duodenum, the Zeissl membrane was present and appears to consist of two layers: the SC and the SG. In the areas between the openings of the pyloric caeca, the SC appears well-developed. Its thickness represents approximately 30% of the thickness of the lamina propria, which is situated between the SC and the basal membrane of the epithelium. The SG was approximately three times thicker than the SC (Figure 5). In areas where the SC is highly developed, the fibers from the SG are quite compact, giving in some cases an aspect of doubling the SC. Between the two bands, fibrous branches with predominantly oblique layouts were observed. Toward the opening orifice of the pyloric caeca, the SC becomes thinner until it disintegrates.

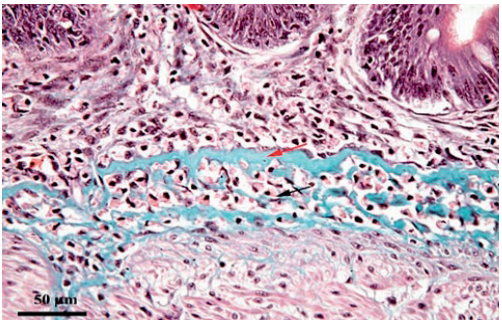

Figure 5. Duodenum, Goldner's trichrome stain; red arrow – *stratum compactum*; black arrow – *stratum granulosum*

The Zeissl membrane was formed only by the SG in the pyloric caeca. The fibrillary component of the SG was disposed quite compactly, forming a structure similar to the SC, but it appears disposed in direct contact with the muscularis externa. From it to the lamina propria, the granular cells typical of this layer were observed. Few granular cells were also observed between the fibrous membrane of the SG and the muscularis externa. The muscularis mucosae are absent and the SG comes in direct contact with the muscularis externa. Therefore, the submucosa is missing in this segment (Figure 6).

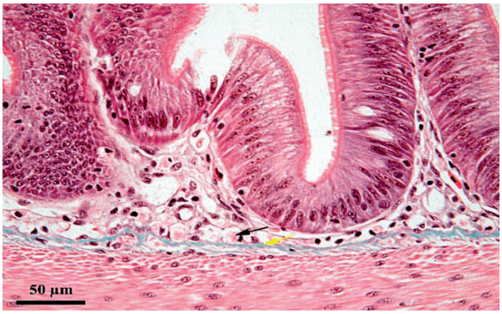

Figure 6. Pyloric caeca, Goldner's trichrome stain; black arrow – *stratum granulosum*; yellow arrow – the fibrillated component of the *stratum granulosum*

In the distal portion of the medium intestine, the Zeissl membrane was very developed and twice thicker than the lamina propria, found between it and the basal membrane of the epithelium. The SC occupies about half of the Zeissl membrane thickness (Figure 7).

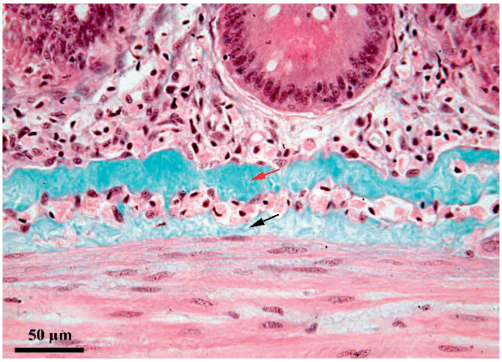

Figure 7. The distal portion of the medium intestine, Goldner's trichrome stain; red arrow – *stratum compactum*; black arrow – *stratum granulosum*

In some areas, where the SC is somewhat thinner, the fibers from the SG are arranged quite compactly to the muscularis externa, giving it the aspect of a double SC, with most of the granular cells disposed between them. Throughout the ileum, the SG comes in direct contact with the inner circular layer of the muscularis externa, so that the submucosa is absent (Figure 8).

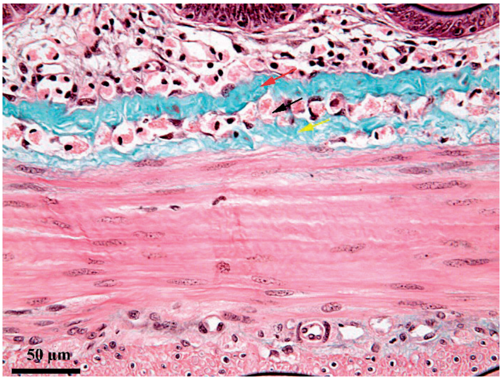

Figure 8. The distal portion of the medium intestine – doubling aspect of the *stratum compactum*, Goldner's trichrome stain; red arrow – *stratum compactum*; black arrow – *stratum granulosum*; yellow arrow – doubling aspect of the *stratum compactum*

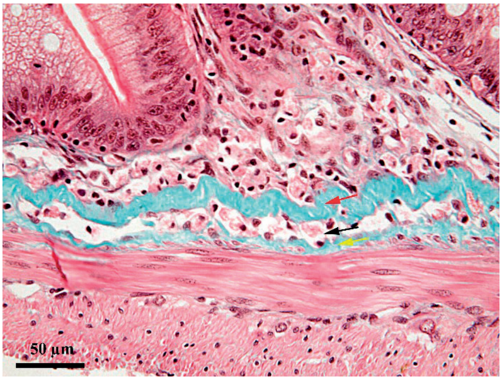

Figure 10. The anterior portion of the rectum – double layered aspect of the *stratum compactum*, Goldner's trichrome stain; red arrow – stratum compactum; black arrow – *stratum granulosum*; yellow arrow – double layered aspect of the *stratum compactum*

In the anterior portion of the rectum, the Zeissl membrane was present on the entire circumference of the wall and is thinner than in the previously described segment. The SC appears more developed than the SG (Figure 9).

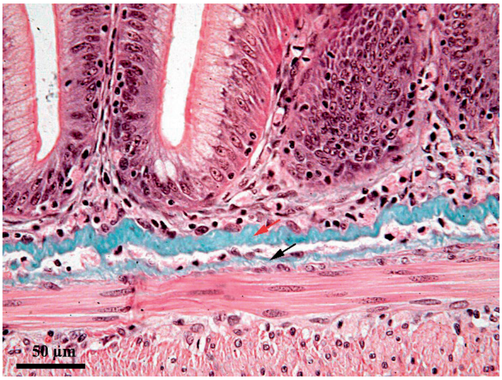

Figure 9. The anterior portion of the rectum, Goldner's trichrome stain; red arrow – *stratum compactum*; black arrow – *stratum granulosum*

In the areas where the SC is thinner, the fibers from the SG are ordered compactly resulting in an aspect of doubling the SC (Figure 10).

In the Danube salmon stomach, the Zeissl membrane presents some differences between the two studied areas. Thus, the SC inside the pyloric area is more developed than in the cardiac area and it is continuous throughout the circumference. As for the SG, it is also more developed in the pyloric segment, where in certain areas the fibril component is compactly arranged giving the aspect of doubling the SC.

The double-layered aspect of the SC was observed in the duodenum, but only in areas where the SC is developed, including the ileum and the anterior portion of the rectum. Some differences between the two areas of the stomach analyzed in the study are also found in the layout of the two layers of the Zeissl membrane.

Thus, in the cardiac area, the SC was discontinuous while in the pyloric region, it was continuous. In the parts where the SC is missing, the muscularis mucosae appear discontinuous, while in areas where the SC is present, the muscularis mucosae is continuous.

Greene (1912) describes SC in the stomach of Chinook salmon as a continuous layer, but not as developed as in the intestine and pyloric caeca. The difference in thickness of the SC between the stomach and the intestine was also reported for brown trout (Burnstock, 1959). The study states that SC limits the elongation of the muscle cells in the stomach wall to 75% and in the gut to 25%. Differences in the thickness of the SC between the stomach and the intestine were also observed during the present study for Danube salmon, with the distinction that, in this case, at the level of the cardiac area of the stomach, SC is discontinuous.

In the pyloric caeca, the Zeissl membrane occurs only as SG, similar to the one described in the intestine of horses (Rajput, 2006). In Chinook salmon, at the level of the pyloric caeca, the SC is continuous and the SG is well-developed (Greene, 1912). The results obtained during this study on Danube salmon are different from those described for Chinook salmon (Greene, 1912). Thus, for the pyloric caeca, the SC is absent and the fibrillary component from the SG is quite compact. This study shows that the muscularis mucosae and the submucosa are missing in the structure of the wall of the pyloric caeca similar to the Chinook salmon (Greene, 1912). Similarly, the muscularis mucosae and the submucosa are missing in the intestine. The structure of the intestinal wall in Danube salmon is similar to the one described for Chinook salmon (Greene, 1912), Brown trout (Burnstock, 1959), and rainbow trout (Khojasteh et al., 2009). In the intestine, the SC is the thickest in the distal portion of the medium intestine, but it does not have the same size over the entire wall circumference. In the areas where the SC is thinner, it has been observed that the fibrillar component of the SG is placed next to the muscularis externa, giving the aspect of a double layer of the SC. The same features appeared in the pyloric section of the stomach, in the duodenum, and the anterior part of the rectum. As a detail, the double-layered aspect of the SC was not described in the literature. According to this, the Zeissl membrane seems to have an important role in reinforcing the digestive tract wall in some voracious predators such as Danube salmon.

CONCLUSIONS

In Danube salmon, the Zeissl membrane is present in all organs assessed in our study, with some regional peculiarities. It is the most developed in the distal portion of the medium intestine and the thinnest in the pyloric caeca, where it appears only by SG. The SG was present in all studied segments, while the SC is the thickest in the distal portion of the medium intestine, discontinued in the cardiac region, and missing in the pyloric caeca. In the areas where the SC is thinner, the fibrillar component on the outer side of the SG is dense, with both layers displaying a similar density. Finally, the structure and distribution of the Zeissl membrane are different among species.

The membrane's unique structure makes it possible for fish to consume a high amount of food at once. Aside from its role in limiting gastrointestinal distension, it may have other implications for the morphophysiology of the digestive tract. Other similar reports are required for a better understanding of the role of the Zeissl membrane in other fish species.

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CONSUMER PERCEPTION ON AQUACULTURE GOODS AND SERVICES IN ROMANIA

Călin LAȚIU[1], Petru-Loredan UJICĂ[1], Radu CONSTANTINESCU[1], Tudor PĂPUC[1], George-Cătălin MUNTEAN[1], Paul UIUIU[1], Daniel COCAN[1], Maria Cătălina MATEI-LAȚIU[2], Cristian MARTONOS[3]

[1]Faculty of Animal Science and Biotechnologies, UASVM Cluj-Napoca, 3-5 Calea Manastur, Cluj-Napoca, Romania
[2]Faculty of Veterinary Medicine, UASVM Cluj-Napoca, 3-5 Calea Manastur, Cluj-Napoca, Romania
[3]Ross University School of Veterinary Medicine, P.O. Box 334, Basseterre St. Kitts West Indies

Corresponding author email: daniel.cocan@usamvcluj.ro

Abstract

Human consumption of fish meat is very heterogeneous, differing from one part of the world to another, from one country to another, from one region to another and is influenced by many factors. In terms of the level of importance of fish consumption globally, the main factor that ranks it among the top important foods is the high amount of protein it provides. In this survey, a series of 22 questions were addressed to people aged between 18 and 76+ summing 1017 respondents. This study reflects consumer perception on aquaculture goods and services in Romania. Respondents purchase fisheries and aquaculture products mostly from supermarkets, consider fish organoleptic properties to be very important, but also, they observe the poor supply of Romanian aquaculture products on the market.

Key words: *fish meat, fish stocks, fisheries, market survey, questionnaire.*

INTRODUCTION

The global production of crude protein from aquaculture and fisheries in 2018 was over 13950 kilotons (Boyd et al., 2022). However, most fisheries are overexploited, and efforts to make them sustainable have made fish farming a sector of great economic growth, aquaculture production exceeding fisheries production ever since 2016 (FAO, 2020). Moreover, in recent years, the captures fisheries production has remained constant. In 2020, the production from aquaculture and fisheries amounted to a value of 424 billion USD, correspondent to 214000 kilotons (FAO, 2022). As the production aspect of aquaculture faces a number of challenges, such as climate change, resistant pathogens, depleting sources of feed ingredients, and others, the sales aspect of aquaculture also presents issues of its own.

There are many factors affecting the buying decision of consumers regarding aquaculture products. The first two attributes that consumers observe and pay attention to are freshness and colour (García-Chavarría & Lara-Flores, 2013). Other factors are also of great importance: safety, nutritional quality, sustainability, price, availability, and also fish welfare, among others (Conte et al., 2014). In some cases, it has been proven that income and education are significant factors affecting the decision to buy fish over other meats (Morales & Higuchi, 2018). The advantages of consuming aquaculture products are evident: the meat is lean and easily digestible, it is a rich source of omega-3 fatty acids, iron, magnesium, zinc, calcium, and fish and other aquaculture products are generally recommended in the diet of pregnant women, children, and ailing people (Yılmaz et al., 2018). However, the perception of low involved consumers is that seafood is not an alternative for terrestrial meats, contrary to highly involved consumers, which rate seafood higher than terrestrial meats (Torrissen & Onozaka, 2017). There are examples of societies, especially insular states, where fish meat is preferred due to its superior nutritional quality, but also because of its high availability. For example, Japanese people have a very high life expectancy (84.79 years in 2021) (www.macrotrends.net), sometimes attributed to Japan's low obesity and heart disease rates,

which can be a consequence of a diet rich in seafood (Tacon & Metian, 2013).
Romania has a long tradition of consuming fish. Its rich water networks, ranging from mountain rivers to lakes, the Danube, and the Black Sea, allows the production of a diverse array of fish and seafood, from trout to carp, sturgeons, and mackerel. About 91% of the population consumes fish, both fresh and processed, but in small quantities, much smaller than the European average (Savin et al., 2021; Mastan et al., 2023). !!! Thus, this study aims to assess the Romanian fish consumption market, using questionnaires, and to describe the profile of fish consumers in Romania.

MATERIALS AND METHODS

Questionnaire elaboration. In general, questionnaires aimed at market research or market segments have complex structures. Usually, the first section of the questionnaire is the demographic and social component (Section 1: Question 1 to Question 5). This structure is essential to be able to analyze the directions and evolution of the markets through the lens of age categories, environment, or level of education. Studies or questionnaires can have several objectives. In the present case, the second component of the questionnaire deals with general preferences and fish consumption (Section 2: Question 6 to Question 9). The third section is intended to collect information on consumers' knowledge about fish meat quality (Section 3: Question 10 to Question 14), while in the next section fish market-related information is collected (Section 4: Question 15 to Question 17). Consumer's knowledge of nutritional information related to fish meat consumption as a healthy food source was also followed (Section 5: Question 18 to Question 20). The last section of the questionnaire was designed to collect information on recreational fishing and fishing license management (Section 6: Question 21 to Question 22). Questions were coded as follows: Question 1 – Q1, Question 2 – Q2, etc.

Survey. The questionnaire was uploaded and configured on the Google Forms platform. Before being distributed online for completion, 3 test sessions were carried out, for the security of receiving the answers and their centralization. The average duration of completing the questionnaire was approximately 3 minutes, an aspect specified at its initiation. The link containing the questionnaire was distributed on social media for 60 days. The answers were recorded and analyzed using Microsoft Excel.

RESULTS AND DISCUSSIONS

The questionnaire was conducted between April and June 2023 and was structured in 6 sections (Table 1), as follows: socio-demographic information of interviewed subjects (Q1 to Q5), general preferences and fish consumption (Q6 to Q9), knowledge on fish quality (Q10-Q14), fish market-related knowledge (Q15-Q17), basic nutritional information related to fish meat (Q18-Q20) and information related to consumer habits related to sport fishing (Q21-Q22). A total number of 1017 respondents participated in the survey.

Socio-demographic results (Q1 to Q5). The 36-50 years old age class was the best represented (32.1%; 326 participants), followed by the 18-25 years old age class (26.5%; 270 participants). A similar percentage (22.6%; 230) was observed in the case of the 26-35 years old age class (Figure 1).

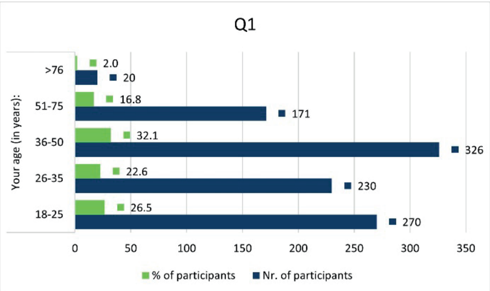

Figure 1. Age class composition for the questioned participants

The lowest percentages of responders belong to 51-75 and >76 years old age class respectively (16.8%; 171 participants and 2%; 20 participants). The sex distribution across the questioned subjects was 43% females and 57% males (Figure 2).

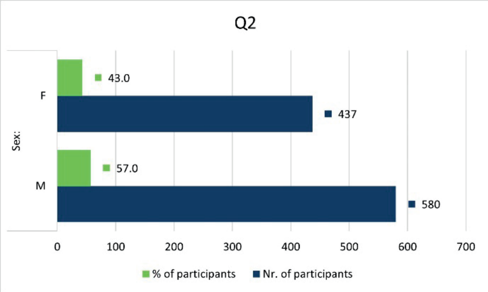

Figure 2. Sex distribution across the participants

According to the website of the National Institute of Statistics from Romania (NIS, 2024), accessed on 01.02.2024, 51.1% was represented by females and 48.9% by males. The "last completed studies" question showed that 35.8% of the subjects had their bachelor's degree, 31.6% finished high school, had a master's degree 20.6%, had a Ph.D. degree 6.8%, and gymnasium degree 1.2%. For the "other studies" category, 4.2% of the subjects had opted (Figure 3).

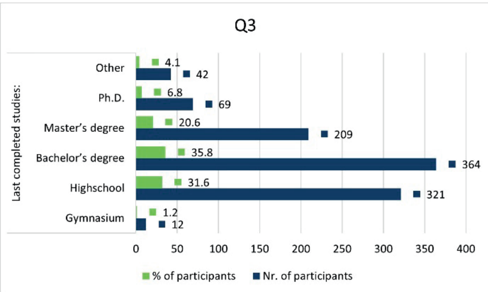

Figure 3. Completed studies of questioned participants

According to NIS, 16% of Romania's population had superior studies in 2021. The area of residence declared by the questioned subjects showed that 70.4% of the participants (n=716) live in urban areas while 29.6% (n=301) live in rural areas (Figure 4).

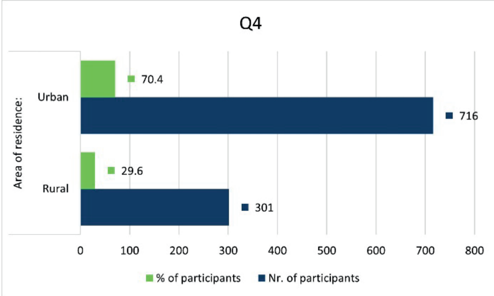

Figure 4. Area of residence distribution of the participants

The monthly net income declared by the participants varied as follows: 32.5% (n=331) had a net income between 400 to 800 €, 25.2% (n=256) had a net income between 800 and 1200 €, 21.9% (n=223) had a net income >1200 € and 20.4% (n=207) declared having a monthly income <400 € (Figure 5).

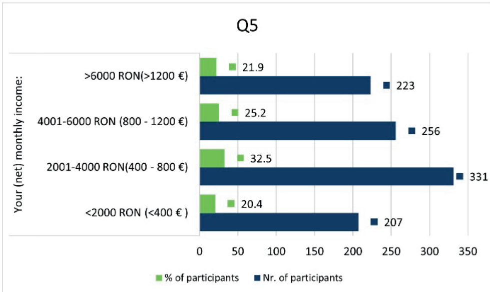

Figure 5. Declared net income of participants

Table 1. Questionnaire addressed in the study

Question No.	Addressed question	Options
Q1	Your age (in years):	18-25
		26-35
		36-50
		51-75
		>76
Q2	Sex:	M
		F
Q3	Last completed studies:	Gymnasium
		Highschool
		Bachelor's degree
		Master's degree
		Ph.D.
		Other
Q4	Area of residence:	Rural
		Urban

Q5	Your (net) monthly income:	<2000 RON (<400 €)
		2001-4000 RON (400 - 800 €)
		4001-6000 RON (800 - 1200 €)
		>6000 RON (>1200 €)
Q6	How often do you eat fish meat?	Daily
		Once a week
		3 to 4 times a week
		Once a month
Q7	Where do you prefer to consume fish?	At home
		At restaurant
		Other location
Q8	Where do you usually buy/procure fish?	Supermarket
		Fish market
		Recreational fishing (angling)
		Fish farms
		Other sources
Q9	Do you prefer:	Freshwater species
		Marine species
Q10	How important is the color of fish meat to you?	Not important
		Important
		Very important
Q11	How important is the odor of fish meat to you?	Not important
		Important
		Very important
Q12	How important is the texture of fish meat to you?	Not important
		Important
		Very important
Q13	How important is it to you to know the source of origin for the fish you buy?	Not important
		Important
		Very important
Q14	Do you consider the labeling of fishery and aquaculture products mandatory?	Yes
		No
		I don't know
Q15	How important is it to you that fish farms, supermarkets, and fish markets have qualified personnel in the field of fish farming and aquaculture?	Important
		Not important
		Very important
Q16	How well do you estimate that fishery and aquaculture products are represented in the Romanian market?	Very well represented
		Poorly represented
		Well represented
Q17	Do you find fisheries and aquaculture products of Romanian origin in most stores/supermarkets?	Yes
		No
Q18	Do you consider fish meat a healthy product?	Yes
		No
		I don't know
Q19	How would you rate your knowledge of the nutritional value of fish?	Elementary knowledge
		Advanced knowledge
		I have no knowledge on the subject
Q20	How important is the nutritional value of fish to you when you buy it?	Not important
		Important
		Very Important
Q21	Do you usually go fishing (angling)?	No
		Yes, occasionally
		Yes, very rarely
		Yes, quite often
Q22	Do you have a fishing license?	Yes
		No

Results on general preferences and fish consumption (Q6 to Q9). More than half of the questioned people (51%, 519 participants) consume fish once a month. The second preference for fish meat consumption according to the participants is once a week (39.2%, 399 participants). A smaller percentage, 8.4% (85 participants) affirmed that they consume fish 3 to 4 times a week. Only a small percentage of the participants, representing 1.2% (12 participants), affirmed that day eat fish daily. Two participants (0.2%) preferred not to answer (Figure 6).

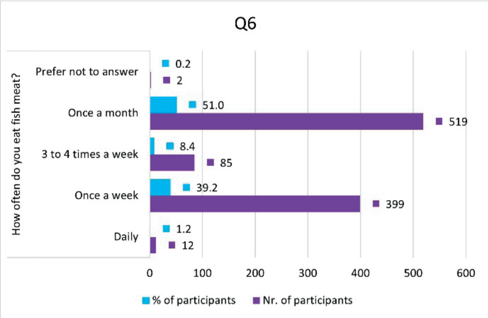

Figure 6. Consumers preferences: frequency of fish meat consumption

The participants prefer to consume fish at home in a very large percentage (82.7%, 841 participants). The second option in terms of consumer preferences is the restaurants (13.3%, 135 participants). A small percentage (3.8%, 39 participants) preferred other locations, while 0.2% (2 participants) preferred not to answer (Figure 7).

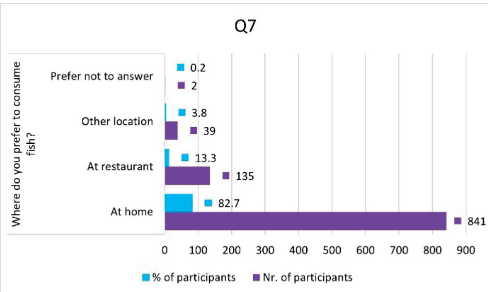

Figure 7. Consumers location preferences for fish consumption

Fish purchasing preferences, according to the data received from the participants in the present questionnaire, showed that 55.6% represented by 565 participants acquire their fish from supermarkets. The second option for fish procurement/acquisition, preferred by consumers, was recreational fishing (14.3%, 145 participants). Under Romanian legislation (OUG 23/2008), anglers can keep specific amounts of the daily capture. The third option in consumers' preferences was fish markets (13.7%, 139 participants). Direct purchase of fish from fish farms was the fourth option among the participants (11.1%, 113 participants). A small percentage of consumers, 5.4% (n=55) procure/acquire their fish from other sources (Figure 8).

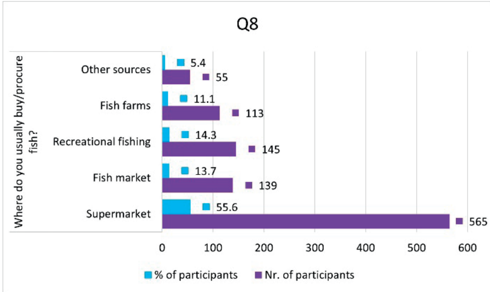

Figure 8. Consumers preferences for place of fish procurement/acquisition

Consumer preferences for freshwater or marine species revealed that 67.6% of the questioned participants (n=687) opted for freshwater species, while 32.4% (n=329) opted for marine species. One participant (0.1%) preferred not to answer (Figure 9).

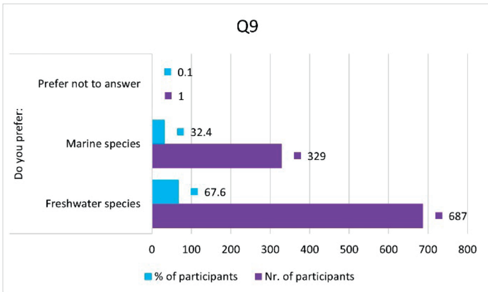

Figure 9. Consumer preferences for freshwater or marine species

Results on consumers' knowledge on fish quality (Q10 to Q14). One of the first qualitative attributes of food evaluation performed by customers is color appreciation (Wu & Sun, 2013; Şengör et al., 2018). When asked about the importance of fish meat color, 43.2% of the participants (n=439) considered fish meat color to be important, 34.8% (n=354) considered fish meat color very important and 22% (n=224) stated that fish meat color is not important (Figure 10).

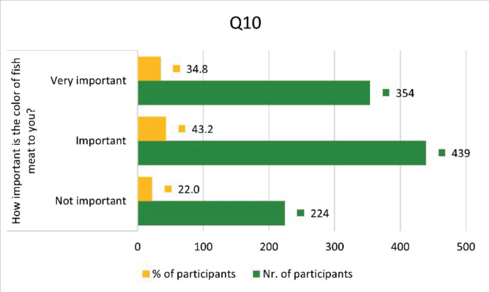

Figure 10. Fish meat color importance according to the participants

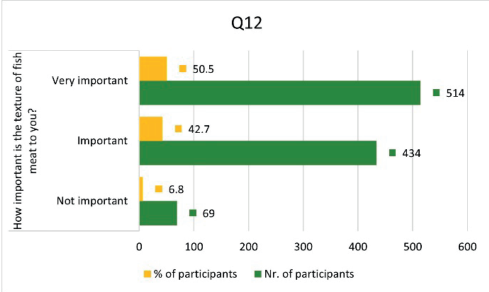

Figure 12. Importance of fish meat texture according to the participants

Meat in general and fish meat in particular is highly subjected to spoilage and contamination and the evaluation may be subjective if only sensory and microbiological methods are used (Hasan et al., 2012). The odor of fish meat was considered very important by 57.8% (n=588) of the participants, important by 34.8% (n=354), not important by 7.3% (n=74) and one person preferred not to answer (Figure 11).

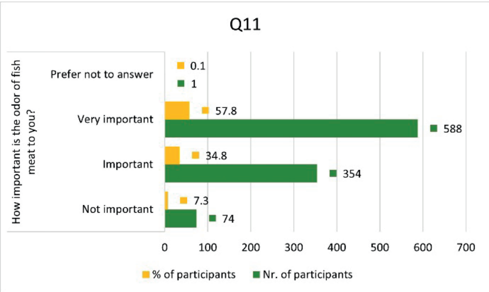

Figure 11. Fish meat odor importance according to the participants

Muscle texture (fish meat texture) is another aspect that can indicate the quality of the product, being affected by age, size, species, nutritional state, and rearing method (Dunajski, 1980). More than half of the participants (50.5%, n=514) considered fish meat texture to be very important, 42.7% (n=434) considered it important while only 6.8% (n=69) of the participants considered texture not important (Figure 12).

"From farm to fork" or "from farm to table" is a concept that embraces a fair, healthy, environmentally friendly sustainable food system (www.food.ec.europa.eu, FAO & WHO, 2024, Moretti et al., 2003). Following or tracing food is important mostly for quality control systems and risk management. Participants mentioned that it is very important to know the source of origin for fish in large percentages (50.5% respectively 41.8%). A small percentage, 7.5% (n=76) did not consider important this issue and one participant preferred not to answer (Figure 13).

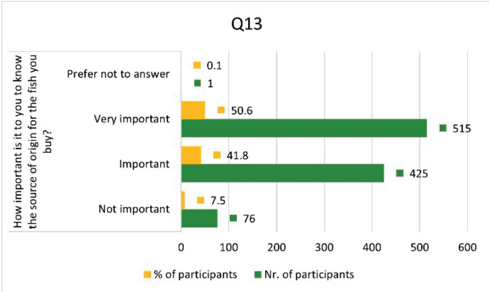

Figure 13. The importance of knowing the origin of fish found on the market

Displaying information on a product (labelling) is in general necessary for product identification, size, composition, nutritive values, expiring date, source/origin of product, possible allergens and many more reasons. In the present study, 85.5% (n=870) of the participants considered mandatory the labelling of aquaculture products while 8.8% (n=90) don't know, 5.5% (n=56) don't consider labelling mandatory. One person preferred not to answer to the question (Figure 14).

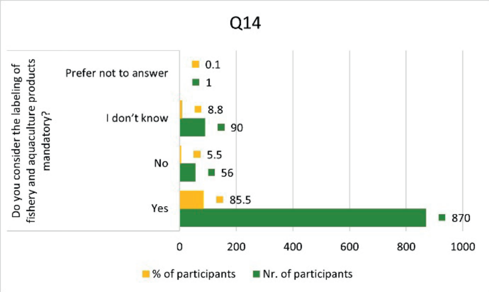

Figure 14. Labelling aquaculture products and customers' perception

Results on consumers' knowledge on fish market-related knowledge (Q15 to Q17). Qualified personnel working in fish farms, in consumers' point of view was very important for 35.8% of participants (n=364), important for 57.5% (n=585), and not important for 6.7% (n=68) (Figure 15).

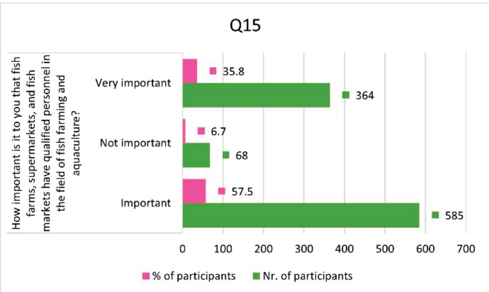

Figure 15. Consumers' view on qualified personnel (fisheries and aquaculture studies) in Romanian markets

The lack of qualified personnel in aquaculture and fish farming is considered one of the main causes of inefficiency in the field (Pillay, 1973). The presence of fishery and aquaculture products in the Romanian market was considered poorly represented by 55.2% (n=561) of the participants, while 39.5% (n=402) considered the opposite. Only 5.2% (n=53) of participants considered that fishery and aquaculture products are very well represented. And one person preferred not to answer (Figure 16).

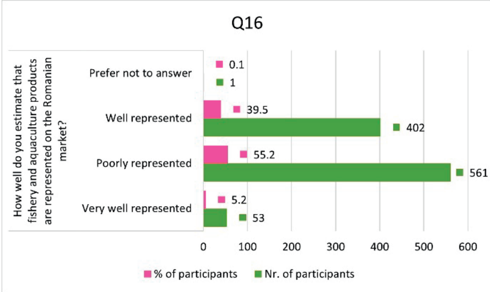

Figure 16. Consumers' view on the representation degree of fisheries and aquaculture products on Romanian markets

Products from fisheries and aquaculture of Romanian origin or production are not to be found in most of the markets and stores according to more than half (58.5%, n=595) of the participants from this study, yet 41.4% (n=421) said the opposite. One person preferred not to answer (Figure 17).

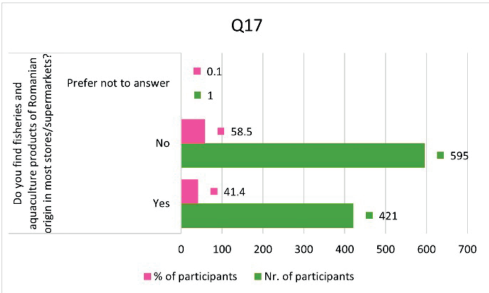

Figure 17. Consumers' view on the presence of aquaculture products of Romanian origin in markets

Results on consumers' knowledge on basic nutritional information of fish meat (Q18 to Q20). Fish meat is a healthy food source, rich in proteins, fatty acids, and minerals, being more available and affordable than other sources of animal protein (Mohanty et al., 2019). Most of the participants (94.4%, n=960) in our questionnaire, consider fish meat a healthy product, 4.3% (n=44) do not have knowledge of the issue, 1.2% (n=12) of the participants do not consider fish meat a healthy product and 1 person preferred not to answer (Figure 18).

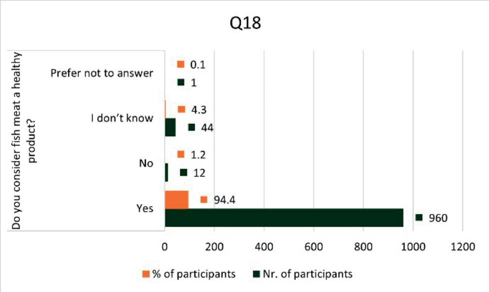

Figure 18. Consumers' view on fish meat as a healthy product

In terms of fish meat nutritional value, 67.7% (n=689) mentioned they have elementary knowledge on the subject, 23.3% (n=237) have advanced knowledge. A small proportion of the participants (8.8%, n=90) said that they do not have knowledge on subject and 1 person preferred not to answer (Figure 19).

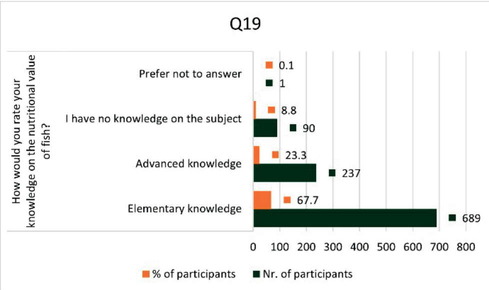

Figure 19. Consumers' self-evaluated knowledge on fish meat nutritional value

When asked about the nutritional value of fish meat at the moment of acquisition, 60.9% (n=619) responded that it is important, 29% (n=295) responded that it is very important and 10.1% (103) responded it is not important (Figure 20).

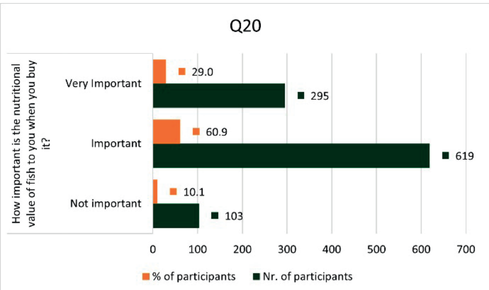

Figure 20. Consumers' view on nutritional value of fish at the moment of purchase

Results on consumers' consumer habits related to sport, recreational fishing (angling) (Q21 to Q22). Recreational fishing is fundamentally different from commercial fishing for different reasons, such as: gear used, size of catch, impact on diversity, economic importance, and socio-cultural. In Romania, the legislation does not distinguish properly the terms "recreational fishing" and "sport fishing". In general, the term sport fishing is associated with competitions (O561/2023; OUG 23/2008) while recreational fishing or angling is often seen as a hobby or recreational activity without involving "competitions, competitors and prizes". In the present study, 42.8% (n=435) participants responded that they do not go fishing, 20% (n=203) go fishing occasionally, 13.9% (n=141) go fishing rarely, and 23.4 % (n=238) go fishing often (Figure 21).

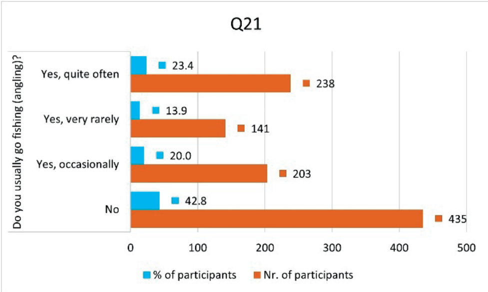

Figure 21. Consumers' preferences when it comes to recreational fishing

The participants also mentioned that 63.5% do not have a fishing license while 36.5 do have a fishing license (Figure 22).

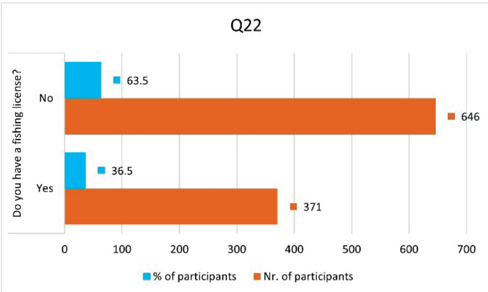

Figure 22. Consumers' option for possessing a fishing license

Analyzing the number of people going fishing in general (from Q21, all yes answers) and the number of people having a fishing license (Q22) it can be observed that 57.3% of the participants go fishing but only 36.5% get a fishing license, meaning that 20.8% go fishing without a license. Private recreational fishing waters do not require by law to have a fishing license. There is a strong possibility that some of the 20.8% of the people going fishing without a fishing license are under this category, while some of them might be fishing on public waters without a license (illegally).

CONCLUSIONS

The present study provides information on customers' perception of fisheries and aquaculture products, on knowledge related to fish meat as a healthy food source and provides feedback as well on the importance of trained personnel in the field in Romania. The context of the survey was based on the European Union's blue economy strategy, where aquaculture is a key element. According to FAO (2020), internal fish production covers less than 20 percent of the total fish consumption in Romania and aquaculture is still based on the semi-extensive culture (common carp and Chinese carps). As a general trend, Romanian consumers prefer to eat fish at home, somewhere between once a week to once a month and they buy fish from supermarkets, preferring mostly freshwater species. They also consider fish organoleptic properties (color, odor, texture) to be very important as well as labelling of fisheries and aquaculture products. Consumers consider qualified personnel in aquaculture to be important, fisheries and aquaculture products are poorly represented according to some and well-represented on the Romanian market according to others (probably depending on the geographical area). Fisheries and aquaculture products of Romanian origin are not found in general very often on the markets. Romanian consumers have elementary knowledge of fish nutritional value, view fish meat as a healthy food source and take into consideration fish's nutritional value at the moment of purchase. According to our data, Romanian consumers go fishing in general and have in most cases a fishing license. This information could be used in the future for strategies in the field of fisheries and aquaculture management, market-customer decisions, and recreational fishing legislation.

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DETERMINATION OF THE MEAT BIOCHEMISTRY OF PONTIC SHAD *Alosa immaculata* (Bennett, 1835) DURING MIGRATION

Cristian Mihael LEONOV[1], Maria Desimira STROE[2], Mirela CREȚU[2, 3], Floricel Maricel DIMA[2, 4], Livia VIDU[1], Carmen Georgeta NICOLAE[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]Institute of Research and Development for Aquatic Ecology, Fishing and Aquaculture, 54 Portului Street, Galați, Romania
[3]Faculty of Food Science and Engineering, "Dunărea de Jos" University of Galați, 47 Domnească Street, Galați, Romania
[4]Faculty of Engineering and Agronomy, 29 Calea Calărașilor Street, 810017, Brăila, Romania

Corresponding author email: cretu.mirela@asas-icdeapa.ro

Abstract

The biochemical analysis of fish tissue is considered a dependable indicator of the fish`s quality, nutritional content, physiological condition, and environmental habitat. Alosa immaculata (Bennett, 1835) is a migratory fish species with great ecological and economic importance, which undergoes a remarkable journey during its migration. As A. immaculata embarks on its migration, the dynamic biochemical changes in its meat composition offer valuable information that contributes to a comprehensive understanding of the species` adaptation to the challenges posed by migration. In this context, this study aimed to investigate the meat biochemistry of Pontic Shad during the period of migration from three different points: Station 1 - Saint Gheorghe Branch, Station 2 - Sulina Branch, and Station 3 – Brăila, km 169 and km 197 of the Danube River. Significant differences (p<0.05) were recorded in the level of water, lipid, and ash, while no differences were recorded in the level of proteins (p>0.05). Notably, the lipid content in the meat samples from the Sulina exhibited the highest percentage, while the protein content in the samples from the St. Gheorghe arm surpassed that of the Sulina and Brăila, indicating differences in nutritional profiles that could be attributed to physiological adaptations during migration.

Key words: *Danube, fish, lipids, proteins, reproduction migration.*

INTRODUCTION

The consumption of fish has attracted considerable attention owing to its wide range of health benefits and nutritional value. Well-known for its easily digestible protein of high biological value (De Smet, 2012; Mocanu et al., 2022), low saturated fat, and abundance of essential omega-3 fatty acids (Zuraini et al., 2006), fish presents an appealing dietary option to enhance overall well-being and mitigate the risk of numerous chronic illnesses (Raatz & Bibus, 2016; Li et al., 2020; Maulu et al., 2021).
Alosa immaculata belongs to the pelagic *Clupeidae* family. It is an anadromous fish, which it lives in winter scattered in the southern regions of the Black Sea, along the Bulgarian coast north of Burgas, at depths greater than 40 m (Niculescu-Duvăz, 1965). It is a commercially important species in Romania, with high-quality protein, omega-3 fatty acids, vitamins, and minerals (Năvodaru, 1997; Mocanu et al., 2022; Savin et al., 2020), essential for human overall health and well-being.
The proximate biochemical composition of a species serves as a crucial indicator for evaluating its nutritional and edible worth about energy units when compared with other species. Fluctuations in the biochemical composition of fish flesh can also be observed within the same species, influenced by factors such as the fishing location, season (Vollenweider et al., 2011), age (Breck, 2014), sex and reproductive condition of the individual (Yousaf et al., 2011). Also, the spawning cycle and food availability emerge as primary determinants contributing to such variations (Love et al., 1980).

The migration pattern of Pontic shad involves extensive journeys from the southern regions to the northern extents along the coastline of the Black Sea in Bulgaria and Romania, culminating at the mouths of the Danube River within Romania. This migratory behaviour reflects the species' adaptation to changing environmental conditions and resource availability throughout its life cycle. Knowing the meat biochemistry of Pontic shad during migration can offer valuable insights into the physiological changes occurring within this species as it undertakes its migratory journey.

In this context, this paper aimed to analyse the biochemical composition and the nutritional condition of Pontic shad during migration of reproduction

MATERIALS AND METHODS

Sample collection. *Alosa immaculata* specimens were collected by scientific fishing from Station 1 - Saint Gheorghe Branch, Station 2 - Sulina Branch, and Station 3 - Brăila (km 169 and km 197 of the Danube River) (Figure 1).

Figure 1. Sampling area of the study (source: https://www.google.com/maps)

All the samples were collected in April 2023. The fish samples (n = 20 fish/each station) were then transported to the laboratory in an icebox with a fish/ice ratio of 1:2, for further analysis. Before collecting the samples, all fish were weighed (g) and measured (cm). Following morphometric measurements, a clean stainless-steel knife dissected the fish. To determine the somatic indices, such as the viscerosomatic, hepatosomatic and gonadosomatic indices, fish viscera and liver were weighed:

Hepatosomatic index (HSI, %) = [liver weight (g)/body weight (g)] × 100;

Viscerosomatic index (VSI, %) = [viscera weight (g)/body weight (g)] × 100;

Gonadosomatic index (GSI, %) = [gonads weight (g)/body weight (g)]×100;

Proximate composition of fish. The biochemical composition of the meat was assessed through conventional analytical techniques (AOAC, 1990). Subsequently, solely the muscle tissue was homogenized using a blender and utilized for subsequent analyses.

To determine the protein content the Kjeldahl method was employed, involving the assessment of nitrogen content, which was then converted into protein equivalent using a multiplication factor of 6.25.

The lipid content (%) was measured using the Soxhlet method with an automated Soxtherm system. For ash content (%), the samples were incinerated in an electric oven at 550 ±1.0 °C for 4 hours, followed by weighing the sample crucibles at room temperature. Moisture content was determined by drying the samples at 105 ± 2°C until a constant mass was achieved.

Fulton coefficient. To estimate changes in the nutritional condition of fish the Fulton coefficient (K) was calculated, according to Froese (2006): $K = W \times 100/Ls^3$, where Ls- standard length of fish (cm), W= fish weight (g).

Statistical Analysis. The results of the biochemical composition and the somatic indices are presented as mean ± standard deviation. To evaluate differences between the biochemical parameters of fish meat, the one-way ANOVA test was employed, with significance established at $p<0.05$. Statistical computations were conducted using SPSS 26.0 for Windows (SPSS, Chicago, IL, USA), and Microsoft Office 2019.

RESULTS AND DISCUSSIONS

Biometric measurements of fish samples taken in analysis are presented in Table 1.

Table 1. Biometric measurements of fish samples

Sample Stations	Mean W (g)	Mean Lt (cm)	Mean Ls (cm)	Mean Lf (cm)	Mean H (cm)	Fulton coefficient
Saint Gheorghe Branch	243.01± 4.60*	30.13±1.13*	27.38±1.07*	26.24±1.07*	6.21±0.34*	1.29±0.32*
Sulina Branch	261.54±46.34*	31.07±1.51*	27.02±1.35*	27.85±1.36*	6.52±0.57*	1.31±0.10*
Brăila (km 169-197 of the Danube River)	213.44±56.77**	29.93±2.53*	25.73±2.30**	28.24±1.21**	5.01±0.49**	1.20±0.11**

Note: N- number of specimens; Mean W- mean weight (g); Mean Lt- mean total weight (cm); Mean Ls- mean standard length (cm); Mean Lf – mean fork length (cm); Mean H- body depth (cm). *- no significant differences between the somatic measurements: ** - significant differences between the somatic measurements

Significant higher ($p<0.05$) weights were registered for fish provided from the station's Saint Gheorghe Branch and Sulina Branch, while at Brăila station, fish weight was significantly lower. Regarding the total fish length (Lt), Standard length (Ls), Fork length (Lf), and body depth (H), significantly lower values were recorded for the fish caught at Brăila (km 169-197 of the Danube River). Concerning the Fulton coefficient, there is a noticeable decrease observed as the migration progresses towards the spawning and egg-laying areas along the Brăila stretch (located between km 169-197 of the Danube River). Specifically, the Fulton coefficient shows values of 1.29 ± 0.32 and 1.31 ± 0.10 at the Saint Gheorghe Branch and Sulina Branch, respectively. At the Brăila station, there is a significant decrease in the Fulton coefficient, with a value of 1.20 ± 0.11. A similar study carried out by Savin et al., 2020 supports these findings, indicating higher Fulton coefficient values at the beginning of the migration, particularly at the St. Gheorghe Branch (1.28), and the lowest value (1.13) at kilometer 197 on the Danube River (Chiscani), while at km 103 (Isaccea village), the recorded value of Fulton was 1.15. Generally, when the Fulton coefficient values exceed or are equal to 1, it indicates a favourable condition within fish populations. In the case of Pontic shad, the Fulton coefficient demonstrates a decreasing trend in correlation with the migration distance to the Danube River and the reproductive process. This decline is attributed to the loss of biomass sustained as energy expenditure for both migration and spawning, as highlighted by Năvodaru (1997).

Figures 1-4 present the biochemical composition of the *Alosa immaculata* muscle. Significant differences ($p<0.05$) were recorded in the level of water, lipid, and ash, while no significant differences were recorded in the content of proteins ($p>0.05$).

