UNIVERSITY OF AGRONOMIC SCIENCES
AND VETERINARY MEDICINE OF BUCHAREST
FACULTY OF ANIMAL PRODUCTIONS
ENGINEERING AND MANAGEMENT

SCIENTIFIC PAPERS
SERIES D. ANIMAL SCIENCE

VOLUME LXVI, NO. 2

2023
BUCHAREST

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BUCHAREST

PUBLISHERS:

University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania – Faculty of Animal Science Engineering and Management
Address: 59 Mărăști Blvd, District 1, 011464, Bucharest, Romania
Phone: + 40 213 182 564, Fax: +40 213 182 888, www.zootehnie.ro

CERES Publishing House
Address: 29 Oastei Street, District l, Bucharest, Romania
Phone: + 40 317 90 23, E-mail: edituraceres@yahoo.com, Webpage: www.editura-ceres.ro

To be cited: Scientific Papers. Series D. Animal Science, Volume LXVI, No. 2, 2023

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ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

International Database Indexing: Web of Science Core Collection (Emerging Sources Citation Index), Index Copernicus, CABI, DOAJ, Ulrich's Periodicals Directory (ProQuest), PBN, Cite Factor (Academic Scientific Journals), Scipio, OCLC (WorldCat), Research Bible, Google Scholar.

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GENETICS AND BREEDING

Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

PARTICULARITIES OF BODY CONFORMATION OF THE MOLDAVIAN KARAKUL LAMBS

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Abstract

The purpose of this research was to reveal the variability of the morpho-productive characters that determine the development of body conformation, as well as to identify the factors that influence its optimization. The researches were carried out on the batches of lambs from the classic Karakul and Moldavian Karakul races, born in the experimental households: the state farm "Kotovschii", Dumbrăveni district and the experimental agricultural station "Tevit", Anenii Noi district, Republic of Moldova. The results of the research demonstrated that the body conformation of the Moldavian Karakul lambs is typical of the classic (Asian) Karakul sheep race, with some particularities related to the development of body mass, body length and constitution. Moldavian Karakul lambs are quite full-bodied at birth, with an average body mass of 4.7-5.0 kg, and in some years over 5.0 kg, this being a biological peculiarity of the new type of sheep. Variability of lamb body mass at birth is hereditarily determined (genotypic correlation coefficient r_{xy} = 0.63; h^2 = 0.36; repeatability coefficient $K_{0\text{-}6\ months}$ = 0.26; $K_{0\text{-}18\ months}$ = 0.23) and influenced environmental conditions, in particular, the nutrition of pregnant ewes. The body mass of the lambs at birth is in a direct-proportional phenotypic relationship with: age of the ewes at calving, body length (r_{xy} = 0.49), skin thickness (r_{xy} = 0.45), fiber length (r_{xy} = 0.22-0.31), furskin surface (r_{xy} = 0.64), loop size and constitution; inversely proportional to: the prolificacy of the ewes in the term and the calving period; curvilinear with: the qualities of own furskin (weight in the flock at calving of the higher class Elita lambs). Lamb body length at birth is in the same similar phenotypic relationships as body mass, including with: skin thickness (r_{xy} = 0.54), fiber length (r_{xy} = 0.15-0.18), furskin area (r_{xy} = 0.78). The constitution of the lamb at birth is in a phenotypic relationship: directly proportional - with its own body mass, body length, thickness and skin reserve; inversely proportional - to skin density.

Key words: *body conformation, Moldavian Karakul lambs.*

INTRODUCTION

The body conformation of the Karakul lamb at rating is of particular importance, because the commercial qualities and the value of the furskin depend on its particularities (the qualities of the fibers and curl, the surface, the thickness and the weight of the skin, the sale price of the furskin), the body development and the weight of the carcass (the production of meat) obtained from the slaughter of the lamb for the furskin. The body conformation is related to the robustness of the body and the degree of manifestation of the race characteristics, which reflects its breeding value as a whole. The notion of body conformation of the lamb includes external features, body mass, body length and constitution.

The Karakul lamb at birth, unlike other sheep races, is quite large. In lambs of the Asian Karakul race, the normal body weight at birth is considered to be on average 4.0-4.5 kg, which is about 8-10% of the body weight of the adult sheep, compared to 7.7% in the race Precocious and 6.4% in the Ghisar race (Ilyev, 1969; Vasin, 1971).

"*The dimensions of the Karakul lamb at birth are of particular importance. The fuller lamb, as a rule, is more developed, has a robust constitution, possesses increased viability, its furskin has a larger surface, which, under equal conditions, is valued at a more advantageous price. But, here, what is required is not a large lamb in general, but one of optimal size and normal development according to all its characters*" (Koshevoy, 1975).

Optimizing the body size of Karakul lambs at birth is achieved through their sustainable selection according to a complex of characters, such as: body mass, body length, constitution

and others. These morpho-productive characters are in phenotypic correlation both with each other and in correlation with other characters that influence their variability and quality, such as: the quality of the skin and the hair cover, the quality of the curls and the type of curl, the commercial qualities and the value of the furskins in assembly.
In order to make the selection process of Karakul lambs according to body conformation more efficient, detailed knowledge of the correlative links between the multitude of morpho-productive characters in tangent with body conformation, their variability and the factors that determine the development of the respective characters is necessary.
In this context, the purpose of this research was to reveal the variability of the morpho-productive characters that determine the development of body conformation, as well as to identify the factors that influence its optimization.

MATERIALS AND METHODS

The researches were carried out on the batches of lambs from both the classic Karakul race and the Moldavian Karakul race, raised in the experimental farms: the state farm "Kotovschii", Dumbrăveni district and the experimental agricultural station "Tevit", Anenii Noi district.
The body conformation of the lambs was evaluated according to the external characteristics, constitution, mass and body length, which were assessed at the rating, according to the methods developed by us (Buzu, 2021).
The external features were appreciated when the lambs were rasing by the visual method. Lambs that had a relatively tall and elongated body, a long neck, an elongated back, a long and tapered rump, the height at the rump was 1-2 cm higher than at the withers, the head was elongated, dry were considered typical for the Karakul race, with a ram (convex) profile, covered with bright embers, the ears were long and pale, or medium long, the tail, at the base, was wide with fat deposit in the shape of a small kurdiuk, and the thin tip curved in the shape of the letter " S", which reached up to the hocks.
The body weight of the lambs at birth was determined by weighing the lambs at the time of rating with a hand scale, with a capacity of up to 7.0 kg and an accuracy of 0.1 kg. For weighing with the hand scale, the lamb was wrapped with a string around the chest on the thin, which was hooked to the hook of the scale held by the hand of the scorer, and the lamb was hanging freely, at the moment when the scorer visually fixed the graduation on the scale. According to the developed methodology, the body mass of Karakul lambs at birth was differentiated into the following categories: *very large* with body mass > 5.0 kg; *large* with 4.5-5.0 kg, *medium* with 4.0-4.5 kg, *reduced* with 3.5-3.9 kg and *small* < 3.5 kg. Lambs with large, very large and medium body mass were the most requested for selection and reproduction. Lambs with reduced and small body mass were not allowed for reproduction.
The body length of the Karakul lamb was determined at rating using the method developed by us (Buzu, 2021), which consists in measuring with a millimeter tape the distance between the base of the neck, from the front edge of the withers and the base of the tail, located on the line drawn at the ischials (Figure 1).

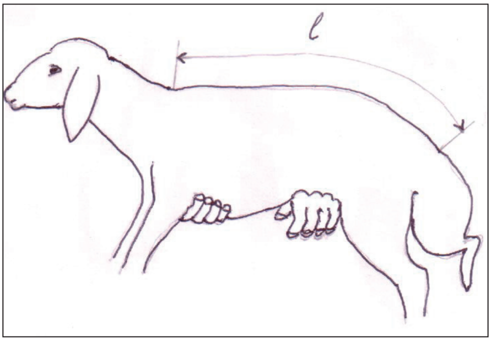

Figure 1. Body length measurement outline at Karakul lamb (l = body length)

When measuring, the millimeter tape was stuck to the body for the entire measured distance. In addition to this condition, the lamb had to be kept with its back straight. In its natural position, the line of the back and rump of the Karakul lamb is slightly convex (bent with the middle up). Body length was measured on the lamb held by the shepherd with one hand under

the chest and with the other - under the abdomen.
According to the developed methodology, the length of the lamb was differentiated into the following categories: *very long* (> 35 cm), *suitably long* (30-35 cm), *reduced* (26-29 cm) and *short* (< 26 cm). Lambs with very long and suitable length were the most requested for selection and breeding.
The constitution of the lambs was assessed at the rating by examining the general condition of the body, the body conformation and the exterior. According to the developed methodology (Buzu, 2021), four types of constitution were differentiated: *coarse, robust, fine* and *weak*.
Coarse constitution was considered when lambs had high body development, coarse bone, with curl usually chewed, curls spread, long fibers with low silkiness, low or dull gloss, bulky head with obvious ram profile, long, broad ears, hanging, thick or thickened skin. Physiologically, they were strong, they stood well on their feet. Lambs with coarse constitution type were partially accepted for breeding.
The robust constitution was found when the lambs had high or medium body development, body regions proportionally developed and typical for the race, well-developed bones, dense, medium-thick skin, thick, silky, glossy hair coat. The ears were long and fluffy. Long head with slightly rammed profile. Wide tail in the shape of a typical kurdiuk and with the tip bent into an "S" shape. The lamb, after birth, was vigorous, stood well after 5-10 minutes after birth and found the mother's nipples for sucking on its own. The lambs with the robust constitution type were the most requested for selection and reproduction.
Fine constitution was found when the lambs had a fine-normal skeleton. Body development was medium or small. The curl, as a whole, was small to medium in size. Loop expansion was excellent or fair. The head was fine, with the profile, as a rule, slightly rammed, the ears of medium length. The skin was thin, the hair cover - dense or reduced, the length of the fibers - as a rule, short. After the birth, they got up harder in search of the nipples. Depending on their physiological state, lambs of fine constitution were partially admitted to reproduction.
Weak constitution was considered when lambs had very little body development, weak-thin bone, very thin skin, sparse, short and thin fibers, very small curls, usually pea-shaped and corkscrew-shaped, small-weak head, with straight profile, short ears, thin triangular tail without fat deposits. The physiological condition of the lambs was sickly. Lambs with a weak constitution were not allowed to reproduce.

RESULTS AND DISCUSSIONS

The research results have shown that the conformation of Moldovan Karakul lambs has a series of specific peculiarities, formed in the process of sustainable selection applied to the creation of this new race of Karakul sheep.
The exterior. Moldavian Karakul lambs, at birth, differ essentially from other types of sheep in appearance, first of all, by the specific shapes of the body regions. They are relatively tall and elongated, the neck is long, the back elongated, the rump long and tapered. The head is elongated, dry with ram (convex) profile, covered with bright dead embers (Figure 2).
The entire surface of the body is covered with a hair covering composed of relatively short (6-9 mm) and medium-short (9-13 mm) fibers, dense (> 40 follicles/mm^2) or fairly dense (35-40 follicles/mm^2), excellent or suitable silky and elastic, forming curls of various types (wave, tube, grain, ribs, manes, dies). In greyish lambs, the hair coat is composed of a mixture of black and white fibers. Depending on the shade and color of the furskin, the mixture of black and white fibers has a certain co-ratio, from 60/40% - for dark shade furskins (of grizzle, mother-of-pearl colors), up to 53/47% (for marble coloration), 47/53% (for bluish coloration), 33/67% (for silver coloration) and 21/79% (for milky coloration).

Figure 2. Purerace Moldavian Karakul lamb

Figure. 3. Purerace Moldavian Karakul lamb, with pointed ears

The most requested colors in the greyish furskins (such as: marble, blue, pearl) are formed from the ratios of 53-42% of black fibers and 47-58% of white fibers (Buzu, 2017).

On the surface of the main body regions (back, rump) of the Moldavian Karakul lamb, valuable curls are obviously observed, such as: long and medium-long waves, tubular, costal, flat or milled mane, arranged parallel-concentrically to each other, parallel-scale or mixed-sinusoidal, which forms an original ornament (drawing), quite aesthetically pleasing.

On the sides of the lamb there are waves of short length and grain, which continue that patterning, forming the specific ornament of the hair covering of the furskin.

The extremities of the lambs (limbs, abdomen, head, tail) are covered with less valuable curls, grain, short manes or with dead and shiny embers.

The ears of Moldavian Karakul lambs, as a rule, are long and flat (hanging down), but there are also individuals with medium-long or even short ears (tips) (Figure 3).

The tail, at the base, is wide with a fat deposit in the shape of a small kurdiuk, and the thin tip is curved in the shape of the letter "S", which reaches to the hock. These external characteristics of the Moldavian Karakul lambs were taken into account in the selection of individuals of the required type in the piggery lots.

Body mass. Our research (Buzu et al., 1989; Buzu et al., 1992; Buzu, 1995; Buzu, 2000a, 2000b; Buzu, 2001; Buzu, 2003a, 2003b; Buzu et al., 2009a; Buzu et al., 2009b; Buzu, 2012; Buzu, 2014) demonstrate that the Moldavian Karakul lambs are quite corpulent at birth.

Their body mass is much higher than the standard of the Asian Karakul race (4.0-4.5 kg) and is on average 4.7-5.0 kg, and in some years even over 5.0 kg. This is a biological peculiarity of the new type of sheep.

The body development of the lamb at birth depends on many factors, among which are the hereditary factors (race, line, genotype of the ancestors) (Vasin et al., 1971; Karymbaev, 2011; Kudrik, 2011; Litovchenko & Esaulov, 1972), environmental factors (sheep nutrition, maintenance technology) (Dyachkov, 1980; Ivanov, 1964c; Koshevoy, 1975; Matter, 1975; Mashtykov, 2010; Nikolaev & Erokhin, 1987; Ombaev, 2010) and bio-productive factors (prolificity, ewe age at calving, calving period, etc.) (Matter, 1975).

The ewe and the ram with a large body mass possess hereditary capacities to reproduce a corpulent offspring. Mating ewes with full-bodied rams contributes to obtaining full-bodied lambs. Research results (Buzu, 2014) demonstrated that, in one and the same herd, under similar conditions of growth and maintenance, from corpulent rams, with a mass of 86-100 kg, the most corpulent offspring with body mass at birth was obtained of 5.16±0.03 kg.

From the rams of batch II, with an average body mass of 71-85 kg, offspring with an average body mass of 4.78±0.04 kg were obtained, and from the rams of batch III, with a low body mass of 60-70 kg, the offspring with the lowest body development of 4.45±0.04 kg was obtained. The progeny of corpulent rams from batch I exceeded their congeners from batch II by 0.38 kg or 7.9% (P<0.001), and those from batch III - by 0.71 kg or 16.0% (P<0.001). The lambs-descendants of the rams from batch II exceeded, according to the body weight at birth, their congeners from batch III by 0.33 kg or 7.4% (P<0.001). This demonstrates that there is an obvious genotypic correlation between the body mass of the father rams and the body mass of the offspring lambs at birth (r_{xy} = 0.63±0.05; t_r = 12.6; h^2 = 0.36).
Researchers from different countries (Brădățan et al., 2001a; 2001b; Kuzembayuly, 2010; Yudin & Kotov, 1951), independently of each other, came to the conclusions that good nutrition of pregnant Karakul ewes positively influences lamb development at birth.
Our research (Buzu, 2017) demonstrates that the body mass of Moldavian Karakul lambs at birth varies in different years, depending on the forage base of the year and twinning (Table 1).

Table 1. Body mass of Moldavian Karakul lambs at birth depending on the fodder base of the year and the type of calving

Type of calving	N	Body mass of lambs, kg		
		M ± m	σ	C_v,%
1997 *(year with good fodder base)*				
Uniparous	615	4.92 ± 0.03***	0.72	14.6
Twin	12	4.00 ± 0.19*	0.67	16.7
1998 *(year with good fodder base)*				
Uniparous	353	4.84 ± 0.05***	0.98	20.2
Twin	36	3.84 ± 0.15*	0.91	23.7
1999 *(year with poor fodder base)*				
Uniparous	304	4.16 ± 0.04	0.76	18.3
Twin	18	3.33 ± 0.20	0.86	25.8
2000 *(year with poor fodder base)*				
Uniparous	222	4.42 ± 0.05***	0.77	17.4
Twin	92	3.21 ± 0.07	0.65	20.2

Note: *- P<0.05; ***- P<0.001, compare with 1999.

In the years with a good fodder base (1997, 1998) the body mass of lambs at birth was higher, compared to the years with a poor fodder base (1999, 2000), by 18.3-9.5% - in uniparous lambs (P <0.001) and, by 20.1-19.6% - in twin lambs (P<0.001). In these years, under equal conditions, lambs born from single births (uniparous) had a body mass at birth higher than those born with twins by 0.83-1.21 kg or 24.9- 37.7% (P<0.001). The weight of the lambs at birth depends on the level of prolificacy of the ewes in herd (Table 2).

Table 2. Body mass of Moldavian Karakul lambs at birth depending on the prolificacy of the ewes

Year	Prolificacy, %	N	Body mass of lambs, kg		
			M ± m	σ	C_v,%
1997	101.0	626	4.90 ± 0.03***	0.73	14.9
1998	104.9	389	4.75 ± 0.05***	1.01	21.3
1999	108.9	322	4.12 ± 0.04	0.78	18.9
2000	117.2	314	4.06 ± 0.05	0.92	22.7

Note: ***- P<0.001 compared to the year 2000.

We found that the higher the prolificacy of the ewes in the flock, the lower the weight of the lambs at birth and, conversely, if the prolificacy is lower, the weight of the lambs is higher.
The body mass of lambs born in the ewes flock, when the prolificacy was 101%, was higher compared to their congeners born in the same flock, when the prolificacy was 104.9%, by 0.15 kg or 3.2% (P<0.01), when prolificacy was 108.9%, with 0.78 kg or 18.9% (P<0.001) and when prolificacy was 117.2%, with 0.84 kg or 20.7% (P<0.001). So, with the increase in prolificacy from 101.0% to 117.2%, the body mass of lambs decreased from 4.90 kg to 4.06 kg or 17.2% (P<0.001).
From the economic point of view, twin calvings in karakultur are convenient to the extent that the genotype of the parents and the nutritional conditions of the ewes ensure the production of offspring weighing at least 3.5 kg at birth, from which furskins with at least the middle surface - 900 cm^2.
Otherwise, when lambs with a body mass of less than 3.5 kg are born in twin lambs, their economic benefit does not reach the level of single lambs, because the furskins obtained from these lambs are assigned, by surface, to the "small" category, and their commercial value is 4-5 times lower, compared to large surface furskins (>1400 cm^2), obtained from lambs with a body weight of over 4.0 kg.
The weight of the lambs at birth depends on the age of the ewes at calving (Table 3).

Table 3. Body mass of Moldavian Karakul lambs at birth depending on the age of the ewes at calving

Age of ewes at calving	N	Body mass of lambs, kg		
		M ± m	σ	C_v, %
13-14 months (early age)	244	4.20 ± 0.05	0.74	17.6
2 years (primary)	342	$4.80 \pm 0.04^{***}$	0.68	14.2
>3 years (adults)	4953	$5.01 \pm 0.02^{***}$	0.83	16.6

Note: ***-P<0.001 compared to 13-14 month old ewes.

For example, in the same flock, the ewes, which were inseminated at an early age (8-9 months) and calved at the age of 13-14 months, produced lambs with a body mass of 4.20±0.05 kg, which which is lower than lambs of primiparas by 0.6 kg or 12.5% (P<0.001). Primiparous ewes gave birth to lambs with a body mass of 4.80±0.04 kg, or smaller than adult ewes by 0.19 kg or 4.4% (P<0.001). Adult ewes (>3 years old) gave birth to the most developed lambs with a body mass of 5.01 ± 0.02 kg, which is higher than lambs obtained from ewes inseminated at an early age (8-9 months) by 0.81 kg or 19.3% (P<0.001), and than in lambs obtained from primiparous ewes by 0.21 kg or by 4.4% (P<0.001).

Research has shown that lambs born in winter lambings (January, February) were more developed than those born in spring (March, April) (Table 4).

Table 4. Body mass of Moldavian Karakul lambs at birth depending on the calving season

Calving season (month)	N	Body mass of lambs, kg		
		M ± m	σ	C_v, %
Rams				
January	357	$5.63 \pm 0.03^{***}$	0.65	11.5
February	365	$5.48 \pm 0.04^{***}$	0.67	12.2
March	325	5.21 ± 0.04	0.73	14.0
April	77	5.12 ± 0.09	0.81	15.8
Ewes				
January	285	$5.40 \pm 0.04^{***}$	0.68	12.6
February	354	$5.18 \pm 0.04^{**}$	0.71	13.8
March	368	$5.10 \pm 0.03^{*}$	0.66	12.8
April	61	5.01 ± 0.04	0.73	14.6

Note: * - P<0.05; ** - P<0.01; *** - P<0.001; Compared to lambs born in April.

For example, rams born in January-February surpassed those born in March-April by 0.42-0.57 kg and 0.27-0.36 kg, respectively, or 8.1-10.0% and 5.2-7.0% (P<0.001). The same situation is found in youtht ewes. With the advance of lambing dates from January to April, the body mass of lambs at birth decreased, in rams, from 5.63 kg to 5.12 kg, or by 0.51 kg (7.8%) (P<0.001) and in ewes - from 5.4 kg to 5.01 kg or by 0.39 kg (7.2%) (P<0.001).

As a hypothesis to explain this phenomenon, we believe that ewes born in the winter months (January, February) better preserve the reserves of nutrients stored in the body from the fall, which ensure the optimal nutrition of the fetus. And, on the contrary, in the spring months (March, April) the reserves of the stores of nutrients in the body of pregnant ewes are exhausted, which negatively influences the development of the lamb.

Some researchers (Bastaev, 2005) state that the body mass of the Karakul lamb and the meat production correlate negatively with the furskin qualities. According to us, these statements do not exactly correspond to reality, at least for the Moldavian Karakul race.

Our research (Buzu, 2014) demonstrated that the lamb's body mass at birth has a curvilinear relationship with the lamb's furskin quality, expressed in the lamb class (Figure 4).

This relationship is manifested by the fact that, with the increase in the body mass of the lambs at birth to an optimal level, specific for each flock (sheep population), their furskin quality improves and, as a result, the share of class lambs increases superior (Elite).

Thus, in the researched herd, with the increase in the body weight of the lambs at birth from 2.75 kg to 5.25 kg, the share of elite class lambs increased from 16.7% to 27.9%, or 1.7 times (P<0.001).

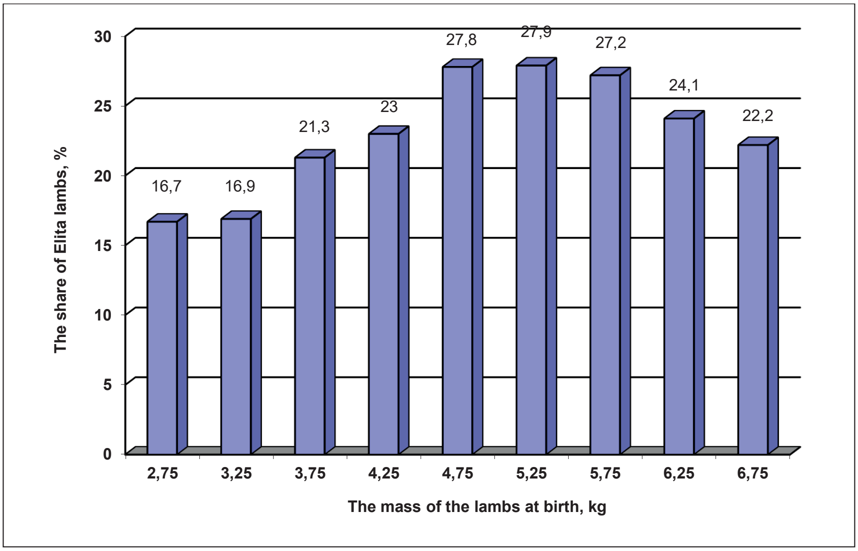

Figure 4. The relationship between the body mass of the lambs at birth and the share of the Elita lambs

The highest percentage of Elite lambs was recorded in those with a body mass in the range of 4.75-5.25 kg. The highest point, 27.9%, was reached in the group of lambs with a body mass of 5.25 ±0.15 kg. We consider that this level of body mass is characteristic for the model lamb of the Moldavian Karakul type.

With the increase in the body weight of the lambs above 5.25 kg, the weight of the higher class lambs decreases.

This is manifested by the loosening of the skin and its thickening. As a result, the length of the fibers increases, the resistance of the loop decreases, the compactness and quality of the loop are reduced (Ivanov, 1964a; Koshevoy, 1975; Bogdanovich et al., 1982).

An obvious correlation is manifested between the body mass of the lamb at birth and the size of the curls, skin thickness and constitution (Table 5).