The moisture content registered the highest value at Brăila station (66.16 ± 2.44 %). At the Sulina Branch (58.88 ± 0.65 %) and Saint Gheorge Branch (58.59 ± 0.72 %) the water content was significantly lower ($p<0.05$).

No significant changes ($p>0.05$) were observed in the protein content among the sampled fish from the three stations. Thus, the protein content recorded a value of 16.95 ± 0.26 % at Sulina Station, 17.42 ± 1.10 % at St. Gheorge Station, respectively of 16.89 ± 1.30 % at Brăila Station. Regarding the lipid content in Pontic shad meat, a significant decrease ($p<0.05$) was recorded at the Braila Station (15.61 ± 2.39 %), in comparation with the Sulina (22.17 ± 1.73 %) and St. Gheorghe (21.35 ± 2.10 %).

The ash content registered significant differences between the selected stations, with higher values at St. Gheorghe station (1.31 ±0.12 %).

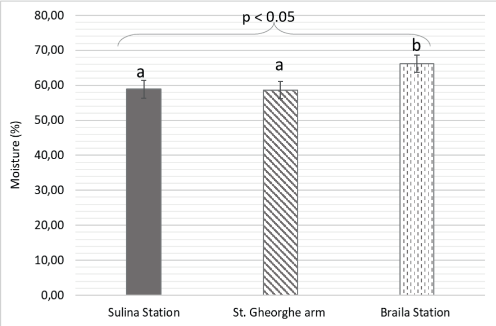

Figure 1. Moisture content of *Alosa immaculata*

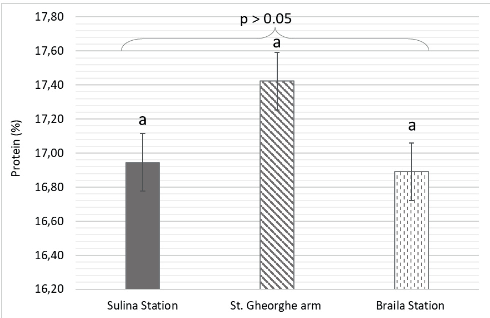

Figure 2. Protein content of *Alosa immaculata*

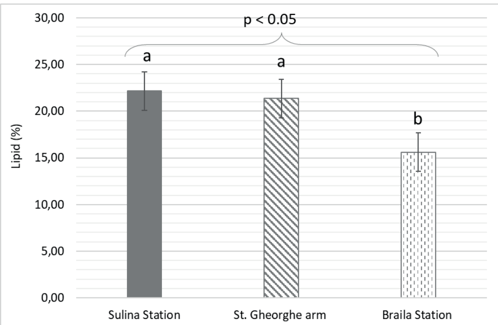

Figure 3. Lipid content of *Alosa immaculata*

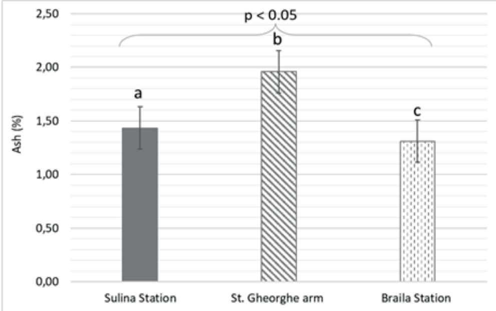

Figure 4. Ash content of *Alosa immaculata*

In general, fish meat exhibits high moisture content, typically ranging between 60-80%, with protein content falling within the range of 15-26 % and fat content ranging from 2-13% (Pearson & Cox, 1976). Notably, *Alosa immaculata* is distinguished among fish species for its remarkable lipid content, which varies between 15.12% and 25.8% (Savin et al., 2020; Ionescu et al., 2006). According to Ionescu et al., 2006, the Pontic shad has a proximate composition of 58.8% water, 25.8% lipids, and 14.2% protein. However, the lipid composition of fish meat is subject to various influencing factors, including species, age, diet, size, and seasonal fluctuations (Všetičková et al., 2020; Popa et al., 2022).

In Romania, for spawning migration, *Alosa immaculata* covers long distances, migrating from the Black Sea to the Danube River. First, it enters from the Black Sea to the Saint Gheorghe Branch, Chilia Branch, and Sulina Branch. After covering a considerable distance, it arrives at Brăila (km 169 - 197 of the Danube River), but the migration continues to the Iron Gate II. Throughout this migration process, numerous physiological and metabolic changes occur. Thus, the lower lipid content from the Brăila station can be explained by consumption of the lipid reserves during the reproductive migration. Generally, the lipid content serves as the primary energy reserve in anadromous fish and plays a critical role in their survival, migration, and reproductive success (Bayse et al., 2018).

Moving upstream in the Danube River requires a significant amount of energy to overcome the water current and for the maturation of the gonads, especially considering that the Pontic shad does not feed. This aspect entails the consumption of nutrients accumulated during feeding, leading to the loss of individuals' body biomass and lipid reserves.

This aspect is further highlighted by the values of hepatosomatic indices. The observed decrease in hepatosomatic indices at Brăila Station underscores the significant energy expenditure required for upstream migration and gonad maturation of *Alosa immaculata* during its spawning migration in the Danube River. This depletion of hepatic reserves emphasizes the critical role of lipid metabolism in facilitating the physiological adaptations necessary for successful reproduction and migration in anadromous fish species. (Table 2).

On the other hand, the increase in viscerosomatic and gonadosomatic indices at Brăila is mainly influenced by the intensification of gonad maturation processes during the reproductive migration of *Alosa immaculata* in the Danube River.

Table 2. Viscerosomatic and hepatosomatic indices of *Alosa immaculata*

Sampling stations	VSI (%)	HSI (%)	GSI (%)	N
Saint George Branch	5.10± 0.66*	2.02 ± 0.47*	2.12±1.14*	20
Sulina Branch	4.76±0.37*	2.14 ± 0.64*	2.37 ±1.14*	20
Braila	9.53±0.16**	1.73± 0.72**	4.51±1.14**	20

Note: VSI - Viscerosomatic index; HIS – Hepatosomatic index; N = number of fish taken in analysis; * - no significant changes were recorded between the stations ($p<0.05$); ** - significant changes were recorded between the stations ($p>0.05$).

CONCLUSIONS

In conclusion, the composition of fish meat, particularly the lipid content, varies significantly between the selected stations. *Alosa immaculata*, known for its high lipid content, undergoes physiological and metabolic changes during its spawning migration in the Danube River, leading to the depletion of lipid reserves. The observed decrease in lipid content at the Brăila station indicates the energy expenditure required for upstream migration and gonad maturation, further supported by the decline in hepatosomatic indices.

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HEPATOPROTECTIVE EFFECT OF *Ulva lactuca* AND *Spirulina platensis* IN *Cyprinus carpio* EXPOSED TO IMAZALIL

Alina Nicoleta MACOVEIU[1], Geanina CONSTANDACHE[1,2], Mirela CREȚU[1], Iulia GRECU[1], Angelica DOCAN[1], Lorena DEDIU[1]

[1]"Dunărea de Jos" University of Galati, Faculty of Science and Environment, 47 Domnească Street, RO-800008, Galati, Romania
[2]Research-Development Institute for Aquatic Ecology, Fisheries and Aquaculture Galați, 54 Portului Street, Galaţi, Romania

Corresponding author email: lorena.dediu@ugal.ro

Abstract

The objective of the current investigation was to assess the hepatoprotective capabilities of two algal species, Ulva lactuca and Spirulina platensis, in carp (Cyprinus carpio) fingerlings subjected to imazalil exposure. Consequently, the experimental groups were simultaneously exposed to the fungicide (5mg/kg feed) and provided with feed containing either Ulva lactuca alone (IMZ-Uv variants – 5 g/kg ulva), Spirulina platensis alone (IMZ-Sp variants – 5 g/kg spirulina), or a combination of both algae (IMZ-Uv-Sp variants – 2.5 g/kg ulva and 2.5 g/kg spirulina). Two control variants were employed in the experiments: a negative control receiving normal feed and a positive control receiving feed with 5mg/kg imazalil. Each variant involved 15 fish per tank, and after the experiment, five fish from each tank were utilized for biological samples. The assessment of biochemical serum parameters and oxidative stress markers for each specimen revealed that imazalil induced alterations in biochemical parameters, resulting in physiological dysfunctions in carp. However, the administration of algae, in particular Spirulina platensis, demonstrated significant potential in mitigating the adverse effects of imazalil.

Key words*: carp, fish blood, imazalil, seaweed, spirulina.*

INTRODUCTION

Along with the increase in the number of the global population, the demand for food quantities also increased. To meet this high demand and to feed the growing urbanized population, both aquaculture and agriculture expanded cultivated areas and shifted technology towards intensive production. Thus, to obtain maximum productivity, intensive agriculture under the protection of crop protection measures, increased the amount of pesticides, thus causing contamination of surface waters (de Souza et al., 2020). Similarly, in the last decades, due to a lack of suitable sites, cage aquaculture has expanded into previously unexplored open-water culture zones in inland, coastal, and marine environments (FAO, 2020). Farming fish in cages offers numerous advantages such as low investment costs, high production, high stock survival, and easy harvesting. However, the fish biomass is continuously exposed to water quality and, in some cases, to different pollutants emerging in the aquatic environment through various pathways such as municipal sewage, industrial wastewater discharges, or agricultural runoff underground contamination

Imazalil (enilconazole) is one of the fungicides frequently used by farmers to combat and treat various pests that appear especially on fruits (citrus and bananas) but also on various seeds before planting (cereals and oilseeds) (Diedhiou et al., 2014; Kellerman et al., 2016). Thus, with its large-scale use, it increased significantly the risks of contamination of the aquatic environment (Chitescu et al., 2015) and therefore the potential to induce severe effects on fish populations (Şişman & Türkez, 2010) (Belenguer et al., 2014; Ccanccapa et al., 2016). With all the benefits that come from the use of pesticides, the risks associated with their use exceed the advantages because they produce a major imbalance in the life of aquatic ecosystems, and also produce harmful effects on human health causing numerous health problems (Kumar Goswami et al., 2018; Fisher et al., 2022).

The studies available so far provide results whose evaluation correlates with the fact that exposure to imazalil of different fish species can produce changes in the locomotor system as well as in the development of zebrafish larvae, they can also cause dysbiosis on the composition of the intestinal microbiota, as well as imbalances on hepatic metabolism in adult zebrafish (Jin et al., 2016; Jin et al., 2017; Huang et al., 2022).

To avoid the risks associated with this excessive dependence on fungicides, new strategies, and alternatives are being sought to encourage ecological practices that mitigate their impact on water and soil quality. Researchers' attention in recent years has been directed to seaweeds, which due to their properties in the treatment of wastewater become promising sources of bioremediation technology (Karthik et al., 2020; Areco et al., 2021). At the same time, it is essential to explore strategies aimed at mitigating the risk of aquatic animal exposure to contaminants or discovering nutritional approaches to enhance the health of aquatic animals and attain higher-quality aquatic feed within the aquaculture industry. From this perspective, including algae in fish diet proved to have the potential for both directions: to effectively replace fish meal and fish oil (Shah et al., 2018) and to ameliorate the negative effects of contaminants in the fish (Abdel-Latif et al., 2022).

Spirulina platensis is a blue-green, microalga that can survive in unfavourable environments (with a high content of heavy metals, organic pollutants, and extreme environmental conditions) also due to its chemical composition rich in vitamins, minerals, essential fatty acids, amino acids, this pigment possesses antioxidant and immunomodulatory qualities, improving the growth and physiological response to stress and diseases of several species of fish (Bortolini et al., 2022; Youssef et al., 2023).

Ulva lactuca is a green macroalga that belongs to the phylum Chlorophyta whose diversification has shown an increasing trend in the last decades, both due to the global distribution and the composition and properties of the bioactive compounds identified in the composition of this macroalga (Øverland et al., 2018; Khora & Navya, 2020). The studies carried out so far, have indicated a high potential for integration into the fish diet, with beneficial effects on growth performance, oxidative stress, and immunity, in terms of improving intestinal microbiota and antimicrobial activity, but at the same time, these valuable compounds can be exploited in sectors such as the pharmaceutical industry, food nutraceuticals, agricultural or biorefineries (Kidgell et al., 2019; Thépot et al., 2021; Negreanu-Pirjol et al., 2022; Vijayaram et al., 2022)

Although those algal species were extensively explored for their potential to improve fish health there are no current studies investigating their role in mitigating the imazalil toxicity in fish. Therefore, the main aim of the present study was to assess the protective potential of the two algal species, *Ulva lactuca* and *Spirulina platensis*, in carp (*Cyprinus carpio*) fingerlings subjected to imazalil exposure. For this purpose, some hematological, oxidative stress, and plasma biochemical markers were considered.

MATERIALS AND METHODS

Experimental design and fish feeding

This experiment was conducted at the Aquaculture Pilot Station of the Faculty of Food Science and Engineering, “Dunărea de Jos” University of Galați, Romania. The fish (*Cyprinus carpio*) were obtained from the Research and Development Institute for Aquatic Ecology, Fishing and Aquaculture, Galați, Romania. All fish were acclimatized for 2 weeks during which were fed with a commercial diet (40% protein, 10% fat, 1.5% fiber). After the acclimatization period, 140 individuals, with an initial body weight of 65.4 ± 0.61 g, were randomly distributed into five experimental groups, as follows: Control (commercial diet), IMZ (commercial diet and 5 mg/kg feed), IMZ+Sp. (IMZ 5 mg/kg feed and *Spirulina platensis* 5 g/kg feed), IMZ+Uv (IMZ 5 mg/kg feed and *Ulva lactuca* 5 g dw/kg feed), and IMZ+Uv+Sp. (IMZ 5mg/kg feed, *Spirulina platensis* 2.5 g/kg feed and *Ulva lactuca* 2.5 g/kg feed). The green macroalgae *Ulva lactuca* were purchased from Algas Atlanticas Algamar S.L., the collection area being the Bay of Biscay

Galicia, Spain. The algal dry biomass was crushed with the help of a mill and later incorporated into the feed. *Spirulina platensis* was purchased from the company Cerasus, China. Imazalil was purchased from Sigma-Aldrich Production GmbH, Switzerland.
The experiment took place over a period of 27 days and was conducted in glass aquariums, with a capacity of 140 L each. Each aquarium has an independent water filtration system. Fish were fed two times per day (09:00, and 17:00) with a daily ration of 2.6% of fish body weight (BW). The survival was surveyed daily.

Hematological analysis and blood biochemistry. At the end of the experimental period, 2 mL of blood was taken by caudal venous puncture, from 7 fish randomly chosen from each experimental unit. For the biochemical analysis, plasma was obtained by centrifuging the blood at 3500 rpm for 10 minutes.
The hematological profile was determined using the routine methodology of fish hematology. Red blood cell count (RBC × 106/µL) was determined by utilizing a Potain pipette and Vulpian diluting solution. The calculation of RBC (10^6/µL) involved the number of cells counted, the number of squares in which they were counted, square volume, and blood dilution (Svobodova et al., 2012). For hematocrit determination (Ht, %), the microhematocrit method was employed following blood centrifugation at 12,000 rpm for 5 minutes. Hemoglobin concentration (Hb, g/dL) was measured using the cyanmethemoglobin method with Drabkin's reagent (DIALAB, Wiener Neudorf, Austria), and the absorbance was read at a wavelength of 540 nm (Hesser, 1960) using a Specord 210 UV–Vis spectrophotometer (Analytic Jena, Jena, Germany).
Hematological indices, including mean corpuscular volume (MCV, fL), mean corpuscular hemoglobin (MCH, pg), and mean corpuscular hemoglobin concentration (MCHC, g/dL), were calculated based on PCV, Hb, and RBC values (Ghergariu et al., 1985; Svoboda, 2001).

The plasma **biochemical parameters** determinations were made with the help of VetTest® Chemistry Analyser, using IDEXX VetTest kits for albumin (ALB, g/dL), Calcium (Ca, mg/dL), Total protein (TP, g/dL), Glucose (GLU, mg/dL) and Globulins (GLOB, g/dL).

Oxidative stress. Lipid peroxidation was quantified by malondialdehyde (MDA, nmol/mL) concentrations. The MDA was assessed using the method outlined by Ohkawa et.al 1979. The optical density of the samples was measured at 532 nm, and the results were expressed as nmol of MDA per mL of plasma or per gram of liver homogenate (nmol/g liver).
Lysozyme activity (LZM, U/mL) was determined through a turbidimetric assay, following the Enzymatic Activity of Lysozyme Protocol (Sigma, EC 3.2.1.17, Sigma-Aldrich, St. Louis, MO, USA). Total antioxidant capacity (TAC, mMol Trolox) was measured spectrophotometrically at an optical density of 734 nm, utilizing the ABTS - (2,2-azinobis 3-ethylbenzothiazoline-6-sulphonic acid) method as described by Re et al. (1999).

Statistical analysis. Statistical analyses were conducted utilizing the SPSS statistical software for Windows, Version 16.0, by SPSS Inc., Chicago, United States. Hematological and serum parameters were presented as means ± S.E. of the replicates. A one-way ANOVA analysis was performed on the data. If significant differences were detected, a Ducan's post hoc test was employed. The significance level for all analyses was established at $p < 0.05$.

RESULTS AND DISCUSSIONS

Growth performance

At the outset of the experiment, there was no statistically significant variation ($p>0.05$) in the initial body mass among the six experimental groups of carp specimens. However, by the end of the trial, the mean weight of the carp exhibited significant differences ($p<0.05$) among the five experimental groups. Thus, IMZ did not affect significantly the growth performance. Notably, the IMZ-Sp experimental variants demonstrated the highest final average weight (FW) and specific growth rate (SGR) at 87.40±11.65 g and 1.04±0.09%, respectively (Table 1).

Table 1. Effect of different treatments on specific growth rate and feed conversion efficiency of *Cyprinus carpio*

Parameters	Experimental groups				
	Control	**IMZ**	**IMZ-Sp**	**IMZ-Uv**	**IMZ-Sp-Uv**
Initial average weight IW (g)	66.14±10.15^{a}	66.92±9.02^{a}	65.78±5.9^{a}	64.64±7.2^{a}	64.92±6.76^{a}
Final average weight FW (g)	83.14±18.61^{a}	84.50±8.22^{a}	87.40±11.65^{b}	84.93±10.16^{a}	83.14±07.65^{a}
Specific growth rate SGR (%)	0.85±0.11^{a}	0.86±.0.07^{a}	1.04±0.09^{c}	1.01±0.12^{c}	0.92±0.03^{b}
Feed conversion ratio FCR	2.04±0.19^{c}	1.98±0.12^{c}	1.64±0.15^{a}	1.71±0.03^{a}	1.91±0.02^{b}

Different letters reflect significant differences between groups

Hematological parameters

At the end of the experiment, fish hematological parameters were determined, and the results are presented in Table 2. Thus, for the fish from IMZ groups, statistical analysis revealed significant ($p < 0.05$) lower values for Ht, Hb, and RBC and higher values for MCV and MCH compared to the control. Nevertheless, the highest values of Ht and MCV were recorded in the IMZ-Uv-Sp groups while the lowest MCV and MCH were observed for IMZ-Sp groups, followed by imazalil and respectively imazalil and ulva, while the highest value of MCH was for the diet with imazalil with ulva, followed by the diet with imazalil and the one with imazalil ulva and spirulina. The higher values for MCV and MCH were observed for the diet with imazalil and spirulina. Regarding the MCHC value, the highest level was for the treatment with IMZ-Uv, followed by IMZ-Sp while the lowest value was observed for IMZ-Uv-Sp treatment.

Table 2. Hematological parameters of *Cyprinus carpio* in different experimental groups

Group experimental	Ht (%)	Hb (g dL^{-1})	RBC (10^6/µL)	MCV (µm^3)	MCH (pg)	MCHC (g dL^{-1})
Control	41.44±3.76^{b}	9.67±0.38 ^{b}	1.87^{b}	223.33 ^{b}	52.13 ^{a}	23.51^{b}
IMZ	39.73±4.63^{a}	9.26±2.07^{a}	1.72^{a}	236.95^{bc}	55.49 ^{b}	23.64^{b}
IMZ-Uv	42.68±7.00^{b}	10.91±2.67^{b}	1.95^{b}	225.53 ^{b}	57.93 ^{b}	25.52^{c}
IMZ-Sp	41.51±6.37^{b}	9.96±0.91^{c}	2.12^{c}	195.75 ^{a}	47.55 ^{a}	24.33^{bc}
IMZ-Uv-Sp	47.32±4.23^{c}	10.22±0.81^{b}	1.92^{b}	249.80 ^{d}	54.12^{bc}	21.64^{a}

Different letters reflect significant differences between groups

Serum Metabolic Profile

At the end of the experiment, several parameters of the metabolic profile of the fish were analyzed, namely glucose (GLU), total proteins (TP), albumin (ALB), calcium (Ca), and globulins (GLOB), represented in Table 3. The highest value of the total protein was in the IMZ-Uv variant (4.26±0.47 mg/dl), while the lowest value was recorded in the control groups (3.08±0.06 mg/dl). The levels of the protein fractions of albumin and globulin varied between the different experimental treatments so that significantly higher values of globulin and albumin were recorded for the IMZ groups (3.20±0.57 mg/dl, respectively 1.12±0.14 mg/dl) comparing with control where TP and ALB had the lowest level (3.08±0.06 mg/dl respectively 0.74±0.07 mg/dl). Regarding the blood glucose concentration, it varied significantly between all treatments, the lowest value being in the treatment IMZ-Sp (133.90±8.14 mg/dl) and the highest for the IMZ treatment (172.80±11.12 mg/dl). The calcium level showed a high value in the case of the IMZ treatment (11.60±0.28 mg/dl), while the lowest value was indicated by the control group (8.62±1.20 mg/dl).

Table 3. Effect of different treatments on serum biochemical parameters for *Cyprinus carpio*

Parameters	Experimental groups				
	Control	**IMZ**	**IMZ-Sp**	**IMZ-Uv**	**IMZ-Sp-Uv**
GLU (mg/ dL)	163.66±9.50^{a}	172.80±11.12^{b}	133.90±8.14^{a}	162.20±44.23^{a}	157.67±19.01^{a}
TP (g/dl)	3.08±0.06^{a}	4.20±0.64^{c}	3.82±0.38^{b}	4.26±0.47^{b}	4.00±0.50^{b}
ALB(g/dl)	0.74±0.07^{a}	1.12±0.14^{b}	0.98±0.07^{b}	1.10±0.07^{b}	1.00±0.07^{b}
GLOB(g/dl)	2.4±0.0 ^{a}	3.20±0.57 ^{c}	2.65±0.35 ^{b}	3.08±0.07 ^{b}	3.00±0.35 ^{b}
Ca(mg/dl)	8.62±1.20^{a}	11.60±0.28^{b}	10.68±1.84^{ab}	11.20±1.63^{b}	10.60±0.64^{ab}

Different letters reflect significant differences between groups

Oxidative Stress Parameters

Biomarkers of oxidative stress showed some changes compared to the control group. Thus, for groups exposed to IMZ both plasma and gut MDA increased significantly ($p<0.05$) compared with the control variant. Although the plasma and gut MDA values were higher in all fish groups exposed to imazalil regardless of the diet, the increase was not significant for groups fed with a diet containing spirulina.

Total antioxidant capacity dropped in all experimental groups but significant differences were found only for IMZ, IMZ-Uv, and IMZ-Sp-Uv groups. Also, the lysozyme (LYZ), which represents an indicator of the immune function evaluated in this study was affected by IMZ. However, the least affected group was represented by IMZ-Sp-Uv (Table 4).

Table 4. Effect of different treatments on oxidative stress markers for *Cyprinus carpio*

Parameters	Experimental groups				
	Control	**IMZ**	**IMZ-Sp**	**IMZ-Uv**	**IMZ-Sp-Uv**
MDA (nmol/ml)	5.49±1.17^{a}	7.28±0.29^{b}	5.54±0.64^{a}	6.66±1.06^{ab}	5.67±0.39^{a}
MDA gut (nmol/g)	7.45±0.43^{a}	8.51±0.37^{c}	7.58±0.29^{a}	8.30±0.41^{b}	7.53±0.27^{a}
TAC (mm Trolox)	20.42±2.94^{c}	15.17±0.85^{a}	20.32±2.71^{c}	19.04±1.08^{b}	17.81±2.59^{b}
Lysozyme (U/mg solid)	223.79±0.78^{b}	204.88±2.86^{a}	207.52±0.94^{a}	206.64±1.58^{a}	212.96±6.51^{b}

Different letters reflect significant differences between groups.

Water pollution has become a real problem and quite difficult to control due to the various harmful substances (insecticides, pesticides, heavy metals, petroleum waste) that are frequently released into the aquatic environment (Khoshnood, 2017; Shah & Parveen, 2020). Part of the polluting substances are the fungicides that are used in agricultural practices, which once in the water can produce unwanted effects on the health of aquatic organisms and implicitly can also affect the health of people through their consumption (Amenyogbe et al., 2021). One of the disturbing factors that involve changes in the morpho-physiological state of the fish is the stress caused by water pollution (Sinha et al., 2022). The hematological and serum/plasma biochemical parameters represent a valuable tool in toxicological research and environmental monitoring due to their high sensitivity in the presence of contaminants, thus producing physiological and pathological changes (Bhatnagar et al., 2017; Kumar Maurya et al., 2019; Ahmed et al., 2020).

The present study aims to evaluate the potential protective effect of the two algae *Ulva lactuca* and *Spirulina platensis* for the *Cyprinus carpio* fish when exposed to imazalil.

The results of our study indicate that the fish exposed to imazalil (5 mg/kg body weight) through the feed registered a decreased number of RBC, Ht, and Hb. Similar results were reported by other authors who found low values of Ht, Hb and RBC in fish exposed to different types of pesticides for species such as *Oreochromis mossambicus* (Ghayyur et al., 2019), *Oreochromis niloticus* (Dawood et al., 2020), *Cyprinus carpio* (Chatterjee et al., 2021), *Cirrhinus mrigala*, (Ghayyur et al., 2021) and *Mystus keletius* (Barathinivas et al., 2022). This suggests that anemia may have been triggered by toxic stress, leading to the breakdown of red blood cells by reactive oxygen species (ROS) generated in reaction to exposure to the fungicide (Bloom & Brandt, 2008).

Similar reports, emphasizing the negative impact of different pesticides (herbicides, insecticides, and fungicides) on oxygen transportation with consequences on fish metabolic rate, are available (Bojarski & Witeska, 2020; Lutnicka et al., 2016).

For the experimental groups, where the diet was supplemented with algae, a slight increase of RBC, Ht, and Hb was observed. However, the significant increase of RBC was associated with IMZ-Sp groups while significantly higher Ht was observed in IMZ-Uv-Sp groups. Therefore, spirulina had a protective role in reducing cytotoxic effects induced by imazalil. Similar studies (Sharma et al., 2005) showed that spirulina could alleviate also the toxicity stress of azo dye-methyl red on the red blood cells (RBCs) of guppies (*Poecilia reticulata*). Moreover, the groups fed with Ulva algae showed the highest level of hemoglobin, which indicates the potential of *Ulva lactuca* to improve the immune response. This ability is supported by the content of various secondary metabolites such as carotenoids, polyphenols, and flavonoids that improve health and increase fish resistance (Abu Hafsa et al., 2021; Pratiwi & Pratiwy, 2022).

An important role in the description of the state of anemia is also played by the blood parameters MCV, MCH, and MCHC, which represent important indicators in determining the size, density, and hemoglobin concentration of red blood cells (Witeska et al., 2023). In our study, significant variations were observed between the different experimental groups, compared to the control group. The elevated MCV and MCH values observed in IMZ-Uv variants may suggest a macrocytic anemia, while the low value of MCV and MCH observed in IMZ-Sp indicates a microcytic anemia, all of which indicate a defense reaction against exposure to pesticides.

These similar effects were also reported in the comparative study of two species of fish, *Clarias gariepinus* and *Oreochromis niloticus* exposed to several types of pesticides on several exposure models, where it was shown that continuous exposure to pesticides is as dangerous as short-term exposure to an increased level of pesticides (Kanu et al., 2023).

To assess the metabolic profile and nutritional well-being of the fish, it is highly beneficial to determine the total content of plasma proteins, including albumins and globulins. This evaluation aids in assessing the health status of the fish and the effectiveness of both specific and non-specific defense mechanisms against harmful agents. Consequently, levels of total protein (TP), albumin (ALB), and globulin (GLOB) increased across all experimental groups compared to the control.

However, it should be mentioned that for all experimental groups where the feed was supplemented with algae, these parameters were significantly higher ($p<0.05$) than the negative control, IMZ group. This suggests that both varieties of algae possess the ability to mitigate the negative impacts of IMZ and restore the concentration of serum proteins

Glucose is also a biochemical indicator that can be used to evaluate the degree of normality of the general physiological state, which can be easily modified under the influence of external or internal factors, having an important role in establishing the level of stress in fish. (Ray & Sinha, 2014).

The data of the current study indicated that the level of serum glucose in carp exposed to imazalil significantly increased ($p<0.05$) compared with the control. In fish exposed to IMZ but fed with algae, the glucose decreased to the level found in control excepting the IMZ-Sp groups which had the lowest glucose level. The reduced level of glucose could highlight the role of the two algae in suppressing the state of stress. Moreover, spirulina has a glucose-lowering effect demonstrated also in other studies. Thus, this effect was emphasized in studies conducted to evaluate the potential of spirulina to mitigate the effect of chlorpyrifos in *Clarias gariepinus* (Mokhbatly et al., 2020) or the effect of diazinon in *Oreochromis niloticus* (Abdelkhalek et al., 2017).

It is well known that both *Ulva lactuca* and *Spirulina platensis* are good candidates for oxidative stress alleviation. Thus, *U. lactuca* contains bioactive compounds such as ulvan or phlorotannins (Holdt & Kraan, 2011) while *S. platensis* contains vitamins, minerals, carotenoids, polysaccharides, and γ-linolenic acid, compounds with a great role in antioxidant defense (Adel et al., 2016).

In our investigation, both types of algae demonstrated the ability to reduce the MDA levels (in plasma and gut tissue) amplified by IMZ. Nevertheless, *S. platensis* proved to have a more pronounced MDA lowering effect compared with *U. lactuca*, since the lowest values were observed in the IMZ-Sp group and, respectively, IMZ-Sp-Uv group. The same pattern was observed also for total antioxidant capacity which was less affected by IMZ in spirulina-fed fish, IMZ-Sp variant.

The primary effect of immunostimulant agents is to enhance the function of phagocytic cells and boost their activity in animals (Sakai, 1999). Previous experiments demonstrated that polysaccharides derived from seaweed exert a beneficial impact on cultured fish by modifying the activity of certain components of the innate immune system (Akbary & Aminikhoei, 2018). In this study, it was observed that by the end of the 4 weeks, fish-fed diets containing Ulva and Spirulina exhibited higher levels of lysozyme and phagocytic activity compared to those fed only Ulva or only spirulina diets.

The adverse impact of imazalil on growth performance parameters was not detected. This could be attributed to the relatively short experimental period during which the tertiary stress level may not have had adequate time to install.

CONCLUSIONS

In our study, imazalil did not influence the growth of *C. carpio*, but it did alter the health condition as indicated by selected hemato-biochemical markers. The inclusion of algae in the carp's diet had a positive impact on enhancing antioxidant levels and bolstering fish immunity. *Spirulina platensis* exhibited superior outcomes concerning the plasma biochemical profile, while both *S. platensis* and *U. lactuca* contributed to improving oxidative stress biomarkers. The potential mutualistic relationship between *Ulva lactuca* and *Spirulina platensis* was highlighted. However, further research is required to substantiate this relationship.

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STRUCTURAL CHARACTERISTICS OF THE PREDATORY MITE POPULATIONS (Acari: Mesostigmata) AT LOCAL SCALE FROM TWO TYPES OF GRASSLAND ECOSYSTEMS IN THE FĂGĂRAŞ MOUNTAINS -ROMANIA

Minodora MANU[1], Roxana Georgiana NICOARĂ[1], Luiza Silvia CHIRIAC (MIHAI)[1], Anca Rovena LĂCĂTUŞU[2], Marilena ONETE[1]

[1]Romanian Academy, Institute of Biology Bucharest, Department of Taxonomy, Ecology and Nature Conservation- Posada Research Station, 296 Splaiul Independenţei Street, Zip code 0603100, Bucharest, Romania
[2]National Research & Development Institute for Soil Science, Agrochemistry and Environment - ICPA Bucharest, Soil Biology Laboratory, 61 Mărăşti Blvd, Bucharest, Romania

Corresponding authors email: marilena.onete@gmail.com

Abstract

Soil mites have been used as bioindicators at local, regional or even at national scales. The present study demonstrated that soil microhabitats are characterised by different structural patterns of soil mite populations. This study was conducted in August 2021, in two types of grassland ecosystems (intensely grazed and moderately grazed with sheep) in the Făgăraş Mountains- Romania. At the local spatial scale, 10 transects were established in each grassland. In total 200 soil samples were analysed, revealing the presence of 33 soil mite species, with 93 individuals. Dominant species were Gaeolaelaps nolli and Gaeolaelaps aculeifer. The population characteristics were analysed for each transect and each grassland, using the following indices: taxa diversity, numerical abundance, dominance, evenness, equitability, Shannon-Wiener index of diversity, Bray-Curtis index of Similarity. Making a comparison, in the moderately grazed grassland the species diversity and the numerical density were higher than those from intensely grazed ecosystem.

Key words*: grazed, mite, soil, structure, transects.*

INTRODUCTION

Grasslands represent one of the most important ecosystems in the world (Vannoppen et al., 2023). This type of ecosystem is involved in soil formation, erosion control, storage of atmospheric carbon, nutrient cycling, biodiversity support and food provision. At the same time it provides a habitat for plants and invertebrates, including soil invertebrates. The main threats to grassland ecosystems are: conversion of native grassland areas to crops and introduced pasture, clearing of native grassland for urban expansion, invasion by exotic plants, overgrazing, soil and habitat disturbance by vehicles, rock removal or rock crushing operations, changes in agricultural practices, poor management of remnant grassland areas, climate change and abandonment of traditional forms of land use. Most of the grassland in the temperate-climate zone in Central and Western Europe developed as a result of human activity and can be considered as semi-natural habitats (Szczęch et al., 2023). Predatory mites are one of the most common and abundant invertebrate groups in soil. They are very sensitive to any environmental disturbance such as the highly intensive grazing of grasslands. Overgrazing decreases the vegetation cover, increases erosion, compacts the soil, and creates favourable conditions for invasive species. Overgrazing influences the proprieties of soil, including its biological activity (Kairis et al., 2015; Manu et al., 2023). There are abundant studies regarding the characteristic structure of predatory soil mites from different type of ecosystem all over the Europe, especially as part of monitoring programmes (Gardi et al., 2009; Dirilgen et al., 2016; Arjen de Groot et al., 2016; Griffiths et al., 2016).

All these studies were made at a large spatial scale, even as great as the European scale, but taking into account the local scales. Based on soil fauna, including the mites, some bioindicators have been selected, taking into account the land use type of ecosystems, climatic zones, and different types of treatments (Gardi et al., 2009; Griffiths et al., 2016). A very complex study made in ten European countries, belonging to four bio-climatic zones (Alpine, Atlantic, Continental and Mediterranean) and 3 land uses (arable, grassland and forestry) demonstrated that these two variables had a significant effect on soil mite communities (Griffiths et al., 2016). Within and between climatic zones, we consider many climatic variables (temperature, humidity, precipitation, wind, etc), and especially those that influence the soil substrate (for soil fauna) at European, national, regional or local scale. Land use type refers to the relationship between people and the land. On the one hand, the edaphic fauna is influenced by the physical and chemical properties of soil, whilst on the other hand, according to the Burton et al., 2022, "soils are a fundamental determinant of plant communities, with soil biota being linked to them directly through symbiosis and herbivory, and indirectly via decomposition and nutrient cycling". These influences are more obvious at the local scale or even at the level of microhabitats (Arjen de Groot et al., 2016; Chiriac et al., 2022; Manu et al., 2022; 2023). Thus, the main objectives of the research were to characterise the structures of the soil mite communities at the local scale from two grasslands, considering their management types (moderately and intensively grazed ecosystems) and the vegetation cover of the samples.

MATERIALS AND METHODS

Study areas

The research was carried out in the Făgăraş Mountains, Romania, which have the status of a protected area under the European ecological network Natura 2000. The study was made in August 2021, in two types of alpine grasslands: Galbena grassland- moderately grazed by sheep and Vemeşoaia grassland - intensely grazed by sheep (Figure 1) (Manu et al., 2023). The complete description of the abiotic (soil) parameters in these investigated areas was presented by Chiriac et al., in 2022.

The Galbena moderately grazed grassland is located at 45^{0}37'47.8"N, 024^{0}23'57.9'E at 1710 m altitude, on a slope of 15^0-20^0 and with a West exposure. The soil type is dystric lithosol and humbric-lithic regosol. The vegetation was dominated by *Nardus stricta* (11.99%), *Festuca rubra* (16.62%), *Agrostis capillaris* (17.15%) and *Vaccinium myrtillus* (28.61%).

The Vemeşoaia intensively grazed grassland is situated at 45^{0}32'44.7"N, 024^{0}26'30.6'E at 1747 m altitude, on a slope of 15^0 and with a South exposure. The soil type is dystric-lithic regosol. The vegetation was dominated by *Nardus stricta* (11.68%), *Festuca rubra* (21.75%), *Agrostis capillaris* (12.11%) and *Vaccinium myrtillus* (2.74%).

In terms of vegetation type, the two grasslands belongs to the dominant association *Violo declinatae –Nardetum* Simon 1966, equivalent to the Romanian habitat R3609 South-east Carpathian grasslands with *Nardus stricta* and *Viola declinata* (Doniţă et al., 2005).

In order to evaluate the structure of predator soil mite populations at the local scale, ten lines/transects, each of 100 m, were established in each grassland (line 1 to line 10). The distance between each line was 20 metres. On each line 10 soil samples were taken at a spacing between samples of 10 metres (Figure 1).

Soil fauna sampling

In total, 200 soil samples were collected, with a Macfadyen soil core (diameter of 5 cm), at 10 cm depth (Macfadyen 1953; 1961). In each grassland, 100 soil samples were taken (10 samples on 10 lines or transects). The mites were extracted for 10-14 days using the Berlese-Tullgren method, using natural light and heat (Berlese, 1905; Tullgren, 1917; Southwood, 1966; Southwood & Henderson, 2000; Krantz & Walter, 2009). Due to the high number of samples collected from the field (200) and to the limited number of samples which could be sorted at one time (75 samples), some samples were kept in a refrigerator (at 4^{0}C) until the next extraction (14 days) (Manu & Honciuc, 2010).

Taxonomic identification and counting were made using a Zeiss stereo-microscope. Soil fauna were preserved in ethyl alcohol (75%-96%) (Krantz & Walter, 2009).

The mites were identified to species level using the published identification keys (Ghilyarov & Bregetova, 1977; Balş, 1981; Karg, 1993; Mašán, 2003; Mašán & Fenďa, 2004; Mašán, 2007; Gwiazdowicz, 2007; Mašán et al., 2008; Mašán & Halliday, 2013; De Moraes et al., 2022).

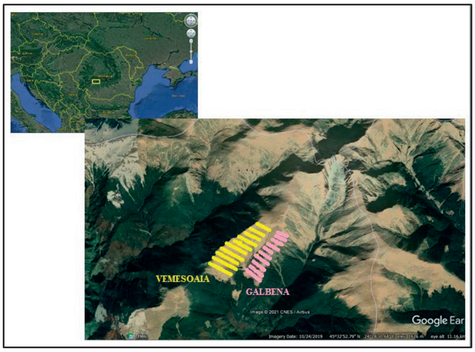

Figure 1. The geographical location of investigated areas at Galbena (pink lines) and Vemeşoaia grasslands (yellow lines), in the Făgăraş Mountains, in 2021

Data processing

Statistical analysis was made using PAST software (Hammer et al., 2001) and the following indices were assessed: Dominance_D; Simpson_1-D; Shannon_H; Evenness_e^H/S; Equitability_J, Bray-Curtis index of Similarity (qBC). The ANOVA test was used for the standard statistical analysis for univariate data (as numerical abundance, density and vegetation coverage).

RESULTS AND DISCUSSIONS

In both types of grasslands 33 soil mite species (93 individuals) were identified, with a density by 4700 individuals/square metre. Of these identified species, 15.15% were common species in both grasslands, 57.57% were characteristic of the Galbena grassland (moderately grazed) and 27.27% for Vemeşoaia (intensely grazed ecosystem) (Tables 1, 2). The common species for both grasslands were *Gaeolaelaps aculeifer, Gaeolaelaps nolli, Lysigamasus lapponicus, Ololaelaps placentula* and *Veigaia nemorensis*. These species have also been identified in other grasslands in Romania (Manu et al., 2022; 2023).

In the moderately grazed grassland from Făgăraş Mountains- Galbena, 24 Mesostigmata species were identified, with a density of 2500 individuals/square metre. The dominant species were *Gaeolaelaps aculeifer, Gaeolaelaps nolli, Alloparasitus oblongus* and *Ololaelaps sellnicki*. The total vegetation cover in this area was 98.55%. Within the ten investigated lines, we observed that lower values for the number of species, numerical abundance and density were recorded in lines 6 and 7, on the opposite being parameters from the lines 3, 8, 9 and 10, with the highest values. The difference of these parameters (numerical abundance and density) between the ten lines is significant (p = 0.0029). The highest values of dominance were recorded in lines 1, 6, and 7, where only one individual of each species was identified. A more balanced structure of mite communities was recorded in lines 2 and 4 (Table 1). Vegetation cover fluctuated between 100% (on lines 1, 7, 8, 9) and 97% or 96.62% (on lines 3 and 6) (Table 1). The differences in vegetation cover between lines are not significant (p = 1)

Table 1. Structural characteristics of soil mite populations from Galbena grassland, Făgăraş Mountains, 2021 (± standard error)

No.	Species	Line 1	Line 2	Line 3	Line 4	Line 5	Line 6	Line 7	Line 8	Line 9	Line 10	Total
1	*Gamasellodes bicolor*										1 ± 0.03	1
2	*Gaeolaelaps aculeifer*			1 ± 0.03					2 ± 0.04	1 ± 0.03	2 ± 0.06	6
3	*Gaeolaelaps nolli*				1 ± 0.03	1 ± 0.03		1 ± 0.03	1 ± 0.03	1 ± 0.03	2 ± 0.06	6
4	*Alloparasitus oblongus*				1 ± 0.03	1 ± 0.03					3 ± 0.06	5
5	*Leptogamasus paracarpaticus*			1 ± 0.03								1
6	*Leptogamasus* sp.								1 ± 0.03			1
7	*Lysigamasus lapponicus*					1 ± 0.03						1
8	*Macrocheles montanus*			1 ± 0.03								1
9	*Olodiscus minima*			1 ± 0.03								1
10	*Ololaelaps placentula*		1 ± 0.03	1 ± 0.03								2
11	*Ololaelaps sellnicki*	1 ± 0.04			1 ± 0.03					2 ± 0.04		5

12	*Onchodellus siculus*		1 ± 0.03									1
13	*Oodinychus obscurasimilis*			1 ± 0.03								1
14	*Oodinychus* sp.									1 ± 0.03		1
15	*Pachylaelaps resinae*			1 ± 0.03								1
16	*Pachylaelaps* sp.								1 ± 0.03	1 ± 0.03		2
17	*Pachyseius humeralis*			1 ± 0.03								1
18	*Prozercon* sp.			1 ± 0.03								1
19	*Rhodacarellus silesiacus*			2 ± 0.06								2
20	*Trachytes aegrota*					2 ± 0.06			1 ± 0.03	1 ± 0.03		4
21	*Trachytes irenae*								1 ± 0.03			1
22	*Trachytes* sp.						1 ± 0.03		1 ± 0.03			2
23	*Urodiaspis pannonica*								1 ± 0.03			1
24	*Veigaia nemorensis*			1 ± 0.03								1
	Total number of individuals	2	2	12	3	5	1	1	9	7	8	49
	Total number of species	1	2	11	3	4	1	1	8	6	4	24
	Numerical density	100	100	600	150	250	50	50	450	350	400	2500
	Dominance_D	1	0.5	0.097	0.333	0.280	1	1	0.136	0.184	0.281	0.07
	Simpson_1-D	0	0.5	0.903	0.667	0.720	0	0	0.864	0.816	0.719	0.93
	Shannon_H	0	0.693	2.369	1.099	1.332	0	0	2.043	1.748	1.321	2.9
	Evenness_e^H/S	1	1	0.972	1	0.947	1	1	0.964	0.957	0.937	0.76
	Equitability_J		1	0.988	1	0.961			0.983	0.976	0.953	0.91
	Vegetation coverage (%)	100	97.5	97	98.9	98.11	96.62	100	100	100	97.32	98.55

In the intensely grazed grassland in the Făgăraş Mountains - Vemeşoaia, only 14 Mesostigmata species were identified, with a density of 2200 individuals/square metre. The dominant species were *Gaeolaelaps nolli, Pachydellus furcifer* and *Zercon carpathicus*. The total vegetation cover in this area was 90.4%. In the ten investigated lines, we observed that the lower values for number of species, numerical abundance and density were recorded on lines 6 and 8, and the highest values of these being parameters in the lines 4, 7 and 10. The differences for these parameters (numerical abundance and density) between the ten lines is significant (p = 0.0448). Comparing the other structural parameters for soil mites on all ten lines, we observed that the highest values of dominance were recorded on lines 5, 6, 7, 8, and 9. The highest values of evenness and equitability parameters were recorded on lines 2 and 4 (Table 2). Vegetation cover fluctuated between 100% (on lines 2, 3, 4) and 69% or 88.5% (on lines 1 and 6) (Table 2). These differences in vegetation cover between lines is not significant (p = 0.9998).

Table 2. Structural characteristics of soil mite populations from Vemeşoaia grassland, Făgăraş Mountains, 2021 (± standard error)

No.	Species	Line 1	Line 2	Line 3	Line 4	Line 5	Line 6	Line 7	Line 8	Line 9	Line 10	Total
1	*Amblyseius* sp.									1 ± 0.03		1
2	*Asca bicornis*					1 ± 0.03						1
3	*Gaeolaelaps aculeifer*				1 ± 0.03							1
4	*Gaeolaelaps nolli*		3 ± 0.06		1 ± 0.03	1 ± 0.03	1 ± 0.03	1 ± 0.03		1 ± 0.03		8
5	*Hypoaspis austriacus*			1 ± 0.03			1 ± 0.03			1 ± 0.03	1 ± 0.03	4
6	*Leptogamasus tectegynellus*	1 ± 0.03						1 ± 0.03				2
7	*Lysigamasus lapponicus*				2 ± 0.06							2
8	*Ololealaps placentula*										1 ± 0.03	1

9	*Onchodellus alpinus*				2 ± 0.04							2
10	*Pachydellus furcifer*	1 ± 0.03	1 ± 0.03	2 ± 0.06	2 ± 0.04			1 ± 0.03				7
11	*Pachydellus* sp.							1 ± 0.03	2 ± 0.06		3 ± 0.06	6
12	*Veigaia nemorensis*			1 ± 0.03				1 ± 0.03				2
13	*Zercon carpathicus*	1 ± 0.03			1 ± 0.03						3 ± 0.09	5
14	*Zercon* sp.			1 ± 0.03		1 ± 0.03						2
	Total number of individuals	3	4	5	9	3	2	5	2	3	8	44
	Total number of species	3	2	4	6	3	2	5	1	3	4	14
	Numerical density	150	200	250	450	150	100	250	100	150	400	2200
	Dominance_D	0.333	0.625	0.280	0.185	0.333	0.500	0.200	1	0.333	0.313	0.11
	Simpson_1-D	0.667	0.375	0.720	0.815	0.667	0.500	0.800	0	0.667	0.688	0.89
	Shannon_H	1.099	0.562	1.332	1.735	1.099	0.693	1.609	0	1.099	1.255	2.39
	Evenness_e^H/S	1	0.877	0.947	0.945	1	1	1	1	1	0.88	0.78
	Equitability_J	1	0.811	0.961	0.968	1	1	1		1	0.91	0.9
	Vegetation coverage (%)	69	100	100	100	79	88.5	89.6	87	93.5	97.3	90.4

In order to highlight the similarities or dissimilarities between soil mite communities from the ten lines in each investigated grassland, the Bray-Curtis Similarity index (qBC) was calculated. In the Galbena grassland (moderately grazed ecosystem), the highest Bray-Curtis similarity index was obtained between populations from line 1- line 4, line 4 - line 5, line 4 - line 7 and line 8 - line 9 (qBC = 0.5 for each) (Figure 2). These similarities could be explained by the complete vegetation cover on these lines, by the presence of common species with quite similar biological and ecological requirements *e.g.*: *Gaeolaelaps aculeifer, Gaeolaelaps nolli, Ololealaps sellnicki* and *Trachytes aegrota*. These species have a wide ecological tolerance, being recorded in similar ecosystems throughout Europe, as well as Romania (Karg 1993; Mašán, 2007; Mašán & Halliday, 2013; De Moraes et al, 2022; Manu et al., 2022; 2023).

In the Vemeşoaia grassland (intensely grazed ecosystem), the highest Bray-Curtis similarity index was obtained between populations from line 1- line 7 (qBC = 0.5), line 2- line 7 (qBC = 0.44), line 3-line 7 (qBC = 0.4) and line 5- line 6 (qBC = 0.4) (Figure 3). Species such as *Gaeolaelaps nolli, Leptogamasus tecegynellus, Pachydellus furcifer* and *Veigaia nemorensis* were common on these lines, with similar numerical abundance. The small differences between vegetation coverage could be a possible explanation for these similarities.

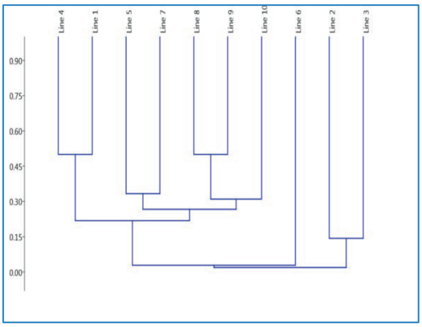

Figure 2. Bray-Curtis Similarity index between soil mite populations from Galbena grassland

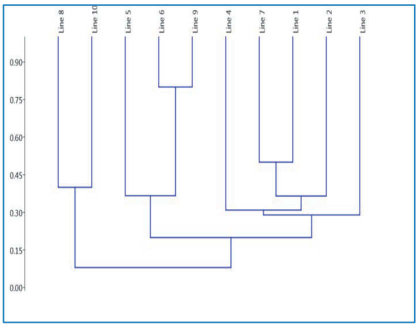

Figure 3. Bray-Curtis Similarity index between soil mite populations from Vemeşoaia grassland

Comparing the two grasslands and considering their management types (moderately and intensive grazed grasslands), we observed that in the Galbena (moderate grazing) the soil mite communities were more abundant, with greater species diversity. Being a moderately grazed ecosystem, the vegetation cover is more complete compared to that of the intensively grazed site. The vegetation layer creates a microhabitat with characteristic environmental conditions (more humid substrate, lower soil temperature, increased soil pH, etc.). Studies have revealed that enhanced species richness of plant mixtures positively affects the diversity of the soil fauna. Habitat/microhabitat loss caused by the deterioration of soil physical-chemical properties is the primary factor affecting soil fauna (Sylvain & Wall, 2011; Kudureti et al., 2023).When results are compared between these sites and similar studies elsewhere in Romania (number of species and numerical abundance), we conclude that the data obtained are comparable with those from grazed and ungrazed grasslands in the Făgăraş Mountains, and with natural, chemical and organically-fertilised ecosystems in the Bucegi Mountains, but lower than pastures from the Central Moldavian Plateau or polluted grasslands from Zlatna-Trascău Mountains (Manu et al., 2022; 2023).

CONCLUSIONS

Soil mite communities were investigated from two management types of grassland (moderately and intensely grazed by sheep), at the local/microhabitat scale, considering vegetation cover. In total, 33 species were identified, with 93 individuals and 4700 individuals/square metre. The type of grassland management influenced the structures of the soil mite communities, phenomena highlighted by the different values of statistical parameters (*e.g.* number of species, numerical abundance, dominance, evenness, equitability, etc.). The moderately grazed grassland was characterised by the highest values of these structural parameters, as well as vegetation cover. There were characteristic species in each type of ecosystem, demonstrating indirectly the local influence of environmental conditions for each microhabitat. Even where we considered the type of grassland management or spatial scale, the environmental biotic and abiotic parameters were very important factors that could influence the soil mite communities and hence we consider that further investigations are needed. The present study demonstrated that anthropogenic impact (in this case grazing) on natural ecosystems influences the structure of mite communities, even at local scale.

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COMPARATIVE ANALYSIS OF HEAVY METALS AND ELEMENTAL PROFILES IN TROUT SPECIES FROM TWO DIFFERENT AREAS

Oana Andreea MASTAN[1], Aurelia COROIAN[1], Aurel DAMIAN[1], Marian MIHAIU[1], Igori BALTA[2], Anca BECZE[3], Adina Lia LONGODOR[1], Ioana SOIMUȘAN[1], Diana MESAROS[4], Ștefania MARIȘ[5]

[1]University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, 3-5 Manastur Street, 400272, Cluj-Napoca, Romania
[2]University of Life Sciences "King Mihai I" from Timisoara, 119 Calea Aradului, 300645,Timisoara, Romania
[3]INCDO - INOE 2000, Research Institute for Analytical Instrumentation Subsidiary, 67 Donath Street, 400293, Cluj-Napoca, Romania
[4]University of Oradea, Faculty of Electrical Engineering and Information Technology, Universitati Street, Oradea, Romania
[5]Universitat de Lleida, UDL, Av Rovira Roure, 191, 25198, Lleida, Spain

Corresponding author emails: oanamastan@yahoo.com, aurelia.coroian@usamvcluj.ro

Abstract

An important concern of man has become to have high-quality food for a healthy life. Fish such as rainbow trout (Oncorhynchus mykiss) and brook trout (Salvelinus fontinalis) are an important bioindicator of pollution. The accumulation of heavy metals represents a potential danger to public health. Trout is preferred and consumed by many people so any possible risk of ingesting metals through food chains should be assessed. The main organs studied according to specialized literature regarding the accumulation of heavy metals are the liver an the kidney, these organs play an important role in preventing the transfer of heavy metals to other organs, for example muscle tissue. As is known, the pollution of aquatic ecosystems has a direct impact on the entire aquaculture activity. Therefore, the purpose of this study was to monitor metals present in the two trout species in different areas.

Key words*: biomonitoring, brook trout, heavy metals, human health risk, rainbow trout.*

INTRODUCTION

The process of human development in relation to the environment has gradually turned into an instrument of destruction with negative effects on the ecosystem, through the participation of "brutal" humanity in the irrational exploitation of nature (Benciu, 2007). Metals in very small quantities are useful to all forms of life, metals enter the cell in a controlled manner in the form of cations. A large amount of any metal causes toxicity to the human and animal body (Elumalai et al., 2023).

According to Kumar et al. (2024), the term heavy metal is commonly used for metals with a density greater than water, so heavy metals are described as elements with densities greater than 5.0 g.cm^{-3} and atomic masses greater than 20, of example: mercury (Hg), copper (Cu), nickel (Ni), cadmium (Cd), chromium (Cr), arsenic (As), zinc (Zn) and lead (Pb).

The term "heavy" also refers to a variety of metals, including certain metalloids, which can be dangerous to both plants and living organisms, even at very low concentrations.

Heavy metals are natural compounds of the earth's crust, they reach the body of animals and humans through various ways: food, water, air. Their effects are bioaccumulation, the concentration of heavy metals increases per mass unit, they block intracellular biochemical processes due to accumulation (Matta et al., 1999).

Heavy metals have mutagenic and carcinogenic properties in the body (Kumar-Sharma et al., 2024).

According to many studies, the deterioration of the ecosystem is primarily due to industrialization.

The Environmental Protection Agency and the World Agency for Cancer Research confirm the damage to the body even at low levels of exposure to heavy metals, the source of contamination being feed, water and air (Abida et al., 2009).

Heavy metals: iron (Fe), manganese (Mn), coblat (Co), nickel (Ni), copper (Cu), chromium (Cr) and zinc (Zn) are necessary for metabolic activities, since they form the cofactor for many enzymes, but their amount is very important, because if the limit is exceeded, they cause harm to the human body and animals (Javed & Usmani, 2017).

According to Zeynali et al. (2009), and other studies in the specialized literature, the factors that influence the accumulation of heavy metals in fish tissue can be the following: nourishment sources, seasonal changes, environmental conditions (temperature, salinity, pH) and biological variations (species, sex, age, size) etc.

Water being essential for life, but its quality has decreased more and more due anthropogenic activities (Koca et al., 2005).

Heavy metals in fish in high concentrations or by accumulation over time, cause acute and chronic diseases, slow metabolism, damage to the epithelial layer, gill disease, damage to liver tissue and ulcers (Market, 2007).

Useful tools in research are: monitoring or evaluation of heavy metals; evaluation of health indices, bioaccumulation, blood biochemistry, enzymes of tissue damage markers, genotoxicity, histopathology, water pollution (Pandey, 2006; Falllah et al., 2011).

Fish is consumed by a large mass of the population, including children, due to the content of high-quality protein and polyunsaturated fatty acids (Malik et al., 2010).

People are more and more cautious about the food they consume, paying attention to the quality of fish and seafood because they represent an important category for a balanced diet (Sneddon et al., 2007).

It is very important to implement remedial approaches to mitigate the negative effects of heavy metals, due to the bioaccumulative power in the fish body, there are some fish species that accumulate higher concentrations of heavy metals, usually fish species at levels higher trophic (Gleick, 1996; Wittmann, 1979).

The trout is a biomonitor in the case of heavy metals confirmed by Barrientos et al., 2019; Market, 2007 and other researchers.

Certain aquatic plants, called macrophytes, are used for remedial purposes to remove heavy metals from aquatic sources as an environmentally friendly technology to reduce heavy metal pollution (Kumar et al., 2024).

Most aquatic organisms, including trout, can form a resistance against heavy metal pollution, and according to Klerks & Weis (1987), we can identify two main mechanisms: acclimatization and adaptation.

According to studies, acclimatization is described as an increased tolerance through exposure to metals, which is acquired at the individual level, through repeated and prolonged exposure to sublethal concentrations of heavy metals.

It should be noted that increased tolerance is not inherited, but acquired through exposure (Wirgin & Waldman, 2004; Hansen et al., 2006).

The most common heavy metals that contaminate the fish are: mercury (Hg), cadmium (Cd) and lead (Pb), and the degree of pollution and the feed mixture used in farms play a major role in heavy metal contamination (Majid et al., 2019).

Regarding the global seafood production, it registers a continuous increase, so that the Food and Agriculture Organization of the United Nations estimates a quantity of 195 million tons around the year 2025, (FAO, 2018).

As for the purpose of this study, it was to monitor metals in brook trout (*Salvelinus fontinalis*) and rainbow trout (*Oncorhynchus mykiss*) harvested from two different areas. These elements can have a major impact on health, so it is very important to know them, to establish their level, because fish meat is fully recommended for the proper functioning of the human body.

MATERIALS AND METHODS

In this study we used trout samples from the Cluj area, Romania. Brook trout (*Salvelinus fontinalis*) was harvested from Marisel, and rainbow trout (*Oncorhynchus mykiss*) was harvested from Gilau. In Transylvania (Romania), there are numerous trout breeding and development systems, and thus the importance of this fish can be seen due to the population's desire to own a farm to support their own trout consumption or for marketing purposes. Obtaining favorable results regarding the level of contaminants-heavy metals in the case of this study, helps us to confirm the quality of the trout and also of the surrounding environment. The impact will be favorable to consumers if the contaminant levels fall within the legal limits.

The trout samples were stored in laboratory conditions in a minifreezer used for research purposes only.

Metals, macro- and microelements from trout samples were analyzed: sodium (Na), magnesium (Mg), aluminium (Al), potassium (K), calcium (Ca), titanium (Ti), chromium (Cr), mangandese (Mn), iron (Fe), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), barium (Br), rubidium (Rb), strontium (Sr), molybdenum (Mo), staniu (Sn), iodine (I), barium (Ba) and phosphorus (P).

The first stage was that of mineralization with microwaves, 0.5 g of trout sample was taken and subjected to microwave mineralization with nitric acid (HNO3)-65% and hydrogen peroxide (H2O2)-30%. In the next stage, the digestive program was carried out (Table 1), followed by the cooling stage.

After this step, the samples were diluted to 20 ml, with ultrapure water for dilution, then the filtration step follows, using a cellulose filter of 0.45 µm.

Table 1. Operating conditions of the microwave digestion system

Specification	Stage				
	1	2	3	4	5
Temperature (°C)	145	170	190	100	100
Pressure (bar).	30	30	30	0	0
Ramp time (min)	5	1	1	1	1
Maitenance time (min)	25	10	15	10	10
Power (%*)	80	80	80	0	0

*100% power corresponds to 1400 W

ICP-MS (Inductively Coupled Plasma Mass spectrometry) / ICP-OES (Inductively Coupled Plasma Optical Emission Spectroscopy) was used to analyze the concentrations of the elements in the solutions that were initially mineralized. A Perkin-Elmer Elan DRC II inductively coupled plasma mass spectrometer ICP-MS was used for liquid sample analysis as well as a quantitative method with single-point calibration and response factor-based exploration, following a double calibration of the detector, to have information about as many elements as possible. All results obtained are expressed in ug/L (micrograms/liter) and the limit of quantification is 1 ug/L.

The normality of all datasets was assessed by the Shapiro-Wilk assay, using GraphPad Prism for statistical analysis. The results are presented as a mean ± standard error of the mean (SEM). The t-test was used to determine statistical significance, and the resulting P-values were annotated on accompanying graphs.

RESULTS AND DISCUSSIONS

The concentration of heavy metals for the two species of trout taken into account from different places of the Transylvanian area, therefore the values of the metals can be seen from the graphs presented below (Figures 1, 2, 3).

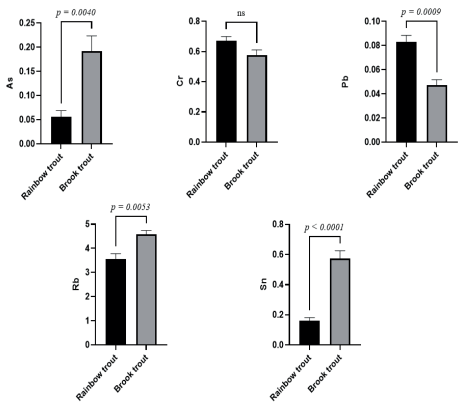

Figure 1. The levels of heavy metals (As, Cr, Pb, Rb and Sn) from Rainbow and Brook trout. Data represents the mean ± SEM (n = 5) expressed in ug/L

According to Figure 1, in the presence of heavy metals, rainbow trout from Marișel commune exhibited significantly reduced concentrations of arsenic (As), rubidium (Rb), and staniu (Sn). Conversely, lead (Pb) levels were substantially elevated in rainbow trout compared to brook trout obtained from the Gilău commune. Additionally, chromium (Cr) concentrations were slightly higher in Rainbow trout but not statistically different to the Brook trout group. Among the five elements considered in Figure 1, the lowest values for rainbow trout were represented for the following two elements: arsenic (As) and staniu (Sn).

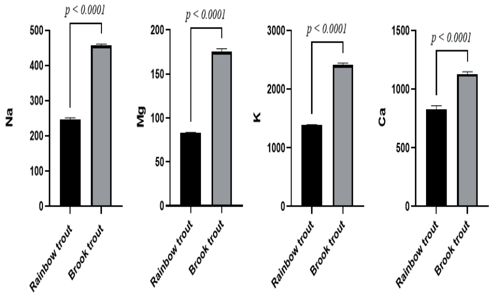

Figure 2. Concentrations of macroelements from Rainbow and Brook trout (Na, Mg, K and Ca) from Rainbow and Brook trout. Data represents the mean ± SEM (n = 5) expressed in ug/L

Differences were also observed in the macroelement profiles of the two fish species (Figure 2). Notably, concentrations of sodium (Na), magnesium (Mg), potassium (K), and calcium (Ca) displayed statistically significant increased trends in Brook trout indicating more nutritional value compared to Rainbow trout groups.

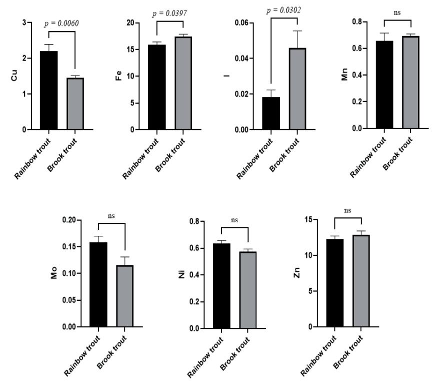

Figure 3. Evaluated microelements (Cu, Fe, I, Mn, Mo, Ni and Zn) from both trout species. Data represents the mean ± SEM (n = 5) expressed in ug/L

Differences in microelement profiles copper (Cu), iron (Fe), and iodine (I) between the two fish species from various locations were also notable (Figure 3). Specifically, copper (Cu) levels were substantially higher in Rainbow trout samples, whereas iron (Fe) and iodine (I) concentrations were significantly elevated in Brook trout samples. Furthermore, the levels of manganese (Mn), molybdenum (Mo), nickel (Ni), and zinc (Zn) exhibited minor variations between the experimental groups, although these differences were not statistically significant.

According to Zeynali et al. (2009), the liver is the organ with higher concentrations of heavy metals (except for Ba and Sr), compared to the muscle tissue of farmed or wild fish. Levels of barium (Ba), chromium (Cr), iron (Fe), manganese (Mn), zinc (Zn) are higher in wild rainbow trout tissues compared to farmed trout, and mercury (Hg) levels in all samples examined were lower than limits accepted by the European Commission, according to the study by Zeynali et al., 2009.

In the study from Chile, higher concentrations were found in trout that were near areas with agricultural and exotic plantations, and this study confirms also the existence of a higher concentration of heavy metals in the liver compared to muscle tissue (Barrientos et al., 2019).

By comparison with other studies, where the concentration of heavy metals in pork from 7 different areas of Romania was analyzed, using the spectrophotometric method, for example the amount of aluminium (Al) varied in the range of 1.68±0.34 µg/L (Cluj) at 9.42±0.21 µg/L (Mureș). Aluminum being considered very toxic for the central nervous system, hematopoietic organs, bone system, etc. (Puia et al., 2019).

Also, in a study for the same locality (Sălaj) Romania, the high degree of concentration of aluminium (Al) and lead (Pb) was found in a pork meat. Al showed a variation of 5.03 mg/kg in the samples from Bistrița-Năsăud and 9.64 mg/kg from Sălaj (Coroian et al., 2017).

Hg (mercury), was found in higher concentrations in liver than in muscle for several fish species, according to several existing studies (Linde et al., 2004).

In liver tissue, heavy metal levels were significantly higher in brown trout than in European eels, and metals levels in the tissue were similar, in both brown trout (*Salmo trutta*) and European eel (*Anguilla anguilla*) (Linde et al., 2004).

According to Jaffal et al. (2011), at the end of winter the levels of copper (Cu) and cadmium (Cd) in the muscle tissue were higher compared to the levels of copper (Cu) and cadmium (Cd) from the end of summer in the liver tissue. Trout farms are a major source of water pollution. The study was carried out in April, July and October.

A control locality and 3 downstream localities were established. According to the working hypothesis, the load with heavy metals was statistically significantly increased especially in sediments but also in E. danica larvae, which proved to be a good indicator for pollution systems (Jaffal et al., 2011).

In a study carried out in Colorado, Brown Trout was exposed for about 2 years to heavy metals: cadmium (Cd), zinc (Zn) and then samples were taken to evaluate the liver and the growth-promoting enzyme (ornithine decarboxylase)-ODC. The results were compared with samples collected from trout that were raised in uncontaminated places.

The activity of the ODC enzyme in both males and females was lower in the trout from the contaminated site (Norris et al., 2000).

CrPic (C18H12CrN3O6) is used in nutritional supplements, which represents a form of organic chromium - Cr (III), because it has a better absorption than other forms of dietary chromium- Cr (III). Also in some studies, CrPic confirms the advantageous role in growth, the improvement of serum protein and the reduction of stress, and in other studies, negative effects such as affecting the function of certain organs are highlighted (Yu Li et al., 2022).

According to Yu Li et al., 2022, in one experiment, the chromium (Cr) content of chromium (Cr) VI in tissues was significantly higher than chromium (Cr) III at 250 mg/kg from 7 to 66 days for most samples, with except muscle tissue, while Cr bioaccumulation of Cr (VI) and Cr (III) in whole fish were similar at 250 mg/kg. Cr (VI) had greater tissue aggressiveness than Cr (III) at a similar level of inorganic Cr bioaccumulation in whole fish, according to the study.

The bioaccumulation of heavy metals in fish according to Kumar et al., 2024, can induce histopathological changes in the essential organs, can also produce oxidative stress at the cellular level and inflammation, respectively the induction of various cellular apoptosis and autophagy pathways.

The most ubiquitous heavy metals are considered by some authors from the specialized literature: chromium, arsenic, mercury, cadmium, lead, copper and nickel, which can lead to pollution of the aquatic environment and also affect the physiology of fish (Kumar et al., 2024).

During an experiment, the authors found that exposure to chromium (Cr) caused major deformations in the liver (apoptosis, vacuolization, necrosis and hypertrophy), (Awasthi & Trivedi, 2019).

According to some authors, to estimate the concentration of heavy metals in various vital organs of fish, the following methods can be used: AAS (Atomic Absorption Spectroscopy), ICP-AES (Inductively Coupled Plasma Atomic Emission Spectroscopy), ICP-MS (Inductively Coupled Plasma Mass spectrometry) and ICP-OES (Inductively Coupled Plasma Optical Emission Spectroscopy) (Sibal &Espino, 2018).

Following a study carried out in Brazil, the authors highlighted the value of heavy metals in water, sediment and fish, during four seasons. The largest concentration of heavy metals was found in sediment, followed by water and the least in fish. According to other studies, suspended sediments adsorb pollutants from water, thus reducing their concentration in the water column (Weber et al., 2013).

In another study carried out in Hungary, where fish were taken from Lake Balaton, metal concentrations in samples taken from different organs (muscle tissue, liver, gills) were between the following ranges: (Cd) cadmium: 0.098; 0.20; 0.106; (Cu) copper: 0.358; 0.327; 1,536; (Pb) lead: 0.3; 0.257; 0.196; (Zn) zinc: 3.00; 7,390; 4.99 μg/g dry weight. The highest concentrations of Cd, Cu, Pb and Zn were detected in the gills and liver of the fish, and the highest concentrations of Hg were measured in the muscle tissue (Farcas et al., 2003).

The results of a study of fish samples taken from the Mexican Pacific coast from two different localities are as follows: in liver tissue were higher concentrations of Cu (Sinaloa 28.3, Guerrero 16.3 $\mu g\ g^{-1}$), Fe (Sinaloa 1098, Guerrero 636 $\mu g\ g^{-1}$) and Zn (Sinaloa 226, Guerrero 186 $\mu g\ g^{-1}$) compared to muscle of

fish from both study areas, according to Spanopoulos-Zarco et al. (2017).
In a study conducted on fish harvested in the Black Sea, Turkey, in different seasons, the samples were analyzed by graphite atomic absorption spectrometry and flame furnace after microwave digestion. According to Mendil et al. (2009), the following metal concentrations were obtained: 25.5-41.4 microg/g iron (Fe), 17.8-25.7 microg/g zinc (Zn), 0.28-0.64 microg/g lead (Pb), 0.64-0.99 microg/g chromium (Cr), 1.3-3.6 microg/g manganese (Mn), 1.4-1.9 microg/g copper (Cu), 0.18-0.35 microg/g cadmium (Cd) and 0.25-0.42 microg/g cobalt (Co) for fish species. In this study, it was observed that the levels of trace elements in the fish species analysed were acceptable for human consumption, while the levels of lead and cadmium in the fish samples were higher than the recommended legal limits.
According to a study carried out in France, certain species of fish were caught in different periods of years (1987-2007), and the results show the decrease of heavy metal concentrations in fish tissue between 1987 and 2007, which reflects the decrease of heavy metal concentrations in environment. According to this study, the results found in 2007 are comparable to those published by another study carried out in the 1990s. It should be specified that the values of the average concentrations of cadmium in fish muscle in 2007 were above the maximum safe level for human consumption defined by the European Commission (Shinn et al., 2009).

CONCLUSIONS

Nowadays, due to various industrial or agricultural activities, heavy metal pollution has become a priority.
The aquatic ecosystem is considered as a final reservoir for the accumulation of heavy metals from various activities.
According to studies, the concentration and distribution of heavy metals is essential for the proper functioning of the aquatic ecosystem, because heavy metals disrupt the aquatic balance, being non-biodegradable and bioaccumulative in nature.
The bioaccumulation of heavy metals leads to various anomalies, structural injuries, functional disorders, cellular dysfunctions, etc. and disrupting the global ecological balance.
To protect the global ecosystem, more regulations must be implemented to conserve water sources, because most of the methods used to treat heavy metals are expensive and also difficult to use.

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GASTROPODS USE AS AN ALTERNATIVE PROTEIN SOURCE IN AQUACULTURE FEEDS - SHORT REVIEW

George-Cătălin MUNTEAN[1], Călin LAȚIU[1], Paul UIUIU[1], Radu CONSTANTINESCU[1], Vioara MIREȘAN[1], Tudor PĂPUC[1], Anca BECZE[2], Daniel COCAN[1], Aurelia COROIAN[1]

[1]Faculty of Animal Science and Biotechnologies, UASMV Cluj-Napoca, 3-5 Calea Manastur, Cluj-Napoca, Romania
[2]National Institute for Research and Development of Optoelectronics - INOE 2000, Research Institute for Analytical Instrumentation, 67 Donath Street, Cluj-Napoca, Romania

Corresponding author email: daniel.cocan@usamvcluj.ro

Abstract

Alternative protein sources to replace fish meal in aquaculture feeds are continuously sought after. The fish meal industry is unsustainable, from an economic point of view and because of its impact on wild aquatic ecosystems. This review presents the use of gastropod species (class Gastropoda) in aquaculture feeds. Gastropods have been studied for their potential use in aquaculture feeds, mainly as an alternative source of protein to fish meal. Gathered studies from various databases encompass gastropods nutritional value, and their performance in rearing aquatic organisms. Fish meal replacement with meals sourced from gastropods is closely analysed in this paper. Synthesized information from this review will benefit future research on this subject.

Key words: *animal protein, fish rearing, Gastropoda, nutrition.*

INTRODUCTION

Aquaculture productions are on a rising trend, according to the Food and Agriculture Organization (FAO 2022). Global needs regarding food and its availability determine the aquaculture sector development and progress. Improving economic efficiency and reducing the impact on the environment is paramount for the aquaculture sector. Feed cost is the biggest expenditure in aquaculture (Baki & Yucel, 2017). A significant part of this cost is represented by the protein sources used in manufacturing the feed. The most utilized protein source in aquaculture feeds is fish meal (FAO 2004). Fish meal is very nutritious, but its price is continuously rising because necessary raw matter becomes harder to procure and the exploitation of small oceanic fish suitable for its production has a direct negative impact on the environment.
The research of alternative protein sources is ongoing for years. Due to better chemical composition, animal protein sources are preferred over plant protein sources. Various animal protein sources are investigated, such as insects, larvae, food processing by-products etc. (Vrabec et al., 2015; Luthada-Raswiswi et al., 2021; Rapatsa & Moyo, 2022).
Gastropoda class encompasses species such as snails and slugs. This class has over 62000 species of aquatic and terrestrial snails and slugs (www.ucmp.berkeley.edu). These invertebrates' potential as an alternative protein source has been investigated in animal husbandry, mostly for poultry and in some instances for swine (Mead, 1961; Creswell & Habibie, 1981; Creswell & Kompiang, 1981; Ali & Leeson, 1995; El-Deek et al., 2002; Diomandé at al., 2008a,b; Ahaotu et al., 2013; Diarra, 2015; Malvar & Agapito, 2020; Dharmawati et al., 2022).
Naturally, gastropods are part of the diet of numerous species reared in aquaculture. Gastropods have been studied as possible candidates in aquaculture for the replacement of conventional protein sources. The aim of this short review was to present research conducted by scientists for the use of gastropods as an alternative protein source in aquaculture feed. This synthesised and centralised information will benefit future research in this direction.

MATERIALS AND METHODS

Studies on the use of gastropods as an alternative protein source have been gathered for this short review. Relevant scientific databases were browsed using predefined search words. Alphabetically, these databases are: CABI Digital Library ebooks; CABI VETMED Resource; Elsevier Ebooks; IEEE – NOW

Foundation ebook Collection; iGroup - American Library Association Ebook Collection; InCites Benchmarking and Analytics; Sage eBooks Collection; ScienceDirect Freedom Collection, Elsevier; Scopus, Elsevier; SpringerLink Journals; Web of Science; Wiley Ebooks; Wiley Journals. Alphabetically, predefined search terms used to browse all the mentioned databases are: gastropod flour; gastropod meal; gastropod meat; hepatopancreas meal; slug flour; slug meal; slug meat; snail flour; snail meal; snail meat; snail viscera flour; snail viscera meal.

Studies relevant to this review were selected and processed based on the following inclusion methodology. Papers that present nutritional information on gastropod species were selected and mentioned in a table structured on the systematics of the Gastropoda class. Valid scientific names for each identified mention are presented and proper identification was conducted using the WoRMS database (www.marinespecies.org). The table contains two categories of citations: unmarked citations with general information about the nutritional value of gastropods including citations that present the use of gastropods in animal husbandry, and citations marked with an asterisk that present the use of gastropods in aquaculture. Studies in which gastropods were used as an alternative protein source in aquaculture feeds were then presented in regard to the taxonomy of used gastropods, nutritional value, and effects on farmed aquatic organisms.

RESULTS AND DISCUSSIONS

Table 1 displays citations to various research conducted on gastropods. These studies include general knowledge on nutritional potential of gastropods, gastropods use in animal husbandry feeds and gastropods use in aquaculture feeds. All research conducted on various species of gastropods nutritional value is relevant, and we brought it forward in this review, because it can help future research on gastropods use as an alternative protein source in aquaculture feeds.

The focus of this short review was to gather available knowledge on the use of gastropods in aquaculture feeds. In continuation, all identified papers related to this topic are briefly presented.

Smoked fish waste and snail (*Pila ampullacea*) meal were utilized in the diet of catfish (*Clarias* sp.) (Nurhayati et al., 2020). When only snail meal was utilized, the feed conversion rate was the highest and the growth performance was the lowest. When using 10% snail meal along 90% smoked fish waste, the results were the best for growth and feed conversion rate.

Pila globosa meal was utilized as a protein source for rearing blue gourami (*Trichogaster trichopterus*) fingerlings (Mohanta et al., 2013). *Pila globosa* proximate composition represented 525 g kg^{-1} dry matter crude protein, 40 g kg^{-1} dry matter ether extract, 160 g kg^{-1} dry matter ash, 275 g kg^{-1} dry matter total carbohydrate and 14.88 MJ kg^{-1} calculated digestible energy. Performance of fish reared using snail meal based diets was not satisfactory compared to other sources of protein analysed, and the authors could not justify the reason for this poor performance.

Mohanta et al. (2015) studied *Pila globosa* meal as a protein source for guppy (*Poecilia reticulata*) fingerlings. *Pila globosa* proximate composition represented 516.2 g/kg dry matter crude protein, 40 g/kg dry matter ether extract, 155.8 g/kg dry matter ash, 288 g/kg dry matter total carbohydrate and 14.95 MJ/kg calculated digestible energy. Authors consider that because they used a high level of incorporation of snail meal (30%), guppies manifested poor growth and nutrient utilization.

In Bombeo-Tuburan et al. (1995) study, different combinations of golden snail meat (*Pomacea canaliculata*), maize and cassava were used to feed tiger shrimp (*Penaeus monodon*). Proximate analysis of snail meat displayed contents (% dry matter) of 54.3% crude protein, 1.4% crude fat, 2% crude fibre, 20.4% nitrogen-free extract and 21.9% ash. *Pomacea canaliculata* represents a good source of linoleic acid, α-linolenic acid, and eicosapentaenoic acid, which are required for the good growth of *Penaeus monodon*. The golden snail had a favourable essential amino acid index of 0.84, which shows it is a good protein source for tiger shrimp rearing.

Jintasataporn et al. (2004) studied *Pomacea canaliculata* use as replacement of fish meal in the diet of giant freshwater prawn (*Macrobrachium resenbergii*). Snail meal had a protein digestibility of 86.36%, which is close to the value of fish meal digestibility of 88.69%.

Table 1. Systematic presentation of identified relevant studies on gastropods nutritional value.

Order	Family	Mentioned species	Accepted name of mentioned species	Reference
		Pila ampullacea	*Pila ampullacea* (Linnaeus, 1758)	1*
Architaenioglossa	Ampullariidae	*Pila globosa*	*Pila globosa* (Swainson, 1822)	2, 3*, 4*
		Pomacea canaliculata	*Pomacea canaliculata* (Lamarck, 1822)	5, 6, 7*, 8*, 9, 10, 11*, 12, 13*, 14, 15, 16, 17*, 24*, 84*
		Pomacea paludosa	*Pomacea paludosa* (Say, 1829)	18
		Pomacea spp.	*Pomacea* spp.	19*, 20*, 21*, 22*, 23*, 25, 26*, 27*, 28*, 29, 30, 31
	Viviparidae	*Viviparus* spp.	*Viviparus* spp.	32*
Caenogastropoda	Cerithiidae	*Cerithium* spp.	*Cerithium* spp.	7*
	Pachychilidae	*Faunus ater*	*Faunus ater* (Linnaeus, 1758)	33*
Neogastropoda	Buccinidae	*Buccinum striatissimum*	*Buccinum striatissimum* G. B. Sowerby III, 1899	34*, 35*
	Muricidae	*Rapana venosa*	*Rapana venosa* (Valenciennes, 1846)	36, 37*, 38*
Stylommatophora	Achatinidae	*Limicolaria aurora*	*Limicolaria aurora* (Jay, 1839)	39*, 40*, 41*
		Limicolaria flammea	*Limicolaria flammea* (O. F. Müller, 1774)	42*, 43*
		Limicolaria spp.	*Limicolaria* spp.	44, 45, 46, 82*
		Achatina achatina	*Achatina achatina* (Linnaeus, 1758)	44, 45, 46, 47, 48, 49*
		Achatina fulica	*Lissachatina fulica* (Bowdich, 1822)	45, 50, 51, 52, 53, 54, 55, 56, 57, 48, 83*
		Achatina spp.	*Achatina* spp.	58
		Archachatina degneri	*Archachatina degneri* Bequaert & Clench, 1936	47
		Archachatina marginata	*Archachatina marginata* (Swainson, 1821)	44, 59, 45, 46, 47, 60, 61, 48, 49*
		Archachatina spp.	*Archachatina* sp.	62
	Helicidae	*Eobania vermiculata*	*Eobania vermiculata* (O. F. Müller, 1774)	63
		Helix aperta	*Cantareus apertus* (Born, 1778)	63
		Helix aspersa, Helix aspersa maxima	*Cornu aspersum* (O. F. Müller, 1774)	64, 52, 63, 65, 66, 67, 68
		Helix lucorum	*Helix lucorum* Linnaeus, 1758	63, 65, 69, 70
		Helix pomatia	*Helix pomatia* Linnaeus, 1758	52, 63, 65, 71, 72
		Helix spp.	*Helix* spp.	73, 74, 77*, 78*
		Otala lactea	*Otala lactea* (O. F. Müller, 1774)	75
		Theba pisana	*Theba pisana* (O. F. Müller, 1774)	75, 76
Species not mentioned				79*, 80, 81*

Note: * Studies in which gastropods were used in aquaculture feeds.

References: 1 - Nurhayati et al., 2020; 2 - Ali & Leeson, 1995; 3 - Mohanta et al., 2013; 4 - Mohanta et al., 2015; 5 - Budiari et al., 2021; 6 - Ghosh et al., 2017; 7 - Hertrampf & Piedad-Pascual, 2000; 8 - Jintasataporn et al., 2004; 9 - Luo et al., 2015; 10 - Malvar & Agapito, 2020; 11 - Pertiwi & Saputri, 2020; 12 – PhilRice, 2001; 13 - Phonekhampheng et al., 2009; 14 - Rattanaporn et al., 2006; 15 - Sumiati et al., 2020; 16 - Ulep et al., 1991; 17 - Visca & Palla, 2018; 18 - Dharmawati et al., 2022; 19 - Bombeo-Tuburan et al., 1995; 20 - Chimsung & Tantikitti, 2014; 21 - Da et al., 2012; 22 - Da et al., 2013; 23 - Da et al., 2016; 24 - Heuzé & Tran, 2017; 25 - Kaensombath, 2005; 26 - Muchlisin et al., 2020; 27 - Santanumurti et al., 2022; 28 - Serra, 1997; 29 - Subhan et al., 2010; 30 - Syaharuddin et al, 2019; 31 - Tami et al., 2017; 32 - Anisuzzaman et al., 2012; 33- Aaqillah-Amr et al., 2022; 34 - Moss et al., 2018a; 35 - Moss et al., 2018b; 36 - Özden & Erkan, 2011; 37 - Sahin et al., 2021; 38 - Sahin & Ergün, 2021; 39 - Adéyèmi et al., 2020; 40 - Sogbesan & Ugwumba, 2008; 41 - Sogbesan et al., 2006; 42 - Imodagbe et al., 2021; 43 - Imodagbe et al., 2020; 44 - Adeyeye & Afolabi, 2004; 45 - Babalola & Akinsoyinu, 2009; 46 - Fagbuaro et al., 2006; 47 - Imevbore, 1990; 48 - Nkansah et al., 2021; 49 - Okanlawon & Oladipupo, 2010; 50 - Creswell & Habibie, 1981; 51 - Creswell & Kompiang, 1981; 52 - Diarra, 2015; 53 - Diarra et al., 2015; 54 - Diomandé et al., 2008a; 55 - Diomandé et al., 2008b; 56 - Garnadi, 1951; 57 - Mead & Kemmerer, 1953; 58 - Jehemat & Koni, 2013; 59 - Amobi et al., 2019; 60 - Imevbore & Ademosun, 1988; 61 - Kalio & Etela, 2011; 62 - Ahaotu et al., 2013; 63 - Dragićevic & Baltić, 2005; 64 - Çağiltay et al., 2011; 65 - Gomot, 1998; 66 - Gorka et al., 2017; 67 - Milinsk et al., 2006; 68 - Milinsk et al., 2003; 69 - Olgunoğlu & Olgunoğlu, 2008; 70 - Olgunoğlu & Olgunoğlu, 2009; 71 - Ikauniece, 2014; 72 - Özogul et al., 2005; 73 - Corda et al., 2014; 74 - Zymantiene et al., 2008; 75 - Caetano et al., 2021; 76 - El-Deek et al., 2002; 77 - Jones & De Silva, 1997; 78 - Jones et al., 1996; 79 - Rana et al., 2022; 80 - Suryadi et al., 2022; 81 - Verhoef et al., 1998; 82 - Ovie & Adejayan, 2010; 83 - Suresh, 2017; 84 - Cagauan & Doria, 1989.

Diets that replaced fish meal with 25% and 50% showed the best results regarding specific growth rate.

In Pertiwi and Saputri (2020) study, *Pomacea canaliculata* meal was utilized as a fish meal substitute in the diets of *Pangasius* sp. (*Pangasianodon* sp. accepted name). Different inclusion levels of snail meal were utilized, the best results being obtained for the diet that incorporates 10% snail meal as fish meal substitute.

Simple golden apple snail (*Pomacea canaliculata*) meal, ensiled golden apple snail meal with 5% citric acid and ensiled golden apple snail meal with 20% sugar-cane molasses was utilized in the diets of *Clarias gariepinus* (Phonekhampheng et al., 2009). Weight gain of *Clarias gariepinus* showed improvements when these ingredients were utilized as fish meal replacements.

Visca and Palla (2018) tested the potential of golden apple snail (*Pomacea canaliculata*) meal in the diet of rabbitfish (*Siganus guttatus*) as an alternative protein source. According to the results, feed with 45% protein content obtained from golden apple snail meal can successfully substitute fish meal for more than two months.

Chimsung & Tantikitti (2014) reared sex-reversed tilapia (*Oreochromis niloticus* x *Oreochromis mossambicus*) using golden apple snail (*Pomacea* spp.) meat meal (GAS) and fermented golden apple snail (FGAS). Proximate analysis of both ingredients showed 49.54±0.40% protein for GAS and 39.11%±0.38% protein for FGAS, 0.83%±0.15% fat for GAS and 0.75±0.04% fat for FGAS, 13.98±0.21% ash for GAS and 3.62±0.03% ash for FGAS. Authors recommend for the best results the use of 75% FGAS replacement of fish meal, but it is also possible to use 50% GAS as a replacement for fish meal.

Da et al. (2012, 2013, 2016) researched golden apple snail (*Pomacea* spp.) meal and other protein sources use as a fish meal replacement in the diet of *Pangasianodon hypophthalmus*. Proximate analysis of golden apple snail meal showed 564 g protein content/kg DM, 16 g lipid content/kg DM, 10 g crude fibre/kg DM, 239 g neutral detergent fibre/kg DM, 118 g ash/kg DM and 12.3 MJ gross energy/kg DM. Total essential amino acid value for golden apple snails was of 174.5 g/kg DM. Golden apple snail meal had the lowest value for gross energy among other tested ingredients but ranked second for the total essential amino acid value. Regarding digestibility, it's concluded that there is no adverse effect on digestibility of feed when golden apple snails are used as a replacement for fish meal. The visceral somatic index ranked highest for the diet containing golden apple snail meal.

Muchlisin et al. (2020) used golden snail (*Pomacea* spp.) meal in the diet of short-fin eel (*Anguilla bicolor*). Crude protein content of snail meal was of 51.8%.

Santanumurti et al. (2022) studied the enrichment of *Moina* spp. with golden snail (*Pomacea* spp.) meal and its effects on the growth and survival of jelawat fish (*Leptobarbus hoevenii*) larvae. Snail meal proximate composition had 55.21% protein, 3% fat and 10.48% ash. Using 4 g/l and 8 g/l of snail meal on *Moina* spp., resulted in good growth and survival rates of *Leptobarbus hoevenii* larvae.

Serra (1997) presents the results of Cagauan and Doria (1989), which studied the use of the golden apple snail (*Pomacea* spp.) meal as feed ingredient for Nile tilapia (*Oreochromis niloticus*). Results indicated better growth for solely using golden snail meal and 3:1 ratio of golden snail meal and rice bran.

Anisuzzaman et al. (2012) studied in Bangladesh the potential of a freshwater species of snails (*Viviparus* sp.) to be used in fish feeds. The results from the culture trial of *Viviparus* sp. showed that freshwater snails are cost effective to be used in the production of fish feed.

Aaqillah-Amr et al. (2022) studied *Faunus ater* biochemical composition as a possible candidate for orange mud crab (*Scylla olivacea*) feed formulation. The analysis showed dry matter contents of 46.26±3.76% protein, 4.6±0.29% lipid, 0.21±0.04% fibre and 26.73±1.33% ash. *Faunus ater* was not used as an ingredient, because of lower lipid and protein content than the mangrove clam (*Polymesoda erosa*), and due to the low meat yields.

Moss et al. (2018a, 2018b) studied snail (*Buccinum striatissimum*) meal incorporation in the diets of *Marsupenaeus japonicus*. Snail meal was utilized for the replacement of squid meal and krill meal in juvenile *Marsupenaeus japonicus* diets. An increase in growth was observed for the replacement of 75% to 100% of squid and krill meals with *Buccinum striatissimum* meal. The shells of *Buccinum striatissimum* were utilized as a source of calcium for *Marsupenaeus japonicus*. Using 10% snail shells powder in the diets of *Marsupenaeus japonicus* showed overall improvements in growth performance and survival rate.

In Sahin et al. (2021) research, rapa whelk (*Rapana venosa*) meal was utilized as a replacement of fish meal in the feed used for rainbow trout (*Oncorhynchus mykiss*) and various reproductive traits, histopathologic aspects and blood parameters were analysed. Crude protein content of *Rapana venosa* meal was of 71.50% and crude fat content of 2.01%. Inclusion of *Rapana venosa* meal of up to 50 g/kg in feed did not affect the studied parameters, but the inclusion of more than 50 g/kg can lead to pathological issues in blood and tissue.

In Sahin and Ergün (2021) study, rapa whelk (*Rapana venosa*) meal was used in the diet of rainbow trout (*Oncorhynchus mykiss*) fry. At 5% inclusion rate along fish meal, rapa whelk meal did not have a negative effect on growth, body composition, survival, hepatosomatic index and digestive enzymes action.

Whole garden snail (*Limicolaria aurora*) nutritional potential as a fish feed ingredient was analysed (Adéyèmi et al., 2020). The proximate composition showed contents of 970.6±5.4 g/kg DM dry matter, 698.0±75.1 g/kg DM ash, 171.3±15.4 g/kg DM protein, 37.3±17.6 g/kg DM fat, 3.2±0.7 g/kg DM fibre, 64±41.5 g/kg DM carbohydrates. Based on comparisons with other ingredients, Adéyèmi et al. (2020) classified the garden snail (*Limicolaria aurora*) as a good mineral source.

In Sogbesan and Ugwumba (2008), the potential as a fish meal supplement in aquaculture of the garden snail (*Limicolaria aurora*) was investigated. Garden snail meal contained (% dry matter) 66.8±3.6% crude protein, 7.9±2.3% crude lipid, crude fibre 4.1±0.9%, 5.8% nitrogen free extract, 6.5±0.5% ash and 2006±3.46 kJ gross energy/100 g. Mineral content of garden snail meal was of 2.32±0.06 g sodium/100 g, 1.13±0.08 g calcium/100 g, 2.23±0.06 g potassium/100 g, 0.15±0.02 g phosphorus/100 g and 0.28±0.04 g magnesium/100 g. Total amino acids from garden snail meal ranked highest (50.06±2.89 g/16 g N) among other tested protein sources of animal origin.

Sogbesan et al. (2006), tested garden snail (*Limicolaria aurora*) meal in the feed of *Clarias gariepinus* fingerlings as a protein source. Garden snail meal proximate composition of dry matter contained 66.76% crude protein, 7.85% crude fat, 5.81% nitrogen free extract, 4.1% ash and 478.35 kcal gross energy /100 g. Optimum growth was noticed for the replacement of fish meal with 25% garden snail meal.

Garden snail meal (*Limicolaria flammea*) meal utilization as feed for *Heterobranchus bidorsalis* was researched by Imodagbe et al. (2020, 2021). Proximate composition of garden snail (*Limicolaria flammea*) meal had 60.13 g crude protein/100 g, 8.5 g crude lipid/100 g, 2.47 g crude fibre/100 g, 8.64 g nitrogen-free extract/100 g and 10.33 g ash/100 g. Weight gain increases were observed for the replacement of fish meal with up to 75% garden snail meal. Best results were observed for the inclusion of 25% garden snail meal. Profit maximization was also observed for the replacement of fish meal with 25% to 50% garden snail meal. At a 25% inclusion rate of garden snail meal, *Heterobranchus bidorsalis* meat biochemical composition showed the biggest protein content and the highest amount of total amino acids.