From the analysis of the data in the table, it appears that the larger the curls, the higher the body mass and, conversely, the smaller they are, the lower the body mass of the lamb at birth.

We found that lambs with large curls exceeded those with medium curls by 0.27 kg (P<0.001), those with small curls - by 0.61 kg (P<0.001) and those with very thick curls. small - by 0.69 kg (P<0.05). As loops decreased from very large to very small size, the body weight of lambs at birth decreased from 5.18 kg to 4.30 kg or 0.88 kg (17.0%; P<0.001).

Table 5. Body mass of Moldavian Karakul lambs at birth depending on the size of the curls, the thickness of the skin and constitution

Specification	N	Body mass of lambs, kg	
		M ± m	C_v, %
Depending on the size of the curls[1]			
Very large (>12 mm)	20	$5.18 \pm 0.14^{***}$	8.3
Large (9-12 mm)	136	$4.99 \pm 0.05^{***}$	12.2
Middle (6-8 mm)	372	$4.72 \pm 0.03^{***}$	14.2
Small (4-5 mm)	88	4.35 ± 0.07	16.6
Very small (< 4 mm)	10	4.30 ± 0.34	26.5
Depending on the thickness of the skin[2]			
Subtle	129	4.31 ± 0.06	15.5
Middle	304	$4.69 \pm 0.04^{***}$	13.2
Thickened	172	$4.98 \pm 0.05^{***}$	13.1
Thick	21	$5.55 \pm 0.18^{***}$	12.6
Depending on the constitution[3]			
Coarse	43	$5.79 \pm 0.10^{***}$	9.8
Robust	503	$4.80 \pm 0.02^{***}$	11.5
Fine	80	3.80 ± 0.07	16.1

Note: ***- P<0.001; 1 - compared to "Small"; 2 - compared to "Thin"; 3 - compared to "Fine".

We elucidated that the thinner the skin of the lambs at birth, the lower the body mass and vice versa, the thicker the skin, the higher the body mass. Lambs with thick skin had a body mass at birth higher by 0.52 kg (t_d=3.25; P<0.01) compared to lambs with thickened skin, by 0.86 kg (t_d=5.54; P <0.001) compared to lambs with medium skin and by 1.24 kg (t_d=7.75; P<0.001) compared to the batch with thin skin. With skin thickening from thin to thick, the body mass of lambs at birth increased from 4.31 kg to 5.55 kg, or 1.24 kg (28.8%) ($P<0.001$). The correlation coefficient between body mass and skin thickness is r_{xy} =0.45±0.05 (t_r=9.0; $P<0.001$). The data included in the table confirm that the body mass of lambs with a coarse constitution was higher, compared to those with a robust constitution by 0.99 kg or 20.6% ($P<0.001$) and, compared to those with a fine constitution, by 1.99 kg or 52.3% ($P<0.001$).

The body mass of the lambs at birth also correlates with other characters, such as the body length, the furskin surface, the length of the fibers on the rump and on the withers (Table 6).

Table 6. Correlation coefficient (r_{xy}) of body mass of Moldavian Karakul lambs at birth with some its morphological characters

Caracters	N	$r_{xy} \pm m_r$	t_r
Body length	859	0.49 ± 0.030	16.3^{***}
Skin thickness	297	0.45 ± 0.057	7.89^{***}
The surface of the skin	164	0.64 ± 0.062	10.3^{***}
To the greyish lambs			
Black fiber length:			
on the rump	115	0.31 ± 0.096	3.2^{**}
on the withers	115	0.30 ± 0.089	3.4^{**}
Length of white fibers:			
on the rump	115	0.22 ± 0.092	2.4^{*}
on the withers	115	0.14 ± 0.093	1.5

Note: *- $P < 0.05$; **- $P < 0.01$; ***- $P < 0.001$.

Of particular importance is the positive correlation between body mass, body length and furskin surface, not only from the genetic (theoretical) point of view, but also from the practical (economic) point of view of raising Karakul sheep. The phenotypic correlation coefficient of the body mass of the lamb at birth with body length is r_{xy} = 0.49±0.03, and with the skin surface is r_{xy} = 0.64±0.06.

This correlation is very important for selection, because it has an additive influence on productive-useful characters, such as furskin surface and carcass mass at slaughter, determining their qualities and marketing prices.

In greyish lambs, both black and white fibers are positively correlated with body mass (r_{xy} = 0.14-0.31). The more developed the lamb, the longer the fibers. From the point of view of selection, this statistically positive correlation is actually "negative" for selection, because as the fibers increase in length, most of the furskin qualities decrease.

The body mass of the lamb at birth is related to its subsequent development at different early age intervals in the postnatal period (Table 7).

The repeatability of this character, starting from birth and later up to different ages in the postnatal period, is quite significant and expressive.

Table 7. The relationship between the body mass of Moldovan Karakul lambs at birth and their development in different intervals of the postnatal age

Masa corporală la naştere, kg	N	Masa corporală la diferite vârste, M ± m, kg			
		20 zile	90 zile	6 luni	18 luni
> 5.1	32	$10.13 \pm 0.40^{***}$	$16.87 \pm 0.52^{**}$	$23.29 \pm 0.80^{***}$	$43.00 \pm 1.07^{***}$
4.6-5.0	39	$8.88 \pm 0.20^{***}$	$16.35 \pm 0.66^{*}$	$22.75 \pm 1.36^{**}$	$41.14 \pm 1.26^{***}$
4.1-4.5	22	$8.14 \pm 0.27^{***}$	15.06±0.87	$22.20 \pm 0.70^{***}$	$41.00 \pm 1.51^{**}$
3.6-4.0	14	$7.07 \pm 0.37^{*}$	13.60±1.02	$21.86 \pm 0.71^{**}$	$38.86 \pm 1.08^{**}$
< 3.5	8	5.90±0.36	11.98±1.70	18.17±0.88	27.50±3.55
$r_{xy} \pm m_r$	115	0.47±0.07	0.39±0.09	0.26±0.08	0.23±0.09

Note: *- $P<0.05$; **- $P<0.01$; ***- $P<0.001$; Compare with "< 3.5 kg".

For example, at the age of 20 days, lambs that had a body mass of 3.6-4.0 kg at birth exceeded those that had a mass of <3.5 kg by 1.17 kg or 19.8% ($P<0.05$); those who weighed 4.1-4.5 kg at birth exceeded them by 2.24 kg or 38.0% ($P<0.001$); those who weighed 4.6-5.0 kg exceeded them by 2.98 kg or 50.5% ($P< 0.001$), and those who weighed >5.1 kg at birth exceeded their congeners, who were <3.5 kg at birth with 4.23 kg or 71.7% ($P<0.001$). Such legitimacy (repeatability) of the body mass of lambs is also observed at the age of 90 days, 6 and 18 months, as well as mature age. Thus, the correlation coefficient (repeatability) between the body mass of the lambs at birth and at 20 days was r_{xy} = 0.47±0.07; at 90 days r_{xy} = 0.39±0.09; at 6 months r_{xy} = 0.26±0.08; at 18 months r_{xy} = 0.23±0.09. Despite the fact that, with the advancing age of the sheep youth, a tendency to decrease the repeatability coefficient of the body mass was observed, however, by selecting from generation to generation, at the validation, well-developed lambs according to the body mass, we can obtain animals corpulent and at a mature age, with increased skills in meat production. From well-developed lambs at birth with a body mass of 5-6 kg, carcasses of 3.5-4.0 kg were obtained, at the age of 3 months - carcasses of 8.3 kg, at 8 months - of 14.1 kg, at 20 months - 21.9 kg, at 32 months - 25.6 kg.

Therefore, knowing the correlations (including negative ones) of the body mass of the lamb at birth with other characters is of great importance in directing the selection process in the direction of obtaining a large surface furskin and a better conformed carcass, in creating the new type of Corpulent Moldavian Karakul sheep.

The body length of the Karakul lamb is one of the most important body dimensions because it clearly characterizes the exterior and linear body development and provides an indirect preliminary information about the possible furskin surface. The body length of the lamb at birth is determined, first of all, by the genotype of the parents and influenced by the nutrition and maintenance of the ewes during the gestation period, especially in the second half. Well-developed lambs with long and very long body length were obtained from the parents, who had a high body development at birth (long and very long body length), in optimal conditions of nutrition and maintenance during the ewes gestation period.

In our research (Buzu, 2001), the repeatability coefficient (K) of this character at birth, 20 days, 3, 6 and 18 months, varies within the limits of 0.16-0.36, being conditioned by many external and internal factors. This means that the targeted selection of lambs to rating according to body length is effective and contributes to the creation of flocks of sheep with large body length.

The body length of the lamb at birth is directly or indirectly related to the furskin surface, body mass, constitution, skin thickness, hair fiber length, etc. (Table 8).

Table 8. Correlation of body length of Moldavian Karakul lambs by birth with some morpho-productive characters

Caracters	N	$r_{xy} \pm m_r$	t_r
Body mass at birth	859	0.49 ± 0.03	16.3***
Surface of the furskin	164	0.78 ± 0.03	26.0***
Skin thickness	282	0.54 ± 0.04	13.5***
Fiber length in black lambs:			
on the rump	199	0.16 ± 0.07	2.3*
on the withers	199	0.15 ± 0.07	2.1*
Fiber length in greyish lambs:			
white fibers: on rump	65	0.15 ± 0.12	1.3
on the withers	65	0.05 ± 0.12	0.4
black fibers: on the rump	65	0.18 ± 0.12	1.5
on the withers	65	0.03 ± 0.12	0.3

Note: *- $P < 0.05$; ***- $P < 0.001$.

The correlation coefficients of these morpho-productive attributes vary within quite wide limits. The highest correlation coefficient was established between the body length and the standard surface of the furskin (r_{xy}=0.78±0.03; t_r=16.3). This indicates that this character conditions, to a large extent, the surface of the furskin and is in direct relationship with this character.

The regression coefficient of body length with furskin surface is R = 101.4. This means that with the increase in the body length of the lamb by 1 linear cm, the useful surface of the furskin will increase by 101.4 cm^2. Significant positive correlation coefficients are also found with body mass (r_{xy} = 0.49±0.03; t_r = 16.3), skin thickness (r_{xy} = 0.54±0.04; t_r = 26.0), fiber length in black lambs on the rump (r_{xy} = 0.16±0.07; t_r = 2.3) and on the withers (r_{xy} = 0.15±0.07; t_r = 2.1). Therefore, with the increase in body length, there is a tendency to increase some characters that cause the decrease of some furskin qualities, such as the thickening of the skin and the increase in the length of the fibers. These positive correlations, from the genetic point of view, are also negative from the point of view of the influence on some curling qualities.

The analysis of the data on the length of the Karakul lambs demonstrates that it is a function of their body mass, the size of the curls, constitution and skin thickness (Table 9).

Table 9. Body length of Moldavian Karakul lambs at birth according to their mass, curl size, constitution and skin thickness

Specification	N	Body length, cm		
		M ± m	σ	C_v, %
Depending on the body mass[1]				
> 5.1 kg	99	37.56 ± 0.16***	1.55	4.1
4.6-5.0 kg	156	36.14 ± 0.12***	1.49	4.1
4.1-4.5 kg	208	35.00 ± 0.11***	1.56	4.5
3.6-4.0 kg	225	33.43 ± 0.12***	1.80	5.4
< 3.5 kg	297	29.95 ± 0.12	2.08	6.9
Depending on the size of the curls[2]				
Very large (>12 mm)	20	34.73 ± 0.38***	1.81	5.2
Large (9-12 mm)	136	32.91 ± 0.17***	1.99	6.2
Middle (6-8 mm)	372	31.68 ± 0.09***	1.77	5.6
Small (4-5 mm)	88	30.29 ± 0.19	1.81	6.0
Very small (< 4 mm)	10	28.60 ± 0.88	2.75	9.6
Depending on the constitution[3]				
Coarse	43	33.44 ± 0.31***	2.07	6.2
Robust	503	31.89 ± 0.09***	1.95	6.1
Fine	80	29.78 ± 0.22	2.00	6.7
Depending on the thickness of the skin[4]				
Subtle	129	30.25 ± 0.18	2.00	6.6
Medium	304	31.78 ± 0.11***	1.94	6.1
Thickened	172	32.80 ± 0.16***	2.03	6.2
Thick	21	34.86 ± 0.38***	1.74	5.0

Note: ***- P < 0.001; 1 - compared to "< 3.5 kg"; 2 - compared to the "Small" loop; 3 - compared to "Fine"; 4 - compared to "Subtle".

We found that the harmonious development of the lamb's body size during the intrauterine period is closely related to weight gain and the general development of the body. Lambs with a body mass of up to 3.5 kg had the shortest body length and those with a body mass of more than 5.1 kg had the longest body length. Along with the increase in body mass of lambs at birth from 3.1 kg to 5.1 kg, their body length increases from 29.95 cm to 37.56 cm or by 25.4% (P<0.001). Full-bodied lambs, which had a birth weight of more than 5.1 kg, exceeded, according to body length, congeners that had a weight of 3.6–4.0 kg, by 4.13 cm or 12.4% (P<0.001).

Therefore, the higher the body mass of the lambs at birth, the longer their body and, conversely,

the smaller the lambs, the shorter their body.

The length of the body in the lamb is related to the size of the loop. We found that lambs with large and very large curls have the largest body length, respectively, 32.91 and 34.73 cm. The smallest body length was found in lambs with small and very small curls, making up 30.29 and 28.60 cm, respectively, being smaller than the first two batches by 4.44-6.13 cm (P<0.001) and, respectively, by 2.62-4.31cm (P<0.001), or by 12.8-17.7% and 8.0-13.1%. It should be mentioned that, with the increase in the size of the loop from very small to very large, the body length of the lambs increases considerably from 28.60 cm to 34.73 cm or by 6.13 cm (21.4%) (P<0.001).

The body length of the lamb depends on its constitution. We found that lambs with a coarse and robust constitution have a greater body length than those with a fine constitution by 3.66 cm and 2.11 cm, respectively, or 12.3 and 7.1% (P<0.001). The largest body length was found in lambs with a coarse constitution. Lambs with a fine constitution had the shortest body length. Lambs of robust constitution are placed, according to this character, in the intermediate position, between the two batches. Therefore, with the growth of robustness of the lambs at birth, their body length increases. It should be mentioned that the values of the body length of the lambs at birth in the researched herd, within the limits of the averages of 29.78-33.44 cm, are quite high. From the lambs of this flock, furskins with a large and very large surface are obtained. The coefficient of variation of the body length of the lambs at birth in profile on batchs with different types of constitution is small. This denotes the fact that the body length of the Karakul lamb at birth is a trait consolidated genetically and largely determined by heredity.

The body length of Karakul lambs at birth is indirectly related to skin thickness. Research has shown that lambs with a large body length also have thicker skin. From the data included in the table, it can be seen that lambs with thick and thickened skin exceeded, according to body length, their congeners with medium and thin skin, by 9.7-15.2% and 3.2-8.4%, respectively (P<0.001). The greatest body length was found in lambs with thick skin (34.86±0.38 cm) and thickened (32.80±0.16 cm). Lambs with thin skin had the shortest body length (30.25±0.18 cm). Lambs with medium skin thickness had a body length of 31.78 ± 0.11 cm and occupied an intermediate position between the other groups.

Knowing the direct and indirect correlative links of the body length of the lamb at birth with the other morpho-productive characters allowed us to conclude that this character is one of the important ones. For these reasons, the selection of lambs at birth according to body length was applied to the creation of the Moldavian Karakul sheep type. Lambs with long and very long body length were retained for reproduction in the farrowing batches.

The constitution of lambs from the Karakul race, as well as from other sheep races, expresses the general state of the organism (morphological, structural and functional), characterized by the body conformation, external and internal (physiological state), which determines the morpho-productive type and the level of productivity. The constitution of lambs at birth is determined by the hereditary capacities of the parents (genotype) and conditioned by external factors (Дъчков et al., 1950; Koshevoy, 1975), by clandestine matings (Borisenko, 1967), by the level of prolificacy of the herd (Koshevoy, 1975), etc.

Based on research, Vasin et al. (1971) affirms that "*The furskin qualities of Karakul lambs, conditioned by heredity, are closely related to the constitutional particularities and, being under the influence of various external actions during the intrauterine period of fetal development, have a great variability*".

The constitution of lambs at birth conditions multiple relationships with different properties of the skin, hair fibers, curls, as well as with their body conformation. The creation of optimal conditions in the respective periods allows the obtaining of vigorous lambs with a robust constitution and the more obvious realization of the potential of furskin qualities, etc. (Vasin et al., 1971; Gigineishvili, 1976; Dyachkov, 1980; Ivanov, 1964b; Ilyev, 1969; Koshevoy, 1975; Yudin, 1943; Kuzembayuly, 2010).

Our research (Buzu, 2017) demonstrated that the constitution of lambs at birth conditions, first of all, the variability of characters related to their body conformation. We found that the constitution is in direct relationship with the body mass of the lambs at birth (Table 10).

Table 10. The relationship between constitution and body mass of lambs Moldavian Karakul at birth

The constitution lambs	N	Rate of lambs (%) with body mass (kg)					
		< 3.5	3.6-4.0	4.1-4.5	4.6-5.0	5.1-5.5	> 5.6
Coarse	43	-	-	4.7^{**}	11.6	18.6^{***}	65.1^{***}
Robust	503	1.0^{***}	9.3^{***}	26.8	32.0^{***}	24.1^{***}	6.8^{***}
Fine	80	37.5	28.7	26.3	7.5	-	-
Total	626	5.6	11.2	25.2	27.5	20.6	9.9

Note: **- P <0.01; ***- P <0.001; Compared to the "Fine" constitution.

Research data show that lambs with coarse constitution have the highest body mass at birth, usually very high.

As a rule, lambs with a robust constitution have a large and medium body mass, and lambs with a fine constitution have the smallest body mass, falling mainly into the reduced and small categories. In the batch of lambs with a coarse constitution, most individuals (83.7%) had a very high body mass at birth (over 5.0 kg), of which 65.1% had a body mass greater than 5.6 kg.

In this batch there were 11.6% of lambs with high body mass (4.6-5.0 kg) and only 4.7% of individuals - with medium body mass (4.1-4.5kg). In the batch of lambs with a robust constitution, most individuals had a high (32.0%) and very high (30.9%) body mass, of which 6.8% had a body mass over 5.6 kg.

At the same time, in this batch, there were also lambs with a medium body weight (26.8%) in a minor proportion. In the batch of lambs with fine constitution, most individuals had low (37.5%) and low (28.7%) body mass. At the same time, in this batch there were, in small numbers, individuals with medium (26.3%) and large (7.5%) body mass. Therefore, based on the analysis of the presented data, we can state that the constitution of Karakul lambs at birth essentially conditions their body mass at this age.

A similar correlation is also observed between the constitution of the lambs and their body length at birth (Table 11).

Table 11. The relationship between constitution and body length of Moldavian Karakul lambs at birth

The constitution of lambs	n	Including, with body length, cm									
		< 26		26 - 29		30 - 33		34 - 37		> 37	
		head	%	head	%	head	%	head	%	head	%
Coarse	43	-	-	-	-	8	18.6***	29	67.4***	6	14.0***
Robust	503	-	-	25	5.0**	336	66.8	139	27.6***	3	0.6***
Fine	80	9	11.3	16	20.0	53	66.2	2	2.5	-	-
Total	626	9	1.4	41	6.6	397	63.4	170	27.2	9	1.4

Note: **- P < 0.01; ***- P < 0.001; Compared to the "Fine" constitution.

Research data demonstrate the fact that lambs with a coarse constitution have the largest body length at birth. As a rule, lambs with a robust constitution have the appropriate long and very long body length, and lambs with a fine constitution have the smallest body length, falling mainly into the short and short length categories. Thus, in the batch of lambs with a coarse constitution, most individuals (81.4%) had a very long body length (over 34 cm), of which 14.0% had a particularly long body length at birth - over 37 cm. In this batch there were also 18.6% of lambs with the suitably body length (30-33 cm). In the batch of lambs with a robust constitution, the overwhelming majority of individuals had the suitably body length (66.8%) and very long (28.2%), of which 0.6% had an especially long body length - over 37 cm. In the batch of lambs with fine constitution, most individuals had the suitably body length (66.2%), reduced (20.0%) and short (11.3%). Therefore, based on the analysis of the presented data, we can state that the constitution of Karakul lambs at birth essentially conditions their body length.

The constitution of the lambs is in direct relationship with the properties of the skin, in particular, with its density (Table 12).

Table 12. The relationship between constitution and skin density of Moldavian Karakul lambs at birth

The constitution of lambs	N	Skin density							
		Very dense		Suitable		Reduced		Loose	
		head	%	head	%	head	%	head	%
Coarse	43	-	-	17	39.5**	19	44.2***	7	16.3***
Robust	503	122	24.3	297	59.0	78	15.5**	6	1.2***
Fine	80	21	26.2	54	67.5	5	6.3	-	-
Total	626	143	22.8	368	58.8	102	16.3	13	2.1

Note: **- P <0.01; ***- P <0.001; Compared to the "Fine" constitution.

Research data shows that lambs with a fine constitution have the densest skin at birth. Lambs with a robust constitution usually have the right skin density, and lambs with a coarse constitution have the loosest skin, mostly falling into the reduced and loose categories. Thus, in the batch of lambs with a fine constitution, most individuals had the suitably skin density (67.5%) and very dense (26.2%). In the batch of lambs with a robust constitution, most individuals had the suitably density (59.0%) and very dense (24.3%). In the batch of lambs with coarse constitution, most individuals had skin with reduced density (44.2%) and loose skin (16.3%). Therefore, based on the analysis of the presented data, we can state that the constitution of Karakul lambs at birth essentially conditions the density of their skin.

The research showed that in the batch of lambs with a fine constitution, the majority of individuals had medium (51.2%) and thin (46.3%) skin thickness. In the batch of lambs with a robust constitution, most individuals had medium-thick (49.7%) and thin (17.9%) skins. In the batch of lambs with coarse constitution, most individuals had thickened skin (44.2%) and thick skin (20.9%). The relationship between the constitution of the lambs and the thickness of the skin is quite obvious (Table 13).

Table 13. The relationship between constitution and skin thickness
in Moldavian Karakul lambs at birth

The constitution of lambs	N	Skin thickness							
		Thick		Thickened		Medium		Thin	
		head	%	head	%	head	%	head	%
Coarse	43	9	20.9^{***}	19	44.2^{***}	13	30.2^{*}	2	4.7^{***}
Robust	503	12	2.4^{***}	51	10.1^{**}	250	49.7	90	17.9^{***}
Fine	80	-	-	2	2.5	41	51.2	37	46.3
Total	626	21	3.3	172	27.5	304	48.6	129	20.6

Note: *- P <0.05; **- P <0.01; ***- P <0.001; Compared to the "Fine" constitution.

Therefore, based on the analysis of the presented data, we can state that the constitution of Karakul lambs at birth essentially determines the thickness of their skin. The relationship between these two attributes is one of the most pronounced.

The constitution of Karakul lambs at birth influences the skin reserve (Table 14).

Table 14. The relationship between constitution and skin reserve
in Moldavian Karakul lambs at birth

The constitution of lambs	N	Skin reserve							
		Pleated		Free		Stretched		Insufficiency	
		head	%	head	%	head	%	head	%
Coarse	43	3	7.0	18	41.8^{**}	15	34.9	7	16.3^{***}
Robust	503	77	15.3^{*}	349	69.4	72	14.3	5	1.0
Fine	80	7	8.8	56	70.0	17	21.2	-	-
Total	626	87	13.9	423	67.6	104	16.6	12	1.9

Note: *- P<0.05; **- P<0.01; ***- P<0.001; Compared to the "Fine" constitution.

Research has shown that in the batch of lambs with a robust constitution, the overwhelming majority of individuals had free (69.4%) and pleated (15.3%) skin reserves. In the batch of lambs with fine constitution, the overwhelming majority of individuals had the skin reserve also free (70.0%). In the batch of lambs with coarse constitution, the majority of individuals had skin with free reserve (41.8%), stretched (34.9%) and insufficient (16.3%). Therefore, based on the analysis of the presented data, we can state that the constitution of Karakul lambs at birth essentially conditions their skin reserve. The increase in robustness of the lambs leads to a decrease in the skin reserve. Analyzing the results of the research on the variability of the constitution of Karakul lambs at birth, we can conclude that with the increase in robustness, from the fine to the coarse, the body mass, body length and skin thickness increase substantially, while the density, suppleness and reserve of the skin decrease. Taking into account the fact that the characters of the body conformation and the skin conditions the development of a series of other properties of the hair fibers and the curl as a whole, knowing the relationships of the constitution of the lambs with the characters and related morpho-productive properties in karakultur acquires a special importance for directing the genetic improvement process of the productive qualities of sheep flocks. Generalizing, in the end, the researches of the degree of manifestation of the body conformation of Moldavian Karakul lambs at birth and the intercorrelation relations of the characters and properties that determine it, we can conclude, in full agreement with the words of the great zootechnician - profesor Panin (1972), that "*The mutual connections of all the characters in the correlational system of the organism are extraordinarily complicated and still very little studied. Without knowledge of these mutual links, it is not possible to correctly determine the direction of artificial selection in the herd or race, it is not possible to scientifically argue the principles of rating and, in particular, the distribution of animals into quality categories - classes, without which any rating loses its practical importance*".