Ovie and Adejayan (2010) used the garden snail (*Limicolaria* spp.) in the diets of *Clarias gariepinus*. The proximate analysis of garden snail meal revealed a content of 66.76% crude protein, 7.85% lipid, 4.10% crude fibre and 6.84% ash. Better growth results in comparison to the control group were showed for diets containing 25%, 50% and 100% garden snail meal replacement of fish meal.

In Okanlawon and Oladipupo (2010) research, snail offal (viscera) meal obtained from *Achatina achatina* and *Archachatina marginata* was utilized in the diet of *Clarias gariepinus*. Based on the proximate composition, snail offal meal contains 50.85 g crude protein/kg dry matter, 9.73 g fat/kg dry matter, 4.27 g crude fibre/kg dry matter, 25.41 g nitrogen free extract/kg dry matter and 9.74 g ash/kg dry matter. Growth performances using snail offal meal diets were not satisfactory, but the use of 50% snail offal meal as replacement for fish meal appears economically profitable.

Giant African snail (*Lissachatina fulica*) was tested as a protein source for common carp

(*Cyprinus carpio*) (Suresh, 2017). Giant African snail proximate composition of dry matter had contents of 57.2% crude protein, 4.2% crude lipid, 1.8% crude fibre, 28.7% nitrogen free extract and 8.1% ash. According to the results, 40% substitution of fish meal with giant African snail meal resulted in 53% bigger weight gain and improved feed conversion rate than for the control diet with fish meal.

In Jones et al. (1996) and Jones & De Silva (1997) studies, Australian freshwater crayfish (*Cherax destructor*) diets were formulated using different protein sources, including snail meal (*Helix* spp.). The best food conversion ratio was obtained for the snail meal diet (0.95). Snail meal diet obtained the highest values for protein efficiency ratio (3.44) and apparent net protein utilization (41.15%). Snail meal diet used for Australian freshwater crayfish (*Cherax destructor*) showed the lowest ash digestibility value (17.3%) compared to other diets.

In Rana et al. (2022) research, cuchia (*Monopterus cuchia*) was reared using different diets, that all contained snail meat (unspecified species).

Verhoef et al. (1998) studied different diets, including freshwater snails (unspecified species), which were tested as alternative diets for rearing Australian crayfish (*Cherax destructor*). Although freshwater snails represent natural prey for *Cherax destructor*, lower growth rate was observed for this diet than for other tested diets.

Previous review-style works on using gastropods as a potential protein source in aquaculture were conducted by Hertrampf and Piedad-Pascual (2000) and Heuzé and Tran (2017). In Hertrampf and Piedad-Pascual (2000) book, the chapter dedicated to snail meal presents literature information on the chemical composition of *Cerithium* spp. and *Pomacea canaliculata*. Heuzé & Tran (2017) focus on the use of apple snails (*Pomacea canaliculata*) as animal feed, including aquaculture applications.

CONCLUSIONS

The versatility of gastropods as an alternative protein source in aquaculture is outlined in the gathered studies. Information synthesised in this short review will help to document future research on the potential use in aquaculture feeds of gastropods.

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TAILORING WESTERN BLACK SEA AQUACULTURE TO IMPENDING CLIMATE CHANGE: LABORATORY TESTING OF GILTHEAD SEABREAM *Sparus aurata* (Linnaeus, 1758) AS A POTENTIAL CANDIDATE

Magda NENCIU, Victor NIȚĂ

National Institute for Marine Research and Development "Grigore Antipa" (NIMRD), 300 Mamaia Blvd, 900581 Constanta, Romania

Corresponding author email: vnita@alpha.rmri.ro

Abstract

Climate change calls for the diversification of aquaculture species, seeking alternatives tolerating warmer summer temperatures. In this context, the research on the adaptability of gilthead seabream Sparus aurata (Linnaeus, 1758) for Romanian marine aquaculture was performed. The experiment demonstrated the possibility of transferring three months old juveniles from a 36‰ salinity into Black Sea water (mean salinity 15‰), without any mortality. The evolution of the stress induced by the difference in salinity was carefully monitored. Glycemic levels between 68-82 mg/dl before, 157-171 mg/dl one hour after the sudden change in salinity and 115-124 mg/dl at 24 hours were documented, respectively. Corroborated with the color changes and the resumption of active feeding and schooling behavior, it can be inferred that the use of the species is feasible in Romania. An average biomass increase comparable to relevant literature was documented: from seven grams initially to 300 grams. The biochemical analysis of the meat also revealed a balanced composition. Overall, S. aurata proved a viable candidate for Western Black Sea cage aquaculture in a rotational system, complementary to colder water species.

***Key words**: aquaculture, adaptability, Black Sea, rotation, seabream.*

INTRODUCTION

The Romanian Black Sea coastline is limited to 245 km, with the Danube Delta accounting for more than half of it. Consequently, traditional Romanian aquaculture has focused primarily on freshwater fish species. However, in recent years, more emphasis has been placed on the potential of mariculture, and research activities have been carried out to stimulate the development of the field, both for finfish (Zaharia et al., 2017; Niță et al., 2018) and shellfish culture (Niță & Nenciu, 2020). Despite environmental and administrative concerns, cold-season cage farming for rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) proved feasible (Nenciu et al., 2022). However, impending climate change requires diversification in order to find alternative species tolerating increasing summer temperatures. Climate change is one of the most severe threats to the environment and society in general. The warming of the climate system is an undeniable reality, according to the Intergovernmental Panel on Climate Change (IPCC, 2019).

Observations indicate increases in global average temperatures of the World Ocean, extensive ice cap melting, and global average sea level rise. Global warming can largely be attributed to greenhouse gas emissions from human activities (Cochrane et al., 2019). The World Ocean is warming, registering, however, some geographical differences and some decadal variability. At least two seas in subtropical latitudes (the Mediterranean Sea and the East China Sea) are continuously warming (Rosenzweig et al., 2007). Salinity is generally increasing in surface marine waters in regions of higher evaporation, while there is a decreasing trend at high latitudes (Antonov et al., 2002). The combined effect of temperature and salinity changes due to climate warming is expected to reduce the surface density of the World Ocean, increase vertical stratification, and alter surface mixing (Sarmiento et al., 2004). Globally, sea levels have risen 21 - 24 cm since 1880. This rise is largely due to a combination of melting glaciers and the ice cap with the thermal expansion of seawater as the ocean warms. In 2021, the global mean sea level was 97 mm above 1993 levels, the highest annual average on record since 1993 to date (Lindsey, 2022). The greatest losses expected to be caused by sea level rise are estimated on the Atlantic and Gulf of Mexico coasts, in the Mediterranean, Baltic Sea and small island regions (Nicholls et al., 2007).

Climate change has also caused seawater acidification and the modification of other chemical properties. Continued absorption of atmospheric CO_2 has lowered surface seawater pH by 0.1 units over the past two hundred years (Caldeira & Wickett, 2005). Changes in pH can also affect marine species in ways other than decalcification. Havenhand et al. (2008), found that low pH reduces sperm motility and fertilization success in the sea urchin *Heliocidaris erythrogramma* (Valenciennes, 1846) and point out that other broadcast spawning marine organisms may be at a similar risk. Other chemical properties subject to trends driven by climate change include oxygen and inorganic nutrients. Oxygen concentration in the oxic thermocline (from about 100 m to 1,000 m) has decreased in most ocean basins since 1970 (Emerson et al., 2004).

As far as biological and ecological changes are concerned, decades of research document that climate variables are the primary factors influencing the distribution and dynamics of marine plankton and fish assemblages (Roessig et al., 2004). Climate change is predicted to displace most species to the poles, expanding the range of marine thermophilic species and reducing that of cryophilic species (Parmesan & Yohe, 2003). Climate-driven changes in species composition and abundance will obviously also alter species diversity, with implications for ecosystem functions such as productivity (Duffy, 2003) and resistance to invasive species (Stachowicz et al., 2002).

Climate change thus affects marine aquaculture through acidification, changes in seawater temperature, salinity and circulation, frequency and severity of extreme events, sea level rise and ecological changes associated with all the phenomena described above. And, moreover, climate change also impacts greatly on the four dimensions of food security (Cochrane et al., 2019): the availability of seafood will vary as a consequence of changes in habitats, stocks and species distribution; the stability of supply will be affected by seasonal changes, increased variation in ecosystem productivity and high production variability and risks; the access to seafood will be affected by changes in livelihoods and opportunities for harvest/fishing and/or farming; and, finally, the use aquatic products will also be affected (Nicolae et al., 2016; Nicolae et al., 2017). For example, some communities will have to adapt to species that are not traditionally consumed, the social acceptability of novel species and/or products being crucial in this adaptation process (Nenciu et al., 2021).

Gilthead seabream *Sparus aurata* (Linnaeus, 1758) is one of the most cultured fish species in the Mediterranean Basin, with an estimated annual production volume of 258,754 tons/year (Mhalhel et al., 2023). In its natural habitat, gilthead seabream lives in environments where temperatures range from 11°C in winter to 26°C in summer (Ibarz et al., 2010). However, aquaculture practices documented that temperatures below 18°C cause changes in the feeding behaviour, while a drastic reduction in food intake has been observed at temperatures below 13°C, greatly affecting fish production (Ibarz et al., 2003; Sánchez-Nuño et al., 2018). In terms of salinity, seabream is more tolerant and thus frequents estuaries and coastal waters, thus it is likely to be cultured in brackish environments (Pavlidis & Mylonas, 2011). In the Black Sea, seabream has only been cultured at the Southern coast, in Türkiye (Öztürk et al., 2020; Öztürk, 2022).

In this broader context, this research aimed at testing the adaptability of gilthead seabream, a widely cultured species all around the Mediterranean Basin, to Western Black Sea conditions, with the view to proposing a rotational farming system in floating cages together with rainbow trout.

MATERIALS AND METHODS

Fish Supply, Adaptation and Transfer

The gilthead seabream fingerlings (N = 218 individuals, aged approximately two months) were purchased in May 2023 from an aquaculture farm and hatchery from Italy (Adriatic Sea) and transported to NIMRD's aquaculture laboratory in oxygen suprasaturated (over 110% dissolved oxygen) plastic bags. Temperature in the transport water was 20°C and the salinity 36‰, similar to the conditions in the hatchery of origin. Prior to the reception of the fish, salinity in the stocking tank was adjusted to 36‰ using Instant Ocean sea salt and kept in a recirculating regime. After a careful temperature check, the fish were gradually released into the stocking tank.

Additional aeration pumps were used to increase the oxygen content of the water. No mortalities were recorded during transportation and/at transfer. The fingerlings were kept in a 900-L stocking tank for 14 days, in order to overcome any stress caused by transport and to guarantee a healthy batch. Throughout adaptation, the fish were fed ad libitum with Skretting Optibream 1P (2 mm) pellets. All food was consumed and faces were removed by syphoning. During the two-week quarantine period, the mean temperature of water in the stocking tank was 20°C. Before performing the transfer to Black Sea water, 2 fish were randomly extracted for blood glucose analysis.

After the initial two-week quarantine period, the gilthead seabream fingerlings were divided in four batches (replicates), each containing 54 individuals, and transferred to the 500-L experimental tanks (Figure 1).

Figure 1. Transfer of gilthead seabream juveniles from the stocking tank (salinity 36‰) to the experimental tanks (salinity 15‰) (*Original photos*)

All fish were weighed and measured individually before transfer. The initial supply of Black Sea water was provided by NIMRD's pump-ashore system (PAS). The water entering the PAS is pumped directly from the Black Sea and is stored in a covered settlement tank for sedimentation and suspended solids reduction before entering the tanks (Niță & Nenciu, 2021). The experimental tanks were fitted with an efficient aquaculture recirculation system (RAS), containing mechanical and biological filtration, UV sterilization, oxygen pumps and a protein skimmer, to ensure proper water quality. Water temperature was not adjusted during the experiment. Upon transfer, water temperature in the experimental tanks was 21°C. The entire experimental period covered seven months, from late May to early December 2023.

Monitoring Environmental Parameters

Temperature (°C), salinity (‰), pH and dissolved oxygen (DO) (%) both in the initial stocking tank and in experimental tanks were measured daily, using a Mettler Toledo Seven Excellence Multiparameter. The experimental RAS tanks were equipped with real-time transmission sensors of the above-mentioned parameters and a trigger for the back-up aeration pump in case of values dropping below the set 75% dissolved oxygen threshold.

Blood Glucose Measurements

In order to investigate blood glucose as a stress indicator, measurements were performed in two replicates/fish. A baseline glucoses reading was made by extracting two fish from the stocking tank before transfer. One hour and 24 hours after transfer into Black Sea water, respectively, two fish were randomly extracted from each experimental tank (total number of extracted fish = 16, remaining fish in each experimental tank = 50) and euthanized by immersion in a 500 mg/ml buffered one third tricaine methanesulfonate (MS-22) and two thirds sodium bicarbonate solution (AVMA, 2013). After cessation of breathing activity, the caudal fin was severed (Witeska et al., 2022) and blood was drawn for reading blood glucose values using an OK Meter Match II automatic glucose reader (Figure 2).

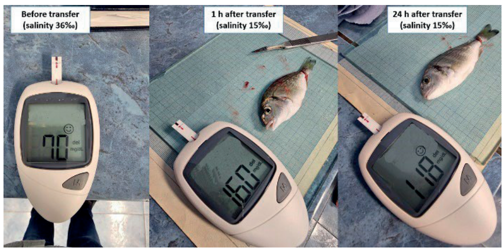

Figure 2. Blood glucose readings on gilthead seabream before, one hour and 24 hours after transfer from 36‰ to 15‰ salinity (*Original photos*)

No treatment was applied to the blood drawn, as it was analysed immediately.

Feeding Protocol

The gilthead seabream juveniles were fed with dedicated Skretting Optibream pellets: Optibream 1P (2 mm) (crude protein 48.5%, crude fat 18%, ash 6.2%, cellulose 2.8%) during the first three months and Optibream 2P (4 mm) (crude protein 44%, crude fat 20%, ash 6.5%, cellulose 3.3%) until completion of the experiment (Ayala et al., 2023). The calculated daily feed ratio was 2% of the biomass (Zaharia et al., 2017), fed in two equal doses during the day (in the morning and in the afternoon). Monthly biomass measurements were performed to adjust the feeding ratio.

Calculating Growth Parameters

Weight and length measurements were performed monthly on all 50 fish from each experimental tank. The total length (TL) of the specimens was measured on millimetric paper to the nearest 0.5 millimetre. The total weight (TW) of the fish was taken on a Kern EW top loading balance. Using the biometric and gravimetric data collected during the 7 months of study, Feed Conversion Ratios (FCR), Specific Growth Rates (SGR%/day), Fulton's Condition Factor (K) were determined for the four batches. The initial and final Length/Weight relationship for the entire lot was also determined (Froese, 2006).

The equations used for calculating the Feed Conversion Ratio (FCR) and the Specific Growth Rate (SGR%/day) are detailed below (Hopkins, 1992):

$$FCR = \Sigma_{fk}/W_t\text{-}W_0 \quad (1)$$

$$SGR = 100[(\ln W_t\text{-}\ln W_0)/t] \quad (2)$$

Where: t = feeding days; W_0 = initial live weight of fish (g); W_t = final live weight of fish (g); L = total length (cm), and fk = weight of feed consumed by fish at each feeding (feed intake) (g).

Fulton's Condition Factor (K) was calculated using the equation below (Reis & Ateș, 2019):

$$K = (W/L^3) * 100 \quad (3)$$

Where: W = total weight (g), L = total length (cm).

Statistical Analysis

For statistical interpretation, data from each group's replicates were pooled for one-way ANOVA analysis, and differences at the 5% level ($p < 0.05$) were considered significant (using the Tukey's significant difference test) (Akbulut et al., 2002).

Biochemical Analysis

Following the completion of the experimental period, a comparative analysis of the proximate composition of the gilthead seabream meat was performed using four replicates of one sample reared in Black Sea water for seven months and one sample reared in an aquaculture farm in the Aegean Sea. The analysis was performed by an accredited laboratory (Biosanivet Ltd.), covering the following parameters: crude fat (%, as per SR ISO 1444:2008), crude protein (%, as per SR ISO 937:2007), total ash (%, as per SR ISO 936:2009), moisture (%, as per SR ISO 1442:2010), carbohydrates (%, as per PA-L-34) and energy values (Kcal/100 g and KJ/100 g, as per PA-L-34).

RESULTS AND DISCUSSIONS

The gilthead seabream lot was carefully observed immediately after reaching NIMRD's aquaculture laboratory. After the transfer into the stocking tank (36‰ salinity), all fish behaved normally, with no visible sign of stress. No mortality was recorded during the entire experimental period. Feeding was started 24 hours after transfer, observing the complete consumption of the pellets. The 2% body mass feeding ratio was applied during the seven-month experimental period.

After transfer into the Black Sea water experimental tanks, temperature, salinity, dissolved oxygen and pH were constantly monitored, showing no significant variations among the two experimental batches ($p \geq 0.05$). During the 7-month period, temperature ranged from 24°C in August to 20°C in November-December, salinity was rather constant, with a maximum of 17‰ in December and a minimum little under 14‰ in peak summer months, dissolved oxygen values ranged between a maximum of 93% in June and a minimum of 79% in August, and pH recorded only small variations throughout the entire experimental period, with a mean around 7.8.

Blood glucose mean values measured as a proxy for indicating stress in gilthead seabream juveniles as a consequence of the sudden change in salinity are shown in Table 1.

Glycaemic levels between 68-82 mg/dl before, 157-171 mg/dl one hour after the sudden change in salinity and 115-124 mg/dl at 24 hours were documented, respectively.

A total number of 18 fish were sacrificed for blood glucose analyses: two from the initial stocking tank, eight from the experimental tanks (two fish from each tank) one hour after transfer and eight from the experimental tanks (two fish from each tank) 24 hours after transfer.

Glucose readings were made in two replicates for each fish and are detailed in Table 1 below.

Table 1. Comparative blood glucose values of *S. aurata* juveniles before, one hour after and 24 hours after transfer to Black Sea water, respectively

Specification	Blood glucose (mg/dl)								
	Before Transfer	1 h after transfer				24 h after transfer			
	Stocking Tank	Tank 1	Tank 2	Tank 3	Tank 4	Tank 1	Tank 2	Tank 3	Tank 4
Mean ± SD	72.25 ±5.67	163.75 ±5.53	163.75 ±4.02	162.25 ±4.96	175.75 ±11.75	119.25 ±1.47	119 ±2.54	119.5 ±3.35	119 ±2.73
	72.25±5.67	166.37±5.44				119.18±0.20			

The growth and biomass gain of gilthead seabream juveniles reared in Black Sea water are summarized in Table 2. In terms of mean fish length evolution, the juveniles experienced a normal linear increase, from an initial length of 7 cm to a final length of 23 cm (Figure 3). No significant differences between the four replicates were recorded. As far as weight gain during the experimental rearing is concerned, all fish grew steadily, from an initial weight around 7 g to a final weight of 300 g (Figure 4, and Figure 5). Similarly, to length evolution, there were no statistically significant differences between the replicates ($p \geq 0.05$).

When referenced to the initial values, monthly percentual increases of both length and weight were high (an increase by more than 200% in length and by more than 4000% in weight, respectively, after seven months compared to the initial value at the beginning of the experiment).

Table 2. Mean values of *S. aurata* growth parameters recorded during the experiment

Parameter	Tank 1	Tank 2	Tank 3	Tank 4	ANOVA
Initial TL (cm)	7.20±0.03	7.18±0.02	7.19±0.03	7.21±0.03	The values among tanks (replicates) were not significantly different ($p \geq 0.05$)
Final TL (cm)	23.23±0.53	23.61±0.36	23.25±0.55	23.43±0.43	
Initial weight (g)	7.07±0.03	7.01±0.02	7.05±0.03	7.06±0.03	
Final weight (g)	301.52±2.93	303.92±2.53	303.54±2.46	302.43±2.72	
K	2.51±0.59	2.55±0.56	2.54±0.58	2.58±0.50	
FCR	1.52±1.40	1.45±1.29	1.42±1.25	1.46±1.29	
SGR (%/day)	1.90±1.11	1.91±1.12	1.90±1.11	1.90±1.11	

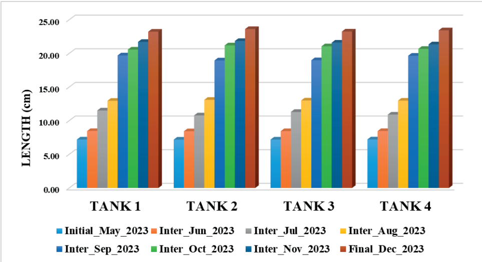

Figure 3. Evolution of fish total length (mean monthly values)

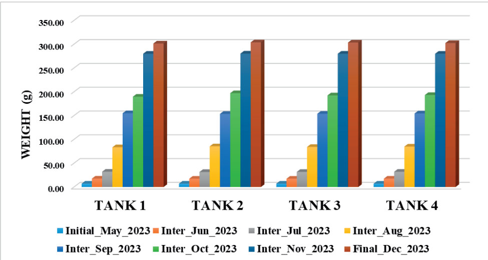

Figure 4. Evolution of fish weight (mean monthly values)

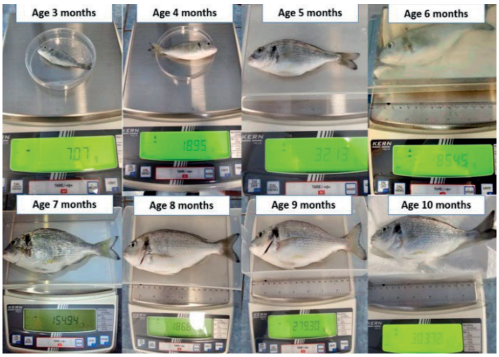

Figure 5. Evolution of gilthead seabream weight during the seven-month experiment in relation to fish age (monthly gravimetric measurements) (*Original photos*)

The calculation of Fulton's Condition Factor (K) for the gilthead seabream juveniles reared in Black Sea water recorded a mean value of 2.54 (Figure 6), while the evolution of the initial (Figure 7) to the final (Figure 8). Length-Weight Relationship indicated a positive allometry (b > 3), the fish increasing in weight faster than in length, corresponding to the rounded shape of adult gilthead seabream.

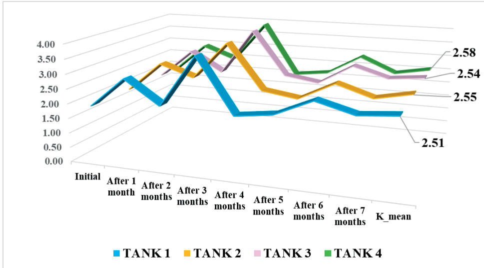

Figure 6. Monthly evolution of the Condition Factor (K)

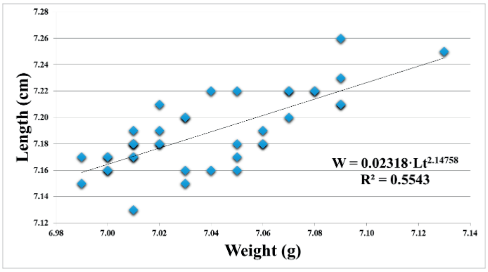

Figure 7. Initial Length-Weight Relationship of gilthead seabream reared in Black Sea water

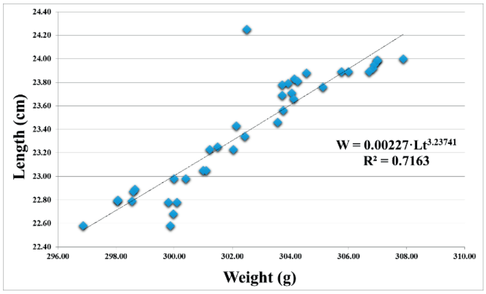

Figure 8. Final Length-Weight Relationship of gilthead seabream reared in Black Sea water (after seven months)

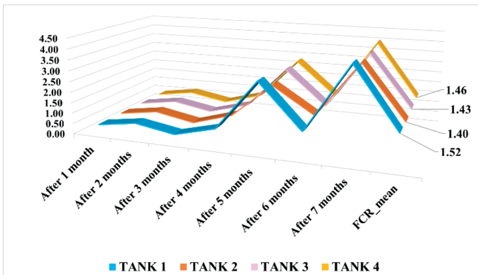

Figure 9. Monthly evolution of the Food Conversion Ratio (FCR)

With reference to feed efficiency and growth, the Food Conversion Ratio (FCR) recorded a mean value of 1.45 (Figure 9), with no significant difference among replicates, while the average Specific Growth Rate SGR (%/day) was 1.90 (Figure 10). Regarding the biochemical parameters investigated, the results of the comparative analysis of the proximate composition of seabream meat (reared in Black Sea water vs. reared in an aquaculture farm in the Aegean Sea) are summarized in Table 3 below (where FW = fresh weight).

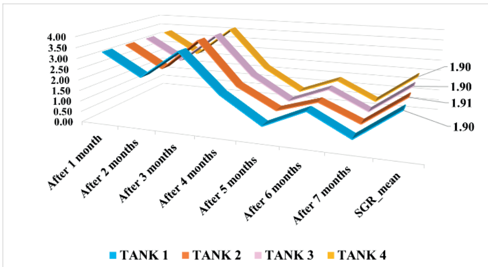

Figure 10. Monthly evolution of the Specific Growth Rates (SGR%/day)

Overall, the values of the two samples were comparable, with only one statistically significant difference in lipid content, which was higher in the Aegean seabream: 10.18% FW compared to 6.14% FW. In the same time, Black Sea reared seabream showed a higher protein content of the flesh, namely 21.15% FW compared to 19.63% FW.

Ash (1.32% FW and 1.41% FW, respectively) and moisture content (68.87 % FW and 71.30% FW, respectively) of the two samples were very similar, while the energy value of the Aegean reared seabream was a little higher, due to the higher fat content. No carbohydrates were detected in any of the samples.

The overall results of the experiment (in terms of growth rhythm, food conversion ratio, condition factor, biochemical composition) suggest that gilthead seabream reared in Black Sea water is comparable in quality to its Mediterranean counterpart.

Table 3. Meat composition of gilthead seabream (reared in Black Sea water vs. in an aquaculture farm in the Aegean)

Culture Environment	Total lipid content (% FW)	Protein content (% FW)	Carbohydrate content (% FW)	Total ash (% FW)	Moisture (% FW)	Energy	
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Kcal/100 g	KJ/100 g
Aegean	10.18±0.02	19.63±0.10	0.00±0.00	1.32±0.005	68.87±0.16	170	710
Black Sea	6.14±0.15	21.15±0.06	0.00±0.00	1.41±0.01	71.30±0.15	140	587
ANOVA	$p < 0.05$	NS	NS	NS	NS	$p < 0.05$	$p < 0.05$

The adaptability of a species to the local environment is of utmost importance when proposing aquaculture developments (Nenciu et al., 2022). Gilthead seabream is a sedentary eurythermal and euryhaline fish that can tolerate wide ranges of temperatures and salinities and thus frequents estuaries and coastal waters (Ibarz et al., 2010; Mhalhel et al., 2023). Moreover, *S. aurata* is documented as occurring in the Black Sea, mostly on the southern coast and only as isolated specimens in the north-western part (Bănărescu, 1964; Aydin & Sözer, 2016). Its tolerance to brackish water (Öztürk et al., 2020) and the continuous warming of seawater at the Romanian coast, with a 2022 average temperature of 14.36°C (2.24°C higher compared to the 1953-2021 annual means) (Vlăsceanu-Mateescu & Lazăr, 2023) suggests that seabream could gradually adapt to this environment after penetration through the Bosphorus.

The primary aim of this research, however, was to test whether a rapid transfer to Black Sea water of *S. aurata* juveniles for aquaculture purposes is feasible. When stocking aquaculture farms (marine cages) with fingerlings coming from hatcheries with higher salinities, there is no time for gradual adaptation and a quick osmoregulation is essential (Mancera et al., 1993; Tandler et al., 1995). The swift adaptation was demonstrated by using blood glucose as stress level indicator. The basic stress response in fish involves catecholamine release and activation of the hypothalamic-pituitary-internal axis. Hypothalamic-pituitary-internal activation causes energy source mobilization, glycogen depletion, and an increase in plasma glucose levels, as well as excessive muscular activity, anaerobic glycolysis, and an increase in plasma lactate (Arends et al., 1999). As a result, the level of glucose in plasma is frequently used to determine stress levels (Fazio et al., 2015). The values measured prior to transfer into brackish water (in the 68-82 mg/dl range) are in line with normal blood glucose levels reported in fish (40-90 mg/dl) (Malini et al., 2018). A sudden increase (157-171 mg/dl) was documented 1 hour after the rapid change of salinity, along with a change of colour and altered swimming behaviour: the fish became darker, swam individually and refused to eat. However, just 12 hours after the transfer, the resumption of active feeding and schooling behaviour was observed, along with the return of the normal colour. The blood glucose measurement performed 24 hours after transfer (115-124 mg/dl) indicated the reduction of stress, which was confirmed by the fact that no mortalities were recorded throughout the entire experimental period.

The second objective of this research pursuit was to assess the growth rate of seabream under local Black Sea water conditions, aiming at scientifically substantiating the species' potential for commercial exploitation in the area. During the seven months of experimental rearing in Black Sea water, the seabream juveniles grew constantly and reached the first commercial size of 300 g, this showing a normal growth rhythm compared to the Mediterranean (Gjije et al., 2022). The mean condition factor (K = 2.54) indicated an excellent condition of the fish (Kop et al., 2019), which displayed a positive allometric growth (b > 3), consistent with the regular growth pattern of seabream Mediterranean (Gjije et al., 2022). The tested seabream lot proved a good feed conversion efficiency, the mean FCR of 1.45 suggesting an optimal intake of the pellets provided (Sadek et al., 2004), which was also confirmed by the high average value of the Specific Growth Rate (SGR = 1.90%/day) (Kraljević et al., 2004).

Upon completion of the experiment, the biochemical analysis revealed a good quality of the meat, with a high protein content (21.15 %FW), in line with documented values of Black Sea reared seabream (Öztürk, 2022). The slightly lower fat content compared to the Aegean seabream (6.14 %FW vs. 10.18 %FW) could be explained by the fact that the commercial counter-sample contained older and larger individuals.

CONCLUSIONS

The research endeavour aiming at laboratory testing of the adaptability of gilthead seabream to Western Black Sea conditions proved to be successful, with good growth parameters and no mortalities, indicating *S. aurata* as a feasible candidate for aquaculture in the region. Moreover, the rapid transfer to brackish water (from 36‰ to 15‰ salinity), mimicking a commercial stocking from a Mediterranean hatchery directly into sea cages, did not cause irreversible stress to the juveniles, which adapted quickly to the new salinity regime.

Despite the documented raise of seawater temperature at the Romanian coast, in the case of open sea farming there are still limitations during the winter. Given the successful testing of rainbow trout during the cold season, the suggested solution is a rotational farming system in floating cages.

This approach would foster the development of Romanian marine aquaculture by maximizing economic profit and using the production facilities all year long. For an efficient outcome, gilthead seabream fingerlings transfer into Black Sea conditions should be made at a larger size (minimum 50 g), to guarantee a higher final commercial value of the harvested fish.

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THE EFFECT OF PRIVATIZATION ON THE SUSTAINABILITY OF THE ROMANIAN ACVACULTURE

Erzsebet OLARIU

Bucharest University of Economic Studies, 6 Piata Romana, District 1, 010374, Bucharest, Romania

Corresponding author email: erji.olariu@gmail.com

Abstract

Privatization emerged as a consequence of nationalization and remains an ongoing contemporary process. After the fall of the communist regime in Romania, the Romanian economy has moved to a competitive market economy from the centralized economy based on the plan-based leadership system. A new economic system based on the requirements of the free competitive market was desired, respecting the principles of economy, sustainability. The paper tries to answer the question of how the sustainability of the aquaculture sector was influenced during this period. The study concludes that the transition that included privatization brought about changes for which the aquaculture sector was not fully prepared. Following the impact, a large part of the companies was liquidated, the sector could not cover the needs of fish consumption in Romania. The way to change the type of property including its management requires the wisdom to learn from the experiences of others, from their own experience, more that the privatization process in Romania still has a lot of potential.

Key words*: economy, fish farming, property right, public goods.*

INTRODUCTION

Humanity for thousands of years has made efforts to improve the communal life, but without touching the property considered sacred. The legal system, from the code of Hammurabi to the XIX century, tried to bring harmony to the community with the effort of preserving the exclusive and absolute character of the property (Nagarajan, 2011).

However, from antiquity to the Middle Ages and during the period of colonial expansion, there are cases in which the state took possession of properties or activities for the purpose of using them for the public benefit, followed by the nationalizations made by the communist totalitarian systems.

Five realities were shown regarding privatization and nationalization in resource-rich economies. The first fact is that "nationalizations and privatizations are repeated, cyclical phenomena, which often come in waves common to several countries". The second fact is that "privatization - nationalization cycles tend to occur more often in the natural resources and utilities sectors". The third fact is that "nationalization of natural resource industries tends to occur when the price of the corresponding commodity is high". The fourth fact is that "contracts for the exploitation of natural resources between governments and private companies are such that commodity price windfalls are mostly appropriated by private firms". The fifth fact is that "nationalization is more likely when inequality is endemic or worsens in the country, and especially when the rents from natural resource or utility companies are perceived as benefiting only a minority" (Chang et al., 2018).

In Romania "the past 30 years were dominated by the economy/society imbalance. It is obsessively considered that economy is the key to the future, the state of the society is just a simple outcome of the economic growth. However, a radical change is needed in the political concept. The social is the main resource of society's development, including of the economy" (Zamfir, 2021).

In Romania, through Law No. 187 of March 23, 1945, for the implementation of the agrarian reform, and later by Decree No. 83 of March 2, 1949, to complement certain provisions of Law No. 187 of 1948, the agricultural holdings of the landlords, including all their inventory, passed into state ownership. On the basis of Law No. 119 of June 11, 1948, for the nationalization of industrial, banking, insurance, mining, and transportation enterprises, the entire property in the industry passed from private ownership to state ownership. Thus, Romania transitioned from a capitalist-type economy to a centralized-type economy. On July 1, 1985, the fish enterprises were established, subordinated to the Central of fish production and industrialization, under the guidance and control of the Ministry of Agriculture and Food Industry - Department of food industry, having as object of activity fish farming, fishing, industrialization, capitalization of fish and other aquatic products. The enterprises were set up by taking over farms, pools, sections and fish processing workshops. The basic units of fish farming, fishing and fish industrialization were: fish production farm, section and factory of fish industrialization, which were organized on three types: big, medium and small. After the change of the political regime in 1989, since 1990 the first steps were taken towards a new economy that included privatization (Chiriac, 2016).

Romanian aquaculture underwent privatization since 1990. This study examines the period from 1990 to 2021 and explores how sustainability and resilience are ensured in the Romanian aquaculture sector.

MATERIALS AND METHODS

The research method used is qualitative research. The research was based on the analysis of documents issued by the Parliament of Romania, the Government of Romania, European Commission, European Parliament, documents from the National Agency for Fisheries and Aquaculture (NAFA), documents issued by the Romanian Court of Accounts, the European Court of Auditors, and literature in the field.

RESULTS AND DISCUSSIONS

In the international market the demand for fishery products is growing, which leads to the development of aquaculture, because it ensures faster growth than fishing. Even if for now the percentage of aquaculture products is small compared to total fishery products is "still gaining in importance" (Jolly & Clonts, 2020).

The National Strategy for Sustainable Development was adopted by the Romanian Government meeting of 9 November 2018, by Government Decision no. 877/2018. Through this strategy, Romania has created national Framework for Supporting the United Nations 2030 Agenda for Sustainable Development implementation of a set of 17 sustainable development goals proposed by it (National Agency for Fisheries and Aquaculture, 2022). To ensure that fisheries and aquaculture are environmentally, economically and socially sustainable and provide healthy food for citizens of the European Union (EU), given that fishing resources in European continental waters are in continuous decline, as a result of habitat degradation and over-exploitation through fishing, at the level of the European Union was adopted the Common Fisheries Policy in 1970 and over time has undergone successive updates. In 2013, the Council and the European Parliament reached an agreement on the new CFP in regulation (EU) No 1380/2013 applicable to all EU member states was adopted (Romanian Court of Accounts, 2021).

In the Regulation (EU) No 1380/2013 of the European Parliament and of the Council of 11 December 2013 on the Common Fisheries Policy the Commission develops strategic guidelines for the Union regarding common priorities and targets for the sustainable development of aquaculture activities, with the aim of promoting sustainability and contributing to food security and supply, economic growth, and employment. These strategic guidelines aim to improve the competitiveness of the aquaculture sector, reduce administrative burdens, encourage economic activity, enhance the quality of life in coastal and inland areas, as well as integrate aquaculture activities into the planning of maritime, coastal, and inland spaces (European Parliament, 2013).

In the Communication from the Commission of to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions- Strategic guidelines for a more sustainable and competitive EU aquaculture for the period 2021 to 2030, it is expressed the need to ensure for EU aquaculture sector a "longer-term sustainability and resilience". The new strategic guidelines establish the objectives such as building competitiveness and resilience, participation in the green transition, ensuring consumer information and social acceptance, increasing innovation and knowledge (European Commission, 2021).

In the Special Program for the Development of Aquaculture, Fisheries, and Fish Processing in the years 1989-1990 and the Five-Year Plan 1991-1995 of the Ministry of Food Industry-Fisheries and Fish Processing Centre issued in May 1989, in the last year of the communist period, is presented the report on the achievements in aquaculture, fisheries, and fish processing for the years 1985-1988, along with the plan for the years 1989, 1990, and the five-year plan for 1991-1995 (Ministry of Food Industry Fisheries and Fish Processing Centre, 1989).

The focus was on increasing fish production in inland waters, particularly to enhance production per unit area in developed drilled basins, from 1300 kg/ha to 1700 kg/ha by the year 1995. In 1985 the physical production of fish caught in inland waters realized was 73.1 Townsend tons, rising in 1988 to 91.3 Townsend tons (Table 1).

Table 1. The agricultural production- fish caught in inland waters, realized in 1985-1988 period

Indicator	UM	1985	1986	1987	1988
Value of fish commodity production	Millions of Lei	491.9	661	452.6	536.4
	Milionis of USD	112.56	153.36	111.20	134.43
Physical production	Townsend tons	73.1	81.3	80.5	91.3

Source: own calculation on the basis of data from NAFA. The value for USD was used the official exchange rate for the Romanian leu/USD dollar set by Order No.4/2003 (Governor of the National Bank of Romania, the President of the National Institute of Statistics, 2003).

The plan of centralized economy in the communist system for 1989 on physical production of fish caught was 180 Townsend tons (Table 2).

Table 2. The agricultural production-fish caught in inland waters, plan for 1989

Indicator	UM	1989
Value of fish commodity production	Millions of Lei	905
	Millions of USD	217.02
Physical production	Townsend tons	180

Source: own calculation on the basis of data from NAFA. The value for USD was used the official exchange rate for the Romanian leu/USD dollar set by Order No.4/2003 (Governor of the National Bank of Romania, the President of the National Institute of Statistics, 2003).

In 1989, employees in fish farms were about 6000 (NAFA). The main law that regulated the privatization process in Romania is: Law no.15/1990 (Parliament of Romania, 1999).

It stipulated that the state units, regardless of the subordinated body, are organized and operate in the form of autonomous administrations or commercial companies. An important aspect is the transmission of property rights on land included in the patrimony of each company (Chiriac, 2016).

The exception was the land in the public domain of the state. The right of ownership derives from the provisions of the Government Decision no. 834/1991 (Government of Romania, 1991). The companies after the procedure of the decision to obtain proof that the asset, exactly delimited, is part of their patrimony, finally obtaining a certificate of ownership attestation. Other regulatory documents in this area were as follows: Law no. 31/1990, which provided that natural persons and legal persons may associate and constitute companies for the performance of trade acts (Parliament of Romania, 1990); Law no.58/1991, which established the legal framework corresponding to the transfer of state property into private ownership of natural persons and legal persons (Parliament of Romania, 1991); Law no.77/1994, which provided that for the acquisition of shares of companies that are privatized according to Law 58/1991, employees and members of the management of these companies may be formed into associations (Parliament of Romania,1994); Law no 55/1995, which provided that the process of acceleration of privatization to be carried out by the actual transfer, free of charge, to the entitled Romanian citizens, of the shares related to the 30% quota% from the share capital of companies with state capital, as well as through the sale of shares issued by these companies (Parliament of Romania,1995).

By Government Emergency Ordinance no. 88/1997, approved by Law no. 44/1998, and amended and completed by Law no. 99/1999, was established the legal framework for the sale of shares issued by companies and owned by the state or by an authority of the local public administration, as well as for the sale of assets belonging to companies to which the state or an authority of the local public administration is a shareholder (Government of Romania, 1997; Parliament of Romania, 1998; 1999).

By the report of audit of Romanian Court of Accounts, the privatization has been considered a beneficial public policy tool because its application can lead to satisfaction of the 'general good'. It is considered that applying a privatization policy can be a solution 'in situations' in which the State is not able to solve the problem through its institutional mechanisms or whose resolution effort can produce major unsustainable economic and social imbalances over time. The principles that argue for the implementation of public policies on privatization are competition, minimizing state responsibility, reducing bureaucracy, increasing budget revenues, increasing productivity, adaptability, flexibility, innovation and performance (Romanian Court of Accounts, 2023).

According to the report, from 1991 to 2019 a total of 11,589 companies were identified that were privatized, of which valid contracts were 9,795 companies, terminated or disbanded were 9,792 companies and for 3 were not found information. The value of the 9792 contracts in the currency in which they were concluded are equivalent to 18,801,708,000 lei or 8,096,126,000 EUR or 9,141,179,000 USD. 9521 contracts (97.20% of the total contracts) were concluded in lei (94.15% in ROL, 3.05% in RON), and the contribution of the value of these contracts, in the total volume of revenues from sales contracts was 1.60% - in relation to the total value volume of sales contracts - share purchase, expressed in lei equivalent; 33.54% - in relation to the total value volume of contracts, expressed in EUR equivalent; 31.35% - in relation to the total value volume of the contracts, expressed in USD equivalent (Table 3, Table 4, Table 5).

The significant difference between these shares is explained by the accelerated devaluation of the national currency in the period 1992-2000, while the accelerated growth of inflation. 90% of the total contracts concluded in lei, the statement said, about 6,500 privatized commercial companies were concluded between 1992-2000.

201 contracts (2.05% of the total contracts) were concluded in USD (of which, 85% concluded between 1992 and 2000) and the contribution of the value of these contracts, the total volume of contract revenue was: 3.34% - reported at the total value of the contracts expressed in lei equivalent; 19.44% - in relation to the total value volume of contracts, expressed in EUR equivalent and 17.95% - reported at the total value of the contracts, expressed in USD equivalent (Table 3, Table 4, Table 5).

49 contracts (0.50% of the total contracts) were concluded in EUR (all concluded after 2000). The contribution of the value of these contracts, in the total volume of revenues from contracts, was: 74.99% - in relation to the total value volume of contracts, expressed in lei equivalent; 46.82% - in relation to the total value volume of contracts, expressed in EUR equivalent; 50.51% - in relation to the total value volume of contracts, expressed in USD equivalent. From the value point of view, the 49 contracts in EUR have a significant share in the volume of revenues obtained (Table 3, Table 4, Table 5).

Table 3. The situation of share sale contracts from 1991 to 2019 in terms of the currency denomination at the time of agreement, equating to thousands of RON

Currency of contracts	Valid contracts		Equivalent- thousands of Lei	
	Number	% total	Number	% total
ROL	9,222	94.15	1,905,340	10.13
RON	299	3.05	276,228	1.47
Subtotal Lei	9,521	97.20	2,181,568	11.60
USD	201	2.05	2,508,475	13.34
EUR	49	0.50	14.099,535	74.99
DEM	18	0.19	11900	0.07
ITL	2	0.02	80	0
CHF	1	0.01	150	0
Total	9,792	99.97	18,801,708	100
Lack of information	3	0.03		
Total contracts	9795	100		

Source: Romanian Court of Accounts (Romanian Court of Accounts, 2023)

Table 4. The situation of share sale contracts from 1991 to 2019 in terms of the currency denomination at the time of agreement, equating to thousands of EURs

Currency of contracts	Valid contracts		Equivalent- thousands EUR	
	Number	% total	Number	% total
ROL	9,222	94.15	2.638,846	32.59
RON	299	3.05	76,411	0.95
Subtotal Lei	9,521	97.20	2,715,257	33.54
USD	201	2.05	1,574,200	19.44
EUR	49	0.50	3,790,459	46.82
DEM	18	0.19	15,920	0.20
ITL	2	0.02	210	0
CHF	1	0.01	80	0
Total	9,792	99.97	8,096,126	100
Lack of information	3	0.03		
Total contracts	9795	100		

Source: Romanian Court of Accounts (Romanian Court of Accounts, 2023).

Table 5. The situation of share sale contracts from 1991 to 2019 in terms of the currency denomination at the time of agreement, equating to thousands of USDs

Currency of contracts	Valid contracts		Equivalent-thousands USD	
	Number	% total	Number	% total
ROL	9,222	94.15	2,759,230	30.18
RON	299	3.05	106,845	1.17
Subtotal Lei	9,521	97.20	2,866,075	31.35
USD	201	2.05	1,640,965	17.95
EUR	49	0.50	4,617,269	50.51
DEM	18	0.19	16,570	0.19
ITL	2	0.02	215	0
CHF	1	0.01	85	0
Total	9,792	99.97	9,141,179	100
Lack of information	3	0.03		
Total contracts	9,795	100		

Source: Romanian Court of Accounts (Romanian Court of Accounts, 2023).

In 1990, based on Government Decision No. 1353, joint-stock companies were established in the food industry. Thus, the joint-stock companies were set up in fishery and fish industrialization. In these companies, the state was the sole shareholder, and the duties of the general meeting of the shareholders were exercised by the council of state proxies. On the date of establishment of joint stock companies, the state economic units were abolished. The assets and liabilities of the disbanded state units were taken over by the established companies (Government of Romania,1990).

The Emergency Ordinance no. 198/1999 establishes the legal framework for the privatization of agricultural companies, which own agricultural land or land permanently under the gloss of water, constituted in accordance with the provisions of Law no. 15/1990. It is foreseen that the privatization of companies, on behalf of the state, to be done by the Ministry of Agriculture and Food (MAF), in collaboration with the State Property Fund (Government of Romania,1999).

In 2000, by Government Emergency Ordinance no. 296/2000 the Authority for Privatization and Administration of State Participation's (APASP) is established, subordinated to the Government, by reorganization of the State Property Fund, which is abolished. MAF received from APASP 38 companies with a fishery profile (Government of Romania, 2000).

In 2001 the Agency of State Domains (ASD) is founded on the basis of Law 268/2001 receiving the attributes of privatization. GEO 198/1999 is repealed and ASD takes over all companies from MAF. The land for which the companies obtained the certificate of ownership of the premises land, issued by the Ministry of Agriculture, Food and Forestry, with the application of the legal provisions regarding the revaluation of tangible assets and the modification of the share capital, they are part of the share capital of companies. Land for which no certificate of ownership attestation has been obtained, which are in the exploitation of companies, state property, and which are not part of the share capital of these companies, will be leased (Parliament of Romania, 2001).

ASD managed the companies until 2004, when it handed them over to the National Company for the Administration of the Fishery Fund (NCAPF). The legal situation of the 38 companies in 2004 was as follows: 4 companies were dissolved and radiated, and 34 companies were handed over to NCAPF. NCAPF was established on the basis of Law 192/200, and took over the companies on the basis of Emergency Ordinance no. 69/2004 (Parliament of Romania, 2001; Government of Romania, 2004).

Of these 34 companies, 17 were privatized, 11 were non-privatized solvent companies, and 6 non-privatized companies were insolvent (NAFA). In 2008 based on the Emergency Ordinance no 23/2008 of NACFP was abolished and, the managed companies were transferred back to ASD. The responsibility for defining and implementing the policy regarding the conservation and management of living aquatic resources existing in maritime and continental waters, in aquaculture, in processing and organizing the market of fishery products, in fishing and aquaculture structures was transferred to the National Agency for Fisheries and Aquaculture (NAFA). NAFA is the central public authority (Government of Romania, 2008).

The managed companies by ASD were to be transferred to NAFA. At inventory has been found that of the 34 companies managed by ASD, 16 was already privatized companies, the state not being a shareholder, and they were not the subject of the transfer. 1 the company was partially privatized, and was included in the transfer. 17 companies were non-privatized companies, all included in the transfer, 5 were in operation, 4 were in insolvency proceedings, 8 were radiated. In total 18 companies were transferred. (NAFA). The concession contracts of the state-owned land were concluded by both ASD and NCAFP, in 2008 being transferred to ASD. Based on Law 317/2009 The National Agency for Fisheries and Aquaculture is subrogated to the Agency for State Domains as regards the rights and obligations arising from contracts concluded by it with contracting agents in operation and in management of fisheries facilities, as well as with those who have concluded joint venture agreements or other types of contracts and will conclude additional acts in this respect. The National Agency for Fisheries and Aquaculture takes over free of charge the shares that the Agency for State Domains owns in companies with fish profile, the lands on which the fish facilities are located, as well as other land related to the fisheries owned by it, based on a handover protocol approved by order of the minister of agriculture, forests and rural development. In 2009 NAFA has taken over from ASD 18 companies and 60 concession contracts for land with a total area of 61,265.13 ha. After solving a privatization contract by ASD, NAFA received another company, a total of 19 companies taken over from ASD. In 2020 of the total 19 companies 2 were in operation, 1 were insolvent, 4 were bankrupt and 12 were radiated. Regarding the concession contracts in 2021, there remained leased land with a total area of 29,905 ha representing the land related to 241 fish farms (Table 6).

Romania, in 2021, had an area of approx. 135,000 ha of freshwater fisheries corresponding to a number of approx. 1,000 fish farms. Of the area of approx. 135,000 ha of fishing facilities, about 62% are surfaces overlapping Natura 2000 protected natural areas, approx. 83,000 ha. The number of units with an aquaculture license has varied insignificant until 2020, with the trend increasing from 2016 to 2020. Thus, if in 2016 there were registered a number of 634 licensed units, in 2020 we find a number of 735 licensed units. The increase was also due to the 34 units established through the Operational Programme for Fisheries (OPF) and Operational Programme for Fisheries and Aquaculture (OPFA). The number of active versus inactive units in the total licensed units followed the reverse trend of the latter, that is, the active units decreased and the inactive ones increased. By percentage, the number of active units decreased from 95% in 2016 to about 90% in 2019.

Table 6. Status of ongoing concession contracts in 2021

No.	County	No. of fish farm	Surface -ha
1	Alba	3	195.6600
2	Arad	2	331.3600
3	Bacau	1	142.0700
4	Bistrita Nasaud	1	134.0200
5	Brasov	2	435.4528
6	Braila	2	829.5200
7	Buzau	2	942.4500
8	Botosani	14	1,951.5650
9	Caras Severin	1	127.7300
10	Calarasi	29	2,592.4847
11	Constanta	16	8,741.8488
12	Dambovita	6	697.3600
13	Dolj	2	103.3100
14	Galati	6	1,256.2000
15	Gorj	27	1,159.1460
16	Ialomita	13	1,159.1460
17	Iasi	11	2,595.5834
18	Ilfov	49	717.9346
19	Mures	4	490.5800
20	Mehedinti	3	795.9000
21	Neamt	2	139.4500
22	Olt	1	19.0000
23	Prahova	6	803.9800
24	Satu Mare	3	655.3557
25	Sibiu	1	169.1500
26	Suceava	8	653.2120
27	Teleorman	12	653.2120
28	Timis	5	380.9500
29	Tulcea	3	1,418.8100
30	Vaslui	4	565.0840
31	Valcea	1	57.0400
32	Vrancea	1	98.5000
	Total	241	29,905.0425

Source: own calculation on the basis of data from NAFA.

The situation of the economic results of the fisheries facilities worsened even more after 1990, when their activity began to face the requirements of the functioning of the market economy and the reconsideration of production costs, as well as management inappropriate for new companies formed in the field. There were not allocated funds for the modernization of fish basins, for the restoration of dams degraded hydro technical. The operating costs have increased greatly in particular due to increase in expenditure on electricity, water, feed, force labour, while the obtained yields remained very small, below 100 kg/ha without the possibility to cover the costs of production. As a result, much of the fish ponds remained unused for fish farming being abandoned or used for other purposes and for especially for agriculture or animal husbandry. In terms of volume of marketed consumer production in recent years, it is noted that after an increase until 2017, supported by the implementation projects carried out through operational programme, followed by even a decrease in production by about 4% in 2018 compared to 2017. In 2019 there is a revival of the marketed production that is based exclusively on reaching the maturity of projects within OPF 2007-2013 (Table.7).

Table 7. Aquaculture production in the 2015-2020 period in Romania

Year	Production (t)
2015	11016
2016	12472
2017	12798
2018	12298
2019	15124
2020	12150

Source: own calculation on the basis of data from NAFA.

As shown in the Special Report 25/2023 of European Court of Auditors, the Scientific, Technical and Economic Committee for Fisheries reported that in 2020 the Romanian EBIT -earnings before interest and taxes, known as "operating profit"- turned negative (European Court of Auditors,2023).

According to the Multi-annual national strategic plan for aquaculture 2022-2030, on the high cost of ensuring aquaculture stocks a given justification has been that a number of environmental and climate issues affecting the sector generates significant economic losses, being it is necessary to establish lines of assistance, which ensure or cover these losses so that they do not endanger sustainability, especially economic. There are no effective tools insurance to cover aquaculture activity (National Agency for Fisheries and Aquaculture, 2022).

Aquaculture is facing labor shortages in mainly due to the lack of attractiveness of aquaculture activity (Table 8).

Table 8. Employees in aquaculture between 2015-2020 in Romania

Year	Number of employees
2015	2047
2016	2035
2017	2364
2018	2065
2019	2303
2020	2332

Source: own calculation on the basis of data from Multi-annual national strategic plan for aquaculture 2022-2030, (National Agency for Fisheries and Aquaculture, 2022)

The staff employed in aquaculture did not very significantly in the period 2015-2020, however in 2017 there is an increase of 15.48% compared to 2015, in 2018, there was a decrease after which in 2019 there is a recovery that is maintained for 2020. Total number of employees reported by aquaculture units for 2020 it is 2,332, of which 451 women and 1,881 men, the share of women being 19.33%.

Compared to 1989, when the number of employees in fish farms was about 6000 (NAFA), the number of employees in 2020 decreased with 61.13%.

Climate change and competition for natural resources are adding pressure additional to the aquatic environment.

Most fish farms have a relatively long history and have fit well into the natural landscape, playing an essential role by creating wet areas, important in the protection and conservation of biodiversity, in strengthening ecological balances, in taking over excess water and nutrients from intensive agriculture in providing and maintaining large areas that function as biological filters.

CONCLUSIONS

The history of Romania's economy has been marked by significant transitions and the nationalization and privatization of the fishing sector is no exception.

In the post communist period, in the Romanian aquaculture there was a fragmented privatization, in the sense that not in all cases all the goods that form a whole of the fishing farms were transferred. In cases where the entire fish farm has been transferred and however not all remained in activity, in all conditions created by privatization, this was not the sufficient condition for durability either.

Privatization was generally perceived as a beneficial tool for economic and social development.

Currently, under the pressure of challenges such as climate change, biodiversity conservation and protection, competition for natural resources, the fishing sector is facing new challenges.

Following the privatization of aquaculture in Romania there is a partial sustainability, but opened new opportunities in this sector.

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CAROTENOIDS IN SALMONID AQUAFEEDS: A REVIEW OF USE AND EFFECTS

Tudor PĂPUC[1], Daniel COCAN[1], Radu CONSTANTINESCU[1], Camelia RĂDUCU[1], Daniela LADOȘI[1], Ioan LADOȘI[1, 2], Călin LAȚIU[1], Paul UIUIU[1], George MUNTEAN[1], Vioara MIREȘAN[1]

[1]Faculty of Animal Science and Biotechnologies, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Mănăștur St., Cluj-Napoca, Romania
[2]Romanian Pork Producers Association, Bucharest, Romania

Corresponding author email: vioara.miresan@usamvcluj.ro

Abstract

This review aims to analyse the use and effects of carotenoids in salmonid aquafeeds. Species of the family Salmonidae are of great interest for aquaculture due to their many properties, like fast growth and nutritional quality. Some species from this family are among the most farmed fish in the world. Thus, there are many studies on different farming technologies, fish biology, market dynamics and others. However, there are always new challenges in the aquaculture of salmonids, being a continuously improving activity. Consumers are becoming more considerate about what they choose to buy and consume. Thus, some aspects emerge as great influencers in the buying decisions of consumers, such as animal welfare, in addition to usual influencing factors, like product freshness and colour. A category of ingredients used in the salmonid aquaculture are carotenoids. They are pigments supplemented to diets for colouring the fish flesh, making it more appealing to consumers. However, some other benefits can also be observed, especially on the health of fishes. Be it synthetic or natural, carotenoids have become a largely used ingredient in salmonid aquafeeds.

Key words*: carotenoids, physiological effects, salmonids, synthetic.*

INTRODUCTION

Aquaculture is one of the fast growing sectors of the farming industry, supplying qualitative and safe food for consumers. The growth of the sector is especially influenced by the limitations on wild fisheries and by the continuous growth of the world population. According to FAO (2023), salmonids have 18% of the value of the total traded aquatic products in 2020, this being the case since 2013, with demand for salmon and trout exceeding that for other fish. The exports of salmon and trout amounted to 18.4% of total exported aquatic products in 2020, with a value of 27.6 billion USD (FAO, 2023). This was possible due to the high nutritional quality of the fish and favourable organoleptic properties, but also because of the advanced technology, logistical capabilities and marketing campaigns of the salmonid farming sub-sector.

High quality products are sought by consumers. Usually, the colour of the fish product is the first perceived immediate indicator of product quality (Shahidi & Brown, 1998), many times deciding the rejection or purchase of the product. For example, the flesh colour is considered the second most important quality indicator in salmonids, after freshness (García-Chavarría & Lara-Flores, 2013). Thus, the use of carotenoids as pigments in aquaculture is fairly common. Wild salmonids have access to feed that naturally contains carotenoids, like crustaceans, bioaccumulating carotenoids in their tissue, colouring it depending on the predominant pigment of the feed. Farmed salmonids are usually administered commercial feed, without pigments, presenting a white flesh, or only slightly coloured. The consumers often perceive the coloured flesh as a sign of health, and ultimately, as an indicator of a better taste in fish. In addition to direct monetary benefits, other benefits on fish health and growth have been observed when administering carotenoid supplemented feeds (García-Chavarría & Lara-Flores, 2013; Nakano & Wiegertjes, 2020). In salmonids, carotenoids are precursors of vitamin A, improve reproduction performances, have antioxidant functions and help the immune system (Garcia-Chavarria & Lara-Flores, 2013), in addition to providing muscular tissue colour. Nowadays, the use of carotenoids in aqua feeds has three main directions: the first is the coloration of the flesh of farmed fish, making it more appealing to consumers; the second is for the health and welfare of farmed fish; and the third is for the external appearance of ornamental fish.

Synthetic carotenoids are mostly used in aquaculture, due to high production compared to natural carotenoids (Li et al., 2011; Koller et al., 2014). However, natural carotenoids are much more effective regarding health benefits, and their market has the potential to grow, especially as consumers are better informed and aware of the after effects of synthetic products, but also of health and environmental impacts (Kaur & Shah, 2017; Novoveská et al., 2019). This review aims to provide a general description of carotenoids and address the effects of some widely used carotenoids in salmonid aquaculture, referring to health benefits, bioproductive performances, and flesh colour of fish.

MATERIALS AND METHODS

A comprehensive literature search was conducted to collect relevant studies and articles on carotenoids in salmonid aquafeeds. The following databases were utilized for the search: PubMed, Google Scholar, Web of Science, Scopus, ScienceDirect. The search strategy incorporated a combination of keywords related to the topic. The primary keywords used included "carotenoids", "salmonid", "aquafeeds", "astaxanthin", "canthaxanthin", "β-carotene", "lycopene".

RESULTS AND DISCUSSIONS

Carotenoid structure, formation mechanisms and sources

Carotenoids are pigments produced by plants, algae, fungi or bacteria. They produce the yellow, orange or red coloration in plants, as well as in some animal organisms. Due to the colours produced, numerous scientists have started to study these pigments at the beginning of the 19th century (Bendich & Olson, 1989). Thus, by 1971, 273 distinct carotenoids had been identified (Isler et al., 1971), the number reaching 563 in 1987 (Straub & Pfander, 1987). Presently, more than 750 carotenoids have been identified (Nakano & Wiegertjes, 2020), with new examples discovered and described annually.

Carotenoids are liposoluble terpenoid compounds, with 40 carbon atoms in the base structure and many conjugated double bonds. Polyenes present conjugation processes that result in special optical properties (Hermann et al., 1973; Götze, 2019). The number of carbon atoms can vary due to various processes. The base structure can be modified by cyclization at one or both ends of the molecule, by hydrogenation, or by the addition of oxygen groups (Britton, 1995). Thus, from the perspective of the chemical structure, there are two distinguishable groups: carotenes, which are carotenoid hydrocarbons that can be cyclized at one or both ends of the molecule, and xanthophylls, which contain oxygen groups.

Two isopropene isomers are required for carotenoid biosynthesis, namely isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). The two isomers can be produced by two pathways, in different cellular compartments of plants (Vranova et al., 2012). The first pathway, of mevalonic acid (MVA), was discovered in the 60s in the biosynthesis of cholesterol from isoprenoids (Katsuki & Bloch, 1967; Lynen, 1967). This pathway involves the formation of IPP, which is then converted to DMAPP by the activity of IPP isomerase. IPP is formed by the decarboxylation of the compound mevalonate diphosphate, which, in turn, is obtained from mevalonic acid (MVA), through various reactions. Acetyl coenzyme A, formed from the synthesis of free acetates, the oxidation of sugars or the condensation of pyruvic acid or fatty acids, is the precursor of MVA (Merhan, 2017). The process takes place in the cytosol. By condensing IPP and DMAPP, geranyl diphosphate (previously named geranyl pyrophosphate) is obtained, and later, by condensing with IPP, farnesyl diphosphate (previously named farnesyl pyrophosphate) is obtained. The condensation of IPP and farnesyl diphosphate produces geranylgeranyl diphosphate (previously named geranylgeranyl pyrophosphate), which is the main precursor of carotenoids. So, carotenoids are formed by the condensation of two molecules of geranylgeranyl diphosphate (Iriti & Faoro, 2009; Nisar et al., 2015).

The second pathway was discovered in the 90s (Rohmer et al., 1993; Rohmer et al., 1996; Arigoni et al., 1997; Rohmer et al., 1999). Methylerythritol 4-phosphate (MEP) involves the simultaneous production of the two compounds (IPP and DMAPP) in plastids (Pulido et al., 2012), starting from the condensation of glyceraldehyde 3-phosphate and pyruvate. Thus, after obtaining geranylgeranyl diphosphate, an intermediate compound called prephytoene diphosphate is produced through its condensation, which then forms the first carotenoid, called phytoene (Altman et al., 1972; Merhan, 2017). By desaturation, phytofluene, the second carotenoid, is obtained. Through a chain of dehydrogenations, lycopene results from phytofluen, which has 13 double bonds. β-carotene, α-carotene and γ-carotene result from lycopene, from which other carotenoids are formed through various reactions. The MAV pathway is only present in plants, but the MEP pathway has also been discovered in protozoa from the phylum Apicomplexa and numerous bacteria, many of which pathogenic (Phillips et al., 2008).

All vegetables and fruits that have colour contain carotenoids (Mezzomo & Ferreira 2016). β-carotene is the predominant carotenoid in nature (Olson & Krinsky, 1995), being found in large concentrations in pumpkins, carrots, nuts, and other vegetables and fruits (Arima & Rodríguez-Amaya, 1990; Godoy & Rodríguez-Amaya, 1998). Lycopene is especially found in red fruits and vegetables, such as tomatoes or watermelons (Park et al., 2020). Lutein and zeaxanthin are found in green leafy vegetables, especially spinach, parsley or broccoli (Chenard et al., 2005; Bunea et al., 2008). Some species of microalgae, bacteria or yeasts produce astaxanthin (Yuan et al., 2002). As primary producers, algae end up in the diet of primary consumers, generally crustaceans, molluscs and even fish. Along the food chain, the astaxanthin produced by the algae is propagated into the body of secondary and tertiary consumers. Thus, the meat of some species of salmon becomes reddish-pink coloured, the lobster has a red colour, and the flamingo bird that has access to molluscs and crustaceans has pink plumage (Fox, 1955).

Carotenoids from natural sources in salmonid aquaculture

Wild salmonids present coloured flesh due to the dietary inclusion of some carotenoid-rich feeds, such as crustaceans or algae. Thus, salmonid farms have tried and sometimes succeeded to obtain the natural coloured flesh through the supplementation of commercial feeds with carotenoids. A distinctive orange-red or pink colour was the initial purpose of using carotenoids in salmonid feeds. Usually, colour is measured visually by using the DSM Salmo fan cards, the Roche colour scale or colorimetrically, with a colorimeter. According to Smith et al. (1992), an optimal colour is correspondent to the values of 13-14 on the Roche scale. The most commonly used synthetic carotenoids in salmonid aquaculture are astaxanthin (pink-red color) and canthaxanthin (orange-red colour) (Akhtar et al., 1999), astaxanthin being the major carotenoid accumulated in the body of salmonids (de Carvalho & Caramujo, 2017).

The higher production price of synthetic carotenoids (Regulation 2018/848 of the European Parliament and of the Council) has made a venue for the use of carotenoids derived from natural sources. Low-cost sources of carotenoids have been studied as dietary supplements for salmonids, such as crustaceans, algae, different vegetables, and others. As salmonids are carnivorous, the supplementation of carotenoids from vegetal sources must be low, to avoid adverse effects on bioproductive performances.

Shekarabi et al. (2020) determined that a dietary supplementation with less than 1% black mulberry (*Morus nigra*) juice powder can significantly enhance the colour of rainbow trout, especially the yellowness and redness of fillet. When using cyanobacteria in the diet of rainbow trout (*Oncorhynchuss mykiss*), Pulcini et al. (2021) obtained an undesirable colour, but the authors noticed a pink pigmentation when the diet had *Procambarus clarkii* meal included. Red pepper can also produce a marketable colour of rainbow trout (Diler et al., 2005; Büyükçapar et al., 2007; Yanar et al., 2016). For a farmed trout to be considered marketable, it should contain minimum 3-4 µg/g total carotenoids (Torrissen et al., 1989). Table 1 presents the effects of different sources of natural carotenoids on the colour of rainbow trout. Although the results presented in the table show an improvement in the colour of rainbow trout, it is important to mention that most experiments had a positive control with synthetic astaxanthin, which performed better in terms of colour and total carotenoid content in the majority of cases.

Table 1. Effects regarding colour of different natural carotenoid sources included in the diets of salmonids

Salmonid species	Dietary carotenoid source	Administered quantity	Trial duration	Effect	Source
Oncorhynchuss mykiss	*Arthrospira platensis*	0, 2.5, 5, 7.5, 10% S. platensis per kg of feed	10 weeks	The 10% S. platensis diet produced the highest carotenoid content in skin and flesh. Authors recommend an addition of 7.5% S. platensis for pigmentation, without effects on growth.	Teimouri et al. (2013)
Oncorhynchuss mykiss	Bee pollen extract	25 and 50 mg carotenoids content per kg of feed	8 weeks	Dietary intake and growth performance were not affected. Commercial scale colour was not obtained.	Sánchez et al. (2019)
Oncorhynchuss mykiss	Red pepper	4.4, 6.6, 8.8% in feed	60 days	Desired coloration obtained. Negative effects on growth in the 2 higher dose treatments	Büyükçapar et al. (2007)
Oncorhynchuss mykiss	Marigold flower	1.2, 2.4, 3.6% in feed	60 days	Yellow colour obtained. Negative effects on growth in the 2 higher dose treatments	Büyükçapar et al. (2007)
Oncorhynchuss mykiss	*Haematococcus pluvialis*	20, 40, 60, 80 mg carotenoids per kg of feed	100 days	The treatments with 20, 60 and 80 mg carotenoids per kg of feed achieved marketable colour.	Sommer et al. (1992)
Oncorhynchuss mykiss	*Chaceon quinquedens*	20% meal; 0.1 and 0.2 mg carotenoid per g of feed	4-23 weeks	The 0.2 mg carotenoid per g of feed produced highly pigmented fish.	Kuo et al. (1976)
Oncorhynchuss mykiss	Black mulberry (*Morus nigra*)	0.25, 0.5, 0.75% in feed	8 weeks	Increased fillet yellowness and redness. Better growth performance parameters.	Shekarabi et al. (2020)
Oncorhynchuss mykiss	Hot or sweet red pepper	0.5, 2, 4.4% dried peppers in the feed	80 days	The 4.4% diet for both peppers produced an adequate accumulation of carotenoids in the flesh and the desired colour.	Yanar et al. (2016)
Oncorhynchuss mykiss	Shrimp waste meal and red pepper meal	60 mg per kg of feed	100 days	Both sources produced a desirable colour, with panellists perceiving a better taste.	Diler & Gokoglu (2004)
Salmo salar	*Calanus* spp. oil	20, 60 mg astaxanthin per kg of feed	181 days	Marketable colouration was not obtained.	Hynes et al. (2009)
Oncorhynchuss mykiss	*Haematococcus pluvialis*	75 mg astaxanthin per kg of feed	6 weeks	No differences in colour between fish fed treatment diets and fish fed control diet with synthetic astaxanthin.	Choubert et al. (2006)
Oncorhynchuss mykiss	Red pepper and shrimp by-products	30, 60 ppm	90 days	The red pepper treatments produced a colour close to the optimal one according to the Roche color scale.	Diler et al (2005)

In some cases, the growth of salmonids does not suffer with the addition of carotenoid sources in the feed. Growth did not very among Atlantic salmon (*Salmo salar*) groups fed diets containing synthetic astaxanthin and *Calanus* oil (Hynes et al., 2009). Teimouri et al. (2013)

did not notice a significant change in the final weight, weight gain, specific growth rate and FCR of rainbow trout fed diets with *Arthrospira platensis* compared to the control. However, the same authors noted that the diet with 10% *A. platensis* addition did produce a significantly higher final weight ($p<0.05$) than the treatment with astaxanthin. Furthermore, the FCR was also reduced to 1.03 in the treatment with 10% *A. platensis*, compared to 1.13 in the control and 1.1 in the astaxanthin treatment (Teimouri et al., 2013). Sanchez et al. (2019) did not determine significant changes in growth performances when adding bee pollen extract in the diet of rainbow trout. Even though not significant, they mention that an addition of bee pollen extract providing 50 mg of carotenoids per kg of feed does provide better results in terms of final weight, total weight gain and specific growth rate for rainbow trout.

According to Kaleshtari et al. (2019), the replacement of synthetic astaxanthin with carrot powder increased the weight gain and decreased the FCR in rainbow trout, at a 75% replacement, the differences being significant ($p<0.05$). The maximum concentration used was 0.1 g/kg of feed.

Thus, some studies observe the effect of different natural supplements rich in carotenoids on salmonids, while others observe the effect of a specific carotenoid, be it natural or synthetic. Overall, the main studied carotenoids are synthetic and natural astaxanthin, synthetic canthaxanthin, natural β-carotene and lycopene. When using natural supplements, in addition to the main carotenoid, other carotenoids are also present, such as lutein, zeaxanthin, α-carotene, and others, which might produce synergistic effects.

Astaxanthin

Astaxanthin, a fat-soluble xantophyll, is the most used carotenoid in salmonid feeds. Nowadays, it is well known that astaxanthin, natural or synthetic, induces the pink-red coloration of fish flesh, bioaccumulating in the tissues. The European Commission classifies natural astaxanthin as a food dye (Roche, 1987). It can be found in algae, yeasts and crustaceans (Abati et al., 2014). The main source of natural astaxanthin is the microalga *Haematoccocus pluvialis*, with 3-5% astaxanthin in dry weight (Orosa et al., 2005; Oslan et al., 2021). Synthetic astaxanthin is mass produced, relatively cost effective, and utilized in many salmonid feeds, while natural astaxanthin is more expensive and more difficult to obtain. However, there are some other differences between natural and synthetic astaxanthin (Villaro et al., 2023), mainly that natural astaxanthin is more stable and more bioavailable, being 20 times more efficient in eliminating free radicals than synthetic astaxanthin (Capelli et al., 2013; Capelli et al., 2019). Structurally, astaxanthin has three optical isomers with all trans configuration of the chain, and the distribution of these isomers differs between natural and synthetic astaxanthin (Moretti et al., 2006).

Astaxanthin acts against reactive oxygen species, it has anti-lipid peroxidation properties, it maintains membrane integrity, helps in reproduction and has roles in the immune system, improving the general health status of fish (Shastak & Pelletier, 2023). It is one of the most used carotenoids in laboratory experiments on salmonids, with many studies highlighting its positive effects on their growth performances and health status. However, flesh colouring remains an important effect of astaxanthin, being the original factor of interest studied. Some studies concentrate on the pigmentation of fish fed dietary astaxanthin under different temperatures, for different life stages of the fish, with different concentrations and sources, and in different time periods (Storebakken & No, 1992; Kurnia et al., 2015; Nickell & Bromage, 1998; Ytrestøyl et al., 2005; Choubert et al., 2006; Zhang et al., 2012). The general consensus is that astaxanthin has great pigmentation properties under various conditions, when the feeding is frequent (Wathne et al., 1998).

In addition, recent studies focus more on the health benefits that the carotenoid has on salmonids. Zhao et al. (2022), in a study comparing the effects of natural and synthetic astaxanthin administered to rainbow trout, determined that synthetic astaxanthin produced the best growth, best pigmentation, best flesh quality, and the trout had the highest level of aspartic and glutamic acids (umami amino acids), while natural astaxanthin achieved the best immunity and antioxidant properties. Compared to rainbow trout fed a negative control, without astaxanthin, all the fish fed experimental diets had a better pigmentation and a higher astaxanthin content in the muscle, a higher final body weight, weight gain rate and specific growth ratio ($p<0.05$). The level of synthetic astaxanthin was 0.1% in the feed, while natural astaxanthin was supplemented to the feed in a higher percentage (1%). The trial lasted for 56 days, and commercial sized rainbow trout was used (251.04±0.91 g). The intestinal morphology of rainbow trout was also improved by astaxanthin diets.

In a trial conducted for 12 weeks on rainbow trout weighing 309 g (initial weight), the fish were fed a normal diet and an experimental diet with a supplementation of 100 mg of synthetic astaxanthin per kg of feed. The study observed that the synthetic carotenoid modulated the effect of the oxidative pentose phosphate pathway (ox-PPP), and positively influenced hepatic health (Kalinowski et al., 2023). In a previous study, the same collective of authors observed that, under stress conditions (hyperoxya), rainbow trout (309±10 g initial weight) fed a diet supplemented with 100 mg synthetic astaxanthin per kg, performed better in terms of reducing oxidative stress. The dietary astaxanthin is believed to have increased glutathione reductase activity. The trial was conducted for 13 weeks, the hyperoxya being induced for 8 hours per day in the last week of experiment (Kalinowski et al., 2019).

In a study where rainbow trout were challenged with paraquat, the authors observed that synthetic astaxanthin improved growth rates and reduced oxidative stress (Hassanzadeh et al., 2022). The upregulation of antioxidant related genes was also observed in rainbow trout fed with astaxanthin-supplemented diets challenged with diazinon, together with better growth performances (Shabanzadeh et al., 2023).

In Atlantic salmon fry (1.75 g weight), dietary synthetic astaxanthin supplementation between 36 and 190 mg kg^{-1} diet significantly increased growth ($p<0.05$), improved survival rate and lipid levels (Christiansen & Torrissen, 1996). Astaxanthin also functions as a vitamin A precursor in Atlantic salmon (Christiansen et al., 1995; Christiansen & Torrissen, 1996), and it is more absorbable when cholesterol (2%) is also supplemented in the diet (Chimsung et al., 2014). A different study concluded that the addition of synthetic astaxanthin and canthaxanthin in the diets of Atlantic salmon does not produce significant differences in growth performance and feed utilization (Baker et al., 2002). The fish used in the trial had an initial weight of 408 g. Another finding of the same study was that the deposition of the pigment in the flesh did not present significant differences, being related linearly to the carotenoid concentration of the feed (Baker et al., 2002).

Canthaxanthin

Canthaxanthin was a widely used keto-carotenoid in aquaculture, with potent antioxidant properties. However, it lost ground to astaxanthin, the latter producing better results. Canthaxanthin occurs naturally in bacteria, algae, and mushrooms and it can also be chemically synthetized. Its main roles involve free radical scavenging, immune-modulating activities and it also helps gap junction communication (Esatbeyoglu & Rimbach, 2017). It produces an orange-reddish colour, and it is approved for use as a feed additive for trout, salmon and other farmed animals in EU, with the specific upper limit of 80 mg kg^{-1} in the feed for salmonids, either alone or with other carotenoids, or 100 mg kg^{-1} when it is administered as a mixture with astaxanthin (European Commission, 2002).

Canthaxanthin is sometimes used in complementarity to astaxanthin to obtain a desirable flesh colour in salmonids. According to Garner et al. (2010), when used in a mixture, astaxanthin and canthaxanthin are better deposited in the flesh of Chinook salmon (*Oncrohynchus tshawytscha*) than when used separately. However, other synergistic effects are questionable. For example, when the oxidative stress biomarkers were studied in the liver and kidney of rainbow trout fed astaxanthin and canthaxanthin mixed and separate diets for 8 weeks, no synergistic effects were observed (Elia et al., 2019). In contrast, Choubert (2010) observed that an increase in the canthaxanthin ratio decreased the total carotenoid retention in the muscle, concluding that diets supplemented only with astaxanthin would provide more reliable results, and that a mix of the two carotenoids may provide non-beneficial results regarding carotenoid retention in the muscle and colour. In a trial on rainbow trout and Atlantic salmon using diets supplemented with astaxanthin and canthaxanthin, Page & Davies (2006) observed that rainbow trout has higher apparent digestibility coefficients and pigment retention efficiency than Atlantic salmon. They also observed that astaxanthin was deposited in higher concentrations in rainbow trout, while canthaxanthin was deposited better in Atlantic salmon, suggesting that carotenoid deposition is dependent on species. The higher deposition of astaxanthin compared to canthaxanthin in rainbow trout was also noted by Tzanova et al. (2022), who analyzed the content of the two xanthophylls in the gonads and liver of the fish. Toan et al. (2021) observed that canthaxanthin- and α-tocopherol-loaded liposomes included in the diet of rainbow trout produced a significantly better growth, and also a more intense colour after two months of trial. In addition, they also observed that, after three months, the difference in colour faded, suggesting a saturation in canthaxanthin accumulation.

Overall, canthaxanthin is mainly used in salmonid feeds together with astaxanthin. When used alone, or in combination with astaxanthin, it generally produces good results in terms of oxidative stress reduction and flesh coloration, but astaxanthin is often preferred.

β-carotene

β-carotene is a carotenoid found in plants, algae, bacteria and some fungi. It is widely found in nature, being one of the most stable carotenoids. Fish are presented with many β-carotene sources in the waters and have a diverse set of β-carotene oxygenases, which help cleave carotenoids for further synthesis of vitamin A, and possibly retinoic acid. Thus, Atlantic salmon has 5 β-carotene oxygenases, while mammals have only 2 (Helgeland et al., 2014).

Amar et al. (2000) note that β-carotene does not influence the growth of rainbow trout, but some immune response parameters are improved, namely total plasma immunoglobulin and serum complement activity. The 45 g fish were administered feeds with 40, 200 and 400 mg β-carotene/kg of feed for 12 weeks. Contradictory results were obtained by Kelestemur & Çoban (2015) in a similar experiment, but with lower doses of β-carotene in the fish body, 30 and 70 mg β-carotene/kg of feed, where the authors obtained a significantly better ($p<0.05$) weight gain, survival rate and specific growth rate for 60 g rainbow trout, and a significantly lower FCR after 12 weeks of trial. Caspian brown trout (*Salmo caspius*) presented significantly better serum lysozyme activity when fed a diet with 100 mg carrot powder supplemented per kg (Farahani et al., 2021).

Ghtobi et al. (2011) conducted a trial to compare the effects of β-carotene and astaxanthin in the diet of 196 g rainbow trout. The supplementations consisted of 50 and 80 ppm for each carotenoid. Growth parameters and food utilization parameters were not affected after the 8-week trial. Dietary β-carotene produced a lower score on the SalmoFan scale, but none of the treatments produced a colour acceptable for commercializing the fish. The authors mentioned that the astaxanthin supplement was 6 times more expensive than the β-carotene supplement.

Thus, although β-carotene is cheaper than astaxanthin and has similar effects, the latter is preferred for better pigmentation properties.

Lycopene

Lycopene is the main carotenoid in tomatoes and other red fruits and vegetables. It relieves oxidative stress and helps the immune system in some fish species (Dawood et al., 2020).

Yonar (2012) observed that rainbow trout (168±24 g) fed with a diet of 10 mg lycopene per kg of fish bodyweight performed better when challenged with oxytetracycline in terms of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase activity (GSH-Px), also increasing the glutathione level. The trials were conducted for 14 days.

Sahin et al. (2014a) also observed a significant increase ($p<0.001$) in SOD, CAT and GSH-Px activities in rainbow trout (18±0.5 g) fed diets supplemented with 200 and 400 mg/kg of feed, under low and high stocking densities. The authors noted a significant increase in the levels of nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and heme-oxygenase 1(HO-1) in the fish muscle, and a significant decrease in malondialdehyde and heat shock protein 70 in the muscle, concluding with the beneficial effects of lycopene against lipid peroxidation and oxidative stress. However, in a similar study conducted by the same authors, a significant decrease in growth performance parameters was observed for rainbow trout fed lycopene-supplemented diets in high stocking densities (Sahin et al., 2014b).

Wang et al. (2019), observed that rainbow trout (29.4±0.1 g) fed diets supplemented with lycopene improved significantly ($p<0.05$) the FCR and protein efficiency ratio, and also improved the antioxidant capacity. As a downside, the authors observed that intestinal amylase activity suffered when lycopene was added. In a similar study, Zhang et al. (2019) observed that the addition of lycopene decreased plasma MDA levels, and improved overall growth. Sheikhzadeh (2013) also noted that carotenoids from red peppers and tomatoes, including lycopene, improve growth performance and lysozyme activity of rainbow trout.

Thus, lycopene has beneficial effects on the health of fish, but the colouring properties are deficient.

CONCLUSIONS

Carotenoids are becoming a very important ingredient in salmonid aquafeeds, not only for the pigmentation of the flesh, but also for the overall welfare of the fish. In most cases, salmonids fed diets supplemented with carotenoids have a better growth performance. Synthetic astaxanthin remains the most widely used carotenoid, due to its absorption and colouration properties, and ease of production. However, natural carotenoids are gaining more interest, as they are much more efficient in improving the health status of the fish, have a favourable market perspective from consumers and may incur a lower cost. Currently, the main disadvantage of carotenoids from natural sources is the lesser effect on flesh colour compared to synthetic astaxanthin and, in some cases, the higher price of production.

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EFFECT OF DIETARY PROTEIN LEVEL ON GROWTH, HEMATOLOGICAL PROFILE, AND MEAT BIOCHEMICAL COMPOSITION OF JUVENILE EUROPEAN CATFISH

Săndița PLĂCINTĂ[1], Mirela CREȚU[1, 3], Marian Tiberiu COADĂ[1, 2], Floricel Maricel DIMA[3, 4], Elena SÎRBU[3], Maria Cristina CHIOVEANU[3]

[1]Faculty of Food Science and Engineering, "Dunărea de Jos" University of Galați, 47 Domnească Street, 800008 Galați, Romania
[2]Cross-Border Faculty, "Dunărea de Jos" University of Galați, 800008 Galați, Romania
[3]Institute for Research and Development in Aquatic Ecology, Fishing and Aquaculture, 54 Portului Street, 800211, Galati, Romania
[4]Faculty of Engineering and Agronomy of Braila, "Dunarea de Jos" University of Galati, 111 Domnească Street, 800008, Galaţi, Romania

Corresponding author email: dima.floricel.maricel@asas-icdeapa.ro

Abstract

This study aimed to evaluate the influence of the protein level from fish feed on the growth dynamics, hematological profile, and meat biochemical composition of juvenile European catfish, Silurus glanis (Linnaeus, 1758). In this context, 508 juvenile European catfish, with an average weight of 33±1.80 g/fish, were reared in a recirculating aquaculture system (RAS). Fish were fed with two different proteins: VE 41- fish feed with 41% protein content, and VE 50- fish feed with 50% protein content. After 35 days, the results regarding the growth performance revealed a better feed conversion ratio (FCR-1.03 g/g) and specific growth rate (SGR - 2.02 %/day) in the VE50P. Also, fish meat's hematological profile and biochemical composition showed a similar trend. In conclusion, the fed protein content plays an essential role in the digestibility and efficiency of nutrient utilization, on the welfare and nutritional quality of the final product.

***Key words**: biochemical composition, European catfish, growth.*

INTRODUCTION

Silurus glanis L. is considered important in aquaculture due to its growth abilities and high nutritional values, such as: high growth rate, white meat with consistency, few bones, pleasant flavor also high feed utilization efficiency (Linhart et al., 2002; Jankowska et al., 2006; Adamek et al., 2015). Also, *S. glanis* has good abilities to adapt to different environmental conditions, and it is suitable for growing in recirculating aquaculture systems (RAS) (Bud et al., 2004). The aquaculture production in ten European countries registered an increase from 600 tonnes in 1993 to 2,000 tonnes in 2002, as reported by Copp et al. (2009), and Linhart et al. (2002).

Several researchers have studied the dietary protein requirements for young catfish in various rearing conditions. Specifically, these studies indicate that for the growing of catfish in troughs, the optimal range for food proteins is between 43% and 45% (Meske, 1987). Similarly, in fishponds, the recommended protein levels range from 40% to 42%, as observed (Krasznai et al., 1980), while for catfish raised in cages and silos, the suggested protein content varies from 40% to 45% (Bogut et al., 2002). Regarding the research on catfish dietary requirements for lipids, there is limited information, with partial investigations conducted by Bogut et al. (2002).

The composition, quality, and quantity of components in fish feed have a crucial role in influencing the growth and survival of fish, affecting both feed costs and meat quality. These factors ultimately have a significant impact on the consumer's experience regarding the fish product. The nutritional profile of fish varies depending on the species, the stage of development of the species, the environmental conditions, etc. (NRC, 2011).

Protein is an important nutrient in fish feeds (Wilson, 2002) necessary for the growth, maintenance as well as production of hormones, enzymes, and antibodies required for many vital processes of fish. Also, lipids are considered an important source of energy and essential fatty acids for fish (Mir et al., 2020; Chen et al., 2023).

Numerous studies have consistently indicated that the optimal dietary protein requirements for young catfish vary depending on the rearing environment. Therefore, in this context, this study aimed to evaluate the influence of the fed protein level on the growth dynamics, hematological profile, and meat biochemical composition of juvenile European catfish, *Silurus glanis* (Linnaeus, 1758), reared in the conditions of a recirculating aquaculture system (RAS).

MATERIALS AND METHODS

Experimental design. The present study was conducted, for six weeks, in the recirculating aquaculture systems of the Faculty of Food Science and Engineering, Dunărea de Jos, University of Galați, România.

Two experimental variants were created: $V1_{50P}$, where fish were fed with commercial feed with a protein content of 50 % crude protein, and $V2_{41P}$ where fish were fed with commercial feed with a protein content of 41 % crude protein (Table 1). The feeding intensity during the period experimental was 3% per body weight per day(BW/day), while the feeding frequency was four meals/day. The photoperiod was maintained on a light/dark (12:12-h) condition during the feeding trial.

In this context, 508 juvenile European catfish with an average individual weight of 33 ± 1.80 g/fish were distributed in the rearing units of the recirculating system. The trial was conducted in duplicate. The constructive description of the RAS system was previously described by Vasilean et al. (2008), Plăcintă et al. (2012).

Table 1. Proximate composition of diets used in the experiment

Composition	UM	Nutra Mp-T	Classic 1P
Crude protein	%	50.0	41.0
Crude fats	%	20.0	12.0
Crude cellulose	%	0.7	3.0
Ash	%	0.7	6.5
Phosphorus	%	1.3	0.9
Digestible energy	MJ/kg	19.7	14.2
Vitamin A	UI/kg	12000	10000
Vitamin D_3	UI/kg	1800	1250
VitaminaE	mg	180	150
Vitamin C	mg	500	75
Lysine	%	-	2.4
Methionine	%	-	0.75
Cystine	%	-	0.6

Water quality parameters. Throughout the experiment, water quality parameters (temperature, pH, and dissolved oxygen) were monitored daily. The nitrogen compounds were measured twice/week with the help of Spectoquant Nova 400 and Merck kits. Mean water temperature, dissolved oxygen, and pH, based on daily measurements, over the experimental periods were: 25.6 ± 1.9 °C, 5.9 ± 1.7 mg/L, respectively 7.58 ± 0.54 unit pH. The nitrogen compounds ($N\text{-}NO_3^-$, $N\text{-}NO_2^-$, $N\text{-}NH_4$) were: 20.16 ± 6.56 mg/L, 0.04 ± 0.01 mg/L, respectively 13.04 ± 1.16 mg/L. These values fall within the established normal range for the optimal rearing conditions of *Silurus glanis*, as indicated by a study conducted by Copp et al., 2009.

Fish Growth Performance. At the end of the experimental period, all the fish were weighed, and the following growth parameters were calculated: fish survival, specific growth rate (SGR), protein efficiency ratio (FCR) protein efficiency ratio (PER), through the following formulas:

- Survival rate (SR, %) is calculated as (Nt/N0) × 100, where Nt represents the fish number at the end of the experiment, and N0 is the number of fish at the beginning of the experiment.
- Weight gain (WG, %) is determined by [(BWf−BWi)/BWi] × 100, with BWi and BWf representing the initial and final average body weight (g) of fish sampled from each tank, and t indicating the experimental period in each trial (days).

- Relative growth rate (RGR, g/g/day) is calculated as (BWf - BWi) / t / BW, where BWf and BWi are the final and initial body weights (g) of fish, and t is the experimental period in days;
- Specific growth rate (SGR, %/day) is determined by [(lnBWf−lnBWi)/t] × 100;
- Feed conversion ratio (FCR) is expressed as FI (g)/BG (g), where FI represents food consumption (food provided – uneaten food), and BG is the biomass gain per rearing unit;
- Protein efficiency ratio (PER) is defined as BG divided by the amount of protein consumed.

Fish proximate composition. The proximate analyses of fish muscle were analyzed according to standard methods (AOAC, 2012) at the beginning of the experiment and the end of the trial.

Dry matter content was determined by heating at a temperature of 105 ± 2 ºC using the Sterilizer Esac oven. The crude protein content was assessed using the Kjeldahl method employing Gerhardt-type equipment. Fats were determined through the Soxhlet method, involving petroleum ether extraction, utilizing Raypa extraction equipment. The ash content was evaluated by combustion at temperatures of 550 ± 20°C using a Nabertherm furnace.

To evaluate the retained proteins and lipids we calculate the retained protein and the retained lipids:

- Retained protein (RP): PR=final individual weight x Pf –initial individual weight x Pi.
- Retained lipids (RL): LR= final individual weight x Lf – initial individual weight, where: Lf=final body lipids (%), Li=initial body lipids (%).

Blood sample collection. Blood samples were extracted from the fish caudal vein using heparinized syringes after deep anesthesia of fish with 0.3 mL/L of 2-phenoxyethanol (Velisek et al., 2004).

Hematological parameters, such as red blood cell count (RBC × 10^6/μL), hematocrit (Ht, %), and hemoglobin levels (Hb, g/dL), were evaluated using established methods. For the RBC we use a Neubauer hemocytometer, glass blood diluting pipette, and Vulpian diluting solution (Svobodova et al., 2012). The cyanmethemoglobin method with Drabkin's reagent was employed for Hb determination, with absorbance measured at 540 nm using a Specord 210 UV–Vis spectrophotometer. Hematocrit (Ht, %) measurements involved transferring 30 μL of heparinized blood into hematocrit microcapillary tubes and subsequent centrifugation. Other hematological indices, including mean corpuscular volume (MCV, fL), mean corpuscular hemoglobin (MCH, pg), and mean corpuscular hemoglobin concentration (MCHC, g/dL), were calculated from the obtained values of Ht, Hb, and RBC.

Data analysis. All data are presented as mean ± standard deviation. The statistical analyses were conducted utilizing the SPSS statistical software for Windows, Version 26.0, Chicago, IL, USA, SPSS Inc. The differences in the means of hematological parameters and proximate composition of fish meat at the two sampling time points (before and after the experimental period) were compared using a T-dependent test ($p < 0.05$) for each treatment diet.

RESULTS AND DISCUSSIONS

Fish growth performance. Feeding fish feeds with different levels of protein had a notable impact on the growth of the fish body. The best results, regarding the fish growth performance, were observed in the group of fish that received a diet consisting of 50% (Table 2).

Table 2. Growth performance of juveniles *of European catfish*

Variant	V150P	V241P
Initial mean weight (g/fish)	32.65 ±0.02[a]	32.63 ±0.02[a]
Final mean weight (g/fish)	66.71 ±2.62[a]	60.12 ±3.71[a]
SGR (%/day)	2.02 ±0.11[a]	1.74 ±0.18[b]
FCR (g feed/g fish)	1.03 ±0.08[a]	1.26 ±0.17[b]
PER (g/g)	1.95 ±0.15[a]	1.96 ±0.27[a]

Values are means ± standard deviation from duplicate groups of fish. Values with different letters on the rows indicate significant differences (T-test, p < 0.05) among experimental variants

No statistically significant differences ($p>0.05$) were found between the mean values of the protein efficiency ratio (PER). The growth rate (SGR) and feed conversion ratio (FCR) were significantly better in the $V1_{50P}$ variant. Thus, the $V1_{50P}$ has obtained an SGR value of 2.02 g%/day and an FCR of 1.03 g fodder/g weight gain, while in variant $V2_{41P}$ value of 1.74 g%/day SGR respectively FCR of 1.26 g fodder/g weight gain. This meant that fish feeding with high-protein diets (50% protein)

use dietary protein more efficiently than fish fed a low-protein diet (41%). Also, regarding the final mean weight of fish, higher values were recorded in the experimental variants where the protein level was higher (50% proteins).
Mares˘ et al., 2003 and Has-Scho¨n et al., 2004, indicate that the optimal level of total protein in the feed for rearing European catfish fry should be within the range of 40–45%. These findings were further supported by Bekcan et al., 2006, who observed that dietary protein content of 40% led to the highest growth performance in European catfish (*Silurus glanis*) compared to other levels such as 30, 35, and 44%.
Florczyk et al., 2014, reported that the fish's growth rate and final body weight were significantly higher when it is used a diet with a protein content of 45% and lipid content of 20% (referred to as the 45/20 diet). The specific growth rate (SGR) in this variant was measured at 2.6% BW/day, highlighting the positive impact of this specific dietary composition on the growth of juvenile European catfish. Similarly, Grecu et al., 2019, identified an analogous outcome in their study. They found that the highest SGR was achieved when the fish were fed a diet containing 54% proteins and 18% lipids, with a feeding level set at 2.5% BW/day. This further supports the concept that specific combinations of protein and lipid content in the diet can significantly influence the growth performance of catfish, as evidenced by the observed higher SGR in the mentioned dietary variant.
Fish proximate composition. The nutritional profile of the feed, the feeding rate, age, size, sex, and habitat features may all play a role in determining the meat composition of the different fish species, and the first indication of the fish's commercial standards that are required for food regulations (Marichamy et al., 2012).
The results of the body proximate composition, at the beginning and the end of the experimental period, are presented in Figure 1.
Regarding the meat's biochemical composition, the obtained results showed significant differences ($p<0.05$) in the levels of crude proteins, lipids, and ash, between the initial moment and experimental variants, respectively between experimental variants ($V1_{50P}$ and $V2_{41P}$). Analyzing the results presented in Figure 1, it is observed that the water content in the $V1_{50P}$ variant is notably lower ($p<0.05$), in comparison with the experimental variant $V2_{41P}$.