CONCLUSIONS

The body conformation of Moldavian Karakul lambs is typical of the classical (Asian) Karakul sheep race, with some particularities related to the development of body mass, body length and constitution.

Moldavian Karakul lambs at birth are quite corpulent, having an average body mass of 4.7-5.0 kg, and in some years even over 5.0 kg, this being a biological peculiarity of this new type of sheep.

The variability of the body mass of the lamb at birth is hereditarily determined (the coefficient of genotypic correlation r_{xy} = 0.63; h^2 = 0.36; the coefficient of repeatability $K_{0\text{-}6\ months}$ = 0.26; $K_{0\text{-}18\ months}$ = 0.23) and influenced by environmental conditions, in particular, by the nutrition of pregnant ewes.

The body mass of the lambs at birth is in a direct-proportional phenotypic relationship with: age of the ewes at calving, body length (r_{xy} = 0.49), skin thickness (r_{xy} = 0.45), fiber length (r_{xy} = 0.22-0.31), furskin surface (r_{xy} = 0.64), loop size and constitution; inversely-proportional to: the prolificacy of the ewes in the flock and the lambing period; curvilinear with: the qualities of furskin (rate in the flock at calving of the higher class Elita lambs).

Lamb body length at birth is in the same similar phenotypic relationships as body mass, including with: skin thickness (r_{xy} = 0.54), fiber length (r_{xy} = 0.15-0.18), furskin area (r_{xy} = 0.78).

The constitution of the lamb at birth is in a phenotypic relationship: directly proportional - with its own body mass, body length, thickness and skin reserve; inversely proportional - to skin density.

AKNOWLEDGEMENTS

The scientific researches were carried out with funding from the State Budget within the research programs and projects, registered with the ciphers: 05.01.03; 07.10.02 and 04.49.02, in which the author was executor and project leader.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

CLIMATIC CHANGES OF ATMOSPHERIC PRECIPITATION AND THE VITAL ACTIVITY OF BEES

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Abstract

The aim of the present work was to reveal the impact of climate changes of atmospheric precipitation on the vital activity of bee colonies. To elucidate this impact, Pearson's linear correlation coefficients were calculated between monthly atmospheric precipitation and the average annual value of each of the 6 main morpho-productive characters of bee families, such as: queen prolificacy, winter resistance, strength and colony resistance to disease, brood viability and honey production. The scientific research was carried out on the families of Apis mellifera bees from the Carpathian race at the "Apibio Regina Mierii" experimental apiary. For the research, the average monthly and annual atmospheric precipitation data for the last 11 years (2010-2020) from the nearest hydrometeorological station, located at a distance of 27 km from the apiary, were used. The results of the research demonstrated that the winter resistance of bee families has an obvious tendency to be positively influenced by atmospheric precipitation in March (r_{xy} = 0.461 ± 0.237; t_r = 1.95; P < 0.1). The prolificacy of queens is influenced negatively - by atmospheric precipitation in June (r_{xy} = -0.582 ± 0.199; t_r = 2.92; P < 0.01) and positively - by atmospheric precipitation in July (r_{xy} = 0.579 ± 0.200; t_r = 2.89; P < 0.01). The strength of bee colonies is positively influenced by atmospheric precipitation in December of the previous year (r_{xy} = 0.571 ± 0.213; t_r = 2.68; P < 0.05), as well as in March (r_{xy} = 0.561 ± 0.206; t_r = 2.72; P < 0.05) and July (r_{xy} = 0.482 ± 0.231; t_r= 2.09; P < 0.05) of the current year. The viability of the bee brood is negatively influenced by atmospheric precipitation in the months of January (r_{xy} = -0.469 ± 0.235; t_r = 2.00; P < 0.05) and May (r_{xy} = -0.577 ± 0.201; t_r = 2.87; P < 0.01) of the current year, and positive - from the atmospheric precipitation in March of the current year (r_{xy} = 0.504 ± 0.225; t_r = 2.24; P < 0.05) and October of the previous year (r_{xy} = 0.599 ± 0.203; t_r = 2.95; P < 0.01). Disease resistance of bee families is influenced negatively - by atmospheric precipitation in January of the current year (r_{xy} = -0.497 ± 0.227; t_r = 2.19; P < 0.05) and positively - by atmospheric precipitation in August of the previous year (r_{xy} = 0.565 ± 0.215; t_r = 2.63; P < 0.05), as well as the annual atmospheric precipitation of the previous year (r_{xy} = 0.560 ± 0.217; t_r = 2.58; P < 0.05). Honey production of bee families is positively influenced - by atmospheric precipitation in the months of September (r_{xy} = 0.711 ± 0.156; t_r = 4.56; P < 0.001), November (r_{xy} = 0.599 ± 0.203; t_r = 2.95; P < 0.01) and annual (r_{xy} = 0.560 ± 0.217; t_r = 2.58; P < 0.05) of the previous year, and negatively - by atmospheric precipitation in February (r_{xy} = -0.706 ± 0.151; t_r = 4.68; P < 0.001) and June (r_{xy} = -0.511 ± 0.223; t_r = 2.29; P < 0.05) of the current year.

Key words: *atmospheric precipitation, bees, climate changes, vital activity.*

INTRODUCTION

Apiculture, in the Republic of Moldova, presents a branch of agriculture of particular importance for the national economy, due to the value and quality of the products offered by it, the fact of creating jobs among the vulnerable layers of the population in rural areas, as well as for maintaining through pollination homeostasis and biodiversity of natural ecosystems.
In total, there are approximately 180 thousand bee families in the country, from which approximately 4.5 - 5.0 thousand tons of honey are obtained annually, of which approximately 4000 tons are exported to different countries (Program National, 2020). Other bee products, quite important, are obtained from bees, such as: wax, pollen, propolis, royal jelly, venom, which are used in various fields of the national economy (food industry, medicine, pharmaceuticals, cosmetics, plastic arts, etc.).
One of the most important benefits brought to man by bees, is the additional product obtained from increasing the productivity of entomophilous plants from cultivated and spontaneous flora, as a result of their pollination, thus ensuring the perpetuation of nature's biodiversity.
In the Republic of Moldova, bees pollinate about 600,000 ha of agricultural land, from which an additional 20-30% of the annual

harvest is obtained, worth more than 3.6-4.0 billion lei. (Statistical Yearbook, 2021).

The honey bee, being the main pollinator of entomophilous crops, is currently facing the impact of climate change and its weather on the world map. In the last decades of the 20th century and the beginning of the 21st century, human society exerts an increasing influence on the climate, in particular, on the Earth's temperature, through the burning of fossil fuels, the cutting of forests and the intensive breeding of animals. These activities generate emissions of enormous quantity of greenhouse gases, which add to those already naturally present in the atmosphere, thus contributing to the greenhouse effect, global warming and hydrological regime change, which have an impact on flora and fauna ecosystems , especially on pollinators, including *Apis mellifera* bees (Ambjerg-Nielsen, 2012; Climate change, 2012; Causes of climate change, 2018; SSC-Raport, 2009; Bojariu et al., 2015; Kremen et al., 2007; Memmott et al., 2007).

Climate change includes changes, in the complex, of air temperature, atmospheric precipitation regime and extreme or more irregular meteorological phenomena, such as: drought, storms, tornadoes, hail, floods, etc. (Bee Decline, 2013; Consequences of climate change, 2018; Econews Infomediu Europe, 2018; Marin et al., 2014). Climate change leads to: an increase in the average global temperature with significant variations at the regional level, a decrease in fresh water resources for the population, a reduction in the volume of the ice caps and an increase in the level of the oceans, a change in the hydrological cycle, an increase in arid surfaces, anomalies in the unfolding of the seasons, an increase in frequency and intensity extreme climate phenomena (Barbu et al., 2011; Themes biodiversity, 2017; Sandu et al., 2010).

Despite the existence of the Paris Agreement - the United Nations Framework Convention on Climate Change (Acordul de la Paris, 2016), greenhouse gas emissions and global warming continue to increase. Thus, in an official publication of the European Environment Agency (EEA), it is shown that the total emissions of greenhouse gases in the European Union (EU) increased by 0.7% in 2017, compared to 2016. The increase of greenhouse gas emissions in the EU was recorded for the fourth consecutive year (Small increase, 2019).

Along with global warming, climate change in atmospheric precipitation is also of major concern. Clouds, which are the more condensed form of tiny water molecules suspended in the air, are the main source of atmospheric precipitation. Tiny water particles play an important role in how and how long clouds form, the amount of solar radiation that clouds can reflect, and determine the type of precipitation generated. The concentrations and composition of water particles determine climate changes, the time and place where precipitation occurs (Busuioc et al., 2006).

Climate changes related to the frequency and volume of atmospheric precipitation have attracted the attention of many specialists and researchers in the field (Busuioc et al., 1999; Ștefan et al., 2004; Ștefănescu et al., 2014; Tomozeu et al., 2007), because they cause real economic and social costs, affecting food production and prices globally.

A report by the European Environment Agency "Climate change, impacts and vulnerability in Europe 2012" reveals rather pessimistic forecasts of climate change, in which Europe will be gripped by higher temperatures, in combination with a decrease in precipitation in the southern regions and increase in precipitation in Northern Europe. In addition, ice sheets and glaciers are melting and sea levels are rising. All these trends are expected to continue (Climate change, 2012). According to the same Report, climate changes in atmospheric precipitation have a direct impact on the physiology, phenology and distributions of biodiversity of fauna and flora, as well as on human society as a whole.

Pessimistic climate change predictions are complemented by other researchers (Birsan et al., 2014; Bojariu et al., 2015), which states that, in the months of the warm season, there is a tendency to decrease precipitation, which will generally increase towards the end of the 21st century. Under these conditions, the trend of predictions is associated with the signal of climate change determined by the increase in the concentration of greenhouse gases in the atmosphere, at a global level, with the regional signal of decreasing precipitation in the area, as

well as with the negative impact on agriculture, natural ecosystems and society human as a whole.
A Greenpeace Research Labs Report (Bee Decline, 2013) states that “climate change, such as rising temperatures, changing precipitation patterns and extreme or more irregular weather events, are impacting pollinator populations. Some of these changes may affect them individually, ultimately affecting their communities, which is reflected in the increased rate of extinction of pollinator species”.
According to the data of some of our previous researches, it was demonstrated that excessively high temperatures in the spring-summer of a dry year caused a drastic decrease in the morpho-productive performance of bee colonies by 20-46% (Cebotari et al., 2013).
In our other research, it was found that climatic changes in air temperature in different months of the year have different impact on the vital activity of bee families depending on the time of year and air temperature values (Cebotari et al., 2019).
Positively appreciating the results of the multiple above-mentioned researches, we can report that they have brought useful information regarding the impact of climate change on ecosystems in general and on pollinators in particular. At the same time, with the exception of our research, in the bibliographic sources accessible to us, there is a lack of information regarding: the concrete influence of climatic changes of atmospheric precipitation on the vital activity of bee families; the evolution of the value of the morpho-productive characters of the bee families according to the monthly and annual atmospheric precipitation; the correlative links between atmospheric precipitation parameters and the level of morpho-productive performances of bee families. Therefore, determining the correlations between the average monthly atmospheric precipitation parameters in different periods of the year and the evolution of the value of the morpho-productive characters of the bee families, presents a scientific and practical interest for mitigating the impact of climate change weather.
In this context, the aim of the present paper was to elucidate the impact of climate changes of atmospheric precipitation on the vital activity of *Apis mellifera* bee families.

MATERIALS AND METHODS

The scientific researches were carried out on the families of *Apis mellifera* bees from the *Carpathian* race at the experimental apiary "Apibio Regina Mierii", located in the Center area of Codrilor Moldovei, Ocolul Silvic Ghidighici, Canton no. 8, Forest sector no. 21. There were a total of 50 bee families in the apiary. During the years 2010-2020, annually, in each family of bees in the apiary, the resistance to wintering was evaluated individually, in the month of March, and in the month of July, the main morpho-productive characters of reproduction and development (prolificity of the queen, strength of the family), of resistance to diseases, viability of the brood, as well as productivity of the quantity of honey accumulated in the nest, according to the methods developed by us (Cebotari et al., 2010) for the Zootechnical Norm regarding the certification of bee families, the raising and certification of bee brood material, approved by the Decision of the Government of the Republic of Moldova no. 306 of 28.04.2011 (Zootechnical norm, 2011). Afterwards, the average value for the entire hive of each of the morpho-productive characters evaluated was calculated.
In order to research the impact of climate change on the vital activity of bee colonies, monthly and annual atmospheric precipitation data were collected for the last 11 years (2010-2020) from the nearest hydrometeorological station, located in Bravicea town, Călăraş district, at a distance of 27 km from the apiary. During this period, for each individual month, Pearson's linear correlation coefficients were calculated between the quantity of atmospheric precipitation and the average value per hive of each of the 6 main morpho-productive characters of the bee families, such as: the prolificacy of the queens, the power bee colonies, colony overwintering resistance, colony resistance to diseases, brood viability and honey production of bee colonies. For the months of the first period of the year (January -

July), the correlation coefficients were calculated between the quantity of atmospheric precipitation and the values of the morpho-productive characters of the bee families, evaluated in the same year at the end of July, with the exception of winter resistance, which was evaluated in March. Given the fact that the climatic factors from the second period of the year (August - December) no longer influence the morpho-productive characters already evaluated in July of the current year, the atmospheric precipitation variable from the months of August-December was calculated in correlation with the value of the morpho-productive of bee families from the following year.

The same correlation coefficients were also calculated for the variable of the annual quantity of atmospheric precipitation and the average values per apiary of the above-mentioned 6 morpho-productive characters.

Pearson's linear correlation coefficient (r_{xy}) was calculated on the electronic computer in the Files/StatSoft/STATISTICA 12 program. For each correlation coefficient separately, the correlation certainty criterion (t_r) and the certainty threshold (P) were calculated according to Student.

The data obtained in the research were statistically processed and their certainty assessed, according to variational biometric statistics, according to the methods of Plokhinsky (1989).

RESULTS AND DISCUSSIONS

The results of the research demonstrated that the climate changes, which took place between 2010 and 2020 in the area where the experimental apiary is located, caused a fairly wide variability in the amount of atmospheric precipitation (Table 1).

Table 1. Monthly and annual atmospheric precipitation recorded at the Station Hydrometeorological "Bravicea", Călărași district, during the years 2010-2020, mm

Month of the year	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**2020**
January	87.3	19.6	18.1	31.4	51.1	26.5	32.8	29.5	50.5	63.2	4.2
February	40.8	45.7	78.1	33.8	5.9	21.3	27.4	22.5	53.2	29.7	29.1
March	18.7	24.8	15.0	65.1	12.9	57.7	25.7	30.8	88.5	11.2	16.3
April	41.4	27.6	84.2	29.1	55.1	41.8	39.8	112.5	5.0	34.5	14.4
May	97.0	22.9	44.1	62.1	88.3	12.6	64.5	42.9	24.1	177.7	68.6
June	147.2	119.8	64.3	142.8	27.4	34.8	188.0	89.7	77.1	81.0	101.3
July	64.0	64.6	44.1	9.5	91.7	83.2	19.6	85.5	70.5	30.3	28.3
August	43.4	50.5	39.7	78.2	18.9	38.0	120.7	33.9	45.9	33.9	1.3
September	49.7	12.2	6.3	114.6	10.3	20.2	9.0	29.7	43.5	36.3	33.7
October	51.7	24.2	36.5	4.9	44.3	42.8	134.5	62.3	4.7	32.9	71.1
November	37.1	4,9	23.3	60.7	91.8	56.6	36.5	31.7	61.4	11.7	25.5
December	56.6	20.2	99.2	6.6	41.3	2.7	11.2	81.2	37.4	28.3	43.7
Annual total	**734.9**	**437.0**	**552.9**	**638.8**	**539.0**	**438.2**	**709.7**	**651.6**	**561.8**	**570.7**	**437.5**

The annual amount of atmospheric precipitation, during this period, varied from a minimum of 437.0 mm in 2011 to a maximum of 734.9 mm in 2010 or by 68.2%. The average annual amount of atmospheric precipitation in the period 2010-2020 was 570.2 mm. The lowest atmospheric precipitation fell in the years 2011 (437.0 mm), 2015 (438.2 mm) and 2020 (437.5 mm). The decrease in atmospheric precipitation, in these years, caused three terrible droughts, which affected not only the harvests of agricultural crops, but had a negative impact on the flora and fauna of the intact ecosystems, especially on the honey flora and, as a result, on the vital activity of bees.

The data show that the monthly amount of atmospheric precipitation varies over the years in quite significant amounts. In the analyzed period (2010-2020), the greatest variability (contrast) of the monthly amount of atmospheric precipitation was recorded in August, oscillating from 1.3 mm in 2020 to 120.7 mm in 2016, the variation constituting 92.8 times (92.80%). The lowest variability in the amount of atmospheric precipitation was recorded in June, oscillating from 27.4 mm in 2014 to 188.0 mm in 2016, the variation being 6.9 times. A fairly large variability in the amount of monthly atmospheric precipitation, during this period, was also found in the months: October, from 4.7 mm in 2018 to 134.5 mm in 2016, with the variation index of 28.6 times; April, from 5.0 mm in 2018 to 112.5 mm in 2017, with a variation of 22.5 times; January, from 4.2 mm in the year 2020 to 87.3 mm in the year 2010, with a variation of 20.8 times, and in November, from 4.9 mm in the year 2011 to 91.8 mm in the year 2014, with a variation of 18.7 times. In the other months of the year, the variability of the amount of atmospheric precipitation, during this period, oscillated from 7.9 times in March to 18.2 times in September.

During the monitored period, four severe droughts were recorded, which started in the years when the amount of annual precipitation was relatively lower compared to other years, but not the lowest. The analysis of the data demonstrates that the terrible droughts manifested in those years, in which the lowest amounts of precipitation were recorded in the warm period (May - August). Despite the fact that in 2011 the lowest annual amount of precipitation was recorded (437.0 mm), the drought did not occur, because during the warm period of this year, sufficient amounts of precipitation fell (257.8 mm). At the same time, the terrible drought was triggered in the years 2012, 2015, 2018 and 2020, in which the amount of atmospheric precipitation in the warm period constituted, respectively, 192.2 mm, 168.6 mm, 217.6 mm and 199.5 mm, being the lower than all the years of the monitored period.

Climatic changes in the amount of atmospheric precipitation, as well as extreme phenomena triggered in the area where the experimental apiary is located, caused a variability, in some cases significant, of the vital activity of bee families, expressed in different levels of development of morpho-productive characters (Table 2).

From the data presented, we observe that the average prolificity of queens per hive varied, during this period, from 1252 eggs/24 hours in 2020, to 1806 eggs/24 hours in 2011. The variability of this character in bee families was 44, 2%.

Table 2. Average morpho-productive indices of families of bees at the Experimental Apiary "Apibio Regina Mierii" during the years 2010-2020

The year	Queens prolifi-cacy, eggs/ 24 hour	Family power, kg	Winter resis-tance, %	Brood viabi-lity, %	Disea-se resis-tance, %	Honey produc-tion, kg
2010	1583	2.83	80.1	85.1	76.8	38.8
2011	1806	2.97	82.5	91.0	89.4	32.8
2012	1740	2.37	86.2	88.6	87.4	23.9
2013	1661	3.03	91.1	91.0	90.5	35.5
2014	1781	3.13	93.3	92.3	91.6	57.4
2015	1711	3.04	88.6	95.8	86.3	44.2
2016	1371	2.20	84.1	95.7	89.2	31.0
2017	1678	2.36	86.8	95.5	92.6	34.2
2018	1781	3.14	88.4	95.4	91.7	39.7
2019	1716	2.38	71.3	88.1	91.6	49.9
2020	1252	2.05	83.4	92.1	91.0	38.8

The strength of the family, expressed by the amount of bees in the nest, fluctuated from 2.05 kg in 2020 to 3.14 kg in 2018, the variability constituting 53.2%. Winter hardiness varied, albeit to a lesser extent, from 71.3 percentage points in 2019 to 93.3 percentage points in 2014, the variability being 30.8%. The lowest variability among the researched morpho-productive characters, in this period, was found in the viability of the brood, fluctuating from 85.1 percentage points in 2010, to 95.8 percentage points in 2015, with a variability of 11 ,2%. The disease resistance (hygienic instinct) of bee families fluctuated, during the nominated period, from 76.8 percentage points in 2010 to 92.6 percentage points in 2017, the variability being 17.1%. Overall, we observe that the climate changes, during this period, caused a fairly obvious variability in the honey production accumulated in the nest, from a minimum of 23.9 kg in 2012, to a maximum of 57.4 kg in 2014, the variability being 58.4%.

In order to elucidate the concrete relationships of climate change impact of atmospheric precipitation on the vital activity of bee families, for each individual month, the linear correlation coefficients (rxy) were calculated between the monthly amount of atmospheric precipitation and the average value per apiary of the morpho characters - productive of bee families (Table 3).

Table 3. The correlation coefficient (r_{xy}) between the monthly quantity of atmospheric precipitation in the first period of the year and the average value of the morpho-productive characters of bee families (N = 11)

Morpho-productive characters	$r_{xy} \pm m_r$	t_r	P
January precipitation			
Winter resistance	-0.321 ± 0.270	1.19	>0.1
Queens prolificacy	0.226 ± 0.286	0.79	>0.1
Family power	0.335 ± 0.267	1.25	>0.1
Disease resistance	-0.497 ± 0.227	2.19	<0.05
Brood viability	-0.469 ± 0.235	2.00	<0.05
Honey production	0.452 ± 0.240	1.88	<0.1
February precipitation			
Winter resistance	-0.152 ± 0.294	0.52	>0.1
Queens prolificacy	-0.150 ± 0.295	0.51	>0.1
Family power	-0.083 ± 0.299	0.28	>0.1
Disease resistance	-0.233 ± 0.285	0.82	>0.1
Brood viability	-0.360 ± 0.262	1.37	>0.1
Honey production	-0.706 ± 0.151	4.68	<0.001
March precipitation			
Winter resistance	0.461 ± 0.237	1.95	<0.1
Queens prolificacy	0.302 ± 0.274	1.10	>0.1
Family power	0.561 ± 0.206	2.72	<0.05
Disease resistance	0.158 ± 0.294	0.54	>0.1
Brood viability	0.504 ± 0.225	2.24	<0.05
Honey production	0.065 ± 0.300	0.21	>0.1
April precipitation			
Queens prolificacy	0.200 ± 0.289	0.69	>0.1
Family power	-0.277 ± 0.278	0.99	>0.1
Disease resistance	0.022 ± 0.301	0.07	>0.1
Brood viability	0.030 ± 0.301	0.10	>0.1
Honey production	-0.240 ± 0.284	0.84	>0.1
May precipitation			
Queens prolificacy	-0.186 ± 0.291	0.64	>0.1
Family power	-0.305 ± 0.273	1.12	>0.1
Disease resistance	-0.032 ± 0.301	0.11	>0.1
Brood viability	-0.577 ± 0.201	2.87	<0.01
Honey production	0.479 ± 0.232	2.06	<0.05
Iune precipitation			
Queens prolificacy	-0.582 ± 0.199	2.92	<0.01
Family power	-0.308 ± 0.273	1.13	>0.1
Disease resistance	-0.270 ± 0.279	0.97	>0.1
Brood viability	-0.426 ± 0.247	1.72	<0.1
Honey production	-0.511 ± 0.223	2.29	<0.05
Iuly precipitation			
Queens prolificacy	0.579 ± 0.200	2.89	<0.01
Family power	0.482 ± 0.231	2.09	<0.05
Disease resistance	-0.087 ± 0.299	0.29	>0.1
Brood viability	0.243 ± 0.284	0.85	>0.1
Honey production	0.363 ± 0.261	1.40	>0.1

The results of the research demonstrated that the atmospheric precipitation in January had a significant negative impact on the resistance to diseases and the viability of the brood. Thus, the coefficient of the linear correlation of the quantity of atmospheric precipitation this month with disease resistance is negative of medium level, having a significance of one certainty threshold according to Student (r_{xy}= -0.497 ± 0.227; t_r = 2.19; P < 0.05) (Figure 1).
The correlation between the quantity of atmospheric precipitation in this month of the year and the viability of the brood is also negative of medium level with the significance of the threshold one according to Student (r_{xy} = -0.469±0.235; t_r = 2.00; P<0.05). In addition, the atmospheric precipitation in January has a tendency of positive influence on honey production (r_{xy} = 0.452±0.240; t_r = 1.88; P<0.1).
Although, the correlation coefficient (r_{xy}) is of medium level, this correlation shows only a tendency, because the certainty criterion (t_r) has a significance, only by the zero threshold according to Student (P<0.1).