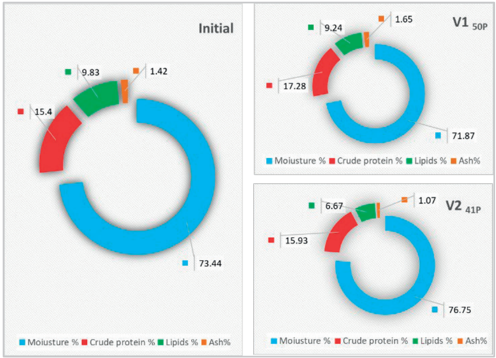

Figure 1. The biochemical composition of *Silurus glanis*, fed with different proteins and lipid level

Regarding the protein content, a significant ($p<0.05$) higher value was obtained in the variant V150P. In terms of the protein content of the $V2_{41P}$ variant and the recorded protein value at the initial moment of the experiment, no significant differences ($p<0.05$) were recorded.
Overall, the obtained results indicate a trend toward higher lipid and protein content in the V150P variant. This variant corresponds to a feed with higher nutrient levels. In comparison with the initial moment of the experiment, the lipid content registered a significantly lower ($p<0.05$) value in $V2_{41P}$.
As shown in Figure 2, the smallest amount of retained protein was recorded in fish from $V1_{50P}$,
where it also retained the largest amount of lipids.

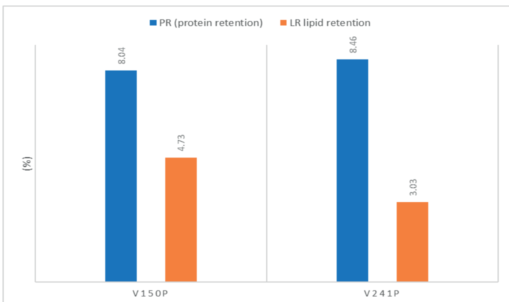

Figure 2. Graphical representation of protein and lipids retention

Similar findings have been reported by other researchers in studies involving other freshwater carnivorous fish species. For instance, Nyina-wamwiza et al., 2005 observed comparable results in pikeperch (*Sander lucioperca*), while Aliyu-Paiko et al. (2010) reported analogous trends in snakehead. Additionally, Bezbaruah & Deka (2021), in their investigation into the variation of protein content in the meat of three catfish species, identified protein content values ranging from 14.49% to 18.14%.

Indeed, the dietary energy provided to fish has a significant impact on their nutrient requirements. Mohanta et al. (2013) underscored the importance of carefully managing energy sources, particularly lipids and dietary proteins, to meet the nutritional needs of fish effectively. The balance and composition of these dietary components play a crucial role in supporting the overall health, growth, and performance of fish.

This meant that the dietary energy of fish has a major impact on the nutrient requirements by proper use of energy sources, particularly lipid and dietary protein (Mohanta et al., 2013).

These findings showed the fact that, although the nutritional profile of fish varies from species to species, size, age, and environmental conditions, the knowledge of the proximate composition of meat of fish can give us an idea regarding developing a more cost-effective and nutritionally balanced feed for the culture of European catfish.

Hematological profile. The hematological profile of fish provides valuable insights into their physiological well-being and overall health. When analyzed in conjunction with other indicators, hematological results can serve as useful tools for identifying and evaluating conditions that induce stress or diseases, ultimately influencing the growth performance of fish.

Table 3 displays the recorded values of hematological parameters at both the beginning and the end of the experiment, offering a comprehensive view of the changes in the fish's physiological state.

The statistical comparison revealed higher values of red blood cell count, PCV, Hb, and MCV at the beginning of the experiment ($p< 0.05$). When we compare the hematological parameter values between the two experimental variants, no significant differences ($p>0.05$) were observed at the end of the feeding experiment.

Table 3. Variation of hematological and biochemical parameters during the experiment

Parameter	Before the growth test	$V1_{50P}$	$V2_{41P}$
RBCc ($x10^6/\mu L$)	$2.15\pm0.18^{*}$	$1.55\pm0.34^{**a}$	$1.28\pm0.37^{**b}$
PVC (%)	$27.46\pm2.37^{*}$	$22.12\pm1.74^{**a}$	$20.25\pm1.79^{**a}$
Hb (g/dL)	$8.51\pm0.43^{*}$	$7.27\pm0.44^{**a}$	$7.16\pm0.89^{**a}$
MCV (μm^3)	$157.72\pm1.6^{*}$	$142.30\pm10.9^{**a}$	$113.63\pm10.65^{**a}$
MCH (pg)	$39.58\pm5.05^{*}$	$46.90\pm8.16^{**a}$	$57.89\pm8.38^{**a}$
MCHC (g/dL)	$31.83\pm3.0^{*}$	$33.96\pm3.96^{**a}$	$36.59\pm3.26^{**a}$

Note: Values with different symbols */** on the row differ significantly at the comparison of initial values and values after feeding with feeds containing different protein content ($p<0.05$). Values with different letters in a row differ significantly in the comparison between the two experimental variants. RBC - red blood cell (erythrocyte) count; Hb - hemoglobin; Ht - hematocrit; MCV - mean corpuscular volume; MCH - mean corpuscular hemoglobin; MCHC - mean corpuscular hemoglobin content.

Overall, the values of hematological parameters obtained in our study ranged with the normal values for European catfish according to the limits found in the literature. In a study conducted by Docan et al. in 2010, focusing on the hematological response of European catfish under various conditions, including different stocking densities and feeds with extruded pellets containing 41% protein and 12% lipid, the erythrocyte count was reported to range between $1.57\pm0.21\times10^6\mu l$, respectively to $1.65\pm0.24\times10^6\mu l$. In our study, no significant differences ($p>0.05$) were recorded in the number of erythrocytes, ranging from $1.28\pm0.37\times10^6\mu l$ in the $V2_{41P}$ variant to $1.55\pm0.34\times10^6\mu l$ in the V1 50P.

Docan et al., 2010, found in a study on the hematological response of European catfish maintained in different conditions stocking density and feed with extruded pellets containing 41% protein and 12% lipid, that the number of erythrocytes in *S. glanis* varies between $1.57\pm0.21\times10^6\mu l$ and $1.65 \pm 0.24 \times10^6\mu l$.

Regarding the values of the erythrocyte constants, there can be a significant decrease ($p<0.05$) in the experimental variants in comparison with the initial moment of the experiment. Also, the statistical comparison of the erythrocyte count between the two experimental variants recorded significant differences, with lower values in the $V2_{41P}$.

In our study, the values of blood hemoglobin content (Hb) of *Silurus glanis* non-significantly differed among the experimental variants ($p>0.05$), but there was a significant decrease ($p<0.05$) of hemoglobin concentration in comparison with the initial moment of the experiment. However, the hemoglobin values fall within the recommended optimal range (7.17 and 7.80 g/mL) recommended by Köprücü et al. (2006), quoted by Docan et al. (2010).

The value of hematocrit percentage showed no statistical differences ($p>0.05$) between the experimental variants recording the highest value in the variant $V1_{50P}$ (22.12±1.74 %) and the lowest value in the $V2_{41P}$ (20.25±1.79%). Comparing the hematocrit values after 6 weeks of feeding with high-protein feeds to the initial moment, a significant decrease ($p<0.05$) can be observed.

The increase of hemoglobin and hematocrit values corresponding to increased dietary protein levels suggested efficient oxygen transport within the body, consequently enhancing the growth performance of fish. This aspect underscores the significance of protein intake and its impact on the growth and health of fish (Ahmed & Ahmad, 2020).

Regarding the values of the Red blood cell constants, obtained after six experimental weeks, it can be observed a significant decrease ($p<0.05$) of MCV, respectively a significant increase ($p>0.05$) of MCH, MCHC in both experimental variants. Higher values of MCH and MCHC were recorded in the experimental variant $V2_{41P}$. The MCV values obtained in the $V2_{41P}$ variant correlate with the lower values of erythrocyte count in this experimental variant, being influenced by the inhibition of erythropoiesis or the destruction of RBC.

CONCLUSIONS

The findings of this investigation reveal the significant impact of dietary protein levels on fish growth, feed conversion ratio, and the hemato-biochemical composition of fish. Based on our results, it is recommended that a dietary protein of 50% crude protein is suitable for promoting the growth and efficient feed utilization of European catfish fingerlings, with a weight of 30-70 g. These data contribute valuable insights for formulating nutritionally balanced diets for the intensive and semi-intensive cultivation of this fish species.

Additional researches are required to determine the ideal protein and lipid levels in dietary content, as these factors are crucial for the successful intensive rearing of *Silurus glanis* in recirculating systems.

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CHARACTERISTICS OF EPIGEIC INVERTEBRATES OF SOME NATURAL FORESTS AND PLANTATIONS FROM THE WESTERN PLAIN (ROMANIA)

Dorina-Marieta PURICE, Minodora MANU, Marilena ONETE

Institute of Biology Bucharest, Romanian Academy, 296 Spl. Independenței, District 6, 060031, Bucharest, Romania

Corresponding author email: minodoramanu@gmail.com

Abstract

The aim of this paper is to highlight some interspecific relationships existing in epigeic invertebrate communities in natural forest ecosystems and plantations in the Western Romanian Plain. Nine sites were studied: 2 natural forests and 7 plantations. The material was collected with Barber traps during the summer season. The fauna was identified at the higher level of the species and Coleoptera: Carabidae - at species level, using specific identification keys. The following aspects were analysed: taxonomic composition, status of the taxa in the local coenoses from the point of view of their constancy classes in the samples, as well as the linear correlation coefficient (r) between the predatory taxa but also between the predators and their food sources. The results of this study highlight the importance of the coenotic heterogeneity in ensuring complex and long-lasting interspecific relationships along the path of ecological succession, resulting in a quality supply of environmental services provided by forests.

Key words: *forests, invertebrates, plantations, populations' structure, Western Plain.*

INTRODUCTION

Deforestation is one of the most dramatic human interventions on the biosphere, with huge consequences on its structure and functions (Palaghianu & Dutca, 2017). In the last decades, with the increase of the climatic changes (and not only), an attempt was made in Romania to compensate the reduction of forest ecosystems by afforestation (Feurdean, 2010; Chivulescu et al., 2021; Munteanu et al., 2016), half of the forest plantations having industrial purposes (Boháč et al., 2007; Stephens & Wagner, 2007). Afforestation is only 8% of the forested surface of Europe (Oxbrough et al., 2010), but it represents an important resource in some countries (even reaching over 70% of their total forested area), used in economic purpose (timber) and also, recreational end ecological (carbon storage) (Budău et al., 2023).

In Romania the natural and plantation forests were subjected to many studies regarding: characterization of the natural (Poole et al., 2003; Neblea et al., 2020) and invasive (Vítková et al., 2020; Ciuvăț et al., 2022) plant species, the native (Varvara & Coldea, 1984; Varvara & Pisică, 1993; Varvara, 1985; 2004; 2005; Cicort-Lucaciu et al., 2020; Ciornei et al., 2020; Prunar et al., 2022; David et al, 2023), and invasive (Olenici et al., 2022) invertebrates species, the forests dynamic (Tudoran et al., 2021) and conservation (Turcu et al., 2016; Albulescu et al., 2022), ecology (Falcă et al., 2000; 2004a; 2004b; 2005a; 2005b; Forio et al., 2020; Hamřík et al., 2023). There are a few subjects focused on afforestation (Burdon et al., 2020; Pérez et al., 2021; Szigeti et al, 2022; Ho et al., 2023).

Invertebrates are important components in all types of ecosystems, due to their role in the integrative trophic networks (Kosewska et al., 2023). In forests, the epigeic invertebrates are present from soil to supraepigeic level as detritivores, phytophagous, predators and, in the meantime are food source for superior trophic level of each of them (Oxbrough et al., 2010; Noreika et al., 2020; Mitchell et al., 2023).

Studying the structural characteristics of the epigeic invertebrates using higher taxa/suprataxa (superior to species level) is not an uncommon practice (Biaggini et al., 2007; Gerlach et al., 2013). However, some invertebrates are special due to their ability to reflect most changes (of natural or anthropogenic origin) in the habitats, through structural variations of their communities, for instance bioidicators such as myriapods, spiders, ants, some of the Coleoptera (i.e. Staphylinidae and Carabidae) (Folgarait, 1998; Rainio & Niemelä, 2003; Pearce & Venier, 2006; Perry & Herms, 2019; Kosewska et al., 2023).

In this study we aimed to find and highlight those structural peculiarities of communities of epigeic invertebrates that make the difference between plantations of different ages and which also, it reports them more structurally and functionally close to mature forests. The comparisons with epigeic invertebrate populations inhabiting adjacent natural forests help to outline ideas related to ecological stage of the forest plantations in the successional ecological context.

MATERIALS AND METHODS

In Western Romanian Plain, into Criș and Mureș river basins, there have been established nine study sites consisting in: three plantations (Uivar, Cermei and Sânmartin) each of them being four years old (hereinafter referred to as "young plantations" and abbreviated U, C and S), four plantations (Adea 1, Adea 2, Secusigiu 1 and Secusigiu 2) each of them being 8-10 years old (hereinafter referred to as "old plantations" and abbreviated A1, A2, S1 and S2), and two natural forests (Adea close to Adea plantations - abbreviated A.n.f. and Sânmartin in the vicinity of Sânmartin ash plantation - abbreviated S.n.f). All plantations differ through the woody species composition, microclimate, and various soil types. 60% from planted species are represented by pedunculate oak (*Quercus robur*), followed by Turkey/Austrian oak (*Q. cerris*) and ash (*Fraxinus excelsior*). In the depression areas the plantations are dominated by poplar (*Populus* sp.), ash and honey locusts (*Gleditsia triacanthos*).

The herbaceous vegetation forms grasslands with mezzophilous and mezzo-hygrophilous plant species and cultivated fields due to various geomorphological aspects at local and regional level. The process of reconstruction the forestry ecosystems was quite incipient in 2012 even the age of the plantations were 4-10 years old demonstrating the fact that the afforestation begun in 2003 and ongoing is working well but slowly in time (Crăciunescu, 2014). We present the study conducted during the summer season (June-August) of 2012 as a contribution at the better understanding of interspecific relationships existing in epigeic invertebrate communities in natural forest ecosystems and plantations, an important subject at national but also at international level. We are aware of the fact that in the interval of 10-12 years, some changes appeared regarding the numerical dominance, structure and constancy classes in epigeic invertebrate taxa and also the dimension of similarities between the invertebrate communities in the nine investigated sites. But our purpose is that our data to be a starting point for similar researches in the areas studied in 2012 or in other areas to highlight long-term structural and functional changes due to anthropogenic impact, climate change and knowledge based restoration of affected areas.

The epigeic invertebrate fauna was collected using Barber traps filled with a mixture (1:1 vol.) of formalin 4% and ethylene glycol. In each site ten Barber traps were installed, at approximately twenty meters from the forest/plantation edge and five meters distance between traps.

The invertebrates were identified suprataxa (superior to species level) using the specific identification keys. The Coleoptera were identified at the Family level. Among the Coleoptera, the ground beetles (Coleoptera: Carabidae) were identified at species level, because data at species level on the structure characteristics and interspecific relationships brings more accuracy regarding aspects related to successional stages of plantations and the conclusions of this study.

The data on epigeic invertebrates offered qualitative and quantitative information on the communities inhabiting both plantations and natural forests: the taxonomic composition, the species richness and specific diversity, the numerical relative abundances and the structure of the numerical dominance, and also the classes of constancy of taxa in the integrative coenoses. We also studied the degree of similarity of the invertebrate communities using the Mann -Whitney test for the degree of statistical significance of these similarities.

To obtain more relevance of the previous information, we calculated the linear regression index (r) for predator taxa and taxa being their potential prey, as well as for pairs of predator taxa known to be in potential competition in habitats.

The correspondence analysis (CA), and Bray-Curtis index of similarity were calculated using the statistical soft PAST (Legendre & Legendre, 1998; Hammer et al., 2001).

The one-way ANOVA test indicates that the differences between investigated ecosystems are significant: $F = 3.148$; $df = 21$; $p<0.05$.

For the Table 1 and 2 the abbreviations are as follows: Numerical dominance: ED - eudominant, D - dominant, SD - subdominant, RCD - recedent, SRCD - subrecedent. Tischler constancy classes: ECT - euconstant, CT - constant, rel. CT - relativ constant, ACS - accessory, ACD - accidental.

RESULTS AND DISCUSSIONS

The number of suprataxa (superior taxa) varies between 13 and 19 in the plantations of 4 years old and between 14 and 19 in the plantations of 8-10 years old respectively (Tables 1 and 2).

The taxonomical composition of epigeic invertebrate communities of the nine sites (without detailed identification of Coleoptera families) is almost identical. Although, the numerical abundances of the superior taxa from the studied communities determine different structure of their numerical dominances, also the frequencies, respective constancy's classes in with the taxa of epigenic invertebrates are framed.

In all plantations it is noted that the taxa considered (eu) dominants vary in proportion apparently randomized. As persistence in habitat, we have a fairly small proportion of (eu)constant taxa in young plantations (e.g. mites, dipterans, spiders, Heteroptera, beetle and Hemiptera). In the old plantations, in the same category of taxa (eu)constants, are also present isopods, Gryllidae and gastropods (Purice & Cioboiu, 2014).

The Coleoptera are subdominant numerically in all plantations. The beetle fauna varies as families' composition and more, as carabid species (Coleoptera: Carabidae).

Table 1. The epigeic invertebrate taxa in the numerical dominance structure and constancy classes, as proportion (%) from the total number of supraspecific / superior taxa/ site in the young plantations and adjacent natural forest

Statistical classes	C	U	S	S.n.f.
ED	7.14	16.67	12.5	28.57
D	21.44	27.77	25	7.15
SD	28.57	5.56	25	21.43
RCD	28.57	5.56	12.5	14.28
SRCD	14.28	44.44	25	28.57
ECT	7.14	55.55	62.5	71.44
CT	0	5.56	6.25	14.28
rel. CT	7.14	16.67	6.25	0
ACS	0	0	25	14.28
ACD	85.72	22.22	0	0

Table 2. The epigeic invertebrate taxa in the numerical dominance structure and constancy classes, as proportion (%) from the total number of supraspecific / superior taxa/ site in the old plantation and adjacent natural forest

Statistical classes	A 1	A 2	A.n.f.	S 1	S 2
ED	5.88	7.14	26.67	33.33	15.79
D	0	0	13.33	20	10.53
SD	11.76	0	0	26.67	15.79
RCD	29.42	7.14	0	6.67	5.26
SRCD	52.94	85.72	60	13.33	52.63
ECT	5.88	0	6.67	6.67	5.26
CT	23.53	0	33.33	33.33	36.84
rel. CT	29.42	7.14	6.67	33.33	15.79
ACS	5.88	50	6.67	13.33	26.32
ACD	35.29	42.86	46.66	13.33	15.79

We found between 4 and 11 families of Coleoptera in young plantations and also 11 families in the natural forest of Sânmartin. Staphylinidae vary in frequency between 60% and 80%, as well as Carabidae which is represented by 6 species, all of them being accidental in the area. The presence of Coleoptera families in old plantations varies between 3 and 12, while in the adjacent natural forest (Adea) we found 11 families. Members of Family Staphilinidae are present in all old plantations, with a frequency ranging between 11.11% (Adea 1) and 62.5% (Secusigiu 2), while members of Family Carabidae (represented by 5 species) has a frequency of 11.11% - 50% (Table 3). These results are quite contradictory. Coleman (2004) emphasized that there are changes in the Carabidae communities inhabiting young forest plantations. On the other hand, Oxbrough et al. (2010) mention that in advanced successional stages a more specialized populations of invertebrates are present, associated with a relative high number of indicator species (as are Carabidae). We also tested the degree of similarities of epigeic invertebrate communities by Bray-Curtis test.

The clear grouping of the sites in three clusters can be observed, based on the similarity in numerical abundances of the superior taxa in epigeic invertebrate communities, as follows: Cermei invertebrate community is detached by the other sites as similarity of the invertebrate community, maybe because here only 4 families of Coleoptera are present (Elateridae with 44.44% frequency in samples and the rest of three families - only 11.11% frequency) (Figure 1).

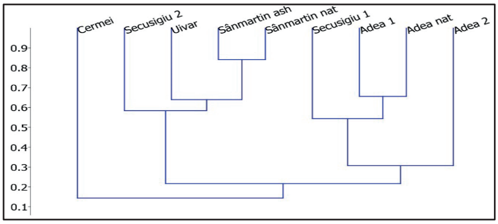

Figure 1. The degree of similarity between epigeic invertebrate communities (according to Bray-Curtis index of similarity, based on numerical abundances of invertebrates)

The second cluster consists in Secusigiu 2 (old plantation), Uivar and Sânmartin ash (young plantations) together with Sânmartin natural forest. The last two sites are very similar regarding invertebrate communities.

We notice that Uivar, Sânmartin ash and Sânmartin natural forest are similar due to the following taxa: Coleoptera, Opiliones, Orthoptera-Gryllidae, Diptera, Homoptera-Aphididae, Collembola, Thysanoptera, Araneae and Myriapoda - Chilopoda. Secusigiu 1 and Secusigiu 2 are similar due to Isopods, Orthoptera var., Diplopoda, cicads, Hymenoptera var., ants and Hemiptera.

Like the previous, Adea 1 and Cermei are similar because of gastropods, Lepidoptera and Acari, while Adea nat. is a distinct community because of Blattodea, Oligochaeta and Zygentoma. The degree of similarity between epigeic invertebrate communities in the 7 plantations and 2 natural forests, in terms of the numerical abundances of suprataxa, is graphically represented in Figure 2.

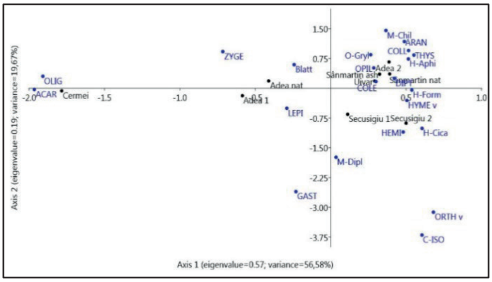

Figure 2. Correspondence analysis (CA) of epigeic invertebrates from all 9 investigated sites (taking into consideration the total numerical abundance/each site)

Legend: GAST (Gasteropoda), OLIG (Oligochaeta), ACAR (Acarina), OPIL (Opiliones), ARAN (Araneae), C-ISO (Crustacea-Isopoda), COLL (Collembola), THYS (Thysanoptera), M-Chil (Myriapoda-Chilopoda), M-Dipl (Myriapoda- Diplopoda), ZYGE (Zygentoma), HEMI (Hemiptera), H-Aphi (Homoptera-Aphididae), H- Cica (Homoptera-Cicadoidea), HYME v (Hymenoptera-var.), H-Form (Hymenoptera-Formicidae), Blatt (Blattodea), ORTH v. (Orthoptera var.), O-Gryl (Orthoptera- Gryllidae), COLE (Coleoptera), DIPT (Diptera), LEPI (Lepidoptera).

The third cluster is composed of the invertebrate communities from Secusigiu 1, Adea 1 and Adea 2 (old plantations) as well as Adea natural forest (Adea nat.). The dimension of these similarities between the invertebrate communities is expressed in Table 3.

Table 3. Matrix of Bray-Curtis index of similarity for the epigeic invertebrates communities of the nine studied sites

	A.n.f.	A 1	A 2	S 1	S 2	C	U	S	S.n.f.
A.n.f.	1	**0.66**	0.26	0.50	0.23	0.23	0.21	0.33	0.31
A 1	**0.66**	1	0.36	**0.58**	0.16	0.21	0.15	0.24	0.23
A 2	0.26	0.36	1	0.31	0.17	0.05	0.12	0.19	0.22
S 1	0.50	**0.58**	0.31	1	0.25	0.16	0.15	0.23	0.27
S 2	0.23	0.16	0.17	0.25	1	0.09	**0.57**	0.61	**0.57**
C	0.23	0.21	0.05	0.16	0.09	1	0.16	0.14	0.12
U	0.21	0.15	0.12	0.15	0.57	0.16	1	**0.65**	0.62
S	0.33	0.24	0.19	0.23	**0.61**	0.14	**0.65**	1	**0.84**
S.n.f.	0.31	0.23	0.22	0.27	**0.57**	0.12	**0.62**	**0.84**	1

In the epigeic invertebrate communities we encounter not only variations in the quantitative aspects of the present taxa, reflected, as we have shown, in different degrees of similarity, but also more or less solid interspecific relationships between taxa. The r linear correlation coefficient was measured between predatory and prey taxa and also, between predatory taxa known to be competitors in forests (Table 4).

Table 4. The r linear correlation coefficient for pairs of taxa in the studied invertebrate communities

Pray-predator relationship	A1	A 2	A.n.f.	S1	S2	C	U	S	S.n.f.
Araneae - predator									
Collembola	0.74	0	0.68	0	-0.58	0.58	0	0.78	0
Acarina	0	0	0	0	0	0.62	0	-0.47	0
Diptera	0	0.58	0.66	-0.56	0.58	0	0	0	0.91
Isopoda	0	0	0	0.73	0	0	0	0	0
Aphididae	0	0	0	0.61	0	0	0.54	0.49	0.88
Carabidae	0	0	0	0	0	0	0.67	0.62	0.46
Coccinellidae	0	0	0	0	0	0	0	-0.43	-0.53
Carabidae - predator									
Collembola	0	0	0	0	0	0	0	0.97	0
Diptera	0	0	0	0	0	0	0	0	-0.83
Aphididae	0	0	0	0.74	0	0	0.88	0	0

The linear correlation coefficient shows how predator taxa use food resources in habitats, as well as potential competition between predators. Although, on average, the number of prey-predator interspecific links is equal in plantations (Verhviläinen et al., 2008), regardless of their age, the food sources used by predators vary from one plantation to another.

For example, in the Adea 1 plantation (A1) the Araneae (spiders) consume Collembola, while in Adea 2 (A2) prefer the Diptera, and in the Adea natural forest (A.n.f.) both resources are consumed. In Secusigiu 1 (S1), the spiders access three food sources, of which, Diptera appear to be limited source (r negative) while aphids are consumed here also by carabids, even to a greater extent. However, between these two predators (carabids and spiders) the competition is not installed. In Secusigiu 2 (S2) Collembola appears to be a limited food resource for Araneae.

In young plantations, competition among predators for food is better noted. This is the situation in Uivar (U) where the carabids consume aphids more than the spiders do and both predators are competing (Table 4). The same situation is presented in Sanmartin Ash (S) for Collembola.

A special situation is in the natural forest of Sanmartin (S.n.f) where the Diptera are consumed by both spiders and carabids, but for carabids the diptera are limited source of food. However, it appears that the spiders compete with Coccinellidae for aphids.

Oxbrough et al. (2010) mentioned that previous studies on carabids in plantations had conflicting results: either high specific richness in the early stages, or, on the contrary, in the advanced stages, or, only minor changes during the ecological succession. The biodiversity-enhancing effects of the vegetation on soil mesofauna (Szigeti et al., 2022) and invertebrates (Chiriac et al., 2020) are documented in recent years, reflecting the importance of habitat and plant species variability. It remains poorly known the impacts of tree diversity on soil fauna (David et al., 2023).

CONCLUSIONS

The studied plantations, as well as the natural forests, prove a relatively high structural heterogeneity, although they have very similar taxonomic composition of epigeic invertebrate communities.

This structural heterogeneity is due both directly and indirectly to the way in which vegetation change. This, over time, after the initial planting moment, develops differently in the sites, even when the initial afforestation formulas are similar, and, because local abiotic factors are involved in the influence of the development of plant communities in a certain way and rhythm. Hence, the emergence and development/persistence of a series of local abiotic and biotic features, generating changes as a domino effect both horizontally and vertically. We are talking about the litter layer generated by the planted species as well as their canopy that varies in their developmental stages. All this determines the existence of different local microclimatic peculiarities, which limit or favour the installation and persistence in these habitats of the other coenotic components (consumers).

It is a great difference between plantations and natural forests in relation to the period necessary to reach maturity (ecological succession), viewed through the prism of epigeic invertebrates and not only. We refer, for example, to the number of predatory taxa and their status as constancy in the habitat. It is worth mentioning that for example, carbides have the status of accidental in all plantations. In addition, the small number of existing predator-predator interspecific links also proves that plantation dynamics are not exactly favourable to predators at that time of study.

The short period of time allocated to this study has prevented us from obtaining a more complex data set, so the results presented are practical, a characterization of the structure of communities of epigeic invertebrates in that short segment of time, especially in relation to the entire successive period they will go through.

That is why we think that it is by far necessary to continue the studies in these plantations, with a complex protocol, in order to obtain more consistent data greatly necessary, at least in Romania, on this segment of research.

We present the study conducted during the summer season (June-August) of 2012 as a contribution at the better understanding of interspecific relationships existing in epigeic invertebrate communities in natural forest ecosystems and plantations, an important subject at national but also at international level. Also, the data we produces 11 years ago we would like to be a starting point for highlighting long-term structural and functional changes of the studied areas or other similar.

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ASSESSMENT OF THE ZOOPLANKTONIC COMMUNITIES IN AQUATIC BASINS IN THE SOUTHERN AREA OF ROMANIA

Silvia RADU, Nicoleta Georgeta DOBROTĂ, Mioara COSTACHE, Gheorghe DOBROTĂ, Daniela RADU, Nino MARICA

Research and Development Station for Fisheries Nucet, 549 Principală Street, Nucet, Dâmbovița County, Romania

Corresponding author email: dobrota19dng@yahoo.com

Abstract

In the present work, the results of the assessment of the state of zooplankton communities are presented from different types of water bodies. For the study, water samples were collected and analyzed from three types of water basins: systematic pond (EC 1 lfov and EC 1 Cazaci - SCDP Nucet), semi-systematic pond (Iaz no. 7 Crevedia) and reservoir (Bunget 2). The research was carried out in 2023, and the results highlighted the fact that the composition of the zooplankton in the aquatic ecosystems studied was made up of species belonging to the taxonomic groups: Rotifera, Copepoda and Cladocera. Based on these aspects, the qualitative and quantitative structure of the zooplankton in the studied water basins was established. Were identified 24 taxa (16 taxa - Rotifera, 3 taxa - Copepoda and 5 taxa - Cladocera). The weight of each taxonomic group is different in the three types of ecosystems: in the systematic pond - type water basins, cladocerans predominate (38.7% in EC 1 Cazaci and 44.0% in EC 1 Ilfov), in semi-systematic pond Iaz no. 7 Crevedia rotifers predominate (69.5%) and in Bunget 2 reservoir copepods predominate (49.5%).

Key words: *reservoir, semi-systematic pond, systematic pond, water basin, zooplankton.*

INTRODUCTION

Zooplankton, by its structure and functions, is indispensable for the normal and efficient development of the circuit of matter and energy in a lentic water basin (Battes, 2018; Battes, 2010). Zooplankton is an ecologically and economically important food source for fish and plays an important role in the transfer of organic matter through food webs. It is one of the basic components of the trophic chain, not only because it represents the trophic base of most filtering organisms, but also because of its qualitative structure. Zooplankton respond rapidly to environmental variability, and changes in population dynamics and species composition often indicate changes within water bodies. Within the appearance and development of organisms with a trophic role, a seasonality related to climatic factors and a variability related to those of the aquatic environment (physico-chemical, biological) can be distinguished (Radu, 2019). This is expressed in the modification of the structure and functional indicators (Lebedenco, 2020). In aquatic ecosystems, filtering zooplankton organisms are the main consumers of vegetable and animal detritus suspended in the water mass and on the bottom of water bodies, directly transforming dead organic matter into biomass for the upper trophic link, thus shortening trophic chains and accelerating the cycle of matter in ecosystems (Pricope, 2011).
Zooplankton can provide useful information on ecosystems and are an ideal indicator for assessing ecosystem health (Richardson, 2008). Any variation in zooplankton biomass has implications for biogeochemical cycling, trophodynamics, fishing and ecosystem services (Caroppo et al., 2013).

MATERIALS AND METHODS

The research on the evaluation of the state of the zooplankton communities in the water basins in the southern area of Romania was carried out in between May-August 2023. Three types of water basins were chosen: systematic pond, semi-systematic pond and reservoir.
The definitions of water bodies used in this study are as follows (Leonte & Leonte, 2005):
- the *systematic pond* is located on a flat land with a low slope, obtained as a result of its

damming. Systematic ponds are made in excavation or filling, surrounded totally or partially by dykes, provided with supply, drainage and perimeter channels, equipped with hydrotechnical constructions and water supply, retention and drainage installations;

- the *semi-systematic pond* is located in the course of a valley, dammed at its narrowest point. The water supply is made from running water, springs, precipitation, pumping stations;
- the *reservoir* is an artificial lake, created by a dam that retains the water of a river, located upstream of a hydroelectric power plant in order to form a water reserve necessary for the production of electricity, but also for the water supply of some localities and for different tourist uses.

Systematic ponds are located within the Research and Development Station for Fisheries Nucet. The EC 1 Ilfov systematic pond (1 ha) is located within the Nucet experimental base and for the research carried out this basin was not fertilized.

The EC 1 Cazaci systematic pond (2.15 ha) is located within the Cazaci experimental base and for the research carried out, this basin was fertilized (8 tones manure/ha).

The ponds within Research and Development Station for Fisheries Nucet are used to grow cultured fish in summer I.

The Iaz no.7 Crevedia semi-systematic pond (4.8 ha) is located in Crevedia commune, Dâmbovița county. The function of the pond is fish farming, with the practice of recreational fishing.

The Bunget 2 reservoir (91.0 ha) is located in the village of Bunget, Văcărești Commune, Dâmbovița county, upstream of the Brătești reservoir and downstream of the Bunget 1 reservoir. The functions of the reservoir are: fish farming, electricity production and flood mitigation.

To evaluate the state of the zooplankton communities, the following structural parameters and biocenotic indices were calculated: average numerical density (N, ex/m^3), percentage numerical abundance (NA, %), total number of taxa (TNT), zooplankton biomass (g/m^3), the dominance index DI (Mc. Naughton & Wolf) and the Shannon-Wiener diversity index (H).

To determine the structure of the planktonic zoocenosis, a total of 20 samples were collected and analyzed. Water samples were taken from three points (from the supply, center and outlet area) and were collected from the surface horizon (0.2 - 0.5 m). After collecting, the sample volume (10 liters) was concentrated by filtration, using the planktonic net made of silk sieve no. 25 (mesh size being 40-50 μ/side).

The zooplankton concentrate was transferred into 100-150 ml glass vials. The sample thus obtained was preserved with 4% formalin. In the laboratory, the samples were concentrated by slow gravitational sedimentation for three weeks, after which the supernatant was removed by siphoning, without shaking the sample.

The zooplankton from the samples thus processed was analyzed qualitatively and quantitatively with a stereomicroscope and a microscope (Axio microscope Vert A.1 -Zeiss) in Kolkwitz type cells. The qualitative determination of the main zooplankton taxonomic groups: Rotifera, Cladocera, Copepoda was made according to: Dussart (1966), Negrea (1983).

Numerical density was calculated by counting all individuals belonging to each species. Finally, the number of individuals for each taxonomic group was added up, from which the total number of individuals in the analyzed sample resulted. The final result was reported in the number of exemplares per m^3, taking into account the amount of initially filtered water. The *average numerical density* was obtained by calculating the arithmetic average of the analyzed samples.

The *percentage numerical abundance* (NA, %) was calculated by relating the number of individuals of a species (ni) to the total number (N) of individuals in the sample.

$$NA = (ni / N) x100$$

The *total number of taxa* (TNT) was calculated for the representatives of the main groups (*Rotifera, Copepoda, Cladocera*).

Zooplankton biomass was calculated in wet matter.

The number of individuals of each species was multiplied by the corresponding individual mean value. Finally, the total biomass per taxonomic group was calculated by summation and then the total biomass per sample, reporting the results in grams per cubic meter.

The dominance index (Mc. Naughton, & Wolf) was calculated according to the formula:

$$DI\ (\%) = \frac{Y1 - Y2}{Y} \times 100$$

where:

Y1 = numerical density of the most abundant species; Y2 = number density of secondary species as number density; Y = total number density. Dominance index shows the degree of influence that the first two species with the greatest numerical development have in a biocenosis.

The Shannon-Wiener diversity index is based on information theory and was calculated according to the formula:

$$H' = -\sum_{i=1}^{S} p_i \ln p_i$$

where:

p_i - represents the numerical, percentage or biomass abundance of species i in the sample;

ln - is the natural logarithm (the logarithm to base e, where e=2.71);

S - the number of species.

The index reflects both species diversity and the evenness of their abundance in the community. The condition of the biotic communities is better in case of higher index value.

RESULTS AND DISCUSSIONS

During the research period, along with the water samples collected to assess the state of the zooplankton communities, water samples were also collected for the analysis of the main physical-chemical parameters of the water (Figure 1).

Figure 1. Water sampling for analysis (own source)

The interpretation of the obtained results was carried out in accordance with the provisions of the "Regulations on the classification of surface water quality", correlated with the data from the specialized literature for waters used for fish farming (OMMGA no. 161/ 2006) (Table 1).

The values recorded during the course of the study shown in table 1, recorded the following characteristics:

The **pH** generally showed a neutral reaction and which falls within the recommended and favorable range for the life of aquatic organisms 7.2 - 7.6 upH.

The **alkalinity** of the water indicates the content of bases, carbonates and bicarbonates. The content of calcium bicarbonate $Ca(HCO_3)_2$ depends on the concentrations of calcium carbonate ($CaCO_3$), magnesium bicarbonate and CO_2 in the water . During the entire monitoring period, the alkalinity recorded values between 3.1-4.20 ml HCl/l, according to Ord. MMGA no. 161/2006 for aquaculture waters.

Concentration of calcium ions (Ca^{2+}) from the surface waters have recorded values that are below the maximum limits allowed for fish waters, and according to Ord. MMGA 161/2006 they fit into the first quality class. The calcium present in the water, expressed in mg/l, represents an element that has a special role for the development of aquatic organisms and the feeding of fish. Calcium ions along with magnesium ions are essential in the development and normal growth of aquatic organisms.

The concentration of magnesium ions (Mg^{2+}) in the surface waters recorded values that are in the optimal range for fish waters of the II-th category of use, according to Order 161/2006.

Ca^{2+}/Mg^{2+} ratio falls within the optimal range for category II aquaculture waters.

Organic substance presented values that fall within the maximum limits allowed, according to the specialized literature (20-60 mg $KMnO_4$/l). In the case of anaerobic decomposition of proteins or odorless compounds, the amount of algae and bacteria affects the aquatic environment.

Dissolved oxygen in water is an important chemical factor that conditions the life of aquatic organisms, facilitates the mineralization of organic substances, influences the photosynthesis of aquatic flora and microflora, influences metabolism, assimilation and the toxicity of some water compounds. The concentration of oxygen in the water depends on the temperature and clarity of the water. The factors that lead to the decrease of O_2 in the water are: increased water temperature, high turbidity, degree of water bloom, etc. Analyzing the dissolved oxygen values from table no. 1, it is found that the water falls into the I and II quality classes.

Ammonia (NH_3^+) can be present in water in molecular form, undissociated (NH_3) or dissociated, in the form of ammonium ions (NH_4^+). The ratio between the two forms of ammonia (dissociated and undissociated) depends on the pH and water temperature. The passage of NH_4^+ into NH_3 is achieved all the more strongly, the higher the temperature and the pH of the water is higher. Ammonium ions are non-toxic for fish, the toxicity of ammonium salts being given by the ammonia molecules. The dissociation depends on the pH of the water, the toxicity increasing proportionnally with the increase in the pH of the water due to the large amount of undissociated molecules in the solution. Ammonia was not present in the analyzed samples.

Nitrates (NO_2^-) recorded values between 0.008 and 0.012 mg/l, falling within the optimal values for the life of aquatic organisms. These values, according to Ord. MMGA no. 161/2006 regarding the classification of surface water quality, classify the waters from the samples taken, in quality classes I-II. The specialized literature also limits the values of nitrites to max. 0.3 mg/l. In quantities outdated nitrites become toxic and affect the health of aquatic organisms, even leading to deaths.

Nitrogen anions (NO_3^-) recorded values between 0.088-1.28 mg NO_3^- /l, falling within the optimal values for fish growth. According to the nitrate content, the water from the analyzed samples is in quality class I, in accordance with Ord. MMGA no. 161/2006.

Phosphorus is a limiting factor of aquatic life. It is found in the form of phosphates in waters. Phosphates determine the productivity of a fish pond, the amount of phosphates measured in the analyzed samples is between 0.082-1.28 mg/l, falling within the limits provided by Ord. MMGA no. 161/2006 for aquaculture waters.

The **chlorines,** determined from the samples taken, presented values that fall within the maximum allowed, according to specialized literature. From the point of view of the content of chlorides in the water, it falls into category I of quality, as stipulated by Ord. MMGA no. 161/2006 regarding the classification of surface water quality.

Table 1. The average values of the main physical-chemical parameters of the water in the studied basins

No. crt.	***The physical-chemical parameter***		***UM***	***Parameter values***				***Optimum according to quality standards***
				EC 1 Ilfov *(systematic pond)*	**EC1 Cazaci** *(systematic pond)*	**Iaz no. 7 Crevedia** *(semi-systematic pond)*	**Bunget 2** *(reservoir)*	
				The average of the results obtained in the analysis				
1	pH		pH units	7.4	7.4	7.2	7.6	7-7.8
2	Temperature		^0C	22.2	23.5	23.3	22.5	20-26
3	Alkalinity		mg/l	190.4	189.1	232.4	256.4	200-400
4	Calcium (Ca^{2+})		mg/l	45.2	44.6	41.8	40.8	90-120
5	Magnesium (Mg^{2+})		mg/l	15.56	15.80	33.56	28.94	10-40
6	Ca^{2+} / Mg^{2+}		mg/l	2.90	2.82	1.24	1.40	5
7	Organic substance		mg $KMnO_4$/l	44.6	32.2	24.6	38.5	20-60
8	Oxygen		mg/l	7.26	7.85	8.56	7.52	05-10
9	Ammonia (NH^+_3)		mg/l	lack	lack	lack	Lack	lack
10	Nitrates (NO^-_3)		mg/l	1.28	0.88	0.92	1.02	2.5-4
11	Nitrites (NO^-_2)		mg/l	0.012	0.008	0.018	0.02	0.03
12	Phosphates ($PO^{3-}{}_4$)		mg/l	0.108	0.086	0.082	1.28	0.05-1.5
13	Chloride	Cl^-	mg/l	6.71	6.36	7.13	8.23	30
		Na Cl	mg/l	11.10	10.52	11.69	13.44	20
14	Ammonium (NH^+_4)		mg/l	0.36	0.24	0.64	0.88	0.5-1
15	Total hardness		(^0D)	9.92	9.88	13.6	12.4	12

Ammonium (NH_4^+). According to the ammoniacal nitrogen content, in accordance with Ord. MMGA no. 161/2006 regarding the classification of surface water quality, the analyzed samples fall into quality class I, with very small variations over the entire studied period.

The total hardness presented values between 9.92 and 13.6° D, values that fall within the limits accepted by the specialized literature.

To evaluate the state of zooplankton communities, the following structural parameters and biocenotic indices were analyzed: average numerical density (N, ind./m^3), percentage numerical abundance (NA, %), total number of taxa (TNT), biomass (g/m^3), the dominance index and the Shannon-Wiener diversity index (H) (Figure 2).

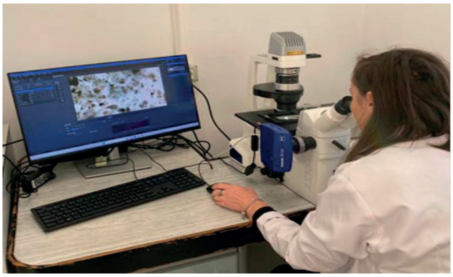

Figure 2. Microscopic analysis of zooplankton (own source)

Average number density (N, ex/m^3) of zooplankton in the analyzed samples varied significantly (Figure 3). The average number of representatives for the main groups (*Rotifera, Copepoda, Cladocera*) was: 345,250 ex/m^3 obtained in EC 1 Ilfov (systematic pond); 1,406,833 ex/m^3 obtained in EC 1 Cazaci (systematic pond); 163,500 ex/m^3 obtained in Iaz no. 7 Crevedia (semi-systematic pond); 400,250 ex/m^3 obtained in Bunget 2 (reservoir).

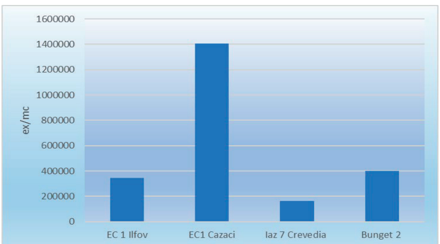

Figure 3. Average numerical density of zooplankton in the analyzed samples

The average with the lowest value was obtained in EC 1 Ilfov and the highest value was obtained in EC 1 Cazaci.

The *average numerical density* of zooplankton for each taxonomic group had minimum and maximum values, as follows:

- *Copepoda*: 24667 ex/m^3 (semi-systematic pond - Iaz no. 7 Crevedia) and 478833 ex/m^3 (systematic pond - EC1 Cazaci);
- *Rotifera*: 72167 ex/m^3 (semi-systematic pond - Iaz no. 7 Crevedia) and 383000 ex/m^3 (systematic pond - EC1 Cazaci);
- *Cladocera*: 7050 ex/m^3 (semi-systematic pond - Iaz no. 7 Crevedia) and 545000 ex/m^3 (systematic pond - EC1 Cazaci).

It is observed that the minimum limits for the three taxonomic groups were reached in semi-systematic pond (Iaz no. 7 Crevedia) and the maximum limits in fertilized systematic pond (EC 1 Cazaci).

The percentage numerical abundance (NA, %), varied in each water body type. In the systematic ponds, the highest values are recorded by the *Cladocera* taxonomic group (38.7% EC1 Cazaci and 44.0% EC1 Ilfov). The abundance of zooplankton in the EC 1 Ilfov is mainly due to the fertilizers distributed as part of the basin preparation works, before the start of the study. In Iaz no. 7 Crevedia the highest value was obtained by the *Rotifera* taxonomic group (69.5%), and in Bunget 2 reservoir the highest value was recorded by the *Copepoda* taxonomic group (49.5%) (Figure 4).