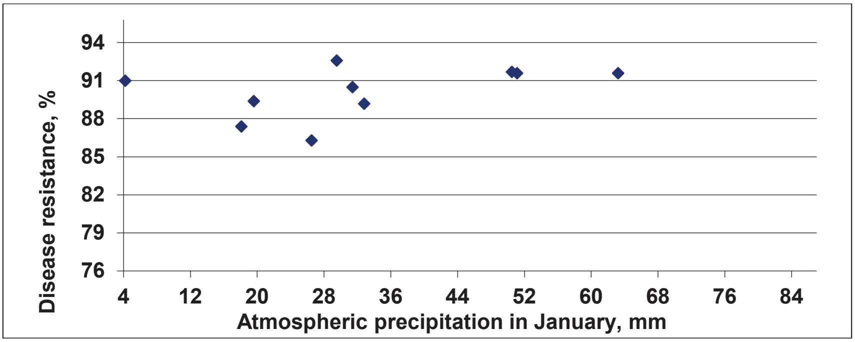

Figure 1. Point correlation between atmospheric precipitation in January and disease resistance

Atmospheric precipitation in February, which usually falls in the form of snow, does not have any significant influence on most of the morpho-productive characters of the bee families, with the exception of honey production. Although the theoretical mechanism is not known, how atmospheric precipitation in February could influence the future honey production evaluated in June-July, but in our research, it was found that they have a significant negative impact on honey production (Figure 2).

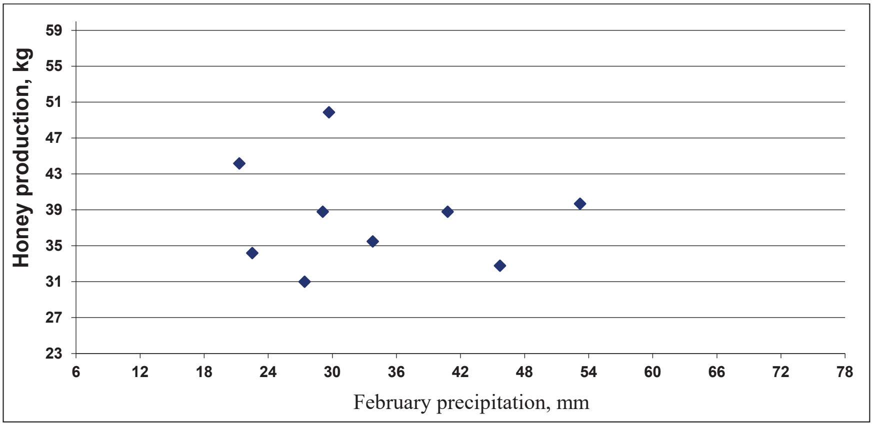

Figure 2. Point correlation between atmospheric precipitation in February and honey production

Thus, the correlation coefficient between these two variables (February atmospheric precipitation and honey production) is significantly negative at a high level (r_{xy} = -0.706 ± 0.151; t_r = 4.68; P<0.001), with the highest threshold of certainty according to the probability theory of prognostications without error according to Student. According to us, the negative impact of these precipitations is an indirect consequence of the increased humidity in the nest of bee families, which has negative influences on the main morpho-productive characters, such as: queen prolificacy, family resistance to wintering, brood viability, disease resistance and family power. The correlation of atmospheric precipitation with these characters is negative, although it is insignificant.

Atmospheric precipitation in March has a general positive influence on the vital activity of bee families. This is confirmed by the existence of positive correlations of different levels with all the research morpho-productive characters, especially on winter resistance, family strength and brood viability.

Thus, the correlation coefficient between atmospheric precipitation in March and wintering resistance is positive at a medium level, with an obvious tendency towards significance close to the threshold of one according to Student (r_{xy} = 0.461±0.237; t_r = 1.95; P<0.1). At the same time, the correlation coefficient between atmospheric precipitation this month and the strength of the bee family is positive above average level and quite significant, falling within the threshold of certainty according to the probability theory of prognoses without error according to Student (r_{xy}= 0.561 ± 0.206; t_r = 2.72; P<0.05) (Figure 3).

The same atmospheric precipitation in March had a significant positive impact on brood viability. Thus, Pearson's linear correlation coefficient between the atmospheric precipitation in March and the viability of the brood is of an above-average level, with the significance of the one certainty threshold according to the probability theory of prognoses without error according to Student (r_{xy} = 0.504±0.225; t_r = 2.24; P<0.05).

The research demonstrated that the atmospheric precipitation in the month of April does not exert any negative or positive influence on the vital activity of the bee families, because the linear correlation coefficients between the amount of precipitation in this month and the morpho-productive characters of the bee families had no values significant (P>0.1).

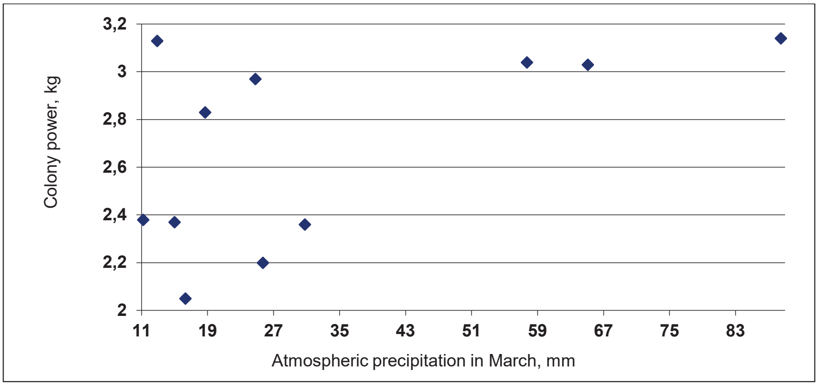

Figure 3. Point correlation curve between atmospheric precipitation in March and colony power

Atmospheric precipitation in May has an ambiguous influence on the vital activity of bee families. On the one hand, the atmospheric precipitation in this month negatively influences the viability of the brood. Between these two variables, an above-average level negative correlation was found (r_{xy} = -0.577 ± 0.201; t_r = 2.87; P<0.01), with the significance of the second certainty threshold according to the probability theory of error-free prognoses according to Student.

On the other hand, it is important to note that the atmospheric precipitation in May had a positive influence on honey production (Figure 4).

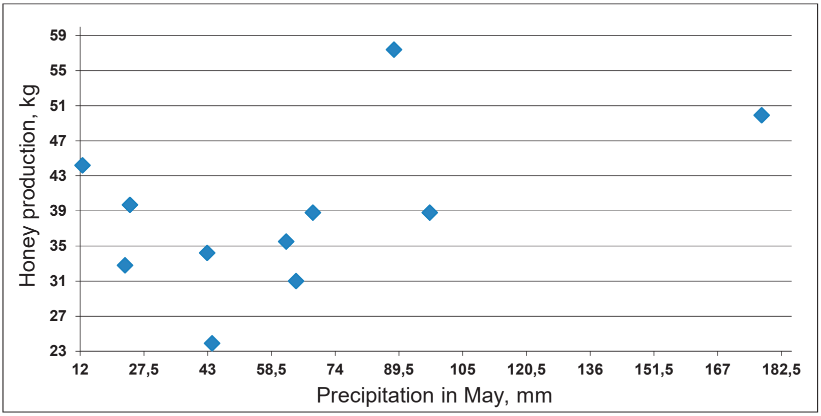

Figure 4. Point correlation between atmospheric precipitation in May and honey production

Thus, Pearson's linear correlation coefficient between atmospheric precipitation in May and honey production is of medium level, with the significance of the first certainty threshold according to the probability theory of prognoses without error according to Student (r_{xy} = 0.479 ± 0.232; t_r = 2.06; P<0.05).

According to us, the positive impact of atmospheric precipitation in May is explained by their stimulating influence on the process of circulation of the sap of honey plants and abundant accumulation of the nectar of their flowers, which directly contributes to the significant increase in the amount of honey accumulated in the nest.

Research has shown that the atmospheric precipitation in June has a negative influence on the development of the main morpho-productive characters. The negative impact of these precipitations is manifested significantly on the prolificacy of queens and honey production (Figure 5).

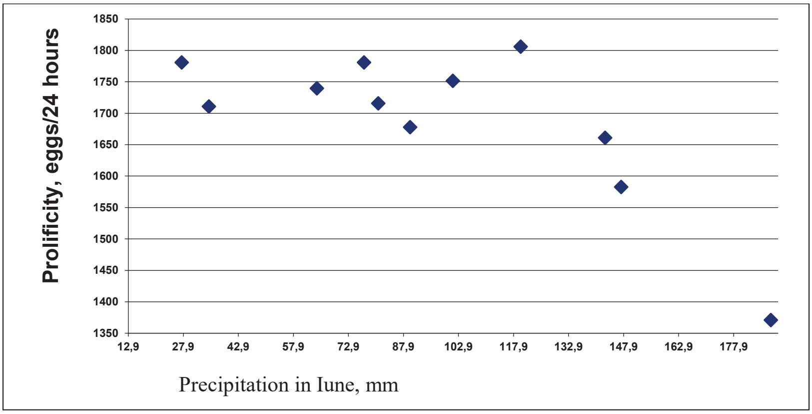

Figure 5. Point correlation between atmospheric precipitation in Iune and the prolificacy of queens

Thus, Pearson's linear correlation coefficient between the quantity of atmospheric precipitation in June and the prolificacy of queens is negative above average level, with the significance of the second threshold according to the probability theory of prognoses without error according to Student (r_{xy} = -0.582±0.199; t_r= 2.92; P<0.01). Between the quantity of atmospheric precipitation in this month and the production of honey, a negative correlation was recorded, also above average, with the significance of threshold one according to the probability theory of prognoses without error according to Student (r_{xy} = -0.511 ± 0.223; t_r = 2.29; P<0.05). At the same time, between the quantity of atmospheric precipitation in Iune and the viability of the young, a medium-level negative correlation trend with zero-threshold significance can be seen (r_{xy} = -0.426 ± 0.247; t_r = 1.72; P<0.1).

Research has shown that atmospheric precipitation in July has a mostly positive influence on the main morpho-productive characters of bee families, especially with significant influence on the prolificacy of queens and the power of bee colonies (Figure 6).

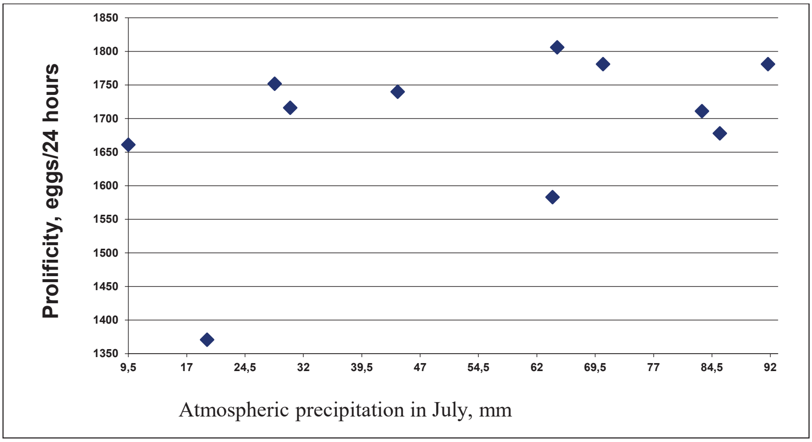

Figure 6. Point correlation between atmospheric precipitation in July and the prolificacy of queens

Thus, Pearson's linear correlation coefficient between the quantity of atmospheric precipitation in July and the prolificacy of queens is positive above average level, with the significance of the second threshold according to the probability theory of prognoses without error according to Student (r_{xy} = 0.579 ± 0.200; t_r = 2.89; P < 0.01). Between the quantity of atmospheric precipitation in this month and the strength of the family, there was a positive correlation of medium level, with the significance of the threshold of one according to the probability theory of prognoses without error according to Student (r_{xy} = 0.482 ± 0.231; t_r = 2.09; P<0.05).

Precipitation in this month has a weak tendency to have a positive influence on honey production.

However, the linear correlation coefficient between these two variables is not significant (r_{xy} = 0.363 ± 0.261; t_r = 1.40; P>0.1).

Generalizing the climate change impact of atmospheric precipitation in the first period of the year, we can deduce that the large amounts of precipitation in this period, especially in February and June, have a negative impact on the vital activity of bee families by reducing the level of development of the main morpho-productive characters. The negative impact is directly caused primarily on queen fertility, brood viability and honey production. Under the action of atmospheric precipitation during this period, the humidity in the nest increases excessively, inhibiting the development of most of the morpho-productive characters of bee families. At the same time, the atmospheric precipitation in the months of January, March, May and July has a mostly positive influence on the main morpho-productive characters, especially on the prolificacy of the queens, the power of the family and the production of honey.

Starting with the second half of the year, the atmospheric precipitation from July - December can no longer have an impact on the morpho-productive characters previously evaluated (in July), but they can have a direct impact on the vital activity of the bee families related to the consolidation of the power of the colonies and their preparation for winter, as well as indirectly on the evolution of the value of morpho-productive characters in the next year (Table 4).

Research has shown that atmospheric precipitation in August has no significant influence on the main morpho-productive characters, except for the resistance of bee families to diseases. It was found that the atmospheric precipitation in this month has a significant positive impact on the disease resistance of bee colonies, evaluated in the following year (Figure 7).

Table 4. The correlation coefficient between the quantity of monthly atmospheric precipitation in the second half of the preceding year and the value of the morpho-productive characters of bee families in the following year

Morpho-productive characters	**$r_{xy} \pm m_r$**	**t_r**	**P**
August precipitation			
Queens prolificacy	0.138 ± 0.310	0.44	>0.1
Family power	-0.158 ± 0.308	0.51	>0.1
Winter resistance	0.152 ± 0.309	0.49	>0.1
Brood viability	0.087 ± 0.314	0.28	>0.1
Disease resistance	0.565 ± 0.215	2.63	<0.05
Honey production	0.052 ± 0.315	0.16	>0.1
September precipitation			
Queens prolificacy	0.166 ± 0.307	0.54	>0.1
Family power	0.299 ± 0.288	1.04	>0.1
Winter resistance	0.098 ± 0.313	0.31	>0.1
Brood viability	-0.192 ± 0.305	0.63	>0.1
Disease resistance	0.349 ± 0.278	1.25	>0.1
Honey production	0.711 ± 0.156	4.56	<0.001
October precipitation			
Queens prolificacy	0.021 ± 0.316	0.07	>0.1
Family power	-0.091 ± 0.314	0.29	>0.1
Winter resistance	0.185 ± 0.305	0.61	>0.1
Brood viability	0.599 ± 0.203	2.95	<0.01
Disease resistance	0.256 ± 0.295	0.87	<0.1
Honey production	-0.397 ± 0.266	1.49	>0.1
November precipitation			
Queens prolificacy	0.307 ± 0.286	1.07	>0.1
Family power	0.325 ± 0.283	1.15	>0.1
Winter resistance	-0.059 ± 0.315	0.19	>0.1
Brood viability	0.385 ± 0.269	1.43	>0.1
Disease resistance	-0.221 ± 0.301	0.73	>0.1
Honey production	0.599 ± 0.203	2.95	<0.01
December precipitation			
Queens prolificacy	0.304 ± 0.287	1.06	>0.1
Family power	0.571 ± 0.215	2.68	<0.05
Winter resistance	0.119 ± 0.311	0.38	>0.1
Brood viability	-0.111 ± 0.312	0.35	>0.1
Disease resistance	0.041 ± 0.316	0.13	>0.1
Honey production	-0.065 ± 0.315	0.21	>0.1
Annual total precipitation			
Queens prolificacy	0.380 ± 0.270	1.41	>0.1
Family power	0.414 ± 0.262	1.58	>0.1
Winter resistance	0.084 ± 0.315	0.27	>0.1
Brood viability	0.165 ± 0.308	0.53	>0.1
Disease resistance	0.560 ± 0.217	2.58	<0.05
Honey production	0.286 ± 0.290	0.99	>0.1

The Pearson's linear correlation coefficient between the quantity of atmospheric precipitation in August and the disease resistance of bee families in the following year is positive above average, with the significance of threshold one according to the probability theory of prognoses without error according to Student (r_{xy} = 0.565 ± 0.215; t_r = 2.63; P<0.05).

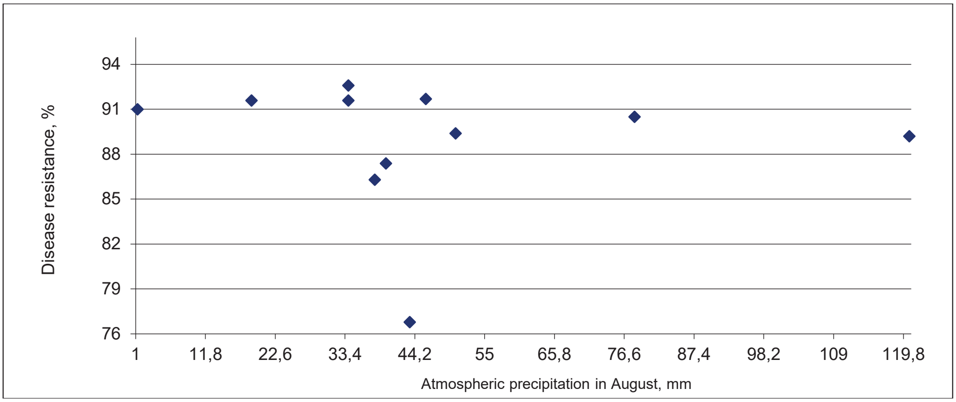

Figure 7. Point correlation between August atmospheric precipitation and disease resistance

Atmospheric precipitation in September tends to have a positive influence on most of the morpho-productive characters of bee colonies in the following year. The positive impact of atmospheric precipitation in September manifests itself significantly on honey production (Figure 8).

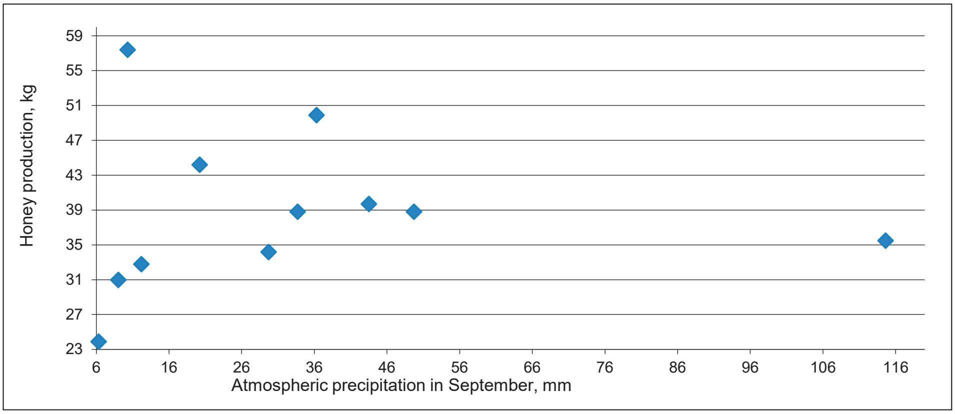

Figure 8. Point correlation between atmospheric precipitation in September and next year's honey production

Thus, Pearson's linear correlation coefficient between the quantity of atmospheric precipitation in September and the honey production of the bee colonies in the following year is obviously high-level positive, with the significance of the third threshold according to Student's theory of the probability of forecasts without error (r_{xy} = 0.711 ± 0.156; t_r = 4.56; P<0.001).

According to us, this influence is indirect and is explained by the fact that the atmospheric precipitation in September favors the process of plant sap circulation, ensuring the successful wintering of honey plants and the production of a considerable quantity of nectar in spring honey flowers.

Atmospheric precipitation in October has a tendency to have a positive indirect influence on most of the morpho-productive characters of bee families in the next year.

In particular, the positive influence is significantly manifested on the viability of the brood. Pearson's linear correlation coefficient between the quantity of atmospheric precipitation in October and the viability of the brood of bee colonies in the year next is above-average level positive, with threshold two significance according to Student's error-free prediction probability theory (r_{xy} = 0.599 ± 0.203; t_r = 2.95; P<0.01).

Atmospheric precipitation in November also tends to have a positive indirect influence on most of the morpho-productive characters of bee colonies in the next year. In particular, the trend of positive influence is more evident on queen prolificacy, bee colony strength and brood viability. Moreover, atmospheric precipitation in November has a significant positive influence on honey production the following year (Figure 9).

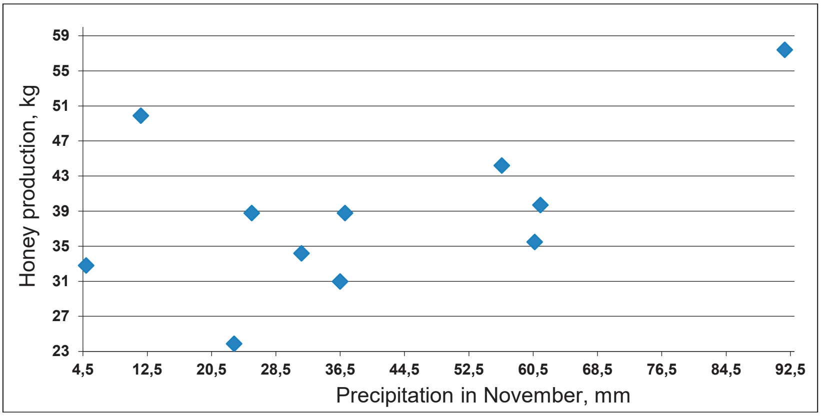

Figure 9. Point correlation between atmospheric precipitation in November and next year's honey production

The Pearson's linear correlation coefficient between the quantity of atmospheric precipitation in November and the honey production of bee colonies in the following year is obviously positive above the average level, with the significance of the second threshold according to the probability theory of Student's error-free forecasts (r_{xy} = 0.599 ± 0.203; t_r = 2.95; $P<0.01$).

Atmospheric precipitation in December also tends to have a positive indirect influence on most of the morpho-productive characters of bee families in the next year. In particular, the trend of positive influence is more evident on the prolificacy of queens, the strength of the bee colony and the resistance to wintering. Through this, the positive impact of atmospheric precipitation in December is significantly manifested on the strength of bee families. Thus, Pearson's linear correlation coefficient between the quantity of atmospheric precipitation in December and the strength of bee colonies in the following year is obviously positive above average level, with the significance of threshold one according to Student's probability theory of forecasts without error (r_{xy} = 0.571 ± 0.213; t_r = 2.68; $P<0.05$).

Generalizing the results of the research of climatic changes of atmospheric precipitation on the vital activity of bee families, we can conclude that the total annual quantity of atmospheric precipitation has a tendency to have a positive influence on the main morpho-productive characters, such as: the prolificacy of queens, the strength of the bee colony, brood viability, disease resistance and honey production. In particular, the positive impact of annual atmospheric precipitation is manifested on the resistance of bee families to diseases (Figure 10).

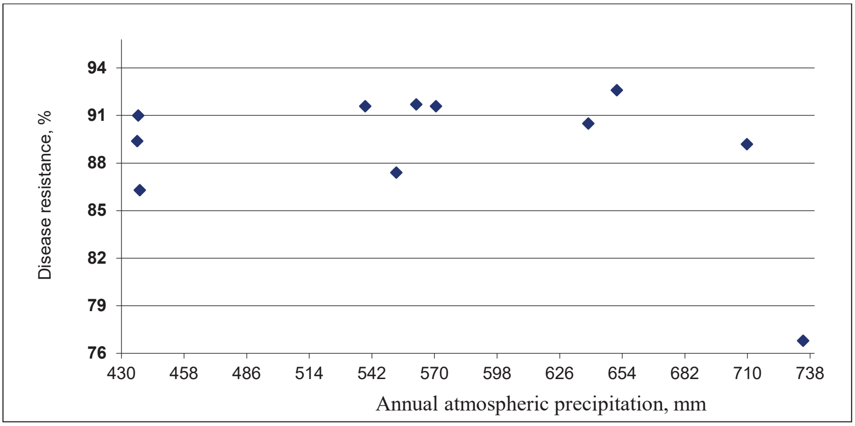

Figure 10. Point correlation between annual atmospheric precipitation and disease resistance of bee families the following year

Thus, the Pearson's linear correlation coefficient between the total annual quantity of atmospheric precipitation and the disease resistance of the bee colonies in the following year is significantly positive above average level, with the significance of the threshold of one according to the probability theory of Student's error-free forecasts (r_{xy} = 0.560 ± 0.217; t_r = 2.58; P<0.05). According to us, atmospheric precipitation, having a general positive impact on honey flora, contributes to the quantitative and qualitative increase of nectar-pollen resources and the strengthening of the immune system of bees, expressed by increasing the resistance of bee colonies to diseases.

CONCLUSIONS

The resistance of bee families to wintering has an obvious tendency to be positively influenced by atmospheric precipitation in March (r_{xy} = 0.461 ± 0.237; t_r = 1.95; P<0.1).

The prolificacy of the queens is negatively influenced by atmospheric precipitation in June (r_{xy} = -0.582 ± 0.199; t_r = 2.92; P<0.01) and positively by atmospheric precipitation in July (r_{xy} = 0.579 ± 0.200; t_r = 2.89; P<0.01).

The strength of bee colonies is positively influenced by atmospheric precipitation in December of the previous year (r_{xy} = 0.571 ± 0.213; t_r = 2.68; P<0.05), as well the ones in March (r_{xy} = 0.561 ± 0.206; t_r = 2.72; P<0.05) and July (r_{xy} = 0.482 ± 0.231; t_r = 2.09; P<0.05) of the current year.

The viability of the bee brood is negatively influenced by atmospheric precipitation in the months of January (r_{xy} = -0.469 ± 0.235; t_r = 2.00; P<0.05) and May (r_{xy} = -0.577 ± 0.201; t_r = 2.87; P<0.01) of the current year, and positive - by the atmospheric precipitation in March of the current year (r_{xy} = 0.504 ± 0.225; t_r = 2.24; P<0.05) and October of the previous year (r_{xy} = 0.599 ± 0.203; t_r = 2.95; P<0.01).

Disease resistance of bee families is influenced negatively - by atmospheric precipitation in January of the current year (r_{xy} = -0.497 ± 0.227; t_r = 2.19; P<0.05) and positively - by atmospheric precipitation in August of the previous year (r_{xy} = 0.565 ± 0.215; t_r = 2.63; P<0.05), as well as the annual atmospheric precipitation of the previous year (r_{xy} = 0.560 ± 0.217; t_r = 2.58; P<0.05).

Honey production of bee families is positively influenced - by atmospheric precipitation in the months of September (r_{xy} = 0.711 ± 0.156; t_r = 4.56; P<0.001), November (r_{xy} = 0.599 ± 0.203; t_r = 2.95; P<0.01) and annual totals (r_{xy} = 0.560 ± 0.217; t_r = 2.58; P<0.05) of the previous year, and negatively - by atmospheric precipitation in February (r_{xy} = -0.706 ± 0.151; t_r = 4.68; P<0.001) and June (r_{xy} = -0.511 ± 0.223; t_r = 2.29; P<0.05) of the current year.

ASKNOWLEDGEMENTS

This work was carried out as part of the Fundamental Scientific Research Project of the Institute of Zoology within the State Program (2020-2023) - Code 20.80009.7007.12

"Diversity of hematophagous arthropods, zoo and phytohelminths, vulnerability, strategies to tolerate climatic factors and the development of innovative procedures for the integrated control of species of socio-economic interest".

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

POSTNATAL DEVELOPMENT OF HEIFER AND MILK PRODUCTIVITY OF UKRAINIAN BLACK-SPOTTED DAIRY COWS OF DIFFERENT TYPES OF CONSTITUTION

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Abstract

Groups of animals were formed at six months based on the physiological and selection index we invented. We studied postnatal development in heifers of the high-enzyme (experimental group) and low-enzyme (control group) constitution type. Thus, due to the main diameters of body parts, which characterize the exterior in postnatal ontogenesis, animals of the high-enzyme type (experimental group) prevailed over their analogues of the low-enzyme type (control group). According to indices of the body structure in postnatal ontogeny, the development of some relative to others is evaluated. In our studies, significant superiority in the experimental group over the control analogues wasn't noted in terms of these indicators. Consonant to the leading indicators characterizing oxidation-reduction processes in the body, animals of the high-enzyme type (experimental group) significantly outnumbered their analogues of the low-enzyme type (control group). Therefore, this indicates that the level of metabolic processes in the experimental group was much more intense compared to the control group. Thus, animals of the high-enzyme type (experimental group) significantly outnumbered their analogues of the low-enzyme type (control group).

***Key words**: Black-Spotted dairy breed, heifers, milk productivity, postnatal development.*

INTRODUCTION

The Black-Spotted breed of cattle is bred on five continents in almost all countries. Out of the total number of cattle worldwide (1.2 billion heads), black-spotted dairy breed cattle account for more than 10% of the herd (Novak, 2012; Ugnivenko et al., 2022). In Western Europe, over the past 10-15 years, the specific weight of black-spotted cattle from the total cattle population has increased by 6% and is 41%, and in the countries of the European Economic Community - 46%. In the EEC countries, between 1970 and 1981, the milk productivity of cows increased on average from 3400 kg to 4127 kg per head, or by 21%, and by 2000, the number of cows in these countries increased one and a half times (Novak, 2012; Kohut et al., 2015). An increase in milk productivity was expected due to the improvement in the selection, breeding work, and feeding conditions. As a result, a change in the breed structure led to a significant increase in black-spotted cattle (Kudlác et al., 1975; McAllister, 2002; Novak, 2012; Tóthová et al., 2017; Sizova et al., 2022).

In Germany and the former GDR, the specific weight of black-spotted cattle is 80%; in Poland and Great Britain - 90%. On the other hand, the number of black-spotted Holstein cattle in the USA and Canada is more than 80% among other dairy breeds (Novak, 2012). As a result, the milk yield per cow in many farms in these countries is 8.0-10.0 thousand kg of milk.

Thus, in many countries of the world, the specific weight of black-spotted cattle is relatively high and increases every year due to the great potential of its milk productivity.

The growth rate of livestock, Black-Spotted cattle, is the second most numerous in the CIS countries. In the total number of cattle, its specific weight (26.9%) is only 1% inferior to the Simmental breed (Novak, 2012).

In Ukraine, Black-Spotted cattle are bred in all its regions, and in terms of the number of livestock, it ranks second after the red steppe breed (Kohut et al., 2015; Sachuk et al., 2019; Zhelavskyi et al., 2020; Stadnytska et al., 2022; Maksymyuk et al., 2022).

The history of breeding black-spotted cattle in the western region of Ukraine dates back to the middle of the 19th century. In the 1950s and 1960s, a small number of black and spotted cattle were brought from Germany to the territory of the Rivne region for the first time (Novak, 2012). However, this did not significantly affect the formation of black-spotted cattle massif. A little later, many animals were imported from Holland and Estonia. In the 970s, the Black-Spotted cattle of the Rivne region largely corresponded to the type of Dutch cattle (Novak, 2012). These cattle were distinguished by high milk productivity and good slaughter performance. Black-Spotted cattle bred in the 50s and 70s of the 20th century are of the combined type of productivity - milk and meat direction.

To increase the milk productivity of the "local" Black-Spotted cattle of the western region of Ukraine, from 1974, they widely began to use the Holstein breed bulls. But, unfortunately, there was no genetic "explosion" from the use of Holstein breed sires. And the intermediate genotypes were at the same level as the mother breed animals in terms of milk productivity, and in low-yielding herds (2000-2500 kg), they were even slightly lower. These negative signs could have been avoided if the selection work had been carried out as a scientific experiment in several farms of the basic level or at a control and testing station with different levels of animal feed. Then, and only after correction, determination of the most optimal variants, and clarification of target standards could such a scheme be recommended for production implementation under compliance with specific selection techniques that reduce contradictions between the genotype and the environment (Novak, 2012; Bomko et al., 2018).

In Ukraine, complex conglomerates of animals with different productivity, body structure, and genotype have been created, which have practically lost the breed's attributes in the classical sense (Novak, 2012; Bomko et al., 2018). Therefore, a national program is needed to regulate the processes of creating breeds and their structures and genetic transformation of complex dairy cattle populations, including those tested as new breeding achievements, into competitive and consolidated breeds. M.S. Pelekhaty (Pelekhatyi & Kucher, 2013) understands by consolidation the high hereditary conditioning of parents' transmission of their qualities to their offspring.

In the 90s of the last century, there were more than 190,000 cattle in the Lviv region, including 100,000 cows. The specific gravity of the black and mottled breed was 91.5% (Pelekhatyi & Kochuk-Yashchenko, 2014). At that time, the specific weight of Black-Spotted cattle was approximately the same in other regions of western Ukraine. By 1970, work was completed in the Western region by structuring its breeding base of black and spotted cattle (Novak, 2012). In the genealogical structure of black and spotted cattle in the Western region, a sufficient number of highly productive animals were rationally used in the breeding process in the breed. Such structural units primarily include the lines of Atleta 4098, Varkumer 4086, Futo-Zenita 3, Edison 801, Klimata 2222, Annas Ademi 30587, Nero 173-4003, as well as the Anker 1608 family group. Further, interbreed breeding improved these lines using highly productive material of many related breeds, primarily Holstein (Novak, 2012).

Currently, Holstein animals from the lines Vis Bek Ideala 1013415, Vis Ideala 0933122, Reflection Sovering 198998, Sealing Tryjun Rokita 252803, Chifa 1427381/502027, and Monteynak are widely used in the massif of Black-Spotted cattle in the western region of Ukraine (Novak, 2012).

The modern massif of the Black-Spotted breed of the Western Ukrainian population in terms of body structure and productivity indicators, its high milk productivity, and good meat qualities should be noted (Novak, 2012; Roman et al., 2020; Slivinska et al., 2020). It is precious that Black-Spotted cattle have a high genetic potential for milk production, as evidenced by the performance indicators of record-breaking cows (Kohut et al., 2015; Mazur et al., 2020). Thus, 10513 kg of milk with an average fat content of 3.75% was milked from Shuta 1375 cow in 5 lactations; 10040 kg of milk with a fat content of 4.14% was milked from the cow Vydra 1103 in 4 lactations. These animals carried the blood of Dutch and Estonian breeding. In addition, 12,681 kg of milk with a fat content of 3.89% was milked from Holstein cow Kiyanka 3386 in 4 lactations. Black-Spotted cattle from the western region of Ukraine, bred in the late 1950s and early 1970s with Dutch and Estonian genotypes, are characterized by excellent slaughter performance. These are animals of the combined milk-meat productivity type (Fedak et al., 2002; Loboda, 2012; Shevchenko & Hmelnichiy, 2014; Griffiths et al., 2018; Foris et al., 2019; Goncu et al., 2019; Gieseke et al., 2022).

The improvement of the Black-Spotted cattle of the Western region in the middle of the 20th century was due to the use of the Dutch and Estonian breeds. And from 1974 to the present - the Holstein breed. When breeding black and spotted cattle in Ukraine as a whole, and in the western region in particular, it is necessary to create a national and regional program of breeding and selection of various breeds. Regional programs should be an integral part of the national program. It is known that Holsteinization over the last 20 years did not increase the productivity of the black and spotted breed (Duff & McMurphy, 2007; Novak, 2012; da Silva et al., 2016; He et al., 2018; VanRaden et al., 2020). Therefore, it is necessary to adjust and differentiate the selection process when breeding this livestock, that is, to use those bloodlines and genotypes adapted to our conditions. These animals should have high milk and meat productivity (Novak, 2012; Borshch et al., 2020; Slivinska et al., 2021; Bashchenko et al., 2021; Mylostyvyi et al., 2021).

The work aims to investigate body weight growth, linear measurements, biochemical blood parameters, pulmonary gas exchange of heifers and firstborns, and milk productivity in cows of the Ukrainian Black-Spotted dairy cow of the western inbred type of different constitution types. The type of constitution was evaluated according to the physiological and selection index we developed.

MATERIALS AND METHODS

Groups of animals were formed at the age of 6 months based on the physiological and selection index developed by us. Postnatal development was studied in heifers with a high physiological selection index (experimental group) and a low physiological selection index (control group). In the process of research, animals of different age groups and different physiological conditions (heifers, cows) were studied: growth of body weight, linear development (we took the primary measurements of the sexes of the body - the height at the withers and sacrum, depth and width of the chest, oblique length of the body, chest circumference behind the shoulder blades, width in the hip joints, wrist circumference, vertical and horizontal semi-girth of the rear) according to methods generally accepted in zootechnical practice Siratskyi et al. (2001) Based on weighing, absolute (kg), average daily gains (g) and live weight growth factors (%), and based on measurements (cm) - body structure indices (%) according to methods generally accepted in zootechnical practice.

In all experimental animals at the age of 6-8 months, depending on the breed and type of constitution, the activity of AST and ALT in blood serum was determined according to the Reitman-Frenkel method as modified by Pashkina. The activity of AST and ALT was determined in the blood serum of heifers at 3, 6, 9, 12, 15, and 18 months, heifers at 8-9 months of gestation, and firstborns - at 2-3 months of lactation. In these experiments, a complex physiological indicator was studied in young animals in postnatal ontogeny.

To study the general level of metabolic processes in the body by periods of growth and development (3, 6, 9, 12, 15, and 18 months, 8-9 months of gestation, and firstborns at 2-3 months of lactation) in the blood of animals in each group, the following were determined:

- hemoglobin content (g/l) and number of erythrocytes (1012l) - on erythrohemometer model 065;

- the content of total, reduced, and oxidized glutathione (mg/l) - according to the Woodward-Free method;
- catalase activity (mg H2O2) - according to the method of Bach and Zubkova;

The following were determined in blood serum:
- total protein content (g/l) - refractometrically and according to the Reiss table;
- the content of sulfhydryl groups (mg/l) - according to the method of Ryzhkova and others

Pulmonary gas exchange was studied in heifers at the age of 6, 9, 12, 15, and 18 months, in heifers at 8-9 months of gestation and 2-3 months of lactating cows - by mask method during three adjacent days before morning and evening feeding; the analysis of exhaled air was carried out on a GHP-100 gas analyzer. Calculations of pulmonary gas exchange were carried out according to the method of Kudryavtsev.

The control slaughter of bulls was carried out at the Rava-Ruska slaughterhouse of the Zhovkva district of the Lviv region.

In three animals from each group, the chemical composition of the average meat sample, the longest muscle of the back, and the morphological and varietal composition of the carcasses were determined according to generally accepted methods.

The study of milk productivity of cows for a separate lactation was carried out employing control of milking every decade. During each month of lactation, the following were determined in milk:
- fat content - acid method according to Gerber;
- protein content - refractometrically;
- dry substance - by drying in a drying cabinet;
- subclinical forms of mastitis of every fourth udder of cows - Whiteside's test (at the beginning, middle, and end of lactation).

Experimental studies were conducted following the Law of Ukraine “On the Protection of Animals from Cruelty Treatment” dated 03/28/2006 and the rules of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes dated 11/13/1987.

The analysis of research results was carried out using the Statistica 6.0 software package. The probability of differences was assessed by Student's t-test.

RESULTS AND DISCUSSIONS

Growth of live weight and linear development of experimental animals. Live weight is one of the integral indicators that characterize an animal's body both externally and internally. In these studies, the task was to study the growth of live weight and the linear development of heifers, heifers, and cows of different types of the constitution of the Western Ukrainian population of the Black-Spotted breed from birth to the end of the third lactation.

Table 1 shows that according to the live weight of newborns at 3, 6, 12, 18, and 21 months, heifers of the experimental group exceeded the control analogs by 1.3, respectively; 0.3; 7.5; 8.5; 5.7, and 2.8%.

The live weight of heifers in the control group was at the level of the breed standard at 18 months, and the analogs of the experimental group in this age period exceeded the analogs standard by 5.79%.

Higher indicators of live weight caused higher average daily increases in the live weight of animals of the experimental group compared to the control analogs (Table 2).

From birth to 3 months of age, the average daily gains in live weight were at the level of 840 and 839 grams, respectively, in animals of the control and experimental groups.

In the age periods of 4-6, 7-12, 0-18, and 0-21 months, heifers of the experimental group outnumbered the control analogs by 19.19, respectively; 9.97; 6.08; 2.99%, and in the age periods of 13-18, 19-21 months, heifers of the control group exceeded the experimental counterparts by 3.87 and 20.28%, respectively, in terms of average daily live weight gains. There was a different intensity of live weight gain in animals of the control and experimental groups at different age periods. This indicates the rhythmic growth of the live weight of heifers in postnatal ontogeny. There is such a pattern: animals that lag in growth in specific age periods try to catch up with their development when feeding is improved, which happens in animals of the control group in the age periods of 13-18 and 19-21 months. From birth to 21 months, heifers of the research group had a live weight gain of 654, and their control counterparts - 635 grams, respectively.

Table 1. Dynamics of the live weight of Ukrainian Black-Spotted heifers dairy breed, kg (M±m)

Age, months	Groups		± before the control
	control (n=10)	experimental (n=10)	
Newborns	28.9 ± 0.67	29.3 ± 0.45	+ 0.4
3	104.5 ± 3.46	104.8 ± 2.93	+ 0.3
6	169.2 ± 4.48	181.9 ± 3.68****	+ 12.7
12	288.4 ± 5.92	313.0 ± 4.09****	+ 24.6
18	375.3 ± 9.23	396.7 ± 7.82**	+ 21.4
21	429.2 ± 8.85	441.5 ± 9.71	+ 12.3

Table 2. Average daily live weight gains of Ukrainian heifers Black-Spotted dairy breed, g (M±m)

Age, months	Groups		± before the control
	control (n=10)	experimental (n=10)	
0–3	840 ± 10.0	839 ± 10.0	-1
4–6	719 ± 11.0	857 ± 11.0	+ 138
7–12	662 ± 12.0	728 ± 10.0	+ 66
13–18	483 ± 14.0	465 ± 11.0	- 18
0–18	641 ± 15.0	680 ± 11.0	+ 39
19–21	599 ± 11.0	498 ± 10.0	- 101
0–21	635 ± 10.0	654 ± 11.0	+ 19

Live weight does not fully characterize the growth and development of animals. This deficiency is complemented by the measurements, which are used to assess the linear growth of individuals. Therefore, in zootechnical practice, basic measurements are used, by which, in general, the volumetric development of animals can be studied in detail (Table 3).

According to the height at the withers of heifers at 3, 6, 12, and 18 months, heifers at 8-9 months of gestation, and cows at 2-3 months of lactation, the experimental group exceeded the control females by 0.43, respectively; 0.40; 2.08; 1.50; 1.60; 1.93%. In the age mentioned above, a similar pattern was noted for such an indicator as the height at the sacrum. In terms of breast depth, heifers at 3, 6, and 12 months, heifers at 8-9 months of gestation, and cows of 2-3 months of lactation of the experimental group exceeded the control females by 0.56, respectively 0.67, 0.72, 0.32, 1.30%, and the animals of the experimental group exceeded the control analogs by 1.49-1.78% in the oblique length of the trunk in all studied age periods.

Experimental heifers at the age of 3, 6, 12, 18 months, at 8-9 months of lactation, and cows at 2-3 months of gestation exceeded the control ones by 2.88, respectively; 0.95; 3.77; 0.95; 0.65; 0.46%. A similar regularity is noted for the width index in the hip joints. In the above research periods, the width of the hips of the animals of the experimental group exceeded the control counterparts by 2.76, respectively 2.37, 2.76, 0.86, 2.89%.

Heifers of the experimental group at 3, 6, 12, 18 months, at 8-9 months of gestation and cows at 2-3 months of lactation exceeded the control females by 0.94, respectively 1.10, 1.66, 0.35, 0.55, 0.44%, and by wrist girth - by 1.44, respectively 1.37, 1.16, 3.28, 2.20, 2.19%.

Thus, according to the main diameters of body parts, which characterize the exterior in postnatal ontogenesis, animals of the high-enzyme type (experimental group) prevailed over their analogs of the low-enzyme type (control group).

According to indices of the body structure in postnatal ontogeny, the development of some sexes relative to others is evaluated. In our studies, no significant superiority of the animals of the experimental group over the control analogs was noted for these indicators (Table 4).

Table 3. Measurements of the body of Ukrainian heifers and cows Black-Spotted dairy breed, cm (M±m)

Measurements	Groups		± before the control
	control (n=10)	experimental (n=10)	
3 months			
Height at the withers	93.7±0.40	94.1±0.50	+ 0.4
Height at the lumbar	96.8±0.30	97.5±0.20*	+ 0.7
Chest depth	35.8±0.20	36.0±0.30	+ 0.6
Oblique length of the body	95.6±0.50	97.3±0.40**	+ 1.7
The width of the chest behind the shoulder blades	24.3±0.30	25.0±0.20*	+ 2.9
Width in hip joints	25.5±0.40	26.5±0.40	+ 1.0
Width at the hips	29.0±0.30	29.8±0.50	+ 0.8
Chest girth behind the shoulder blades	106.2±0.10	107.2±0.15*	+ 1.0
Wrist girth	13.9±0.10	14.1±0.12	+ 0.2
6 months			
Height at the withers	99.9±0.13	100.3±0.17*	+ 0.4
Height at the lumbar	104.2±0.19	104.7±0.18**	+ 0.5
Chest depth	45.0±0.20	45.3±0.15	+ 0.3
Oblique length of the body	103.9±0.17	104.8±0.18**	+ 0.9
The width of the chest behind the shoulder blades	31.5±0.20	31.8±0.25	+ 0.3
Width in hip joints	31.6±0.21	31.8±0.24	+ 0.2
Width at the hips	33.7±0.30	34.5±0.31	+ 0.8
Chest girth behind the shoulder blades	127.2±0.14	128.6±0.17***	+ 1.4
Wrist girth	14.6±0.11	14.8±0.12	+ 0.2
12 months			
Height at the withers	110.6±0.70	112.9±0.50**	+ 2.3
Height at the lumbar	118.7±0.10	118.7±0.12	0
Chest depth	55.1±0.11	55.5±0.13	+ 0.4
Oblique length of the body	121.9±0.18	123.5±0.20***	+ 1.6
The width of the chest behind the shoulder blades	37.1±0.30	38.5±0.30**	+ 1.4
Width in hip joints	39.3±0.15	41.1±0.19****	+ 1.8
Width at the hips	39.8±0.13	40.9±0.18***	+ 1.1
Chest girth behind the shoulder blades	156.7±0.25	159.3±0.30***	+ 2.6
Wrist girth	17.2±0.40	17.4±0.20	+ 0.2
18 months			
Height at the withers	119.0±0.30	120.8±0.35***	+ 1.8
Height at the lumbar	125.5±0.20	127.8±0.30***	+ 2.3
Chest depth	61.6±0.40	61.6±0.20	0
Oblique length of the body	133.3±0.40	135.8±0.41***	+ 2.5
The width of the chest behind the shoulder blades	42.0±0.20	42.4±0.15	+ 0.4
Width in hip joints	45.3±0.17	45.6±0.18	+ 0.3
Width at the hips	44.0±0.44	44.0±0.50	0
Chest girth behind the shoulder blades	172.5±0.30	173.1±0.20	+ 0.6
Wrist girth	18.3±0.17	18.9±0.18**	+ 0.6
Cows for 8-9 months. corporeality			
Height at the withers	124.9±0.30	126.9±0.20***	+ 2.0
Height at the lumbar	130.7±0.15	133.3±0.20***	+ 2.6
Chest depth	63.0±0.11	63.2±0.17	+ 0.2
Oblique length of the body	137.6±0.19	138.5±0.17**	+ 0.9
The width of the chest behind the shoulder blades	44.6±0.40	45.3±0.30	+ 0.7
Width in hip joints	49.5±0.10	49.9±0.11**	+ 0.4
Width at the hips	46.5±0.15	46.9±0.14*	+ 0.4
Chest girth behind the shoulder blades	181.3±0.13	182.3±0.14***	+ 1.0
Wrist girth	18.2±0.17	18.6±0.16	+ 0.4
Cows for 2-3 months of lactation			
Height at the withers	124.4±0.30	126.8±0.15***	+ 2.4
Height at the lumbar	132.0±0.17	134.4±0.18***	+ 2.4
Chest depth	61.4±0.20	62.2±0.19**	+ 0.8

Oblique length of the body	141.2±0.30	143.3±0.25***	+ 2.1
The width of the chest behind the shoulder blades	43.5±0.1	43.7±0.18	+ 0.2
Width in hip joints	47.9±0.20	49.6±0.21***	+ 1.7
Width at the hips	45.0±0.17	46.3±0.13****	+ 1.3
Chest girth behind the shoulder blades	182.7±0.18	183.5±0.14**	+ 0.8
Wrist girth	18.3±0.11	18.7±0.12**	+0.4

Table 4. Indexes of body composition of Ukrainian heifers and cows black and spotted dairy breed, %

Indexes	Groups		± before the control
	control (n=10)	experimental (n=10)	
3 months			
Long-legged	61.8	61.7	- 0.1
Stretching	102.0	103.4	+ 1.4
Thoracic pelvis	83.8	83.9	+ 0.1
Pectoral	67.9	69.4	+ 1.5
Beating	111.0	110.2	- 0.8
Overgrowth	104.0	102.8	- 1.2
Ossification	14.8	15.0	+ 0.2
6 months			
Long-legged	54.9	54.8	- 0.1
Stretching	104.0	104.5	+ 0.5
Thoracic pelvis	93.4	92.2	- 1.2
Pectoral	70..0	70.2	+ 0.2
Beating	122.4	122.7	+ 0.3
Overgrowth	104.3	104.4	+ 0.1
Ossification	14.6	14.7	+ 0.1
12 months			
Long-legged	49.8	51.2	+ 1.4
Stretching	110.2	109.4	- 0.8
Thoracic pelvis	93.2	94.1	+ 0.9
Pectoral	66.8	69.9	+ 3.1
Beating	128.5	129.0	+ 0.5
Overgrowth	107.3	105.1	- 2.2
Ossification	15.5	15.4	- 0.1
18 months			
Long-legged	48.2	49.0	+ 0.8
Stretching	112.0	112.4	+ 0.4
Thoracic pelvis	95.4	96.3	+ 0.9
Pectoral	68.2	68.8	+ 0.6
Beating	129.4	127.4	- 2.0
Overgrowth	105.4	105.8	+ 0.4
Ossification	15.4	15.6	+ 0.2
Cows for 8-9 months. corporeality			
Long-legged	49.4	50.3	+ 0.9
Stretching	110.1	109.1	- 1.0
Thoracic pelvis	97.4	95.1	- 2.3
Pectoral	71.1	70.8	- 0.3
Beating	132.5	130.9	- 1.6
Overgrowth	104.5	105.0	+ 0.5
Ossification	14.6	14.6	0
Cows for 2-3 months of lactation			
Long-legged	50.9	50.6	- 0.3
Stretching	113.0	113.5	+ 0.5
Thoracic pelvis	93.9	95.4	+ 1.5
Pectoral	69.9	71.2	+ 1.3
Beating	128.0	129.4	+ 1.4
Overgrowth	104.0	108.0	+ 4.0
Ossification	14.7	14.7	0

According to the long-leggedness index, in the postnatal ontogeny of experimental animals, the depth of the chest increased more intensively than the height at the withers, as the value of this index decreased from 61.70 to 50.9%.