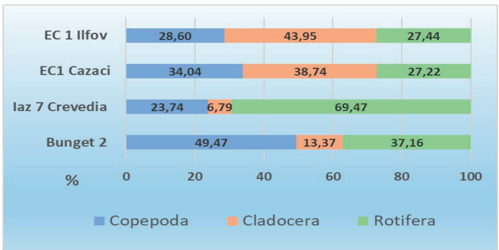

Figure 4. Numerical percentage abundance of zooplankton in the analyzed samples

The total number of taxa (TNT) is an important indicator of the diversity of hydrobiological communities. Following the qualitative and quantitative analysis of the zooplankton, the presence of a number of 24 taxa, which belong to the systematic groups: *Rotifera, Copepoda* and *Cladocera.* A number of 16 taxa belong to the taxonomic group *Rotifera*, 3 taxa belong to the taxonomic group *Copepoda* and 5 taxa belong to the group *Cladocera*. The highest number of taxa was found in EC 1 Cazaci (20), and the lowest number was found in EC 1 Ilfov (15). The number of taxa encountered for Iaz no. 7 Crevedia was 17, and for Bunget 2 reservoir it was 18 (Figure 5).

Figure 5. Total number of taxa (TNT) recorded in the analyzed samples

The taxonomic structures of zooplankton species identified for each water basin in the research are presented in Figures 6, 7, 8 and 9.

Following the qualitative and quantitative analysis of the zooplankton in **EC 1 Ilfov**, the presence of a number of 15 taxa is found (Figure 6). The systematic group *Rotifera* is represented by 10 species, *Copepoda* by 2 species and *Cladocera* by 3 species. The largest number of specimens was recorded by the species *Daphnia magna* (*Cladocera*) with 68288 ex/m^3 and the lowest number was obtained by the species *Euclanis* sp. (*Rotifera*) - 1885 ex/m^3.

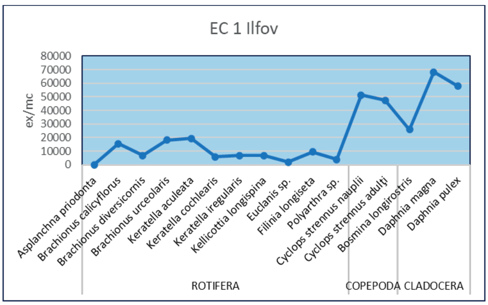

Figure 6. Taxonomic structure of the species identified in the zooplankton from the EC 1 Ilfov (fertilized systematic pond)

Following the qualitative and quantitative analysis of the zooplankton in **EC 1 Cazaci,** the presence of 20 taxa was found (Figure 7). The systematic group Rotifera is represented by 14 species, Copepoda by 2 species and Cladocera by 4 species. The highest number of specimens was recorded by the species *Cyclops strennus nauplii* (Copepoda) with 335183 ex/m^3 and the lowest number was obtained by the species *Polyarthra sp* (Rotifera) – 3830 ex/m^3.

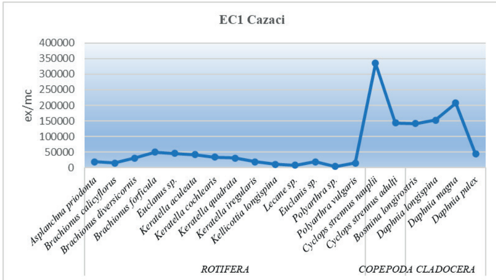

Figure 7. Taxonomic structure of the species identified in the zooplankton from the EC 1 Cazaci (unfertilized systematic pond)

The zooplankton analysis in **Iaz no. 7 Crevedia** showed the presence of 17 taxa (Figure 8). The systematic group Rotifera is represented by 11 species, *Copepoda* by 3 species and Cladocera by 3 species. The highest number of specimens was recorded by the adult *Cyclops strennus species* (*Copepoda*) with 15540 ex/m^3 and the lowest number was obtained by the *Daphnia cucullata* (*Cladocera*) species - 1622 ex/m^3.

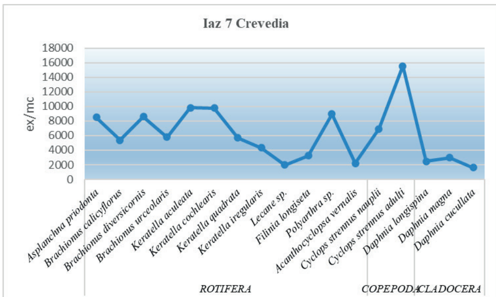

Figure 8. Taxonomic structure of the species identified in the zooplankton from the Iaz no. 7 Crevedia (semi-systematic pond)

The zooplankton analysis in **the Bunget 2 reservoir** showed the presence of 18 taxa (Figure 9). The systematic group Rotifera is represented by 12 species, *Copepoda* by 2 species and Cladocera by 4 species. The highest number of specimens was recorded by the adult *Cyclops strennus* species (*Copepoda*) with

108900 ex/m^3 and the lowest number was obtained by the *Euclanis sp.* (*Rotifera*) species – 120 ex/m^3.

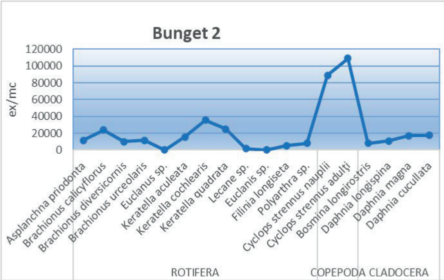

Figure 9. Taxonomic structure of the species identified in the zooplankton from the Bunget 2 (reservoir)

Average biomass for the main taxonomic groups (*Rotifera, Copepoda, Cladocera*) had different values, depending on the average individual weight of each taxonomic group.
The total zooplankton biomass recorded for the 3 taxonomic groups is presented as follows:

- EC 1 Ilfov 45,940 g/m^3;
- EC1 Cazaci 76,779 g/m^3;
- Iaz 7 Crevedia 6,309 g/m^3;
- Bunget 2 - 11,004 g/m^3.

Thus, if for the taxonomic group *Rotifera* the lowest value was recorded in Iaz no. 7 Crevedia (semi-systematic pond) - 0.202 g/m^3, and the highest value was recorded in EC 1 Cazaci (unfertilized systematic pond)-1.048 g/m^3, for the *Cladocera* group, the average biomass had the minimum value recorded in the Bunget 2 (reservoir) - 5.046 g/m^3 and the highest value was recorded in EC 1 Cazaci-66.88 g/m^3. For the *Copepoda* taxonomic group, the average biomass had the minimum value recorded in Iaz no. 7 Crevedia -1.060 g/m^3 and the highest value was recorded in EC 1 Cazaci - 8.846 g/m^3 (Figure 10).

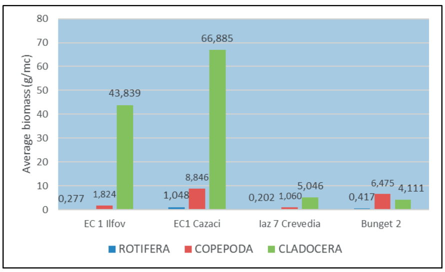

Figure 10. Average biomass for the main taxonomic groups of zooplankton in the analyzed samples

Average Biomass is closely correlated with average weight for each individual taxonomic group.
The systematically non-fertilized pond (EC 1 Ilfov) recorded a zooplantonik biomass with 40.2% less than a systematically fertilized pond (EC 1 Cazaci).
The non-fertilized semi-systematic pond (Iaz 7 Crevedia) recorded a zooplantoik biomass with 91.8% less than a systematically fertilized pond (EC1 Cazaci).
The non-fertilized reservoir (Bunget 2) recor-ded a zooplantonik biomass with 85.7% less than a systematically fertilized pond (EC1 Cazaici).

The Dominance Index (Mc. Naughton, & Wolf) (DI). Through the analysis of the dominance index, the degree of influence of the first two species with the greatest numerical development in an aquatic biocenosis was shown. So, following the results obtained, the best value was obtained in EC 1 Ilfov (fertilized systematic pond) - 19.78% and the lowest value was obtained in Bunget 2 (reservoir) - 4.95% (Figure 11).

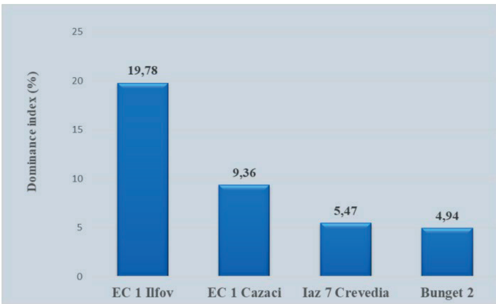

Figure 11. The Dominance Index
(*Mc. Naughton & Wolf*)

Shannon-Wiener diversity index To analyze the alpha-diversity of zooplankton communities based on numerical abundance, the Shannon-Wiener diversity index was calculated. Thus, according to the data obtained, the best condition of the zooplankton is at the Iaz no.7 Crevedia - 2.66, and the weakest condition was obtained in the Bunget 2 reservoir - 2.28 (Figure 12).

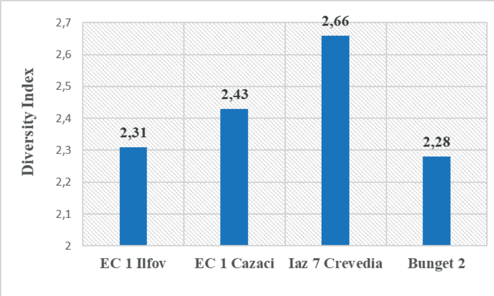

Figure 12. Diversity index (*Shannon-Wiener*)

CONCLUSIONS

The research on the evaluation of the state of the zooplankton communities in aquatic basins in the Southern area of Romania was carried out in year 2023 and studied three types of water basins: systematic pond, semi-systematic pond and reservoir.

From a chemical point of view, most of the parameters in the analyzed samples fall into the II class of surface water quality, according to Order 161/2006.

The ***average numerical density*** of zooplankton for each taxonomic group had minimum and maximum values. Following the results obtained, it can be said that the average numerical density of zooplankton in the analyzed samples varied significantly for the three types of water basins. This fact was influenced by several factors, such as: the type of basin, technological interventions on water bodies, the time of day when the sample was taken, meteorological conditions.

Average Biomass - Among the 3 non-fertilized basins (EC 1 Ilfov, Iaz no. 7 Crevedia and Bunget 2) the highest zooplankton biomass was recorded in EC 1 Ilfov (systematic pond), respectively 45,940 g/m^3. Compared to the systematically unfertilized pond (EC 1 Ilfov), the semi-systematic unfertilized pond (Iaz 7 Crevedia) produced a zooplantonik biomass with 13.7% less, and the unfertilized reservoir (Bunget 2) with 24% less.

Dominance index. The best value was obtained in EC 1 Ilfov -19.78% and the lowest value was obtained in the Bunget 2 reservoir - 4.95%. EC 1 Cazaci indicated a value of 9.36% and Iaz no. 7 Crevedia – 5.47%.

Analyzing the values of the ***Shannon-Wiener diversity index***, the better condition of the zooplankton was registered in Iaz no.7 Crevedia - 2.66, and the lowest condition was obtained in the Bunget 2 reservoir - 2.28.

EC 1 Cazaci indicated a value of 2.43 and EC 1 Ilfov - 2.31.

Zooplankton, as the most dynamic component of aquatic invertebrates, in the researches carried out, is characterized by an uneven development, disturbances of the population both in terms of quantitative and qualitative structure.

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OVERALL EFFECTS OF CLIMATE CHANGE ON ECONOMICALLY VALUABLE FISH POPULATIONS IN THE DANUBE RIVER

Maria Desimira STROE, Angelica DOBRE, Mirela CREȚU, Magdalena TENCIU, Floricel Maricel DIMA, Neculai PATRICHE

Institute of Research and Development for Aquatic Ecology, Fishing and Aquaculture Galati, 54 Portului Street, 800211, Galati, Romania

Corresponding author email: sdesimira.icdeapa@gmail.com

Abstract

The ecosystems of the Danube River basin exhibit remarkable biodiversity, housing over 2,000 plant species and more than 5,000 animal species. This places the Danube among Europe's most valuable rivers in terms of aquatic life diversity, with a total of 103 identified species. The Danube is increasingly affected by climate change, impacting the hydrologic cycle and the availability of food and water resources. The effects of climate change are already observable and projected to intensify, notably in increased frequency and intensity of extreme weather events such as heatwaves, drought periods, and floods. In this paper, we aim to present the impact of climate change on economically valuable fish within the Danube River. We have determined the abundance of these species and analyzed the correlation between recorded temperatures and the river's water level. Given the Danube's unique variety of species, we will highlight the importance of protecting and conserving this valuable ecosystem amidst the escalating climate threats.

Key words: *abundance, aquatic ecosystems, carp population, environmental factors, predatory species.*

INTRODUCTION

Global climate change is one of the greatest challenges of the 21st century (Sarwar, 2008), with significant implications for aquatic ecosystems and fishing resources worldwide (Hoegh-Guldberg & Bruno, 2010). Such climate alterations have a negative impact on fish migrations and habitats, leading to increases in fish abundance in certain areas and decreases in others (Hannesson, 2007).

According to Aadland (1993), river level and water temperature, as well as their modifications, are key variables influencing fish species' habitats and behavior. The natural diversity of fish communities can be explained by variations in water level, water temperature, physical habitat, water quality, and other significant environmental characteristics (Poff & Allen, 1995; Gebrekiros, 2016).

The effect of temperature on fish species distribution has long been recognized, manifesting at a broad geographic scale (Shuter, 1980). Temperature changes can lead to increased physiological demands and stress, reduced water oxygen saturation levels (Byrne, 2011; Matthews & Berg, 1997), limit fish species distribution, and alter their biological cycles, behavior, and distribution.

The interaction between temperature and precipitations is one of the key factors influencing the hydrological cycle within a watershed. Temperature and precipitation changes, caused by climate change, become essential elements for the transformation of the hydrological cycle. In the case of the Danube River, the hydrological regime is influenced by its geographical location on the continent at the intersection of the temperate-oceanic climate in the west, the temperate-continental climate in the east, and Baltic influences in the north (Chioveanu et al., 2020; Stroe et al., 2023). The evolution of water temperature is shaped by the distinct characteristics of the traversed regions.

Water temperature represents a central parameter in determining the overall health status of aquatic ecosystems, considering that aquatic organisms have specific temperature ranges they can tolerate. Additionally, air temperature significantly influences water temperature, as water warms and cools more slowly than air, generating linear variations in water temperature compared to abrupt changes in air temperature.

Water level represents a measure of the water height in a body of water, such as a river, a lake, or an ocean, relative to a certain reference point (Scrădeanu & Gheorghe, 2007). The Danube River level is a crucial parameter, holding major implications for fish habitat. The variability in water level can affect the availability of breeding and feeding areas for fish species.

In these conditions, aquatic ecosystems are subject to increasing pressure, and the Danube River, Romania’s main hydrological artery, is no exception. The Romanian region of the Danube River holds special significance for fish populations, essentially constituting a central wetland area (Ibănescu et al., 2016). The Danube Basin region encompasses a fascinating diversity of ecological territories, providing shelter for numerous plants and animals with unique characteristics.

The lower segment of the Danube River covers a distance of 1072 km, representing 38% of its entire length, from the Iron Gates to its mouth in the Black Sea. Over time, the Danube’s ichtyofauna has undergone profound changes determined by embankments and hydrotechnical works, water pollution, increases in fishing effort, as well as improvements in fishing methods and tools. Research on the Romanian sector's ichthyofauna of the Danube has led to the identification of 66 fish species, systematically classified into 19 families (Bacalbașa et al., 1984).

The fishery resource supporting the fishing activity is ensured by a small number of fish species. Thus, even though the Danube’s ichtyofauna includes 66 species, mostly freshwater fish or migratory species, only 18 species are commercially fished. The main economically valuable fish species caught annually are: pontic shad, perch, catfish, carp, pike. These species represent not only important resources for the fishing industry but also sensitive indicators to environmental changes. Understanding fish population dynamics and processes, including recruitment, growth and mortality is fundamental for improving fish stock evaluation and more efficient fisheries management (Sparre & Venema, 1998). Furthermore, Pauly (1983) considered that growth parameters and mortality rates represent key aspects of fish stock assessment. This importance derives from their ability to provide crucial information about how fish size varies over time and about the reduction in populations due to fishing and/or natural factors.

This paper focuses on exploring trends recorded by environmental factors (water level and temperature) during the period 2018-2022, highlighting the fluctuation and significant changes that can directly influence the annual catches of economically valuable fish species, as well as the total allowable catch, to provide a clear imagine of fishing sustainability and how climate change can affect the population of these species.

Additionally, the paper analyzes whether there are significant discrepancies between reported catches and allowable limits, identifying potential pressures on these exploited fish populations. This analysis will contribute to formulating recommendations for more efficient and sustainable resource management, considering climate change and its impact on economic species.

Through a holistic approach, we aim to contribute to a detailed understanding of complex interactions between climate changes, environmental factors and the exploitation status of valued species in the Danube River, thus providing a useful framework for decision-making in the sustainable management of aquatic resources.

MATERIALS AND METHODS

Data collection. The analysis of the influence of environmental factors was based on processing information regarding the daily water levels and temperatures of the Danube River from the period 2018 to 2022, obtained from the National Institute of Hydrology and Water Management (I.N.H.G.A.). Moldova Veche - km 1048, Calafat - km 795, Giurgiu - km 493, Braila - km 170, and Tulcea - km 71 were established as reference stations. Their processing involved calculating the annual average values for each analyzed station.

The comparative analysis of reported fish catches for the period 2018-2022 was conducted based on official data obtained from the National Agency for Fisheries and

Aquaculture, for the Romanian sector of the Danube, from the entry into the country at Baziaș to the Black Sea. The data were statistically processed using the Excel program from the Office 365 package.

Fishing area. The fish samples subjected to analysis were collected by research personnel from the Research and Development Institute for Aquatic Ecology, Fishing and Aquaculture Galați at the five mentioned stations (Figure 1), during scientific fishing sessions from May to August each year during the period 2018 to 2022.

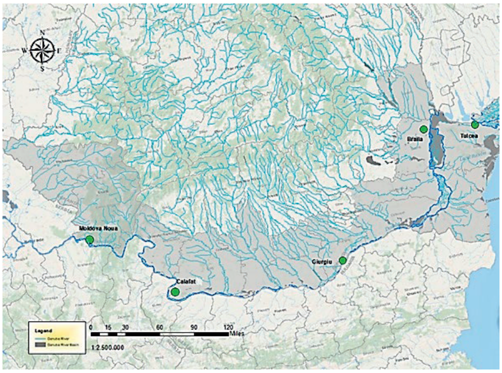

Figure 1. Fishing area in Danube River Basin km 71-km 1048, Romania (Source: original map)

Fishing was carried out using gear such as fixed nets and floating nets. The dimensions of the fishing gear were as follows: For fixed nets, the length of the set (Lp) ranged from 100 to 200 m, the vertical height (Hp) varied between 2.5 and 3.5 m, and the mesh size (a) measured between 40 and 60 mm.

In the case of floating nets, the length of the set (Lp) ranged from 150 to 200 m, the vertical height (Hp) could be between 2.5 and 4.0 m, while the mesh size (a) varied between 40 and 80 mm.

Utilizing an ichthyometer with a precision of ±0.01 cm, we gathered precise data on the total length (cm), standard length (cm), and height (cm) of the fish specimens.The weight of the specimens was determined with high precision using a scale with an accuracy of ±0.001 g.

The growth parameters and mortality rates. Growth parameters were estimated using the von Bertalanffy growth function (VBGF) (Von Bertalanffy, 1938) within the FISAT II program, using length-frequency data (class interval of 5 cm).

The specimens captured from the Danube River were grouped into length classes of 5 cm, and for each year of the analyzed period, the asymptotic length ($L\infty$; cm), growth coefficient (k; $year^{-1}$), and growth performance index (ϕ') were calculated.

According to Santos et al (2022), the asymptotic length represents the length that fish in a population would reach if they were to grow indefinitely, and k is the growth coefficient that expresses the rate at which the asymptotic length is approached.

The growth performance index (Ø') is a measure of the efficiency of individual fish growth, calculated using the formula $Ø' = \log_{10}(K) + 2*\log_{10}(L\infty)$ (Munro & Pauly, 1983).

Data regarding the length of the harvested biological material were separated by sexes, but growth parameters were estimated for both sexes. The purpose of estimating growth parameters is to gain a deeper understanding of how individual organisms or populations grow in size or weight depending on factors such as environmental conditions and food resource availability. These estimates can provide valuable information for assessing the health and stability of ecosystems and anticipating the impact of environmental changes on biodiversity.

The total annual mortality rate (Z; $year^{-1}$) for economically valuable species captured was estimated using length-based catch curves (Ricker, 1975) in FISAT II.

Natural mortality (M; $year^{-1}$) was calculated based on Pauly's empirical formula (Pauly, 1980), while fishing mortality (F; $year^{-1}$) was obtained from the relationship: $F=Z-M$ (Gulland, J.A., 1971). The annual average water temperature (T) was set at 15°C, based on the average annual water temperature of the Danube River.

The exploitation rate of the species was obtained based on the relationship $E=F/Z$ and reflects the degree of fishing exploitation of these species. Estimating mortality rates aims to quantify and understand the impact of factors leading to the death of individuals or populations within a certain time interval.

The growth parameters of fish population dynamics (asymptotic length L∞ and growth coefficient k) are critical for stock assessment and fisheries management (Santos, 2022).

RESULTS AND DISCUSSIONS

In recent years, the intensification of the drought phenomenon, closely linked to the increased frequency of extreme temperatures during the summer and the reduction in precipitation level has a negative impact on our territory, with significant repercussions, especially in the southern and southeastern regions of the country.

The time interval from 2018 to 2020, characterized by precipitation deficits, marked the onset of prolonged drought in Romania. At the same time, the period between 2019 and 2029 stands out as the warmest in the last 11 consecutive years, which is confirmed by the sustained increase in air temperature in our country.

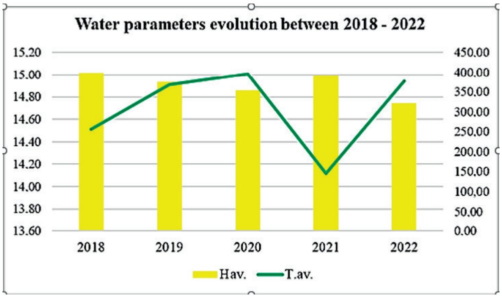

Figure 2. Evolution of Danube parameters 2018–2022 (Source: original)

The analysis of the relationship between the average annual water temperatures and the water level of the Danube River over the past five years indicates an inversely proportional relationship with reciprocal influences (Figure 2). For example, the increase in water temperatures in 2020 and 2022 affects the hydrological regime, resulting in a decrease in the river's water level. The variability of these parameters affects aquatic ecosystems, navigation, and the management of water resources for various uses. Changes in water temperature influence the biological cycles of aquatic species and affect water quality, with a direct impact on biodiversity and even human health. Current research by Madeira et al. (2016) and Linderholm et al. (2014) present the vulnerability of early life stages of aquatic organisms to increases in water temperature.

Thus, a fluctuation in average annual temperature and average annual water level can be observed over the analyzed period. The variation in average annual water temperature of the Danube River ranges from 14.12°C in 2021 to 15.01°C in 2020, with a standard deviation of 0.37°C. Additionally, the average annual temperature varies relatively easily from year to year, ranging from 13.98°C in Calafat in 2021 to 15.72°C in Giurgiu in 2020.

The values of the average annual water level vary on average by 3.43 cm within the interval of Hmin=320.96 cm in 2022 and Hmax=398.14 cm in 2018. Extrapolating the results from the period 2018-2022 to the analyzed stations (Figure 3), it can be observed that each station, except Moldova Veche, whose level remains relatively constant, records significant increases in the average level. The average values range from 71.66 cm at the Giurgiu hydrological station in 2022 to 748.73 cm at Moldova Veche, also in 2022, highlighting the repercussions of the 2022 pedological drought.

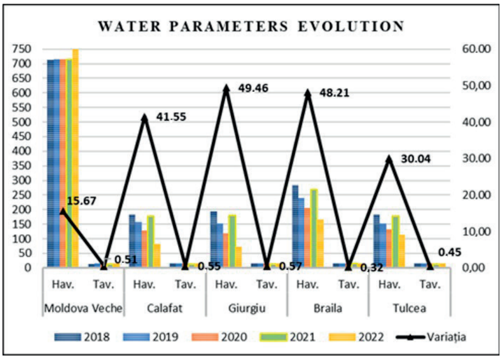

Figura 3. Danube River paramaters variation - on hydrological stations - between 2018-2022 (Source: original)

According to the National Meteorological Administration, the year 2022 - ranked third among the hottest years in Romania and tenth among the driest years in terms of precipitation - was highlighted as a year with pedological drought, caused by low water levels and high temperatures, affecting the availability of food and spawning grounds for fish species.

Analyzing the parameters for each month (Figure 4), it can be concluded that the warmest **January** was recorded in 2021, with high temperatures at all five analyzed stations (T.max = 4.52°C ± 0.79°C). The coldest January was in 2019 (Tmin. = 2.35°C ± 0.79°C). The highest water level was recorded in 2018 (Hmax. = 414.07 cm ± 78.71 cm), while the lowest was in 2020 (Hmin. = 229.79 cm ± 78.71 cm), due to the low precipitation level in that month (January 2020 ranks 3rd in the list of the driest January months).

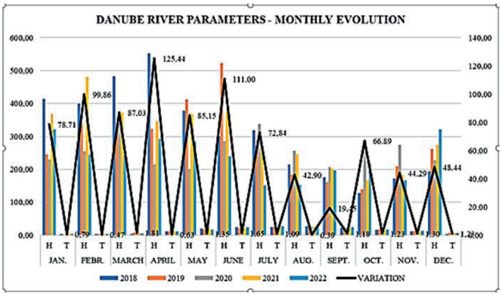

Figure 4. Danube River paramaters variation between 2018-2022 (monthly mean values) (Source: original)

Throughout the period 2018-2022, the relationship between the two parameters was strongly positive (Pearson's coefficient = 0.81).
For **February**, the water level ranged from Hmin = 244.25 cm ± 99.86 cm in February 2022 to Hmax = 480.94 cm ± 99.86 cm in February 2021. The temperature range of the Danube for February is between Tmin = 3.67°C ± 0.47°C (in 2022) and Tmax = 4.90°C ± 0.47°C (in 2020). Throughout the period 2018 - 2022, the correlation between the two parameters was positive (Pearson's coefficient = 0.69).
The warmest **March** in terms of Danube water temperatures was recorded in 2020 with Tmax = 8.11°C ± 181°C, while the coldest was in 2018 (Tmin = 3.80°C ± 1.81°C). Water temperature is one of the central parameters determining the overall health of aquatic ecosystems because aquatic organisms have specific temperature ranges they can tolerate, and variations of nearly 2°C from year to year have negative consequences on populations. The water level in this month ranged from Hmin = 243.20 cm ± 87.03 cm in 2022 to Hmax = 482.92 cm ± 87.03 cm in 2018. The Pearson coefficient value for March<0.5 indicates a lack of correlation between the two parameters.
The water level of the Danube in **April** fluctuated greatly from one year to another, with differences of 125.44 cm compared to the mean values for this month. The boundaries of the water level interval were Hmin = 215.23 cm (in 2020) and Hmax = 552.05 cm in 2018. The minimum level recorded in 2020 was due to extremely low precipitation in that month (the monthly precipitation amount for April 2020 was 12.8 mm, a deviation of -73% from the median of the 1991 - 2020 reference interval). Also, the lowest water temperature recorded during the analyzed period was Tmin = 10.43°C ± 0.63°C. The highest water temperature in April was recorded in 2019, Tmax = 12.03°C ± 0.63°C. The Pearson coefficient = 0.64 highlights the existence of a positive correlation.
In **May**, Tmin = 16.74°C ± 0.63°C, and Tmax = 20.27°C ± 0.63°C, mainly due to high air temperatures (according to the National Meteorological Administration, May 2018 ranks 2nd in the list of the hottest Mays in Romania, from 1961 to 2022). The water level ranged from Hmin = 201.46 cm ± 85.15 cm in 2020 to Hmax = 412.34 cm ± 85.15 cm in 2014. A strong positive correlation between the parameters is highlighted in this month as well (Pearson's coefficient = 0.77).
The water temperature in **June** ranged between 20.49°C and 24.51°C, with fluctuations of 1.65°C from year to year compared to the mean values for this month. The highest water level recorded in June was in 2019, Hmax = 523.20 cm ± 111 cm, and the lowest was in 2020, Hmin = 286.03 cm ± 111 cm. A positive correlation between the parameters is also evident for this month (Pearson's coefficient = 0.62).
July stands out as the hottest month of the year. In July, the Danube's water level ranged from Hmin = 151.07 cm ± 72.84 cm in 2022 to Hmax = 338.16 cm ± 72.84 cm in 2020. The average temperature values range from Tmin = 24.79°C ± 1.09°C in 2020 to Tmax = 27.08°C ± 1.09°C in 2021. There is a strongly positive correlation between the parameters (Pearson's coefficient = 0.73).

The water level in **August** reaches lows of 152.70 cm ± 42.90 cm (in 2023) and highs of 256.23 cm ± 42.90 cm (in 2020), while the temperature ranges between Tmin = 26.21°C ± 0.39°C (2020) and Tmax = 27.24°C ± 0.39°C (2022). A Pearson coefficient value of 0.86 indicates a strong positive correlation between the two parameters.

A strong positive correlation is also evident in **September**, with a Pearson coefficient of 0.96. The temperature range is between Tmin = 21.03°C ± 1.18°C (2020) and Tmax = 23.90°C ± 1.18°C (2022), while the water level ranges from Hmin = 160.65 cm ± 19.45 cm (2019) to Hmax = 206.61 cm ± 19.45 cm (2020).

In **October**, the monthly average for the Danube's water level ranged from 127.71 cm ± 66.89 cm (in 2018) to 290.70 cm ± 66.89 cm (in 2020). The minimum water temperature was recorded in 2021, Tmin = 14.60°C ± 1.23°C, while the maximum was 17.63°C ± 1.23°C in 2019. Pearson's coefficient = 0.67.

In **November**, Tmin = 10.49°C ± 1.30°C (2020), Tmax = 13.36°C ± 1.30°C (2019). Hmin = 167.12 cm ± 44.29 cm (2022), Hmax = 275.01 cm ± 44.29 cm (2020). Pearson's coefficient = 0.76.

In **December**, Tmin = 3.93°C ± 1.23°C (2018), Tmax = 7.27°C ± 1.23°C (2019). Hmin = 193.88 cm ± 48.44 cm (2018), Hmax = 321.70 cm ± 48.44 cm (2022). Pearson's coefficient = 0.94.

Monthly analysis of the Danube's parameters has shown high Pearson coefficient values in the majority of the months, with strong positive correlations in January, August, September, and December >0.8, highlighting the close relationship between the two parameters in these months and the significant influence they exert.

As ectothermic organisms (Favero et al, 2022), for reproduction (Donelson et al., 2010; Pankhurst & King, 2010), growth (Viadero, 2005; Akhtar et al., 2013), and even for survival, most fish species require specific ranges of water temperature and level (Fernandes et al., 2018; de Barros et al., 2019). Large year-to-year differences in both water temperature and level negatively impact river fish populations.

Comparative analysis of TAC and reported catches

Fisheries management is a strategic and practical approach for sustainably managing fish resources, ensuring the conservation of fish species and maintaining the ecological balance of aquatic ecosystems (Lopes, 2021). This field involves the implementation of policies and practices aimed to prevent the overexploitation of fish resources and promoting their sustainable use.

Constant monitoring of fish populations is essential to understand their dynamics. This process involves collecting data on population size. Based on stock assessments, authorities can establish fishing quotas that limit the quantity of fish that can be harvested from a particular area or for a certain species in decline. These quotas are set to prevent overexploitation and to maintain sustainable exploitation of resources.

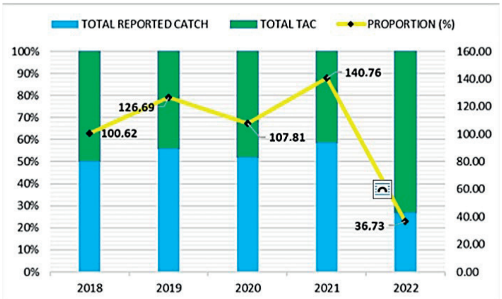

Figure 5. Analysis of Total Allowable Capture vs. Reported Catch (Source: original)

From Figure 5, it can be observed that the reported catch varied from year to year during the analyzed period. The lowest reported catch was in the year 2022 – 160.75 t, indicating either possible changes or limitations in fishing dynamics or resource management policies, or decreases in the migration behavior of aquatic species due to environmental factors.

Throughout the analyzed period, the reported catch exceeded the allowable quota, except in 2022, when the reported values were 36.73% of the Total Allowable Catch (TAC). Exceeding the allowable quotas by over 20% (in the years 2019, 2021) indicates overexploitation of fish populations, which can be concerning from a sustainable management perspective.

Analysis of the status of economically valuable species exploitation

The total of economically valuable species captured in the lower sector of the Danube River during the period 2019-2023 reached a count of 3,608 specimens. The most productive year of scientific fishing was in 2021, with a total of 831 specimens, including 467 carp, 105 perch, 200 catfish, and 59 pike. This was a year in which the analyzed environmental parameters, the temperature and water level of the Danube River (Tav. = 14.12°C and Hav. = 391.83 cm), favored the development of breeding and feeding habitats.

The proportion of each species and their distribution by gender is depicted in Figure 6, revealing a slightly higher presence of male specimens in the catches each year.

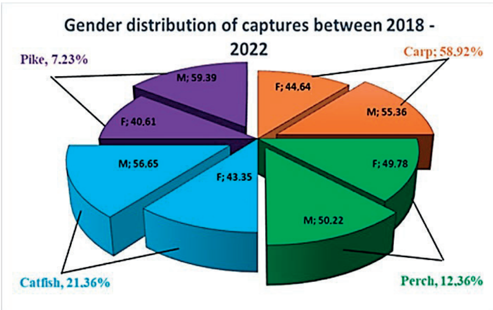

Figure 6. Gender distribution of captures between 2018-2022 (Source: original)

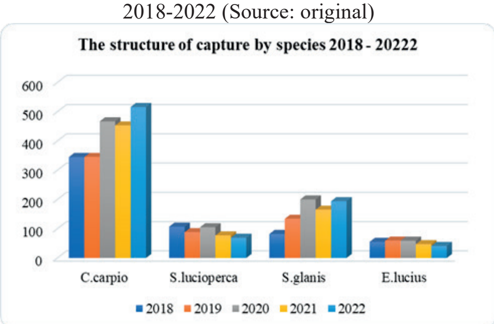

Figure 7. The structure of capture by species between 2018-2022 (Source: original)

From Figure 6 and Figure 7, the dominant presence of the carp species can be observed each year, accounting for 58.92%, followed by catfish with 21.48%, perch with 12.36%, and pike with 7.23%. The dominance of carp in the catches each year indicates that the fishing area provides specific breeding and feeding habitats for peaceful species. Additionally, years with high water levels, such as 2018 and 2021, favor the growth of aquatic vegetation, which provides suitable spawning substrates for phytophilic and phytofilic cyprinids, such as carp, etc. (Janac et al., 2010).

On the other hand, catfish and perch species occupy secondary positions, with percentages of 21.48% and 12.36%, respectively, indicating a significant but lesser presence compared to carp. The increase in water temperature may be conducive to predatory fish species such as catfish, perch, and pike by stimulating the growth rate of juveniles, increasing individual metabolic rates, and extending the growing season (Szczepkowski, 2006; Winfield et al., 2008). However, Hassler, 1982 reported for the northern pike that in the case of eggs incubated at temperatures higher than 15°C, premature hatching may occur, leading to anomalies in larvae that hinder the functional development of yolk sac larvae, adherence, and sinking to the bottom of the water, where toxic conditions are often encountered (low oxygen levels, presence of hydrogen sulfide). This phenomenon could increase larval mortality by 40%.

The pike species recorded the lowest presence, representing only 7.23% of the total catches, suggesting a more limited presence but indicating an ecosystem balance.

From year to year, a decrease in the size range of individuals can be observed, from 35-91 cm (Lt) in 2019 to 30-71 cm in 2023 (Table 1), which may indicate a change in the demographic structure of the fish population or may result from external influences, such as or changes in the aquatic habitat. Within the same species, variations in the parameters $L\infty$ and k can be attributed to a wide range of causes, including changes in water conditions, food availability, metabolic rate, fishing pressure, and pollution levels.

Additionally, the growth performance indices (ϕ') of carp, perch, and pike vary significantly over time and may reflect changes in growth rates and estimated maximum length of the analyzed fish species, while for catfish, it remains relatively stable.

Table 1. Parameters of the von Bertalanffy growth equation for catches from the period 2019–2023

SPECIES	NO. OF FISH	LTMIN.	LT.MAX	L∞	K	M	Z	F	E	M/K	Φ'
CYPRINUS CARPIO											
2019	345	35	91	94.5	0.91	0.91	1.07	0.16	0.15	1.00	3.91
2020	345	35	91	94.5	1.2	1.09	0.37	-0.72	-1.96	0.91	4.03
2021	467	41	90	94.5	0.45	0.58	1.34	0.76	0.57	1.29	3.60
2022	453	30	80	84	1.5	1.31	1.71	0.4	0.24	0.87	4.02
2023	516	30	71	73.5	0.61	0.75	1.67	0.92	0.55	1.23	3.52
TOTAL	2126										
SILURUS GLANIS											
2019	82	52	91	94.5	0.56	0.66	0.64	-0.02	-0.04	1.18	3.70
2020	134	56	98	99.75	0.62	0.7	1.82	1.12	0.62	1.13	3.79
2021	200	45	90	94.5	0.56	0.66	0.85	0.19	0.22	1.18	3.70
2022	165	38	92	99.75	0.62	0.7	1.91	1.21	0.63	1.13	3.79
2023	194	30	91	94.5	0.56	0.66	0.97	0.31	0.32	1.18	3.70
TOTAL	775										
SANDER LUCIOPERCA											
2019	107	28	57	57.75	1.4	1.39	2.91	1.52	0.52	0.99	3.67
2020	88	28	57	57.75	1.4	1.48	3.95	2.56	0.65	1.06	3.67
2021	105	28	63	63	0.89	1.01	1.24	0.23	0.19	1.13	3.55
2022	77	28	50	52.5	1.1	1.22	5.29	4.07	0.77	1.11	3.48
2023	69	30	76	68.25	1.5	1.39	1.96	0.57	0.29	0.93	3.84
TOTAL	446										
ESOX LUCIUS											
2019	55	25	54	52.5	0.55	0.77	3.11	2.34	0.75	1.4	3.18
2020	59	25	54	52.5	0.55	0.77	3.25	2.48	0.76	1.4	3.18
2021	59	25	54	52.5	1.1	1.22	1.38	0.16	0.12	1.11	3.48
2022	47	25	52	42	0.75	1.01	1.43	0.42	0.29	1.35	3.12
2023	41	32	59	57.75	1.4	1.39	0.85	-0.54	-0.63	0.99	3.67
TOTAL	261										

Cyprinus carpio

The analysis of the growth parameters and mortality rates of the species *Cyprinus carpio* during the period 2019-2023 highlights significant and relevant variations for the fish population status. The variation in the species' growth rate is influenced by external factors such as environmental conditions and exploitation through fishing. During the analyzed period, fluctuations in the growth rate are observed.

From Figures 8, 9, and 10, it can be observed that in the years 2019, 2020, and 2022, the growth rate was faster, while in 2021 it was slower.

In general, the high values of natural mortality (M) in these years indicate underexploitation of the species. This conclusion is also supported by the low values of the exploitation coefficient (E), indicating a reduced proportion of the fish population being captured by fishing. The low values of fishing mortality (F) in years with high growth rates (2019, 2020, and 2022) suggest low fishing pressure on the population. These aspects can be advantageous for maintaining fish stocks at healthy levels.

The exploitation coefficient (E) indicates the proportion of the fish population that is caught by fishing. The low values of E in these years (especially in 2019 and 2020) support the hypothesis of species underexploitation.

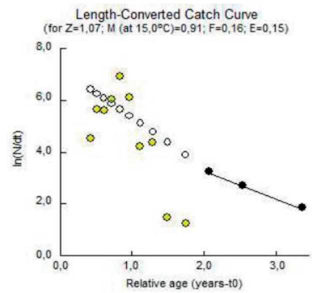

Figure 8. Growth parameters and mortality rates for *C. carpio* - 2019 (Source: original)

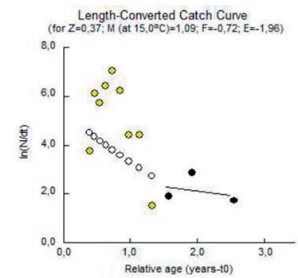

Figure 9. Growth parameters and mortality rates for *C. carpio* - 2020 (Source: original)

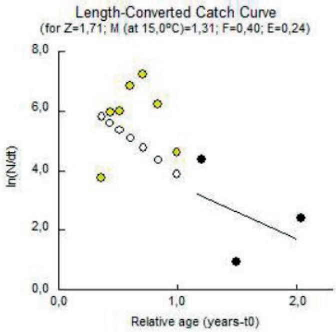

Figure 10. Growth parameters and mortality rates for *C. carpio* - 2022 (Source: original)

Regarding the years 2021 and 2023, where the exploitation coefficient E takes values of 0.57-0.55, indicating slight overexploitation. Similar results are also reported in the literature by Gheorghe et al (2011), for the carp species caught at Braila, Km 170-196.

The analysis indicates a complex relationship between growth rates, natural mortality, and fishing pressure. Understanding these interactions is essential for the sustainable management of fish resources. The changes observed in growth and mortality parameters can have consequences for population dynamics and may require adjustments in fishing management and conservation policies.

Silurus glanis

The data analysis for the species *Silurus glanis* in the period 2019-2023 reveals important information about the population dynamics and the exploitation status of the species. The growth rate (K) varies slightly from year to year, generally ranging around the values of 0.56 or 0.62.

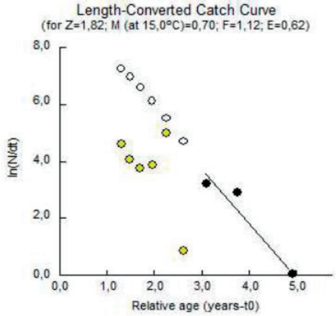

Figure 11. Growth parameters and mortality rates for *S. glanis* - 2020 (Source: original)

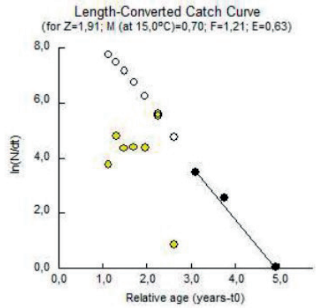

Figure 12. Growth parameters and mortality rates for *S. glanis* - 2022 (Source: original)

Natural mortality (M) remains relatively constant, with relatively high values, close to 0.66 or 0.7. The total mortality rate (Z) registers values ranging from 0.64 to 1.91, being influenced by both components, natural mortality, and fishing mortality. The slightly elevated exploitation rate in 2020 and 2022 (Figures 11 and 12) indicates a tendency of overexploitation of the species, a situation that is rectified in 2023, indicating the need for monitoring and evaluation in the future to ensure sustainable management of fish resources.

The growth parameters and mortality rates indicate a relatively stable population of the catfish during the analyzed period. The positive values of the exploitation coefficient suggest that the species is subject to moderate fishing pressure, but not at an alarming level.

Sander lucioperca

For the perch species, growth parameters and mortality rates show significant variation between the analyzed years ($p < 0.05$), reflecting possible changes due to environmental pressure and fishing activities.

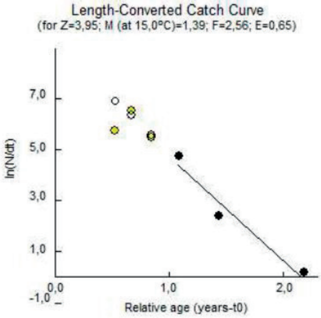

Figure 13. Growth parameters and mortality rates for *S. lucioperca* - 2020 (Source: original)

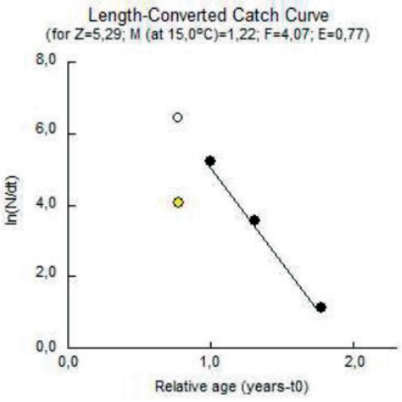

Figure 14. Growth parameters and mortality rates for *S. lucioperca* - 2022 (Source: original)

The growth rate (K) exhibits significant variations between years ($p<0.05$), ranging from 0.89 to 1.5. Natural mortality (M) remains relatively constant in the analyzed years, with values between 1.01 and 1.48.

The total mortality rate (Z) is highly variable, suggesting a significant influence from external factors on population mortality. The variable values of the exploitation coefficient indicate the need for continuous monitoring and sustainable management of fish resources for the *Sander lucioperca* species.

The analysis indicates that in the years 2020 and 2022, the growth parameter values and mortality rates for the perch species are higher compared to other years (Figures 13 and 14). Therefore, these years could be considered significant for the perch species. Specifically, in 2020 and 2022, the growth rate (K) and total mortality rate (Z) are generally higher, and the exploitation coefficient (E) indicates greater fishing pressure on the perch population.

Thus, these years can be considered significant in terms of the dynamics of the perch population and the pressure exerted by fishing activities on it. Results indicating overexploitation of the *Sander lucioperca* species in the Danube River have also been reported by Ibănescu et al., (2019) for the river section near the locality of Brăila, km 170-197.

Esox lucius

The significant variations in growth parameters and mortality rates suggest a fluctuating dynamic of the *Esox lucius* population during the analyzed period. The growth rate (K) varies significantly between the analyzed years, with values ranging from 0.55 to 1.4. Natural mortality (M) is also variable, with values between 0.77 and 1.39. The total mortality rate (Z) is variable, with values between 0.85 and 3.25. Fishing mortality (F) is also variable, with values between -0.54 and 2.48.

The exploitation coefficient (E) is variable and indicates the fishing pressure on the population. The values are generally positive, except for the year 2023, indicating moderate to high fishing pressure on the species.

The years 2019 and 2020 recorded higher values of mortality rates and exploitation coefficients, indicating greater pressure on the population due to fishing activities (Figures 15 and 16).

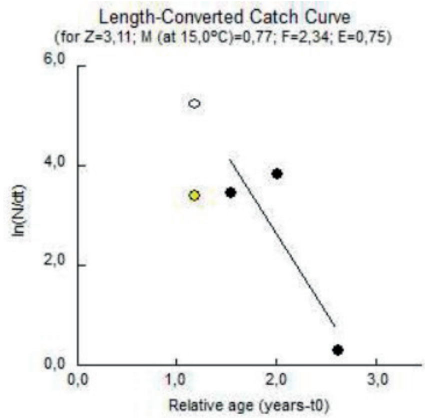

Figure 15. Growth parameters and mortality rates for *E. Lucius* - 2019 (Source: original)

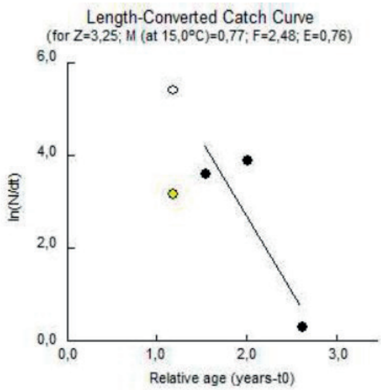

Figure 16. Growth parameters and mortality rates for *E. lucius* - 2020 (Source: original)

The year 2023 stands out with negative values of fishing mortality and exploitation coefficient, which may indicate underexploitation of the species in this year. (Figure 17)

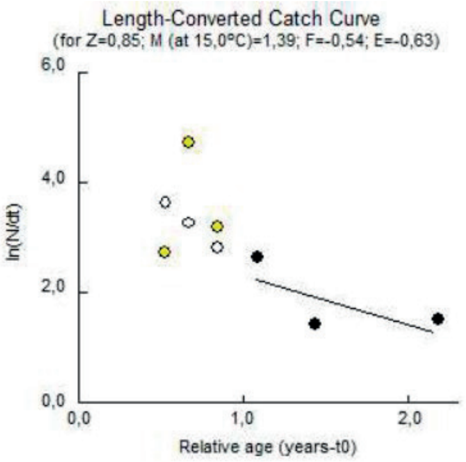

Figure 17. Growth parameters and mortality rates for *E. lucius* - 2023 (Source: original)

The data analysis for *Esox lucius* shows significant variation in growth parameters and mortality rates in the studied period (2019, 2020, 2023) can be considered important years due to significant changes observed in these parameters.