The overall (volumetric) development of animals in terms of age increased from the distension index, which grew from 102.0 to 113.5%.

From 3 months of age to 8-9 months of gestation, the pelvic index increased in animals of the control and experimental groups. This indicates that the front and back of the body developed proportionally. However, compared to 8-9 months of gestation, the pelvis-thoracic index slightly decreased during the lactation of firstborns.

Chest index characterizes the volumetric development of the animal's chest. In the postnatal ontogeny of experimental animals, the value of this index has a wave-like character of change. In some age periods of growth and development, it is higher, and in others - lower. This indicates the rhythmic development of the chest in animals of the control and experimental groups.

The weight loss index increased from the 3-month age of the heifers to the 8-9 months, and it slightly decreased during the 2 cows' 3 months of lactation. This indicates that in the postnatal ontogeny, the volume of the chest and the length of the body of the experimental animals developed proportionally.

The height measurements of the experimental animals in the postnatal ontogeny had a linear character of increase. The overgrowth index evidences this. Thus, it was 104.0-102.8% in 3-month-old animals and 104.0-108.0% in cows in 2-3 months of lactation.

Due to the ossification index, no significant fluctuations were found in the postnatal ontogeny of the experimental animals.

Thus, the indices of body structure indicate that the animals developed harmoniously and proportionally in the postnatal ontogeny.

Morphological and biochemical indicators of the blood of experimental animals. Blood is a constantly circulating liquid connecting various animal body structures (Grymak et al., 2020; Lesyk et al., 2022; Martyshuk et al., 2022). The main physiological functions of blood include alimentary, excretory, respiratory, protective, mechanical, and others. These functions are performed by a complex of enzymes and proteins, as well as morphological indicators of blood (Kulyaba et al., 2019; Martyshuk et al., 2020).

The content of erythrocytes and hemoglobin in the blood. The erythrocytes perform vital functions in the body, including respiratory (Slivinska et al., 2019). The Table 5 gives an indicator of age-related changes in the number of erythrocytes in the blood of experimental animals.

Table 5. The number of erythrocytes in the blood of heifers and cows of the Ukrainian Black-Spotted dairy breed, 10^{12}/l (M±m)

Age, months	Groups		± before the control
	control (n=10)	experimental (n=10)	
Heifers: 3	6.12 ± 0.22	6.38 ± 0.05	+ 0.26
6	6.86 ± 0.25	7.54 ± 0.38	+ 0.68
12	4.58 ± 0.17	5.03 ± 0.04**	+ 0.45
18	5,16 ± 0.09	5.20 ± 0.11	+ 0.04
Cows for 8-9 months. corporeality	6.15 ± 0.05	6.83 ± 0.10***	+ 0.68
Cows for 2-3 months. lactation	6.31 ± 0.12	6.67 ± 0.08**	+ 0.36
On the average	5.81 ± 0.15	6.27 ± 0.13**	+ 0.46

The erythrocytes number in the blood of heifers at 3, 6, 12, 18 months, at 8-9 months of gestation, and cows at 2-3 months of lactation of the experimental group was higher than that of the control group by 4.25, respectively; 9.91; 9.82; 0.77; 11.06; 5.70; 7.92%. On average, during the entire growing period, the animals of the experimental group outnumbered the control analogs by 7.92%.

Another essential component of blood, part of erythrocytes, is hemoglobin, which carries oxygen from the lungs to the tissues and transports carbon dioxide back to the lungs (Table 6).

According to the content of hemoglobin in the blood, experimental heifers at the age of 3, 6, 12, 18 months, at 8-9 months of gestation, and cows at 2-3 months of lactation, exceeded control females by 6.67, respectively; 8.20; 10.83; 3.62; 6.35; 10.90%. On average, during the entire growing period, the animals of the experimental group outnumbered the control analogs by 7.64%.

Table 6. Hemoglobin content in the blood of heifers and cows of the Ukrainian Black-Spotted dairy breed, g/l (M±m)

Age, months	Groups		± before the control
	control (n=10)	experimental (n=10)	
Heifers: 3	105.0 ± 2.0	112.0 ± 0.7***	+ 7.0
6	122.0 ± 4.1	132.0 ± 2.1**	+ 10.0
12	109.0 ± 3.8	120.8 ± 3.0**	+ 11.8
18	115.9 ± 2.1	120.1 ± 0.3*	+ 4.2
Cows for 8-9 months. corporeality	110.2 ± 1.9	117.2 ± 1.1***	+ 7.0
Cows for 2-3 months. lactation	105.5 ± 2.4	117.0 ± 0.7***	+ 11.5
On the average	111.3 ± 2.7	119.8 ± 2.6**	+ 8.5

Thus, according to the main morphological parameters of the blood, animals of the high-enzyme type (experimental group) significantly outnumbered the analogs of the low-enzyme type (control group) in postnatal ontogenesis. This shows that the level of metabolic processes in the body was more intense in the animals of the experimental group than in the control counterparts.

The level of protein metabolism in the body of animals. One of the main components of protein metabolism in the body of animals is the content of total protein in blood serum, the activity of peramination enzymes in blood serum, as well as the index for assessing the type of constitution of animals, which is calculated based on the activity of AST and ALT in blood serum and economically useful characteristics of animals (Gutyj et al., 2019; Gutyi et al., 2019).

The content of total protein in the blood serum of heifers at the age of 3, 6, 12, 18 months, at 8-9 months of gestation, and cows at 2-3 months of lactation of the experimental group exceeded the control analogs by 1.81, respectively 6.25; 9.77; 7.47; 15.23; 8.64% (Table 7).

On average, during the entire growing period, the animals of the experimental group outnumbered the control analogs by 8.09%.

Table 7. Content of total protein in blood serum of heifers and cows of the Ukrainian Black-Spotted dairy breed, g/l (M±m)

Age, months	Groups		± before the control
	control (n=10)	experimental (n=10)	
Heifers: 3	66.4 ± 1.7	67.6 ± 2.1	+ 1.2
6	68,8 ± 1.2	73.1 ± 1.7**	+ 4.3
12	68.6 ± 2.5	75.3 ± 1.3**	+ 6.7
18	70.9 ± 0.9	76.2 ± 1.4****	+ 5.3
Cows for 8-9 months. corporeality	66.3 ± 1.9	76.4 ± 1.2****	+ 10.1
Cows for 2-3 months. lactation	74.1 ± 0.8	80.5 ± 1.4****	+ 6.4
On the average	69.2 ± 1.50	74.8 ± 1.52**	+ 5.6

Another important indicator that characterizes protein metabolism in the animal body is the activity of aminotransferases in blood serum. Regarding AST activity in blood serum, on average, over the entire period of rearing, the animals of the experimental group exceeded the control group by 11.78% (Table 8).

We found a similar pattern in the activity of ALT in blood serum. Thus, according to this indicator, the heifers of the experimental group at 3, 6, 12, 18 months, at 8-9 months of gestation and cows at 2-3 months of lactation were superior to the same age control group by 4.72, respectively 22.55, 26.12, 8.13, 8.01, 10.28% (Table 9).

Table 8. AST activity in the blood serum of heifers and cows of the Ukrainian Black-Spotted dairy breed, units/l^3 (M±m)

Age, months	Groups		± before the control
	control (n=10)	experimental (n=10)	
Heifers: 3	34.14 ± 0.58	35.47 ± 0.44*	+ 1.33
6	29.70 ± 0.44	33.03 ± 0,58****	+ 3.33
12	30.36 ± 1.07	39.12 ± 2.24****	+ 8.86
18	37.02 ± 0.38	44.33 ±0.32****	+ 7.31
Cows for 8-9 months. corporeality	44.73 ± 0.41	47.26 ± 0.48****	+2.53
Cows for 2-3 months. lactation	33.47 ± 0.51	34.80 ± 0.23**	+ 1.33
On the average	34.89 ± 0.56	39.00 ± 0.71****	+ 4.11

Table 9. ALT activity in blood serum of heifers and cows of the Ukrainian Black-Spotted dairy breed, units/l^{-3} (M±m)

Age, months	Groups		± before the control
	control (n=10)	experimental (n=10)	
Heifers: 3	18.84 ± 0.81	19.73 ± 0.80	+ 0.89
6	15.74 ± 0.58	19.29 ± 0.38****	+ 3.55
12	16.96 ± 0.83	21.39 ± 0.26****	+ 4.43
18	21.90 ± 0.29	23.68 ± 0.40***	+ 1.78
Cows for 8-9 months. corporeality	28.48 ± 0.73	30.76 ± 0.58**	+ 2.28
Cows for 2-3 months. lactation	25.38 ± 0.29	27.99 ± 0.73***	+ 2.61
On the average	21.22 ± 0.59	23.81 ± 0.52***	+ 2.59

Due to this indicator, the average difference in favor of experimental animals was 12.20% during the growing period.

A comprehensive indicator of the body's protein metabolism in combination with economically advantageous traits is the index of evaluation of the type of constitution of heifers of the Western Ukrainian population of the Black-Spotted breed, which we determined at the age of 3, 6, 12, and 18 months (Table 10).

The animals of the experimental group at 3, 6, 12, 18 months of age exceeded the control analogues by 4.52, respectively, due to the constitution type assessment index, 23.81, 39.06, 22.00%. On average, over the entire growing period, the heifers of the experimental group outperformed the control heifers by 25.05% according to the constitution type evaluation index.

Therefore, according to the leading indicators of protein metabolism, the animals of the research group significantly outnumbered the control analogues, which indicates a more intense level of metabolic processes in their body.

Table 10. Physiological and selection index of evaluation of the type of constitution of heifers of the Ukrainian Black-Spotted dairy breed

Age, months	Groups		± before the control
	control (n=10)	experimental (n=10)	
3	20.81	21.75	+ 0.94
6	28.90	35.78	+ 6.88
12	51.20	71.20	+ 20.00
18	83.13	101.42	+ 18.29
On the average	46.01	57.54	+ 11.53

Sulfhydryl groups are part of sulphur-containing amino acids and also characterize protein metabolism in the animal body. Therefore, residual and protein SH-groups are generally determined in physiological and biochemical practice.

In experimental animals, the change in the content of total and protein groups had a wave-like character, which is obviously due to the rhythmicity of animal growth.

Analysis of the indicators is given in the Table 11, shows that the content of total sulfhydryl groups in the blood of heifers of the experimental group at 3, 6, 12, 18 months, at 8-9 months of gestation, and cows at 2-3 months of lactation exceeded the control analogues by

10.85, respectively 27.50, 23.44, 18.01, 22.08, 8.86%.

It was established that the content of residual SH-groups decreased with age in the body of both groups of animals.

Due to the content of residual SH-groups in the animals of the experimental group in the postnatal ontogeny, they prevailed in the control group by 24.34% (Table 12).

Table 11. The content of total sulfhydryl groups in the blood of heifers and cows of the Ukrainian Black-Spotted dairy breed, g^{-3}/l cysteine (M±m)

Age, months	Groups		± before the control
	control (n=10)	experimental (n=10)	
Heifers: 3	605.7 ± 8.7	671.4 ± 50.5	+ 65.7
6	459.3 ± 5.0	585.6 ± 13.13***	+ 126.3
12	689.0 ± 28.7	850.5 ± 54.6**	+ 161.5
18	504.7 ± 17.9	595.6 ± 22.0***	+ 90.9
Cows for 8-9 months. corporeality	651.3 ± 35.8	795.1 ± 21.8****	+ 143.8
Cows for 2-3 months. lactation	578.0 ± 15.3	629.2 ± 12.1**	+ 51.2
On the average	581.3 ± 18.6	687.9 ± 29.0***	+ 106.6

Table 12. The content of residual SH-groups in the blood of heifers and cows of the Ukrainian Black-Spotted dairy breed, g $^{-3}$/l cysteine (M±m)

Age, months	Groups		± before the control
	control (n=10)	experimental (n=10)	
Heifers: 3	157.4 ± 1.4	173.6 ± 4.0	+ 16.2
6	135.3 ± 5.3	178.7 ± 5.3****	+ 43.4
12	131.2 ± 7.2	159.5 ± 5.3***	+ 28.3
18	123.1 ± 8.1	149.3 ± 4.0***	+ 26.2
Cows for 8-9 months. corporeality	118.2 ± 7.6	171.7 ± 2.0****	+ 53.5
Cows for 2-3 months. lactation	126.2 ± 2.7	151.4 ± 3.5****	+ 25.2
On the average	131.9 ± 5.4	164.0 ± 4.0****	+ 32.1

Heifers of the experimental group at 3, 6, 12, 18 months, at 8-9 months of gestation and cows at 2-3 months of lactation exceeded the control analogues by 4.98, respectively, in terms of the content of protein sulfhydryl groups in the blood; 18,11; 13.22; 8.78; 0.86; 2.69% (Table 13).

Therefore, according to the content of total, residual, and protein SH-groups, animals of the high-enzyme type (experimental group) in the postnatal ontogeny significantly outnumbered the analogues of the low-enzyme type (control group). This indicates that according to the given indicators of protein metabolism in the body of the animals of the experimental group, metabolic processes were more intense than in the control analogs.

Table 13. The content of protein SH-groups in the blood of heifers and cows of the Ukrainian Black-Spotted dairy breed, g^{-3}/l of cysteine (M±m)

Age, months	Groups		± before the control
	control (n=10)	experimental (n=10)	
Heifers: 3	6855.5 ± 158.9	7197.1 ± 610.8****	+ 341.6
6	4712.3 ± 62.2	5565.7 ± 246.4***	+ 853.4
12	8105.3 ± 576.2	9176.5 ± 764.4	+ 1071.2
18	5378.9 ± 187.7	5851.2 ± 136.9*	+ 472.3
Cows for 8-9 months. corporeality	8090.0 ± 830.0	8160.0 ± 210.0	+ 70
Cows for 2-3 months. lactation	5940.0 ± 210.0	6100.0 ± 290.0	+ 160
On the average	6513.7 ± 337.5	7008.4 ± 376.4	+ 494.7

Redox processes in the blood of experimental animals. The respiratory pigment hemoglobin, catalase, and glutathione, are contained in erythrocytes and play an important act in redox processes in the cells and tissues of the body. In addition, glutathione in the blood performs a redox function and generally participates in the body's respiratory process. Therefore, the

content of total, reduced, and oxidized glutathione in the blood indicates the intensity of redox processes in the body of animals.
Experimental heifers at 3, 6, 12, 18 months, at 8-9 months of gestation, and cows at 2-3 months of lactation exceeded the control analogues by the content of total glutathione, respectively, by 8.38, 20.25, 7.44, 5.48, 18,11, 1.80% (Table 14).
A similar pattern was observed in the reduced glutathione content in the animals' blood. The individuals of the experimental group over the entire period of cultivation prevailed over the control females of the same age by an average of 9.56% (Table 15).
According to the content of oxidized glutathione in the blood of heifers at 3, 6, 12, 18 months, at 8-9 months of gestation, and cows at 2-3 months of lactation, the experimental group exceeded the counterparts of the control group by 7.92, respectively 15.13, 7.77, 6.52, 46.88, 4.01% (Table 16).
On average, during the entire growing period, the animals of the experimental group exceeded the control animals by 10.98% in terms of the oxidized glutathione content in the blood.
It was established that experimental heifers at 3, 6, 12, 18 months, at 8-9 months of gestation and cows at 2-3 months of lactation exceeded the control heifers by 0.89, respectively, in blood catalase activity, 15.57, 4.53, 3.93, 22.61, 3.68% (Table 17).

Table 14. Content of total glutathione in the blood of heifers and cows of the Ukrainian Black-Spotted dairy breed, g^{-3}/l

Age, months	Groups (M+m)		± before the control
	control (n=10)	experimental (n=10)	
Heifers: 3	366.3 ± 17.5	397.0 ± 4.9*	+ 30.7
6	313.1 ± 7.0	376.5 ± 5.4****	+ 63.4
12	375.2 ± 6.9	403.1 ± 2.6***	+ 27.9
18	428.7 ± 4.5	452.2 ± 5.3***	+ 23.5
Cows for 8-9 months. corporeality	396.0 ± 7.7	467.7 ± 3.7****	+ 71.7
Cows for 2-3 months. lactation	455.3 ± 2.7	463.5 ± 5.3	+ 8.2
On the average	389.1 ± 7.72	426.7 ± 4.53****	+ 37.6

Table 15. The content of reduced glutathione in the blood of heifers and cows of the Ukrainian Black-Spotted dairy breed, g^{-3}/l

Age, months	Groups (M+m)		± before the control
	control (n=10)	experimental (n=10)	
Heifers: 3	339.7 ± 22.8	368.3 ± 7.1	+ 28.6
6	279.4 ± 12.4	337.7 ± 3.5****	+ 58.3
12	334.8 ± 9.4	360.1 ± 3.7**	+ 25.3
18	385.2 ± 3.8	412.4 ± 3.7****	+ 27.2
Cows for 8-9 months. corporeality	359.2 ± 9.2	413.4 ± 5.4****	+ 54.2
Cows for 2-3 months. lactation	379.6 ± 1.0	384.7 ± 2.7*	+ 5.1
On the average	346.3 ± 9.8	379.4 ± 7.3**	+ 33.1

Table 16. The content of oxidized glutathione in the blood of heifers and cows of the Ukrainian Black-Spotted dairy breed, g^{-3}/l

Age, months	Groups (M±m)		± before the control
	control (n=10)	experimental (n=10)	
Heifers: 3	26.5 ± 5.4	2.6 ± 4.1	+ 2.1
6	33.7 ± 5.3	38.8 ± 4.1	+ 5.1
12	39.9 ± 2.6	43.0 ± 2.8	+ 3.1
18	39.9 ± 3.1	42.5 ± 3.4	+ 2.6
Cows for 8-9 months. corporeality	36.9 ± 1.8	54.2 ± 5.1***	+ 17.3
Cows for 2-3 months. lactation	75.7 ± 1.5	78.8 ± 4.1	+ 3.1
On the average	42.53 ± 3.3	47.2 ± 3.9	+ 4.7

On average, over the entire experiment period, the experimental group's animals exceeded the control analogues by 7.60% in terms of catalase activity in the blood.

Therefore, according to the leading indicators characterizing oxidation-reduction processes in the body, animals of the high-enzyme type (experimental group) significantly outnumbered the analogues of the low-enzyme type (control group). Furthermore, the level of metabolic processes in the experimental animals was much more intense than that of the counterparts of the control group.

Table 17. Catalase activity in the blood of heifers and cows of the Ukrainian Black-Spotted dairy breed, g^{-3} H_2O_2 /l

Age, months	Groups (M±m)		± before the control
	control (n=10)	experimental (n=10)	
Heifers: 3	6.77 ± 0.27	6.83 ± 0.22	+ 0.06
6	6.23 ± 0.34	7.20 ± 0.05	+ 0.97
12	7.07 ± 0.06	7.39 ± 0.13	+ 0.32
18	6.62 ± 0.05	6.88 ± 0.30	+ 0.26
Cows for 8-9 months. corporeality	5.13 ± 0.12	6.29 ± 0.19****	+ 1.16
Cows for 2-3 months. lactation	9.24 ± 0.20	9.58 ± 0.05	+ 0.34
On the average	6.84 ± 0.17	7.36 ± 0.16**	+ 0.52

Pulmonary gas exchange in animals. The growth and development of animals are based on complex processes of assimilation and oxidation of nutrients in the body. The intensity of growth and development in different age periods is not the same, and the level of metabolic processes in the body of animals is also different.

In the process of growth and development, the metabolic processes in an animal's body are influenced by the external environment and hereditary factors.

The intensity of complex metabolic processes in the animal body can be tested by the level of gas-energy exchange, which is an integral indicator of the body's complex biochemical and physiological processes.

Inspiratory capacity and pulmonary ventilation. In the growth and development of young animals, pulmonary breathing is characterized by the frequency and depth of breathing and pulmonary ventilation. With age, the indicators of pulmonary respiration in animals have a corresponding pattern.

The characteristics of the breathing rate of experimental animals are given in Table 18.

Table 18. Frequency of air inhalation per minute by heifers and cows of the Ukrainian Black-Spotted dairy breed

Age, months	Groups (M±m)		± before the control
	control (n=10)	experimental (n=10)	
Heifers: 3	28.8 ± 1.94	32.4 ± 0.91*	+ 3.6
6	26.9 ± 0.03	25.5 ± 0.79	- 1.4
12	21.8 ± 0.47	19.8 ± 0.87*	- 2.0
18	20.7 ± 0.50	17.8 ± 1.44*	- 2.9
Cows for 8-9 months. corporeality	18.0 ± 0.14	15.9 ± 0.48****	- 2.1
Cows for 2-3 months. lactation	11.0 ± 0.08	9.9 ± 0.43**	- 1.1
On the average	21.2 ± 0.53	20.22 ± 0.82	- 0.98

Table 19. The depth of air inhalation by heifers and cows of the Ukrainian black and spotted dairy breed, Jr

Age, months	Groups (M±m)		± before the control
	control (n=10)	experimental (n=10)	
Heifers: 3	867 ± 71.88	787 ± 5,78	- 80
6	1276 ± 34.12	1575 ± 41.60****	+ 299
12	2114 ± 34.12	2638 ± 185.30**	+ 524
18	3012 ± 77.88	3695 ± 319.57*	+ 683
Cows for 8-9 months. corporeality	3065 ± 61.85	3760 ± 50.59****	+ 695
Cows for 2-3 months. lactation	7457 ± 383.58	9049 ± 484.12**	+ 1592
On the average	2965 ± 110.0	3584 ± 181.2***	+ 619

Heifers of the control group at 6, 12, 18 months, at 8-9 months of gestation and cows at 2-3 months of lactation prevailed over experimental counterparts by 5.49, respectively 10.10, 16.29, 13.21, 11.11%. It should be noted that at the age of 3 months, heifers of the experimental group outnumbered the control counterparts by 12.5%. On average, over the entire experiment period, the animals of the control group exceeded the experimental ones by 4.95% in terms of breathing frequency.

Thus, the animals' breathing frequency in the experimental group's postnatal ontogeny was lower than that of the control counterparts.

If the breathing frequency of the animals in the experimental group was lower than that of the control group, on the contrary, according to the index of the depth of breathing, they significantly exceeded the control analogues (Table 19). Heifers at 6, 12, 18 months, at 8-9 months of gestation, and cows at 2-3 months of lactation, the experimental group outnumbered the control analogues by 23.43, respectively 24.78, 22.68, 22.67, 21.35%. At the age of 3 months, heifers of the control group outnumbered the experimental counterparts by 10.16%.

According to the indicators of pulmonary ventilation in all age periods, the animals of the experimental group significantly exceeded the control peers (Table 20).

Table 20. Pulmonary ventilation of heifers and cows of the Ukrainian Black-Spotted dairy breed, l/min

Age, month	Groups (M±m)		± before the control
	control (n=10)	experimental (n=10)	
Heifers: 3	24.78 ± 0.34	24.85 ± 0.33	+ 0.07
6	34.30 ± 0.87	39.80 ± 0.43****	+ 5.50
12	48.11 ± 0.70	51.57 ± 0.98**	+ 3.46
18	62,20 ± 0.15	64.70 ± 1.30*	+ 2.50
Cows for 8-9 months. corporeality	67.10 ± 1.16	69.10 ± 0.33***	+ 2.00
Cows for 2-3 months. lactation	93.82 ± 3.42	102.80 ± 1.63**	+ 8.98
On the average	55.38 ± 0.97	58.47 ± 0.97**	+ 3.09

Heifers of the experimental group at the age of 3, 6, 12, 18 months, at 8-9 months of gestation, and cows at 2-3 months of lactation exceeded the control counterparts by 0.28, respectively 16.03, 7.19, 4.02, 2.98, 9.57%. On average, during the entire growing period, the animals of the experimental group outnumbered the control animals by 5.58%.

Thus, according to the depth of breathing and pulmonary ventilation, the high-enzyme type (experimental group) animals significantly outnumbered the low-enzyme type (control group) analogs.

Gas-energy exchange in animals. The intensity of oxidation-reduction processes in the animal body depends on the digestion level and assimilation of nutrients supplied with feed. The level of oxygen consumption in growth and development essentially characterizes the intensity of metabolic processes in the animal body. Important indicators of gas exchange are the consumption of oxygen and the release of carbon dioxide by the body in absolute and relative units.

In terms of total oxygen consumption, test heifers at 6, 12, and 18 months, at 8-9 months of gestation, and cows at 2-3 months of lactation exceeded the control analogs by 6.93, respectively 9.22, 2.34, 20.69, 1.48% (Table 21).

Table 21. Absolute oxygen consumption by heifers and cows of the Ukrainian Black-Spotted dairy breed, l/min

Age, month	Groups (M±m)		± before the control
	control (n=10)	experimental (n=10)	
Heifers: 3	0.66 ± 0.018	0.65 ± 0.005	- 0.01
6	1.01 ± 0.007	1.08 ± 0.02***	+ 0.07
12	1.41 ± 0.07	1.54 ± 0.02*	+ 0.13
18	1.71 ± 0.02	1.75 ± 0.01	+ 0.04
Cows for 8-9 months. corporeality	1.45 ± 0.08	1.76 ± 0.11**	+ 0.31
Cows for 2-3 months. lactation	2.70 ± 0.01	2.74 ± 0.11	+ 0.04
On the average	1.49 ± 0.03	1.59 ± 0.05	+ 0.10

At the age of 3 months, the animals of the control group had a slight advantage over experimental peers in terms of total oxygen consumption. A similar pattern was observed in the relative oxygen consumption of the experimental animals (Table 22). According to this indicator, the animals of the experimental group exceeded the control analogs by 6.10% on average during the entire growing period.

Table 22. Relative oxygen consumption by heifers and cows of the Ukrainian Black-Spotted dairy breed, l^{-3}/min/kg

Age, month	Groups (M±m)		± before the control
	control (n=10)	experimental (n=10)	
Heifers: 3	5.61 ± 0.14	6.12 ± 0.27	+ 0.51
6	6.02 ± 0.16	6.32 ± 0.24	+ 0.30
12	5.12 ± 0.08	5.12 ± 0.16	0
18	4.41 ± 0.31	4.83 ± 0.11	+ 0.42
Cows for 8-9 months. corporeality	3.16 ± 0.02	3.67 ± 0.23**	+ 0.51
Cows for 2-3 months. lactation	6.18 ± 0.10	6.28 ± 0.29	+ 0.10
On the average	5.08 ± 0.13	5.39 ± 0.22	+ 0.31

Therefore, in terms of absolute and relative oxygen consumption in the postnatal ontogeny, the animals of the experimental group probably exceeded the control counterparts.

According to the absolute indicators of carbon dioxide excretion, heifers of the experimental group at 6-, 12- and 18 months of age, heifers at 8-9 months of gestation, and cows at 2-3 months of lactation prevailed over peers of the control group by 15.28, respectively 4.59, 7.20, 14.65, 1.79% (Table 23).

Table 23. Absolute release of carbon dioxide by heifers and cows of the Ukrainian Black-Spotted dairy breed, l/min

Age, month	Groups (M±m)		± before the control
	control (n=10)	experimental (n=10)	
Heifers: 3	0.50 ± 0.01	0.50 ± 0.01	0
6	0.72 ± 0.09	0.83 ± 0.02	+ 0.11
12	1.09 ± 0.03	1.14 ± 0.02	+ 0.05
18	1.25 ± 0.02	1.34 ± 0.01***	+ 0.09
Cows for 8-9 months. corporeality	1.16 ± 0.04	1.33 ± 0.04***	+ 0.17
Cows for 2-3 months. lactation	2.24 ± 0.11	2.28 ± 0.02	+ 0.04
On the average	1.16 ± 0.04	1.24 ± 0.02*	+ 0.08

During the entire growing period, according to the above indicator, the animals of the experimental group exceeded the control ones by an average of 6.90%.

The animals of the experimental group also outperformed the control peers in relative carbon dioxide excretion. Thus, heifers at 3, 6, 12, 18 months, at 8-9 months of gestation and cows at 2-3 months of lactation of the experimental group prevailed by this indicator of the analogues of the control group, respectively, by 13.73, 11.37, 0.53, 9.14, 9.88, 7.41% (Table 24).

Table 24. Relative excretion of carbon dioxide by heifers and cows of the Ukrainian Black-Spotted dairy breed, l^{-3}/min/kg

Age, month	Groups (M±m)		± before the control
	control (n=10)	experimental (n=10)	
Heifers: 3	4.15 ± 0.07	4.72 ± 0.15***	+ 0.57
6	4.31 ± 0.22	4.80 ± 0.19*	+ 0.49
12	3.80 ± 0.16	3.82 ± 0.12	+ 0.02
18	3.39 ± 0.12	3.70 ± 0.10*	+ 0.31
Cows for 8-9 months. corporeality	2.53 ± 0.03	2.78 ± 0.14*	+ 0.25
Cows for 2-3 months. lactation	5.13 ± 0.32	5.51 ± 0.27	+ 0.38
On the average	3.89 ± 0.15	4.22 ± 0.17	+ 0.33

The advantage of the experimental group over the control group in terms of the relative release of carbon dioxide was 8.48%.

Therefore, according to the absolute and relative release of carbon dioxide in the postnatal ontogeny, animals of the high-enzyme type (experimental group) probably outnumbered the analogs of the low-enzyme type (control group). This indicates that the oxidation-reduction processes level in the animals of the experimental group was more intense than that of the control counterparts.

According to the respiratory coefficient, no significant difference was found between the animals of the experimental and control groups (Table 25). In heifers at 3-, 6-, 12-, and 18 months of age and heifers at 8-9 months of gestation, the respiratory coefficient was within 0.71-0.80. In this range, experimental animals undergo fat metabolism.

In firstborns at 2-3 months of lactation, this indicator was in the range of 0.86-0.84. This testifies that protein exchange was more intense in the animal's body during this period.

Table 25. The respiratory ratio in heifers and cows of Ukrainian Black-Spotted dairy breed

Age, month	Groups (M±m)		± before the control
	control (n=10)	experimental (n=10)	
Heifers: 3	0.76 ± 0.005	0.77 ± 0.008*	+ 0.01
6	0.71 ± 0.02	0.76 ± 0.006**	+ 0.05
12	0.75 ± 0.05	0.74 ± 0.01	- 0.01
18	0.74 ± 0.007	0.77 ± 0.005	+ 0.03
Cows for 8-9 months. corporeality	0.80 ± 0.15	0.76 ± 0.04	- 0.04
Cows for 2-3 months. lactation	0.86 ± 0.02	0.84 ±0.04	- 0.02
On the average	0.77 ± 0.05	0.78 ± 0.02	+ 0.01

Table 26. Total energy allocated by heifers and cows of the Ukrainian Black-Spotted dairy breed, kJ/h

Age, month	Groups (M±m)		± before the control
	control (n=10)	experimental (n=10)	
Heifers: 3	792.4 ± 13.67	779.1 ± 7.09	- 13.3
6	1187.8 ± 55.51	1295.1 ± 22.15***	+ 107.3
12	1726.2 ± 30.25	1791.4 ± 29.34	+ 65.2
18	2024.2 ± 28.73	2097.3 ± 17.73**	+ 73.1
Cows for 8-9 months. corporeality	1760.2 ± 89.26	2150.8 ± 105.22**	+ 390.6
Cows for 2-3 months. lactation	3199.4 ± 11.75	3331.0 ± 10.73****	+ 131.6
On the average	1781.7 ± 38.19	1907.4 ± 32.04**	+ 125.7

Energy exchange in the body of animals in postnatal ontogenesis is characterized by the total energy released by animals and the relative accumulation of heat production.

According to the unconditional release of energy, experimental heifers at 6, 12, 18 months, 8-9 months of gestation, and cows at 2-3 months of lactation exceeded the control analogues by 9.03, respectively 3.78, 3.61, 22.19, 4.11% (Table 26).

At the age of 3 months, heifers of the control group exceeded the heifers of the experimental group by 8.68% in terms of absolute energy release.

During the entire growing period, the animals of the experimental group exceeded the control analogs by 7.06% in terms of absolute energy release.

The heifers of the research group, according to the relative indicators of heat production at 3-, 6-, and 18 months of age, 8-9 months of gestation, and cows at 2-3 months of lactation prevailed over the control heifers by 9.40, respectively 6.19, 5.86, 14.70, 5.18% (Table 27). At 12 months, according to this indicator, a particular advantage of heifers of the control group was observed. Over the entire growing period, the advantage of the animals of the experimental group over the analogues of the control group in terms of relative indicators of heat production was, on average, 6.07%.

Table 27. Relative indicators of heat production in heifers and cows of the Ukrainian Black-Spotted dairy breed, kJ/h/kg

Age, month	Groups (M±m)		± before the control
	control (n=10)	experimental (n=10)	
Heifers: 3	6.70 ± 0.17	7.33 ± 0.30*	+ 0.69
6	7.11 ± 0.22	7.55 ± 0.29	+ 0.44
12	6.09 ± 0.21	6.18 ± 0.21	+ 0.09
18	5.46 ± 0.15	5.78 ± 0.14	+ 0.32
Cows for 8-9 months. corporeality	3.81 ± 0.01	4.37 ± 0.26*	+ 0.56
Cows for 2-3 months. lactation	7.33 ± 0.32	7.71 ± 0.21	+ 0.38
On the average	6.10 ± 0.15	6.47 ± 0.23	+ 0.37

Thus, according to indicators of energy metabolism in postnatal ontogenesis, animals of the high-enzyme type (experimental group) significantly outnumbered their analogues of the low-enzyme type (control group). This indicates that the energy exchange in the animals of the experimental group was much more intense than in the control counterparts.

Milk productivity of cows. Milk productivity of cows is one of the leading indicators that characterize the growth of live weight, linear development, and redox processes of young animals in the postnatal ontogenesis during the formation of economically valuable traits.

As noted above, the heifers of the control and experimental groups were kept on the same feed rations. At the same time, in the process of growth and development, the heifers of the experimental group were characterized by higher indicators of body weight growth, linear development, and the level of synthetic processes compared to control females of the same age. Therefore, according to the National Academy of Sciences norms, we also set ourselves the task of determining the future milk productivity of cows of different types during the I, II, and III lactations at a moderate feeding level. Indicators of the milk productivity of cows and the content of fat and protein in milk are given in Table 28.

Table 28. Milk productivity of experimental cows per lactation

Indicators	Groups (M±m)	
	control (n=10)	experimental (n=10)
I lactation		
A cow's milk yield, kg	3066±141	3642±193**
Fat content in milk, %	3.58±0.07	3,66±0.02
Amount of milk fat, kg	104.74±10.0	133.20±12.4
Protein content in milk, %	3.24±0.05	3.32±0.02
The amount of protein in milk, kg	99.34±7.20	120.91±10.11
II lactation		
A cow's milk yield, kg	3403±156	4043±214*
Fat content in milk, %	3.61±0.08	3.70±0.04
Amount of milk fat, kg	122.51±9.40	149.54±8.40
Protein content in milk, %	3.30±0.05	3.40±0.02*
The amount of protein in milk, kg	112.30±8.50	137.46±11.00
III lactation		
A cow's milk yield, kg	4078±187	4844±257**
Fat content in milk, %	3.63±0.06	3.75± 0.01*
Amount of milk fat, kg	148.02±8.40	181.6±15.00
Protein content in milk, %	3.31±0.03	3.42±0.01**
The amount of protein in milk, kg	134.98±6.55	165.66±9.47

The cows of the experimental group exceeded the control analogs by 18.70, respectively, in terms of milk yield for the I, II, and III lactations; 18.81 and 18.79% (P>0.90...95), and according to the fat content in milk - by 2.2, respectively 2.5 and 3.3% (P>0.90).

It is known that at the current stage in the world, work is being carried out on selecting cows for milk protein. Therefore, we want to pay attention to the fact that the cows of the experimental group exceeded the control analogs by 2.5, respectively, in terms of protein content in milk during the I, II, and III lactations; 3.0 and 3.3% (P>0.95).

CONCLUSIONS

Thus, the cows of the research group in the section of three lactations at a moderate level of feeding following the norms of the National Academy of Sciences in terms of milk yield per lactation, and the fat and protein content in milk, probably exceeded the control counterparts. This indicates that in the body of the animals of the experimental group, both young and mature age, redox processes were more intense than in the control counterparts. Therefore, the high-enzyme type (experimental group) cows should be left to the tribe and carry out the selection process. In contrast, cows of the low-enzyme type (control group) should be sorted into the industrial group. Animals classified as industrial should be crossed with meat breeders of domestic or foreign breeding to create an array of meat cattle in the western region, which will make it possible to increase the production of high-quality beef.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

MARKER SELECTION IN ANIMAL HUSBANDRY AND POULTRY FARMING

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Abstract
This article provides data on candidate genes CS3, CS2, BLG, LALBA as molecular markers for predicting milk productivity, technological properties of milk in cattle of Red Estonian, black-mottled Belarusian and Moldovan breeds, Karakul sheep breed, and also, OV, TFR loci in chickens of various breeds. The marker selection makes it possible to identify animals with valuable genes and preserve them in the population, as well as to look for a relationship with economically useful traits. The disadvantage of this method today is the high cost of research. Analyzing the relationship of candidate genes with productive qualities, it can be seen that the genotypes beneficial for one breed are not those for another breed. This is due to the degree of absorption of the breed - founder/bull in crosses and breeding `in itself`, etc. Nevertheless, in our opinion, valuable kappa-casein alleles are introduced by individuals of the Holstein and Simmental breeds, which are of the greatest value for breeding.

Key words*: genotype, loci QTL, marker genes, productivity, selection.*

INTRODUCTION

The formation of genomics in the 80's of the XX century led to the development of marker selection based on the identification and targeted selection of genes/alleles that influence quantitative traits important for agriculture. Thanks to the development and application of molecular marker technologies and statistical methods of data analysis, a large-scale research was carried out for quantitative trait loci (QTL) - DNA sequences containing genes or regions linked to them that have significance on the effect of a quantitative trait. Such loci were genetically mapped in all farm animals and poultry for a large number of quantitative traits characterizing productivity, fertility and the evolution of various genetic diseases (Kiser et al., 2017; Cai et al., 2019).
Marker selection is a promising branch in breeding, which makes it possible to reliably determine not only the genotype of animals and their productivity, but also to identify pathological mutations in genes, in connection with which it is possible to assess the risk of hereditary diseases, to make it possible to carry out specific reactions of the body to foreign cells, and to determine the causes of infertility of females (Shushpanova, 2021).
In particular, in cattle, QTL were mapped, which have a significant impact on the development of leukocyte adhesion deficiency syndrome, leading to the death of young animals as a result of immune deficiency, leukemia, scrapes, spongiform encephalopathy encephalopathy and many others (Hu et al., 2019).
In poultry farming, modern breeding technologies use DNA markers (single nucleotide polymorphisms (SNPs), which help identify QTL associated with egg-laying traits. For example, in the publication of Barkova (2021), data obtained using genome-wide association search (GWAS) for the selection of QTL and candidate genes that affect egg production, weight, strength and thickness of eggshell are presented to create a QTL system that is responsible for egg productivity of laying hens and egg quality.
Introducing DNA markers into the practice of animal husbandry were gradually identified

possible growth points in this direction. It shows how the genomic assessment of breeding value is constructed, what are the key conditions necessary for its implementation, as well as the advantages and limitations of genomic and marker selection. Thus, there was a real possibility of rational use of the genetic potential of farm animals (Stolpovsky et al., 2020).

In sheep breeding, for example, it is possible to increase the accuracy of the assessment and selection of animals, and hence the effect of breeding, only if there are reliable methods for determining economically useful traits. (Selionova et al., 2020). It was found that the milk productivity of Lacon sheep of the *GDF9ag* genotype is higher compared to animals carrying homozygous genotypes. The sheep with the homozygous *GDF9GG* genotype surpass sheep carriers of the *GDF9Ag* and *GDF9A* genotypes in terms of the amount of fat and protein in milk (Selionova et al., 2021).

The article presents a synthesis of molecular studies for the period 2000-2020 in the research for promising marker genes in cattle breeding, sheep breeding and poultry farming.

MATERIALS AND METHODS

The studies were conducted on the Holstein, Estonian Red, Belarusian Black and White, and Simmental cows; then in the sheep of the Karakul breed; further in the chickens of the Marans, Orpington, and Plymouth Rock breeds. Polymorphic systems *CS3*, *CS2*, *BLG*, *LALBA*, *OV*, *TFR* were determined by horizontal method of electrophoresis according to Smithies (1955) and Zhebrovsky (1979), and by method PCR.

The assessment of gene polymorphism was carried out by amplification of gene fragments *CSN3* using primers:

CAS1 5´-ATA GCC AAA TAT ATC CCA ATT CAG T-3´
CAS2 5´-TTT ATT AAT AAG TCC ATG AAT CTT G- 3´;
gene *LALBA*:
LAC 1: 5´- AAGAGTTGGATGGAATCACC - 3´;
LAC 2: 5´- TTCAAATTGCTGGCATCAAGC- 3´
gene *BLG*:
LG 1: 5´-TGTGCTGGACACCGACTACAAAAAG - 3´
LG 2: 5´-GCTCCCGGTATATGACCACCCTCT- 3´

RESULTS AND DISCUSSIONS

Among the many genes that control milk productivity and milk quality, we can single out a group of major genes that make the greatest contribution to the formation and functioning of this quantitative trait. Between such genes include the kappa casein gene *CSN3*, which is one of the genetic markers associated with qualitative and quantitative signs of dairy productivity of animals.

It is known that animals with the $CSN3^{BB}$ genotype are characterized by high protein-milk content, their milk has good cheese-making qualities (Zyryanova, 2021).

In our experiments, a *B*-allele variant of kappa casein was established in all cattle populations, which is associated with higher protein content in milk and with higher cheese yield, as well as better coagulation properties of milk. This is due to the different levels of glycolysis and the smaller diameter of micelles in the milk of animals with *BB* genotype. Cheese made from the milk of animals with the *BB* genotype contains more protein and less fat (24.7% and 33.18%, respectively), compared with *AA* homozygotes (24.22 and 33.71%).

The *CSN3* locus is correlated with the amount of milk produced, fat content and milk fat yield. The productivity per lactation in heterozygous $CSN3^{AB}$ cows of the red Estonian breed is higher (with 4746.3 liters of milk) than in homozygous $CSN3^{AA}$ (with 4229.3 liters). The average fat content is also higher in heterozygous individuals - 4.03%, and exceeds, on average, by 0.11% homozygous relatives (≈3.92%). The heterozygous genotype was also predominant in terms of milk fat yield by 25.5 kg.

In cows of the Belarusian Black and White breed, the desired genotype in terms of protein content $CSN3^{BB}$ exceeded the genotypes $CSN3^{AA}$ and $CSN3^{AB}$ in terms of fat content by an average of 0.2 %, and in terms of protein by 0.03 % - 0.11 %, respectively. It should be noted that this genotype is quite rare, in the populations of cows of the Belarusian black-and-white breed, its frequency is 1.1% (Ganja et al., 2019), and in Simmental cows it may be absent altogether (Zyryanova, 2021).

Numerous studies have shown that the frequency of occurrence of the desired *CSN3B* allele in black-and-white cattle populations is very low and systematically continues to decline, according to some experts, due to the widespread use of the Holstein breed.

Genetic variants of alpha-S1-casein (*LALBA*) influence the technological properties of milk. (Tyulkin, 2019). Association analysis showed that polymorphism g.-1001T > C in the promoter region of the *LALBA* gene has an effect on milk productivity in Polish Holstein-Friesian cows. High daily milk yield and dry matter yield, as well as high lactose yield and concentration are associated with the *TT* genotype. Cows of the *TT* genotype also had a smaller number of somatic cells in milk, which can be considered as an indicator of healthy state of udder (Ostrowska et al., 2021).

According to the *LALBA* gene in the Simmental breed, cows with the $LALBA^{BB}$ genotype were superior to cows with $LALBA^{AA}$ in fat content ($p<0.05$), in the «Sibiryachka» breed, higher fat content was observed in cows with the $LALBA^{AB}$ genotype (Unzhakova et al., 2021).

In other studies, no significant effect of *LALBA* on milk production was found on Holstein Friesian cows (Soyudal et al., 2019).

LALBA polymorphism and association with signs of milk lactation were also found in Chinese Holstein dairy cows (Yang, 2020).

In our studies, this locus has shown the presence of correlations with the amount of milk produced, the fat content in it and the yield of milk fat. It was found that animals with the homozygous $LALBA^{BB}$ genotype (with 3332.5 and 9368.2 liters of milk per lactation) have higher productivity in Moldovan and Belarusian Black and White cows, whereas the $LALBA^{BC}$ genotype (of 4648 liters) has an advantage in the Estonian Red breed. The most advantageous in terms of fat content in milk in black-and-white individuals of the Moldavian type is the heterozygous $LALBA^{AB}$ genotype (of 3.66 %), which exceeds the *BB* genotype by 0.02 %. In the Estonian Red breed, a higher fat content was found in cows with the heterozygous $LALBA^{BC}$ genotype - 4.02%, and in Belarusian black-and-white with the heterozygous $LALBA^{AA}$ - 4.17%. According to the yield of milk fat in black-and-white cows of the Moldavian type, the $LALBA^{BB}$ genotype turned out to be a priority and amounted to 121.5 kg, which was 1.2 kg higher than in the cows of the $LALBA^{AB}$ genotype.

The beta-casein locus *CSN2* can also serve as a marker in animal selection for milk productivity. Analysis of the effect of this gene on the productivity of Estonian Red cows showed the superiority of the *AB* genotype (4977 liters of milk) over the *AA* genotype (4210.5 liters). In terms of fat content, there is a slight superiority - by 0.07% of the heterozygous genotype *AB* over the homozygous *AA*.

The inclusion in the traditional breeding rules for the selection of cows with another marker - betalactoglobuline (*BLG*) makes it possible to achieve significant success in increasing milk yields and improving its quality in specific herds of black-and-white cattle in a shorter time.

The *BLG* locus on cows of the red Estonian and black-mottled breed of the Moldavian type showed that animals with homozygous genotype *BB* have the highest productivity (4303 liters of milk per lactation and 3339.2 liters, respectively). The heterozygous *AB* genotype (of 3.69 %), which exceeds the *AA* genotype by 0.08 %, is advantageous in terms of fat content in milk in Black and White cattle of the Moldavian type.

In the Estonian Red breed, a higher fat content was found in *AA* homozygotes - 4.08%. According to the yield of milk fat in Black and White cows of the Moldavian type, the *AB* genotype is predominant (123.09 kg), whereas for the red Estonian breed, the homozygous *BLGAA* (169.36 kg).

Thus, analyzing the influence of the genetic polymorphism of QTL loci on the milk productivity of Estonian Red cows, it can be concluded that heterozygous genotypes in *LALBA, CSN2, CSN3* loci are advantageous for the selection of animals according to such traits as the amount of milk produced, fat content and milk fat yield. At the BLG locus, a homozygous *BB* genotype is desirable in terms of milk production, whereas the *AA* genotype is desirable in terms of fat content.

In the course of the research, the features of protein systems were studied in connection with the association with leukemia and mastitis, which cause great economic damage to animal husbandry, especially to the breeding genetic fund of highly productive livestock.

Diagnosis of infection of cattle with bovine leukemia virus (BLV - Bovine Leuxemia Virus) is mainly performed by assessing the presence of antibodies to BLV antigens in animals using REED diagnostics. The method is characterized by low accuracy, it is known that a READ-positive analysis is confirmed only by a pathoanatomic analysis in 1-8% of cases.

It is proved that the sensitivity to leukemia in cattle is determined by postalbumins of type *BB* and transferrins of type *AD*. It follows from this that during breeding work it is necessary to constantly monitor the accumulation of these alleles in the population, and breeding bulls should be typed according to these genes.