CONCLUSIONS

The studied species are important, both economically and ecologically, which is why the estimated population parameters serve as a benchmark in the development of stock assessment.

The impact of climate changes on the environment, especially on the water temperature and level of the Danube River has been evident in recent years. Rising temperature and water level variability have a significant influence on the aquatic ecosystem and fish species behaviour.

Changes in water temperature and level determined fluctuations in fish habitat and distribution, affecting biological cycles, migration and availability of reproduction and feeding areas.

Comparative analysis of reported catches and allowable fishing limits has revealed a trend of overexploitation of commercially valued fish species in the lower Danube sector. Although there are regulations and fishing quotas established to maintain sustainable exploitation of resources, reported catches have consistently exceeded allowable limits in the analyzed period.

Economically valued fish species such as carp, catfish, pikeperch, and pike have shown significant variations in growth parameters and mortality rates during the analyzed period. These variations can be attributed to multiple influences, including changes in environmental conditions, fishing pressure, and alterations in aquatic habitats.

The study has highlighted the need for more efficient and sustainable management of Danube fish resources, considering the impact of climate change and fishing pressures. Recommendations for the future include continuous monitoring of fish stocks, adjusting fishing quotas based on population dynamics, and implementing sustainable fishing practices.

In conclusion, climate change poses a significant threat to fish populations in the Danube, and proper management of these resources becomes increasingly urgent in the context of environmental fluctuations and anthropogenic pressures. Continuous monitoring and conservation actions are ways to ensure the sustainability of fishing and aquatic ecosystems in the future of the Danube.

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QUALITY ASSESSMENT OF TRADITIONAL SMOKED ARCTIC CHAR, *Salvelinus alpinus* (Linnaeus, 1758)

Paul UIUIU[1], Alexandru SAVA[1], Camelia RADUCU[1], Radu CONSTANTINESCU[1], Călin LAȚIU[1], Cristina CRAIOVEANU[2], Andrada IHUȚ[1], George-Cătălin MUNTEAN[1], Tudor PAPUC[1], Vioara MIREȘAN[1], Daniel COCAN[1], Anca BECZE[3]

[1]Faculty of Animal Science and Biotechnologies, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, 3-5 Calea Manastur, Cluj-Napoca, Romania
[2]Sociobiology and Insect Ecology Laboratory, Department of Taxonomy and Ecology, Faculty of Biology and Geology, Babes-Bolyai University, 44 Gheorghe Bilascu Street, Cluj-Napoca, Romania
[3]National Institute for Research and Development of Optoelectronics - INOE 2000, 67 Donath Street, Cluj-Napoca, Romania

Corresponding author email: cocan.daniel@usamvcluj.ro

Abstract

Regular consumption of fish can contribute to a more balanced and healthy diet. Incorporating fish into one`s diet is important for maintaining a healthy and sustainable lifestyle. The processes of traditional smoking undergo a complex series of steps that significantly influence its quality, nutritional composition, and sensory characteristics. To address this issue, a study was conducted to evaluate the meat quality and salt concentration of traditionally smoked Arctic char at different stages of the smoking process (fresh meat, salted meat, desalted meat, and smoked meat). All the physicochemical measured parameters varied significantly during the smoking processes, with the quality of the products remaining high. The final product, traditionally smoked trout, experiences a substantial decrease in water content through drying, salting, desalting, and smoking at high temperatures, increasing dry matter content. Furthermore, there is a significant boost in protein content in smoked trout compared to fresh, salted, and desalted meat.

Key words: *human health, salmonids, smoked trout, traditional smoking.*

INTRODUCTION

Global fish consumption has steadily increased in recent years mainly due to its nutritional and health benefits (Chen et al., 2022; Hei, 2020). Regular consumption of fish can contribute to a more balanced and healthy diet (Pieniak et al., 2010; Swanson et al., 2012; Utri-Khodadady and Głąbska, 2023), offering various health benefits and reducing the risk of chronic diseases (Karimi et al., 2020; Zhao et al., 2016). Approximately 50% of the seafood humans consume consists of fresh and live fish. The term "fresh" refers to fish that have not undergone the freezing process, including still-alive fish, as well as those kept in the cold (but not frozen) or those packed in a modified atmosphere (McManus et al., 2014).

Usually, the common preservation methods used for fish products to extend their shelf life maintain quality, and ensure consumer safety are cooling and refrigeration, drying and dehydration, use of different types of packaging, salting, and use of different types of preservatives (Mahmud et al., 2018).

Besides the usual consumer choice of either fresh or chilled/frozen fish, other forms as smoked, sun-dried, brined, and canned fish products (in oil, water, or sauces), occupy a significant place in the market and are seen as valued delicacies. When it comes to fish and seafood products, consumer safety is of major importance due to the potential risks associated with microbial, chemical, and biotoxin contamination (Biji et al., 2016; Mohanty et al., 2019; Novoslavskijv et al., 2015). Ensuring the safety of fish products involves a combination of good manufacturing practices and regulatory oversight (Hidayat et al., 2019).

The crafting of traditional smoked trout is a particularly meticulous process that involves the careful selection of fish specimens and ingredients, followed by different cleaning methods, brining (immersing fish in a salt solution), and hot smoking (70-85°C) to enhance flavor, texture, and preserve its freshness over time (Belichovska et al., 2019). The processes of traditional smoking undergo a complex series of steps that significantly influence its quality attributes, nutritional composition, and sensory characteristics (Belichovska et al., 2019, Sava et al., 2020). Each step in the production process contributes to the unique characteristics that make smoked trout a cherished food item among consumers.

The majority of research conducted on smoked meat focuses solely on the end product. There is limited analysis of the particular steps involved in the production process. Thus, this study approaches the quality assessment of smoked meat of Arctic char, *Salvelinus alpinus* (Linnaeus, 1758) species in each step of the traditional way of smoking (fresh, salted, desalted, and smoked meat). The main objective was to determine the physico-chemical properties of traditional smoked trout meat, and an analysis of the salt content used in the smoking process.

MATERIALS AND METHODS

All procedures involving animals were conducted following Romanian and European Legislation (Law no. 43/2014; EC-Directive 2010/63/EU). The Animal Ethics Committee of the UASVM Cluj-Napoca approval (No. 145/2019) was obtained before the study started and followed all the bioethical rules and guidelines applicable to animal studies described by them.

Fish and Experimental Protocol

The fish specimens used in this study were sampled from a trout farm located in Râșnov, Brașov County, named Trecătoarea Ursului. One hundred specimens of Arctic char were used in the study, with an average weight of 251.3 ± 0.63 g, and an average total length of 28.5 ± 0.68 cm. The specimens used in the study were clinically healthy.

The traditional smoking stages were: harvesting, stunning (mechanical stunning percussion in the dorsal-aboral region of the head), evisceration (removing of the internal organs), washing (removal of mucositides and impurities adhering to the fish surface, elimination of blood and viscera), salting (prepare a curing mixture using salt and water, salting time is at least 16 hours, under refrigeration conditions, 2-4°C) desalting (washing), drying (the washed fish is left to dry, between 2 and 4 hours), hot smoking at 70-85°C (the fuel used for smoking is made of beech and cherry wood, stifled when necessary, with nettle and fir satin), followed by cooling and resting phase (the fish needs to cool to room temperature before refrigerating) (Sava et al., 2020; Sava et al., 2023).

Physicochemical Analysis

Five samples from different places in the smoking chamber were taken and analysed during the different smoking stages. Samples were examined as fresh, salted, desalted, and smoked meat. Moisture content was determined by the reference method SR ISO 1442:2010. The dry matter content was determined according to Mertens et al., 2004. Fat content was determined by the Soxhlet method based on the reference method SR ISO 1444:2008. The total nitrogen content was determined by the Kjeldahl method based on the reference method SR ISO 937:2007, with the subsequent calculation of the protein content. The non-nitrogenous extractive substances (SEN) content was determined by the standard method, according to Odagiu and Porca, 2003, and the ash content according to the method used by Perez and Andujar (1981).

Data analysis

The following fish meat parameters were measured in percentages: moisture, dry matter, fat, protein, non-nitrogenous extractive substances (SEN), ash, and supplemented salt. Stages of meat preparation were defined as treatments with the following steps: 1_fresh (the non-treated fish meat), 2_salted, 3_desalted, and 4_smoked. We compared fish meat parameters between treatments with the help of a paired t-test or with the Wilcoxon test in case data was not normally distributed (just the case of ash percentage in fresh samples).

To place the nutritional values of *S. alpinus* after this traditional processing methodology in the context of a wider fish meat palette available on the market, we compared the data from our study with values obtained in another study of our research team, written by Sava et al. in 2020, in a similar study from two other

species: *Salvelinus fontinalis* (Mitchill, 1814) and *Oncorhynchus mykiss* (Walbaum, 1792).
We compared our data to the data obtained in the Sava et al. 2020 study with the help of a one-way ANOVA or a Kruskal-Wallis test, depending on the data distribution. Normal distributions of all parameters were checked with a Shapiro-Wilk test. All statistical analyses and graphs were performed in the program RStudio (Posit team, 2023). We assembled graphs in the form of Boxplots that represent median values (line inside the box), interquartile interval (box), minimum and maximum values (whiskers), and outliers (circles). Letters above or under parameter values express differences from pairwise t-tests or Wilcoxon test comparisons. Different letters between treatments represent significant differences at the $P<0.05$ threshold.

RESULTS AND DISCUSSIONS

Moisture varied significantly with treatment: through salting the moisture percentage sank significantly from 72% to 70%, followed by an increase to approximately fresh levels in the desalting stage, and then dropping to approximately 62% after smoking (Tab. 1, 2, Fig. 1).
Dry matter followed a complementary pattern to moisture, significantly varying between treatments. Dry matter increased through salting, decreased after desalting to approximately fresh levels, and increased again by approximately 10% after smoking (Tables 1, 2, Figure 1).
Fat percentage was lowered by one percent after salting, however, the percentage returned to a similar value of the fresh meat after smoking (Tables 1, 2, Figure 1).

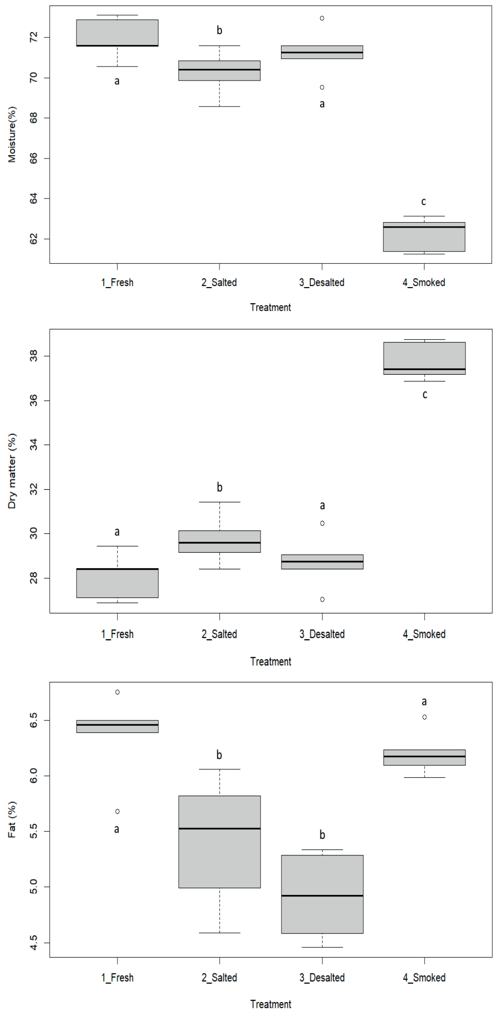

Figure 1. Percentages of moisture, dry matter, and fat measured in samples of *S. alpinus* meat in different stages of processing (treatments)

Table 1. Mean and Standard Deviation values for measured parameters of *S. alpinus* meat samples in different stages of processing (treatments)

Parameter	Treatment	Mean	Standard deviation
Moisture % (N=5)	Fresh meat	71.94	1.05
	Salted meat	70.26	1.13
	Desalted meat	71.26	1.23
	Smoked meat	62.23	0.86
Dry matter % (N=5)	Fresh meat	28.06	1.05
	Salted meat	29.74	1.13
	Desalted meat	28.74	1.23
	Smoked meat	37.77	0.86
Fat % (N=5)	Fresh meat	6.36	0.40
	Salted meat	5.40	0.60
	Desalted meat	4.92	0.40
	Smoked meat	6.20	0.20
Protein % (N=5)	Fresh meat	16.93	0.68
	Salted meat	15.43	0.73
	Desalted meat	16.79	0.80
	Smoked meat	22.06	0.65
SEN % (N=5)	Fresh meat	0.28	0.08
	Salted meat	0.24	0.02
	Desalted meat	0.43	0.07
	Smoked meat	4.10	0.52
Ash % (N=5)	Fresh meat	4.49	0.30
	Salted meat	8.67	0.55
	Desalted meat	6.60	0.58
	Smoked meat	5.41	0.32
Salt % (N=5)	Salted meat	3.77	0.42
	Desalted meat	1.93	0.10
	Smoked meat	1.12	0.19

Table 2. Pairwise comparisons with paired t-tests or Wilcoxon-test (df = 4 in all comparisons) of parameter mean values in successive stages of processing (treatments)

Parameter	F vs Sl	F vs D	F vs Sm	Sl vs D	Sl vs Sm	D vs Sm
Moisture %	t = 2.88 P = 0.045	t = 1.16 P = 0.310	t = 13.29 P < 0.001	t = -9.63 P < 0.001	t = 20.18 P < 0.001	t = 20.97 P < 0.001
Dry matter %	t = -2.88 P = 0.045	t = -1.16 P = 0.310	t = -13.29 P < 0.001	t = 9.63 P < 0.001	t = -20.18 P < 0.001	t = -20.97 P < 0.001
Fat %	t = 6.05 P = 0.004	t = 5.12 P = 0.007	t = 0.69 P = 0.530	t = 1.21 P = 0.294	t = -2.85 P = 0.046	t = -5.99 P = 0.004
Protein %	t = 4.06 P = 0.015	t = 0.29 P = 0.787	t = -9.33 P < 0.001	t = -2.83 P = 0.047	t = -18.07 P < 0.001	t = -13.22 P < 0.001
SEN %	t = 0.95 P = 0.397	t = -3.19 P = 0.033	t = -14.83 P < 0.001	t = -5.62 P = 0.005	t = -16.49 P < 0.001	t = -17.04 P < 0.001
Ash %	W = 0 P = 0.008	W = 0 P = 0.008	W = 1.00 P = 0.016	t = 41.35 P < 0.001	t = 10.55 P < 0.001	t = 3.75 P = 0.020
Salt %	-	-	-	t = 12.15 P < 0.001	t = 23.76 P < 0.001	t = 15.99 P < 0.001

*Significant differences are marked with a grey background. (F – fresh; Sl – salted; D – desalted; Sm – smoked)

Protein percentage was lowered after salting, but increased back to fresh levels after desalting and increased significantly by 5% after smoking (Tables 1, 2, Figure 2).

Non-nitrogenous extractive substances (SEN) did not differ between fresh meat and salted meat; however, after desalting, the percentage of SEN was higher and increased even more in smoked samples of fish meat.

Ash percentage increased significantly after salting; however, ash percentage was lowered after desalting, and got even lower after smoking to approximately 1% higher than in the fresh samples.

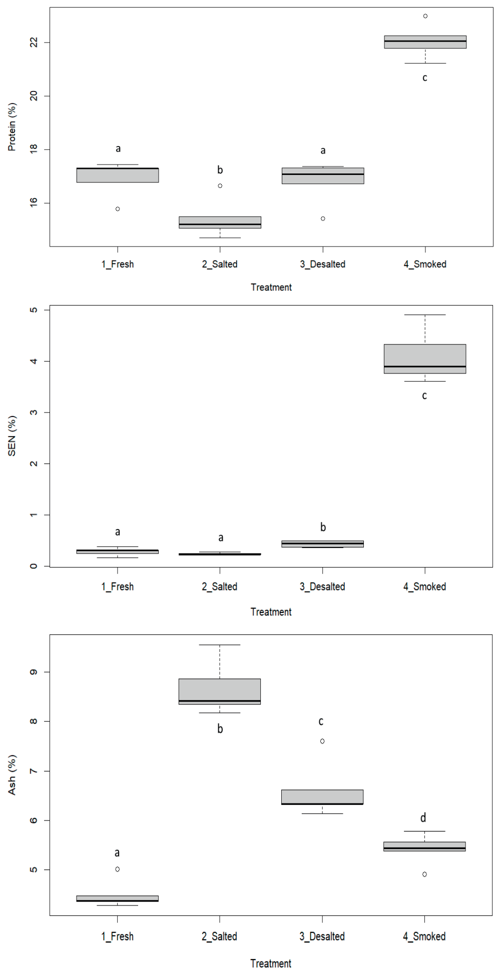

Figure 2. Percentages of protein, non-nitrogenous extractive substances, and ash measured in samples of *S. alpinus* meat in different stages of processing (treatments)

For the supplemented salt percentage, we also recorded significant differences between treatments. After salting, the salt content was high in the samples, with a mean of 3.7%. After desalting and smoking, the salt percentage went down to a mean of 1.1% (Tables 1, 2, Figure 3).

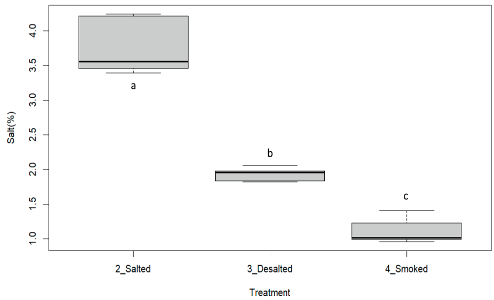

Figure 3. Percentages of salt measured in samples of *S. alpinus* meat in different stages of processing (treatments)

When comparing species' parameters, we observed significant differences for the following parameters: Fat % (df = 2, F = 24.91, P < 0.001), Protein % (df = 2, F = 5.07, P = 0.025), SEN % (df = 2, F = 11.65, P = 0.02), Ash % (df = 2, F = 138.60, P < 0.001). *S. alpinus* had higher fat and protein percentages than *O. mykiss*, but similar percentages to that of *S. fontinalis*. SEN percentage was lower in *S. alpinus* than in both other species. Ash percentage was higher in *S. alpinus* than in both other species. All mean values of the measured parameters in all three species are presented in Figure 4.

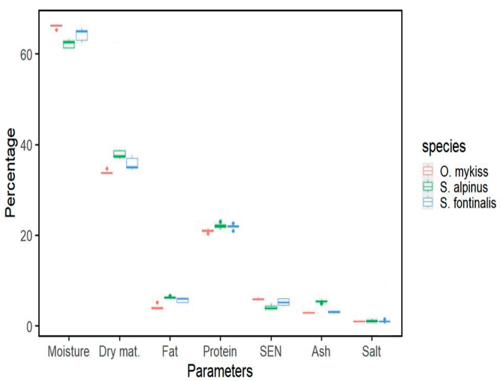

Figure 4. Mean values of measured parameters (in %) in *S. alpinus* compared to two other species, *S. fontinalis* and *O. mykiss*

CONCLUSIONS

During the traditional smoking process, the trout undergoes several stages that lead to significant changes in its chemical composition. The trout is first washed, salted, then desalted, and finally, it is dried and smoked at high temperatures. As a result of this process, the trout loses a substantial amount of water content, which increases its dry matter. Additionally, the protein content of smoked trout is significantly higher than that of fresh, salted, and desalted fish due to changes in the amino acid structure that occur during the hot-smoking process.

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HOW ONE HEALTH AND ONE WELFARE CAN STRENGTHEN THE EVIDENCE OF A MANAGEMENT PROCEDURE - A CASE STUDY OF EYESTALK ABLATION IN FARMED SHRIMP

Laura URDES[1], Chris WALSTER[2], Julius TEPPER[3], Leo FOYLE[4]

[1]Faculty of Management and Rural Development, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]The Island Veterinary Associates, Stafford ST17 4AH, United Kingdom
[3]Long Island Fish Hospital Manorville, 1 Saddlebrook Lane, Manorville, NY 11949, United States
[4]College of Public Health, Medical and Veterinary Sciences, James Cook University, 1 James Cook Dr, Douglas QLD 4814, Australia

Corresponding author email: urdeslaura@gmail.com

Abstract

One Health is connected with the One Welfare through links between animal and human welfare, and with sustainable animal-keeping systems. This connection fosters interdisciplinarity, and helps ensure human and animal wellbeing, while addressing more effectively current societal challenges in a more sustainable way. There is a knowledge gap regarding some species-specific operational welfare indicators in some aquaculture species such as crustaceans with potential to impact human wellbeing, and justifying a One Welfare approach. A review of the scientific literature based on PRISMA protocols has been carried out within this study. The review focuses on pain indicators and nociception, and the potential impact on the welfare of shellfish, as well as on the physiological and molecular mechanisms associated with the use of eyestalk ablation (EA) in adult female shrimp in aquaculture facilities. Through the One Health and One Welfare approaches, an assessment of whether the EA procedure is valid for use in shrimp management protocols was also carried out. The case study concluded that alternatives to EA should be sought to ensure compliance of this practice with the One Health and One Welfare concepts.

Key words: *aquaculture shrimp, eyestalk ablation, one health*, *Penaeus spp.*

INTRODUCTION

The concept of One Health has been practiced since ancient times, yet we have come a long way to the One Health concept as we understand it today. One Health has been recently defined by the One Health High Level Expert Panel (OHHLEP) as the concept which "recognizes that the health of humans, domestic and wild animals, plants, and the wider environment (including ecosystems) are closely linked and interdependent" (Adisasmito et al., 2022). One Health is contributing to sustainability, addressing collective needs, such as the requirement for safe and nutritious food, as well as other needs (WOAH/OIE, 2019). One Health, used for the human, animal and environment components of health, partially overlaps with One Welfare, thus extending One Health's approach to a much broader view (Pinillos et al., 2016). Consumer protection, food safety and public health are intrinsic components of One Health, wherein traceability of animals and animal products in animal farming and the food production industry are of paramount importance, aligning One Health with One Welfare. As food provenance and farm assurance gain traction, consumers are increasingly seeking information on conditions under which their food is produced (Nicolae et al., 2017). Among known good practices, wellbeing of the producers of animal-derived food, and welfare of the farmed animals (including humane handling, and the prevention of animal disease), are required (WOAH/OIE, 2019). The importance of welfare of farmed animals is now well known, although there are still many conflicting aspects arising from its translation into practice. Identifying all (farmed) sentient non-human animals to whom strict welfare criteria should be applied is a daunting task. Ideally, a gold standard should be available to benchmark sentience amongst different species. Such a standard is currently lacking (Regan, 1985; Diggles et al., 2024). This paper discusses the practice of eyestalk ablation (EA) in penaeid shrimp, currently applied in some shrimp (and other shellfish) maturation facilities to help overcome captivity induced to resolve the inhibition of maturation in females (Meng et al., 2020). Using the example of EA, the authors explore existing links between One Health and One Welfare, while assessing whether EA is appropriate for use in shrimp management protocols. Based on the assumptions of Preferred Reporting Items for Systematic review and Meta-Analysis Protocols (PRISMA protocols) (Moher et al., 2015), in order to be more sensitive and capture as many relevant references as possible, this review used the term shrimp interchangeable with prawn, and shellfish with crustacean. Pain was first used to describe a human emotional negative experience, and is defined by the International Association for the Study of Pain as an "unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage" (Yue, 2008; Raja et al., 2020). Invertebrates demonstrate behavioural and neural plasticity that is similar to that of vertebrates. There is an increasing number of studies providing evidence of sentience in cephalopods due to their cognitive ability and response to noxious stimuli and pain killers (Powell, 2022). Although pain may not be directly measurable to observers of invertebrates, pain-like states can be inferred in these animals based on animal behaviour, underlying nervous activity in the nociceptive systems that process information related to an injury, sound and "vocalization", which approximately 100 invertebrate species are reported capable of producing to communicate among themselves (Walters, 2018; Miles et al., 2022). Pain has survival and adaptive values, helping to increase the chance of passing on genetic makeup to future generations, and leading to species preservation. Diggles (2019), argues that the scientific literature of welfare and pain in crustaceans is immature, based largely on a few dubious and disputed studies conducted in a small number of decapod species. Despite recognition that there is a growing focus on invertebrates such as cephalopods and crustaceans, further research is needed to provide more insight into the sentience of these animals, and in this case, particularly in penaeids (Proctor et al., 2013). Nociception research has been conducted mostly on the search for nociceptors, or pain receptors, the sensory neurons responsible for signalling potential damaging stimuli (Zimmerman, 1986; Taylor et al., 2004; Tobin & Bargmann, 2004; Kristiansen & Bracke, 2020; Olsson et al., 2021;). In this paper, the authors chose the criterium of pain because it has a direct impact on the welfare of commercially farmed animals. This is the reason that eyestalk ablation (EA) is discussed here. The specific objectives of this study are to review the scientific literature about pain indicators and nociception, and the potential impact on the welfare of crustacea, review the physiological and molecular mechanisms associated with the use of EA in adult female shrimp in aquaculture facilities, try to determine through the One Health and One Welfare concepts whether this procedure is valid for use in shrimp management protocols, and provide suggestions for alternatives to EA.

MATERIALS AND METHODS

A review of the scientific literature on pain indicators and nociception, and the potential impact on the health and welfare of shellfish following EA was performed based on the assumptions of PRISMA-P (Moher et al., 2015). PRISMA uses a set of items for reporting in systematic reviews and meta-analyses, focusing primarily on the reporting of reviews evaluating the effects of interventions and critical appraisal of published systematic reviews. The information sources were identified through the National Library of Medicine databases [PubMed, PubMed Central (PMC)] and Web of Science. Google Scholar, which is a much broader, yet less focused search, was not included with this study. The search strategy for this review consisted in use of the term 'shrimp' interchangeable with 'prawn', and 'shellfish' with 'crustacean' to be more sensitive, and to capture as many relevant references as possible. These terms are used interchangeably across the globe with unclear distinction between them. Shellfish and shrimp were replaced with crustacea* and prawn respectively, in some searches. The eligibility criteria for the main search were the findings based on the following keywords: ‘eyestalk’, ‘ablation method’, ‘eyestalk ablation’, ‘shrimp’, ‘shellfish’, ‘pain indicators’, ‘welfare’, ‘nociception’, ‘consciousness’ and ‘emotions’. Figure 1 shows the examples of the search methodology used for this research.

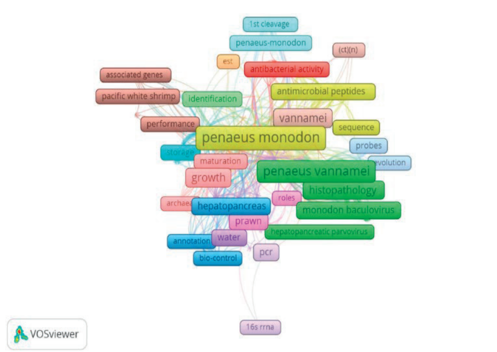

Figure 1. Search methodology by key words - adapted from PRISMA-P 2020 (Moher et al., 2015)

This search was indicative of potential issues with EA but not sufficient for it to provide conclusive evidence of pain in penaeids. Therefore, we initiated an additional generic search to include ‘one health’, ‘one welfare’, ‘Specific Pathogen Free AND shrimp / prawn’, ‘Genomic selection AND White Spot Syndrome Virus AND Pacific white shrimp / crustacea*’, ‘ablation method AND shellfish / crustacea AND prawn / shrimp’.

RESULTS AND DISCUSSIONS

When the words ‘ablation method AND shellfish AND shrimp’ were used, one paper was obtained revealing that EA-induced responses of the neuroendocrine-immune system in *Penaeus vannamei*, unique differentially expressed genes are observed in the eyestalk, brain and thoracic ganglia (Liu et al., 2020). When the words ‘pain indicators AND shellfish AND shrimp’ were used, only one paper was found, and it was not related to the topic of interest (Rapala et al., 2005). When the words ‘pain indicators AND welfare AND shellfish AND shrimp’ were used in PubMed, 3 of the 10 papers retrieved were related to shrimp and one to crayfish (Sakaew et al., 2013; Adams et al., 2019; Chandararathna et al., 2021; Passantino et al., 2021). When the same keywords were used in PMC, 3 of 10 papers were related to shrimp and antimicrobial resistance (Sakaew et al., 2013; EMA, 2017; Chandararathna et al., 2021). When using the words ‘pain indicators AND welfare AND shellfish AND shrimp’ to search the PMC database, five papers were listed. These were physiological and behavioural indicators to measure crustacean welfare, the need to enact legislation to protect crustaceans, potential pain in fish and decapods, animal welfare issues in capture-based aquaculture and Entomophagy (the practice of eating insects and invertebrates (Adams et al., 2019; Elwood, 2021; Pali-Schöll et al., 2019; Chandararathna et al., 2021; Passantino et al, 2021;). A search using the words ‘shrimp AND sentient’ provided 1 and 30 papers in Pubmed and PMC, respectively, but only 1 paper referred to ‘sentient’ (Browning & Birch, 2022). The original keyword search, although using appropriate terms (in the opinion of the authors), returned few relevant papers on the subject, although these papers indicated that the EA procedure might be problematic from a management and welfare perspective and there was some evidence that shrimp might be considered sentient (Wyban & Sweeney, 1991; Bray & Lawrence, 1992; Hoang et al., 2002; Almeida et al., 2004; Taylor et al., 2004; Sainz-Hernández et al., 2008; Chung et al., 2011; Uawisetwathana et al., 2011; Asusena et al., 2012; Diarte-Plata et al., 2012; Pamuru et al., 2012; Shen et al., 2013; Burrell, 2017; Rowe, 2018; Aguiñaga-Cruz et al., 2019; Sathapondecha & Chotigeat, 2019; Liu et al., 2020; Zhang et al., 2020; Jin et al, 2021; Laphyai et al., 2021; Albalat et al., 2022; Ortiz-Gullién et al., 2022; Walters, 2022; Zhang et al., 2022; Mood et al., 2023). However, this information was far from conclusive to form a robust opinion, or as a guide for policymakers or the public. It was decided to expand the literature search to a more generic one to include wider areas such as welfare, veterinary, regulatory, public health, genetics, and to assess eyestalk ablation (EA) under One Health and One Welfare concepts. One Health had to be searched for as one word (One-Health rather than One Health, with WOS for example, yielding 143 for the former but 3,394 for the latter). Including One Welfare allows for additional consideration of, and reflection on human and animal wellbeing, consumer opinion and food production policy making, but there were no relevant returns for including One Welfare with aquaculture and shrimp / crustacea*. These latter searches returned several additional results. The search using the key words ‘ablation method AND crustacea AND prawn’ yielded two publications of relevance, one paper on the role of methyl farnesoate in growth and maturation of the ovary, and another paper about the mechanisms of eyestalk ablation-induced ovarian maturation in the swimming crab (Ayanath & Raghavan, 2020; Xianliang et al., 2020). The search using the key words ‘impacts of EA on molting’ returned seven papers of relevance (Almeida et al., 2004; Sainz-Hernández et al., 2008; Chung et al., 2011; Diarte-Plata et al., 2012; Pamuru et al., 2012; Shen et al., 2013; Aguiñaga-Cruz et al., 2019). The search ’pain in invertebrates’ was far too generic but returned nine papers of relevance (Buckingham et al., 2005; Marder, 2007; Blitz & Nusbaum, 2011; Crook et al., 2013; Mason et al., 2014; McMackin et al., 2016; Burrell, 2017; Crook, 2021; Walters, 2022;). The search for ’Genomic selection AND White Spot Syndrome Virus AND Pacific white shrimp’ returned seven papers of relevance describing genetic evaluation of shellfish and genetic tests in relation with White Spot Syndrome Virus in shrimp (Zwart et al., 2010; Lillehammer et al., 2020; Hernández-Montiel et al., 2021; Onihary et al., 2021; Trang, 2021; Parrilla-Taylor et al., 2022; Medrano-Mendoza et al., 2023) (Table 1).

Eyestalk ablation (EA) refers to the removal or cutting of one (unilateral) or both (bilateral) eyestalks from an adult female shrimp. Another practice which induces the same effect is eye ligature by tying a thread around the base of the eye stalk then burning through the base of the eye stalk with a surgical forceps (Browdy & Samocha, 1985). EA is the most expedient method used for the induction of ovarian maturation and spawning in penaeid and non-penaeid shrimp (Primavera, 1983; Browdy, 1992; Vaca, 1999). This procedure was a major breakthrough in shrimp farming and commercialization, as it increased maturation and fertility of the eyestalk ablated shrimp brood stock females, making these hatcheries profitable (Primavera, 1985). Eyestalk ablation was routinely practiced on adult female shrimp in almost every research and commercial marine shrimp maturation or reproduction facility in the world, in both research and commercial settings. Commercial maturation of female penaeids used to rely almost exclusively on the technique of unilateral EA (Fingerman, 1997). It gave predictable peaks of maturation and spawning, but problems were reported with its use in penaeids and non-penaeids, like reduced reproductive performance, and deterioration in spawn quality and quantity over time and conflicting results on spawn size, hatch success and other variables (Emmerson, 1980; Tsukimura & Kamemoto, 1991; Kannan et al., 2015; Anand et al., 2019; Rodrigues et al., 2022). Today, several large-scale shrimp maturation facilities no longer use this procedure because non-ablated females live longer and produce eggs and nauplii of higher quality. The most economically important species are currently the giant tiger prawn *Penaeus monodon* and *P. vannamei*. Availability of fast-growing, specific pathogen-free (SPF) *P. vannamei* has contributed to make this species the most important aquaculture species worldwide. In most cases, female shrimps raised in captivity suffer from inhibition of ovarian maturation. Following uni- or bilateral EA, female shrimps develop ovaries and spawn in captivity, as complete ovarian development often ensues within 3 to 10 days. The presumed mechanism of the EA-induced fertility in shrimp female brood stock is that, following EA, the gonad inhibitory hormone (GIH) is not released from the eyestalk neurosecretory complexes, thereby lessening the inhibitory effect on the ovaries. GIH releases naturally in the non-breeding season. The fact that ovaries do not reach maturity in captivity is correlated with elevated levels of GIH in these females. EA lowers the haemolymph titter of GIH. The exact mechanism of EA on ovarian maturation is not known. This practice is of welfare concern as the EA technique is often applied without anaesthesia, while impaired vision and/or blindness is debilitating and secondary infection can ensue (Albalat et al., 2022). Through a participatory approach and trans-disciplinarity, One Health aims to achieve outcomes not achievable by silo mentality (Zinsstag et al., 2022). From a holistic perspective, and taking into account all inputs into the whole-of-chain approach, consumer protection is an intrinsic component of One Health. Traceability is a priority in the whole-of-chain approach to food safety. Competent authorities assure consumers of transparency and security at all stages of the food production continuum, as they are entitled to wholesome, and nutritious food.

Table 1. Relevant papers describing genetic evaluation of shellfish and genetic tests in relation with White Spot Syndrome Virus in shrimp

Key words used	**PubMed**	**PMC**	**WOS**
eyestalk AND pain AND shrimp AND shellfish	0 (0)	17 (2)	0
eyestalk ablation AND shrimp AND shellfish	4	19	10
eyestalk AND ablation AND shrimp AND shellfish / crustacea	4 / 55	19 / 175	51.817[a]/ 42
eyestalk AND ablation AND shrimp OR crustacea	50.157	14.176	31.993
Eyestalk ablation AND crustacea* OR prawn	2.271	4.567	9.921
ablation method AND shellfish / crustacea AND shrimp / prawn	1 / 4	57 / 172	1 / 1[g]
pain AND shrimp AND shellfish / crustacea*	1 / 4	370 / 453	0[c]/ 9
eye peduncle[b] AND shrimp AND ablation	2	130	0
eyestalk AND shrimp AND ablation	61	202	224[d]
nociception AND sentience AND shrimp AND shellfish	4	77	0[d]
pain indicators AND sentience AND shrimp, shellfish	6	160	0[d]
pain indicators AND nociception AND shrimp	0	28	0[d]
pain indicators AND welfare AND shrimp / crustacea*	0 / 3	70 / 62	0 / 3
Shrimp / crustacea* welfare	35 / 77	1566 / 2064	246 / 396
welfare AND shellfish AND shrimp	6	316	42
welfare AND crustacea AND prawn	4	84	11
welfare AND prawn OR crustacea*	22.221	34.326	64.397
nociception AND welfare AND aquatic	2	102	4
nociception AND emotions AND shellfish / crustacea*	0 / 1	3 / 24	0 / 0
nociception AND consciousness AND shellfish	227	10	1[e]
Ablation AND Welfare	184	9516	968
Genomic selection AND White Spot Syndrome Virus AND Pacific White Shrimp / crustacea*	2 / 5	239 / 293	7 / 5
One-Health AND aquaculture	1743	20.717	3394
Pain in invertebrates	3423	9712	148
Impacts of Eyestalk ablation on molting	1	16	1
One-Health AND aquaculture AND shrimp / crustacea*	128 / 56	4524 / 3025	17 / 6
One-Welfare AND aquaculture AND shrimp / crustacea*	0 / 0	1 / 3[f]	0 / 0
Shrimp aquaculture practices	171	1,571	454
Specific Pathogen Free AND shrimp / prawn	79 / 5	4349 / 712	139 / 15

[a]Results were too vague and returned same number with eyestalk ablation AND shrimp OR shellfish, or replacing shellfish with Crustacea*
[b]Searches performed by using the words "*eye peduncle*" instead of "*eyestalk ablation*", used by the shrimp industry in Ecuador
[c]Replacing AND with OR shellfish yielded over 32,000 articles, many on seafood safety
[d]Same result substituting prawn for shrimp, or crustacea* for shellfish
[e]Valente, 2022 but with crustacea* instead of shellfish
[f]No relevant articles

Food traceability is also important during primary production, as it ensures the traceability of the raw material inputs into the food chain, based on quality, certification and accreditation of their products. Despite facing global challenges, such as the requirement to develop sustainability in food production for future generations, recognizing and acting upon existing and/or emerging animal welfare issues should not be neglected. Eyestalk ablation is an example of a technique used to support intensive production of some aquacultured crustacea, originally meeting increasing consumer demand for these products. It could be argued that food security was being addressed by applying techniques that are potentially stressful and harmful to these farmed species. This would seem contrary to any One Welfare, One Health paradigm discussed earlier. There are indications that, through better management, non-EA female brood stock can reach a comparable level of productivity, providing they are adequately fed The problem the authors perceive with EA is threefold: as consumers are more aware of food provenance, including the welfare applied to animal food producing systems, and similar to the drive for free range hen eggs in some sectors, those consumers investigating their food supply chains may be unwilling to purchase and consume shrimp obtained via EA protocols (Sampson et al., 2021). Secondly, most consumers are not aware of EA that is applied to some seafood, which may be translated into a lack of transparency in the food traceability process. This raises the question of whether industry should pre-empt any perceived lack of communication with public discourse. Consumers indicate a higher willingness-to-pay for improved animal welfare (Van Loo et al., 2014). Calls to include details about the way farmed seafood is obtained in the existing traceability requirements would not only meet what we perceive as the need for more transparency of this production chain to inform the consumer, and to empower their decision making, but it would also motivate possibly higher costs of production of welfare-based seafood, for which consumers would be willing to pay (Żakowska-Biemans & Tekień, 2017; Balzani & Hanlon, 2020). Thirdly, EA may not just relate to the wellbeing of these animals and the social and mental wellbeing of consumers, but it may also impact the mental health of staff working in the industry, such as shrimp farmers/producers and veterinarians/health care assistants of these farms. The attitude of those working on the EA shrimp farms towards animal welfare is based on many factors, including the ability of those workers to bond with their stock (Balzani & Hanlon, 2020). Involving farmers in their view and value of farm animal welfare can lead to better management and animal health outcomes. The shrimp EA protocol is for most, if not all welfare advocators, a non-necessary, stressful procedure, with a high potential to cause distress in the animal subjected to EA. As observed by animal welfare regulators, cases where animal welfare conditions are not met may cause the development of mental health issues of individuals working on such farms, and potentially affect their families. The Canadian Farmed Animal Health and Welfare Council recognizes the existence of a wide range of "unique occupational stressors" in the animal food producing industry, calling for "an effective mental health support system to recognize these unique and specialized stressors", suggesting the need for appropriate intervention to ensure the welfare of the affected animals, and to facilitate the recovery of those experiencing distress from animal welfare incidents. The fact that it is impossible to actually know which of invertebrate species are sentient makes invertebrate sentience an important issue (Mood et al., 2023). To prove sentience in these species, a clear understanding of the way consciousness occurs, and identification of the structures allowing for consciousness to take place would be necessary. While such knowledge is currently lacking science-based proofs, different indicators that help estimate which invertebrates are sentient are being used. There are different attitudes towards different animal uses. Some are driven by the fact that the use of animals by humans is often associated with animal welfare costs. Sometimes, these are due to deliberate harm, but most often, they are due to inadequate husbandry, breeding and management protocols. Animal welfare is an interdisciplinary field where many have a role to play. One way to ensure animals are provided good welfare is to use the Five Freedoms as benchmark for meeting animals' needs (Van Loo et al., 2014). It is recognized that little is currently known about some of the five dimensions as they relate to penaeid shrimp, and despite being regarded as outdated by some, the Five Freedoms may be a good place to start (Webster, 2008; McCulloch, 2012; Mellor, 2016). They include, among other freedoms, the freedom from discomfort (by providing adequate environment), freedom from physical problems (pain, injury and disease), and freedom from fear and distress (treatment and conditions which avoid animal suffering). Building consensus over the utilization of a shared working definition for welfare, pain and sentience is of paramount importance, especially in those animals raised to enter the food chain. This is a strong aspect of One Welfare. Pain is an emotion which evolved by natural selection and it may be shared by many animal species (Wadiwel, 2016). It is unlikely that pain arose *de novo* in humans, but rather it is a product of the evolutionary process, developed under selection pressures. Chordates, of course, share a common ancestor with many invertebrate phyla, such as molluscs and arthropods. While most research studies addressing pain have focused on humans and other mammals, current findings in invertebrate species are shedding light on nociception and pain-related functions, indicating that these may be ancient adaptive mechanisms present across phylogenetically related taxa, and that their welfare should be considered (Walters, 2018). Operation Welfare Indicators for capture-based aquaculture (CBA) protocols and provisions of the Animals (Scientific Procedures) Act 1986 should also be taken into consideration. Research on the ability of invertebrates to feel pain is both controversial and relatively rare, and response to noxious stimuli might be one way of investigating further. Invertebrates are underutilized in nociception studies, although there is perceived potential that these studies could promote development of novel therapies of pain in humans and other species. It is also expected that nociception studies conducted in invertebrates will reveal new insights into the mechanisms of those stimuli causing behavioural changes (Burrell, 2017). Insights into the fundamental mechanisms of nociception are provided by comparative approaches which use a wider range of animal species as model systems. Despite the increasing number of studies on pain, it is still difficult to be defined, as it cannot be soundly, scientifically proven, and it seems in most cases impossible to reproduce in other individuals. This indicates the "broad" feeling of pain, and may explain why experimental results are not relevant in these cases. Perhaps a preliminary definition of pain should be accepted in shellfish and other non-human species, to have the welfare concept applying to all animal species. From our literature research conducted on pain definitions and characterization, we suggest the definition proposed by Walters (2018): "Pain is a complex phenomenon, which is different in every individual and involves both a sensory (nociception) and affective (emotional) component." Behaviour can be interpreted as associated with pain but since we accept "our" interpretation of behaviour as indicating pain in more familiar mammals e.g., dogs and cats, why do we dispute behaviour in fish and invertebrates?

CONCLUSIONS

Eyestalk ablation, used widely in decapod crustaceans to expedite ovarian maturation and spawning, has a negative impact on the immunocompetence of the eyestalk-ablated females; state-of-the-art genetic findings may lead to new ways to induce ovarian maturation without having to perform EA.

Adopting more welfare-friendly handling techniques such as the LSDOT (Low Salinity and Diet, Optimal Temperature), or using topical anaesthetics, such as XylocaineR, prior to EA may reduce stress. Application of a coagulating agent (FibraseR) and antibiotics, diminish the time required for haemolymph clotting at the ablation site, minimizing haemolymph loss, and helping to prevent potential infections after the procedure.

From the animal welfare perspective, it is necessary to amend welfare assessment platforms, such as the Animal Welfare Assessment Grid (AWAG), currently used in mammals and birds, to achieve objective outcomes for welfare of decapods. In light of new developments in pain and sentience, novel approaches to scientifically explore these concepts in animals should be proposed in the future.

Species-specific operational welfare indicators for all farmed Crustacea should be developed, and appropriate intervention to facilitate the recognition and recovery of those potentially experiencing distress due to welfare incidents in EA shrimp farms must be considered.

To assess the validity of the study (i.e., bias, confidence in estimates etc.), further systematic reviews on the topic are required.

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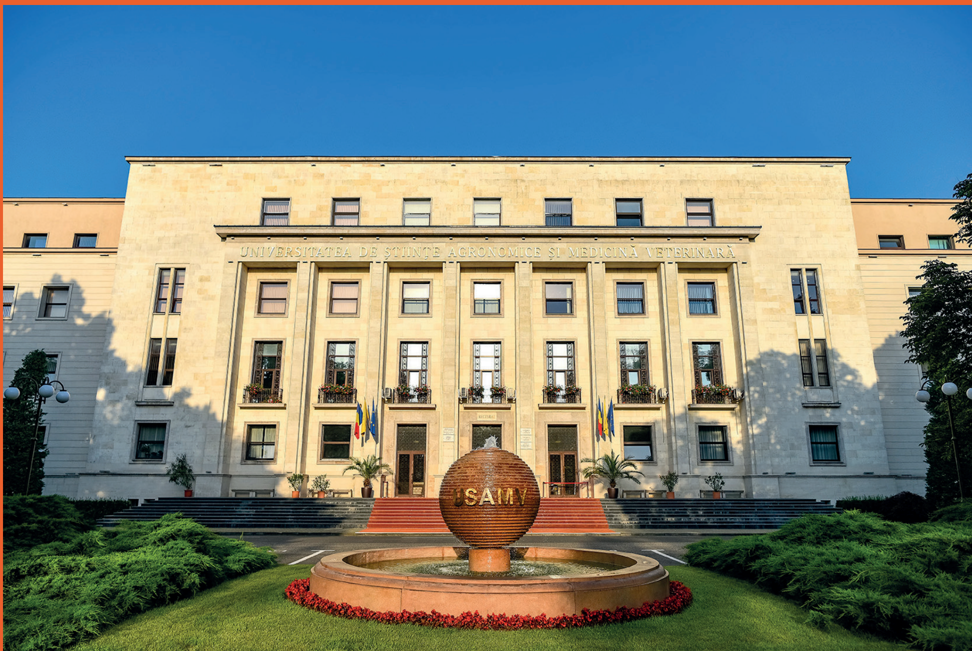

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