In sheep, the locus that controls the synthesis of *BLG* is localized on the second chromosome and it is believed that it gives taste to milk. The data on the relationship of *β*-lactoglobulin with the milk productivity of sheep are contradictory (Klimanova & Tarasenko, 2021).

In our studies, in Karakul sheep, the BLG^{AA} genotype is interrelated with high milk productivity, they produce 0.24 % more dry matter compared to heterozygous *AB* individuals. *BB* homozygotes contain 0.79 % more dry matter in milk than heterozygotes. Comparing the percentage of fat, it was found that 0.13% more fatty milk (8.55 ± 2.14 %) can be obtained from sheep with the genotype BLG^{BB}, compared with the genotype BLG^{AA} (8.07 ± 1.041%) and 0.61 % more, compared with heterozygotes BLG^{AB} (7.94 ± 1.14 %).

The highest percentage of 4.82 % of protein was found in the milk of individuals with the *AB* genotype, which is 0.16 % higher than in the *AA* genotype and 0.45 % higher than in heterozygous animals.

As for the percentage of casein in the milk of Karakul sheep, it turned out that individuals with the genotype BLG^{BB} have the lowest casein content of 3.45 %, which is 0.25 % less than the casein content in the milk of homozygous *AA* individuals 3.7 % and 0.28 % lower than in heterozygous animals 3.73 %. As you can see, the most advantageous genotype for such traits as: the protein and casein content in the *BLG* locus is the heterozygous ones.

Thus, the highest percentage of fat content in the *BLG* locus is noted for the BLG^{BB} genotype, and in the case of protein and casein, the heterozygous *AB* animals are prioritized.

To assess the gene pool of agricultural chickens, 5-6 systems can be used, in which a stronger genetically determined polymorphism is detected. These include albumins, globulins G3 and G2, transferrins, etc.

In our studies on populations of chickens in the Marans and Orpington breeds, the *OV* locus showed a pleiotropic effect, influencing the fertilization and hatchability of eggs. The homozygous *AA* chickens had increased egg production, with the lowest live weight, egg weight and low hatchability of chickens, compared with the heterozygotes birds by 3-10 %. Chickens carrying the OV^{A} allele outperformed chickens with the OV^{B} allele in live weight and egg weight. In homozygous OV^{BB} hens, the chickens are more viable compared to heterozygous ones.

The transferrins are associated with the egg mass. The homozygous *BB* genotype in Marans chickens determined the lowest egg mass of 46.54 ± 0.55 g. The *AA* genotype had the highest embryonic mortality of 4.9% in the Orpington breed. The *AB* genotype is interrelated with the live weight of ♀ Orpington × ♂ Moldavian Naked Neck hybrids (2.58 ± 0.09 kg).

Immunogenetic indicators can also be used as markers to study the genetic structure in order to determine the common origin of breeds. For example, the genetic similarity of cows of the Estonian Red and Red Steppe breeds was r = 0.9976 and between Jersey and Black and White Cow breeds r = 0.8907. The Moldavian type of Black and White cattle is genetically similar with the Holstein breed by 77.14%, which indicates their commonality (the common ancestor of the Holstein) in lactoproteins. At the same time, a genetic change was established between them (D = 0.2595), i.e. on average, for every 26 out of 100 loci, complete allele substitutions occurred. This is due to the reproductive and isolating mechanism, namely, the breeding of a population of Black and White breed of cows "in itself".

CONCLUSIONS

Analyzing the relationship of candidate genes with productive qualities, it can be seen that the genotypes beneficial for one breed are not those for another breed. This is due to the degree of absorption of the breed - founder/bull in crosses and breeding "in itself", etc. Nevertheless, in our opinion, valuable kappa-casein alleles are introduced by individuals of the Holstein and Simmental breeds, which are of the greatest value for breeding.

Thus, the obtained research results allow us to draw the following conclusions.

In cattle breeding:

For the production of protein products with a high protein content, we recommend considering the *CSN3* locus of the BB genotype as an economically important selection criterion for dairy cattle of the Belarusian Black-and-White breed.

The *LALBA* gene can be used as a genetic marker in the selection of animals according to the following indicators: the amount of milk produced, the fat content in milk, the yield of milk fat (for the Estonian red breed of the BC genotype; for black-and-white cows of the Moldavian type and Belarusian black-and-white breed - genotype BB).

The *BLG* locus is associated with milk yields (the advantage of the BB haplotype in the populations of the Estonian red breed and the Moldovan black-mottled cattle breed has been proven; the AA haplotype in cows of the Belarusian black-mottled breed).

To increase the fat content of milk in the *BLG* locus, a heterozygous genotype AB is desirable in cows of the Moldavian Black-and-White type, genotype AA in the Estonian Red breed, homozygous BB in the Belarusian Black-and-White breed.

To constantly monitor the accumulation of alleles that determine leukemia and mastitis, we suggest using marker-dependent proteins, postalbumins and transferrins.

In sheep breeding:

The *BLG* locus is recommended as a genetic marker for increasing milk the productivity and fat content in Karakul sheep (genotype AA for high milk productivity and genotype BB for increasing the percentage of fat in milk).

To evaluate the gene pool of agricultural chickens, we recommend using ovalbumin proteins as markers. To stabilize the lines by quantitative characteristics of productivity, it is recommended to give preference to homozygous AA genotypes, as well as to select chickens with homozygous OV^{BB} genotype for the production of the next generation and increase their viability.

Thus, it is necessary to continue monitoring the allele found of local breeds in order to preservation of optimal genetic diversity (polymorphism of the level of 5%) for further search of the most representative markers for each breed of animals separately.

ACKNOWLEDGEMENTS

The results of the research were implemented in two scientific projects: 20.80009.51.07.12 “Strengthening the food-livestock chain through the use of new feed resources, innovative methods and health care schemes” and 20.80009.5107.20 “Genetic potential management and production of breeding animals, reproduced and operated in the soil and climatic conditions of the Republic of Moldova”.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

ESTIMATION OF THE HERITABILITY FOR PRODUCTION AND REPRODUCTION TRAITS IN THE PALAS – PROLIFIC LINE SHEP, USING BLUP METHODOLOGY

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Abstract

The purpose of this study is to estimate the heritability in Palas - Prolific Line Sheep, for milk and prolificacy. This objective was undertaken applying the BLUP methodology, to a random regression animal model, for milk yield and, also, to an ordinary animal model for the number of offsprings/ewe/lambing. For milk yield, the data set consisted of 4610 test day records from the first lactation of 485 ewes, with records, sired about 97 rams. Totally, there were 1049 animals, including the ancestors. The fixed effect in the model were fixed lactation curves and flock-test-date. The random effects of animal and permanent environmental effects were modelled using orthogonal polynomials of order three. The curve of daily heritabilities for milk yields were 0.216, 0.133, 0.151, 0.219, 0.280, 0.314, 0.318, 0.292, 0.243, 0.199 and 0.210 for 50, 64, 78, 92, 106, 120, 134, 148, 162, 176, 190 days in milk. For the number of offsprings/ewe/lambing (prolificacy) the heritability was estimated using an animal model. The fixed effect was the year and season of lambing. Random effects was represented by animals. The heritability of prolificacy was 0.177.

Key words*: animal model, heritability, Palas - Prolific Line Sheep, production and reproduction traits, random regression model.*

INTRODUCTION

The Best Linear Unbiased Prediction (BLUP) and animal model are widely used in the genetic evaluation of milk production traits in sheep. This method uses a statistical model that takes into account the relationships between animals and the genetic and environmental factors that affect milk production (Jurado et al., 2006).

The characteristics of dairy sheep with the greatest economic value are fecundity and milk production (Slavova et al., 2015). Sheep milk is highly nutritious and is an excellent source of protein, calcium, and vitamins. The profitability of the sheep is predicated on the rise in reproduction indices, irrespective of the type of exploitation technique used. The focus of the interest is mostly on fecundity, fertility, and prolificacy indices that are increasing. Because it results in an increase in the number of animals and meat production, intensifying prolificacy is a key goal in the exploitation of all sheep breeds (Bulmaga et al., 2021). Breeders begin by defining the traits they want to enhance in their population, gather data on the animals' performance and genetic relationships, identify the animals with the best genetic potential, determine the ratio of animals to be mated in order to achieve a specific genetic gain in the following generation (Blasco, 2022).

Animal breeding is founded on the idea that a parent's characteristics are more or less reflected in their offspring, which means that a parent's heritable ability to pass on an animal's characteristics to its offspring. In animal breeding, prospective parents are chosen based on specific characteristics, and the best ones are actually employed as parents (Oldenbroek & van der Waaij, 2014). Any breeding program that aims to improve the genetics of livestock must include the estimation of genetic parameters and the breeding value prediction of animals.

The purpose of this paper is to estimate the heritability for milk and prolificacy in Palas - Prolific Line Sheep. The importance of the heritability for any trait is evident in its being an assistant to the breeder in determining both the method of selection and the mating system to be followed, and that determining the expected genetic improvement depends partly on the estimation of the heritability.

MATERIALS AND METHODS

To estimate the heritability of the characters milk quantity and prolificacy, the data from a number of 485 sheep, descendants of a number of 170 fathers and 393 mothers, were used. The average size of the father's family was 2.85 demi-sisters.

For milk production, the phenotypic information was represented by the quantities of milk measured and recorded during the official performance control, in lactating ewes, after the lambs were weaned. In the analysis, only the first lactation, achieved by 485 ewes, in the period 1993-2001, was taken into account. The interval between two controllers was 14 days, and the total number of recorded performances was 4610 checks, with an average of 9.5 controller /sheep.

BLUP methodology was used to estimate heritability, applied to two separate biometric models. Thus, to estimate the heritability of the milk quantity, the test day model with random regressions (random regression test day model) was used, and to estimate the heritability for prolificacy, the individual animal model with repeatability (Repeatability animal Model) was used.

RESULTS AND DISCUSSIONS

Phenotypic structure of the analyzed population. Table 1 shows the average performances of the animals taken in the study, for the two analyzed characters.

Table 1. Average performances for daily milk yield and prolificacy for sample data analysed

Specification	Unit of measurement	n	$\bar{X} \pm s_{\bar{X}}$	Limits (min-max)
Daily Milk Yield	ml.	4610	441.74 ± 3.38	20-2000
Number of lamb/ewe	nr.	485	1.15 ± 0.016	1-2

From the data included in Table 1 it can be observed that the average daily milk production, during lactation after the lambs are weaned, is within normal limits, this being characteristic of a prolific sheep population with a good lactation capacity, Murphy (2016) found that the average daily milk production was within the normal range. This result is consistent with another study (Sutera et al., 2021).

Several studies indicated that there is a common effect of genetic and environmental factors on milk production in sheep, as Capistrak et al. (2002) indicated that environmental factors greatly affect the variation in season length and daily milk quantity, especially among members of the same breed, and this was confirmed by Oravcova et al. (2005), which indicated that 90% of the variation in milk production within a single breed is mainly due to environmental reasons and only 10% is due to genetic reasons, which means the need to involve as many environmental factors as possible or modify them while determining genetic effects, despite genetic control It depends on the factors that contribute directly or indirectly to the production of milk, but these factors are greatly affected by the level of protein in the diet as well as the number of feeding times, which means that there are differences in the amount of milk.

Also, the prolificacy of 115% shows the fact that for every one hundred ewes, a number of 115 lambs are obtained, which places the respective line in the group of populations with good prolificacy. Where the results of the study were observed through a study conducted on Polish sheep, Peter et al. (2017) found that the fertility was approximately 133.86%. Fertility was higher in another study, amounting to 1.52 (Hadeel et al., 2021). The prolificacy rate at birth was 1.15 live births/ewes, which is somewhat low, but the result is similar to what Jawasreh et al. (2010) reached in his study on Awassi sheep, and the rate obtained in the current study is within the range for the breeds of neighbouring regions and countries. The studies unanimously agreed that the reproductive characteristics in sheep (including prolificacy) are complex characteristics and have a low heritability coefficient and are affected by a number of genes. Responsible for the degree of prolificacy changes according to the season, type of nutrition, health status and sex ratio (Jafari et al., 2014).

Genetic structure of the analyzed population. The causal components of the phenotypic, genotypic and environmental variance for each analyzed character are presented in Table 2.

The amount of phenotypic and genetic variation estimated for daily milk production in this study was 2080 and 582, while the remainder that reflects the environmental influences that were not included in the mathematical model amounted to 1498 (Table 2). The high phenotypic variation (sum or sum of genetic and environmental variation) is attributed to the high milk production It is one of the quantitative characteristics of great variation because it is affected by many environmental factors, whether temporary, such as nutrition, temperature, humidity, or fixed factors such as the type of birth, sex of the newborn, season and year of birth, as well as the breed, as well as the number of records or observations that have been approved.

Table 2 shows that the percentage of phenotypic and genetic variation estimated for the prolificacy rate or the number of lambs produced per ewe in this study amounted to 0.119 and 0.021, respectively, which are low estimates (despite its importance) given that this characteristic originally had small rates as well as The low prolificacy rate in this study, which amounted to 1.15 births / ewe, and the estimated variations will reflect or determine the heritability of the trait, as will be shown in the subsequent tables (Tables 3 and 4).

These variance components provide important information on the sources of variation for these traits. The genotypic variance indicates the potential for genetic improvement through selective breeding, while the residual variance can be used to identify areas for improvement in management practices or measurement techniques. Understanding these variance components can help farmers and researchers to develop more effective strategies for improving these performance measures.

Based on the causal components of the variance, the heritabilities of the two analyzed characters were estimated, according to the data in Table 3.

Table 2. Variance causal components for daily milk yield and prolificacy for sample data analised

Specification	Variances		
	Phenotypic (V_P)	Genotypic (V_A)	Residual (V_E)
Daily Milk Yield	2080	582	1498
Number of lamb/ewe	0.119	0.021	0.098

Table 3. The heritability values for daily milk yield and prolificacy

Specification	n	$h^2 \pm s_{h^2}$
Daily Milk Yield	4610	0.280 ± 0.25
Number of lamb/ewe	485	0.177 ± 0.16

It is evident from Table 3 the estimates of the heritability for the two traits studied, and the heritability of daily milk production was 0.28, and this estimate is considered average, but it is considered the upper limit of the majority of previous studies, meaning that there is an important genetic variation in milk production, and therefore it is possible to improve this trait In several ways, if the selection was based on the fact that there is an important genetic variation and that it is one of the traits that are affected by a large number of genes, or by improving the environmental conditions, given that there is a wide variation of up to 0.72, as well as the possibility of improving this trait by cross-crossing with specialized purebreds, This result can be compared with previous studies in the Czech Republic, Bauer et al. (2012)

conducted research on Lacaune and East Friesian ewes using a data collection comparable in size to the Quebec population. They discovered that milk production had a heritability of 0.28. The heritability of daily milk yield was low in a study of Slovakian Lacaune sheep, amounting to 0.15 (Oravcova, 2007). Another study on Awassi had low heritability of daily milk production 0.11 (Al-Dabbagh, 2011).

The estimate of the heritability for the prolificacy in this study was 0.177, and the trait of prolificacy is one of the traits with a very low heritability (0.02-0.05), the result in this study is higher when compared with most studies, Schaeffer & Szkotnicki (2015) noted through their genetic evaluation of sheep in Canada they found that the heritability of prolific is 0.14. This indicates the heritability value is low for prolificacy. The percentage of prolificacy were: 0.30 (Hadeel et al., 2021). The research was carried out on Polish Merino ewes heritability values indicate that the influence of genetic assumptions on ewe fertility is low 0.104 ± 0.024 (Piwczynski and Kowaliskyn, 2013). In a comparative study between the percentage of twins for the ewes of the Iraqi Awassi and the Turkish Awassi under the conditions of the research station, the researchers Ishaq and Ajeel (2013) found that the percentage was 17.42% for the local Awassi and 22.5% for the Turkish Awassi. Therefore, it can be used in genetic improvement programs.

Estimates of the heritability of the same trait differ from one study to another depending on the sample size, the method of estimation, the mathematical model, the place and time of conducting the study, and whether or not to test the matrix of variance components.

Additive genetic and permanent environmental variances, as well as associated heritabilities, for lactation days, 50, 64, 78, 92, 106, 120, 134, 148, 162, 176, 190 days, are presented in Table 4.

Table 4. Additive genetic, permanent environmental variances and associated heritabilities for 50, 64, 78, 92, 106, 120, 134, 148, 162, 176, 190 test days

Variance	Lactation day										
	50	64	78	92	106	120	134	148	162	176	190
V_A	0.074	0.036	0.040	0.062	0.085	0.100	0.100	0.088	0.069	0.056	0.067
V_{per}	0.067	0.037	0.024	0.020	0.019	0.018	0.015	0.013	0.015	0.025	0.053
h^2	0.216	0.133	0.151	0.219	0.280	0.314	0.318	0.292	0.243	0.199	0.210

Table 4 shows the data pertains to the variance in daily milk yield across different days of lactation, as well as estimates of the additive genetic variance (V_A) permanent environmental variance (V_{per}) and heritability (h^2) for each day of lactation. The values (V_A) of and (V_{per}) represent the proportion of variance in milk yield that is due to additive genetic effects and permanent environmental effects, respectively.

The heritability (h^2) is the proportion of the total variance in milk yield that is due to genetic factors and it ranges from 0 to 1.

The values of (V_A) (V_{per}) and h^2 vary across different days of lactation, which suggests that the genetic and environmental factors influencing milk yield may change as lactation progresses. In general, the heritability estimates are moderate to high, with values ranging from 0.133 to 0.318, for lactation days 64 to 134, then it begins to decrease. This indicates that genetic factors play a significant role in determining milk yield in this population. The genetic variances are bigger in the middle of lactation and smaller at the end of the lactation. Other studies confirmed this (Sutera et al., 2021; Popa et al., 2020). Because the genetic variances and the heritabilities associate are high in the middle of the lactation, we can conclude that the number of test days per ewe could be reduced in order to take into account only those that could give high genetic progress.

CONCLUSIONS

Heritability plays a significant role in the milk production and prolific of dairy animals. Milk production has moderate to high heritability, which means that offspring of high-yielding sheep are more likely to produce high amounts of milk.

The heritability is the main genetic parameters used for prediction the breeding values and genetic progress. In the present study, milk yield trait had an intermediate value for heritability, for the whole lactation (0.28), but varied between 0.151 and 0.318, depending the stage of lactation. Because the high values of heritability is in the middle of the lactation, in order to maximize the genetic progress we can reduce the number of test days, only those taken in the middle of lactation.

Fertility have lower heritability, meaning that genetic factors have less influence on these traits than environmental factors.

Dairy farmers can select animals with high milk production and fertility to breed, which can lead to improved genetics in the offspring.

The best model for evaluating the genetics of milk output in sheep is the random regression model, which also allows for selection of lactation curves with high persistency while minimizing costs and improving estimations of breeding value.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

THE BOOROOLA SHEEP BREED AS A GENETIC RESOURCE WORLDWIDE IN BULGARIA

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Abstract

The Booroola Merino Breed, developed in Australia, is a valuable genetic resource for increasing biological fecundity in sheep worldwide. The purpose of this study was to summarize information about the Booroola Merino breed and its use worldwide and in Bulgaria for increasing the reproductive potential of Merino sheep and other breeds. The positive effect of the introduction of the FecB gene for high fecundity in sheep has been confirmed by a number of authors over a period of over 30 years. It has been established that crosses with Booroola achieve up to 30% higher ovulation rates and 20% more lambs after weaning compared to the purebred animals. The positive effect of introducing the FecB gene from Booroola in the Bulgarian fine-fleece breeds of sheep was established in terms of increasing the ovulation rate and the number of lambs born, with an insignificant negative impact on weight development and wool productivity. Negative effects can be reduced by choosing appropriate crossing schemes. Achieving a balance between benefit and risk in the introduction of the FecB gene from Booroola is an important condition for increasing the economic effect of animal husbandry and creating sustainable production in sheep farms.

Key words: *Booroola Merino Breed, fecundity, FecB gene, sheep.*

INTRODUCTION

Booroola, the breed with high fecundity rates, was created in Australia. Under the conditions specific to this range, the sheep are reared year-round in large flocks, make long journeys, suffer losses from predator attacks, inseminate naturally, and under these realities, an average of 78.5 lambs are obtained from 100 ewes. In farms with better feeding and rearing conditions, 110-115% weaned lambs obtained at 120-130% biological fecundity are reached (Bojkovski et al., 2009). The breeding process of the Booroola breed began in 1919 in New South Wales - Australia, in the "Kumi" area. Farmer Berg Sayres found that one of his Merino sheep was giving birth to three lambs each year and decided to form a flock from her offspring. In 1944, brothers Dick and Jack Seares purchased two sheep from this flock for their Booroola farm. Through targeted selection of progeny from multiparous lambs, they created a flock with an average fecundity of 1.9 lambs born per ewe and this is the first flock of the Booroola breed. It is the only merino breed that is characterized by high biological fecundity. Initially, the Siares brothers teamed only female animals born from multiparous mothers or family selection. The breeders working in the multiparous flock are from other Australian Merino flocks with breed-normal fecundity. The history of the Booroola breed is based on a gradual increase in the frequency of individuals carrying a specific gene that largely determines the fecundity trait (Tzonev, 2014). Genetic analysis (Bindon, 1984) of sheep of this breed shows, that responsible for increasing the number of ovulated eggs and the number of lambs born in one birth is a major gene F (FecB). This was confirmed in subsequent scientific experiments when it was found that its occurrence was due to a mutation (BMPR-1B) in a chromosome no. 6. This mutation was later found in indigenous sheep breeds in India, China and Indonesia. Fogarty (2009) confirmed the hypothesis of the appearance of the Australian FecB gene after the importation of Garole (also known as Bengal) sheep from northeastern India in 1792 and 1793. Davis (2005) also found the presence of the gene for high fecundity in local Indian sheep breeds, found a high degree of similarity and considered the Booroola Merino to be a direct descendant of the Garole breed. The F (FecB) gene determines higher fecundity in other sheep breeds - Finnish Landrace, Romanovska, British Milk Sheep (Abraham & Thomas, 2012). In male Booroola breeders, the FecB gene can be detected after judging the fecundity of their daughters. In the Australian Merino, the birth of three lambs in one lamb is a very rare phenomenon (0.1%), therefore the registration of multiple births in daughters is considered as evidence that the father is a carrier of the FecB gene. The F (Fecundity) designation of this gene is analogous to publications related to mouse studies (Lyon, 1977). The same author found that in mice it determines fecundity and it is accepted to be marked with the letter "F", and the other type of alleles with the alternative symbol (+). According to Turner (1978), one of the problems with the use of this FecB gene is that, even in the heterozygous state, it can increase fecundity more than the desired level and this leads to delayed weight development and higher than average lamb mortality.

The purpose of this review is to summarize the information available in the scientific literature regarding the Booroola breed and the possibilities for the introduction of the FecB gene for high fecundity in other breeds worldwide and in Bulgaria, with the aim of increasing the reproductive potential and the economic effect in sheep breeding.

INTRODUCTION OF THE F GENE (FecB) FROM BOOROOLA

In 1973 in New Zealand the Ministry of Agriculture bought two rams of the Booroola breed. In 1974 and 1977 Heldon Station, MacKenzie Province made large imports of multifertile Merino rams and ewes from Australia and established an elite flock of 200 ewes with an average fecundity of 3.6 born and 2.9 weaned lambs a sheep. Since 1981, the Heldon station has been actively producing and exporting rams, ewes, embryos and deep-frozen semen of the multi-fertile Booroola breed to Brazil, Chile, Hungary, Uruguay, and later to England, the USA, Canada, the Czech Republic, Scotland, Bulgaria.

Fecundity of F1 crosses with Booroola have been well studied by Robertson (1974), Allison et al. (1978). Daughters of homozygous Booroola (BB) rams can produce twice as many lambs as Merino ewes in Australia and New Zealand, where they produce an average of 0.85 lambs per ewe. In Hungary, two programs are being worked on to obtain thin-fleece sheep with longer wool and higher fecundity and milk yield based on the Hungarian Worsted Merino. Fine fleece ewes are crossed with Booroola and F1 crosses with Suffolk or German Blackheads (Veress, 1983). The same scientist reports that when crossing the Hungarian fine-fleece breeds with homozygous Booroola (BB) rams, fecundity increases to 150-170%. When transferring the FecB gene from the Booroola breed in the Czech Republic, they were able to increase the fecundity of fine-fleece sheep to about 150% (Horak et al., 1989). Davis et al. (1981) found that 88% of the progeny of a ram (BB) homozygous for the fecundity gene ovulated 3 or more eggs, but only 56% gave birth to 3 lambs. In the heterozygous (B+) ram progeny, 62% had 3 or more ovulated eggs and 61% lambed with three or more lambs. Bindon (1984) found that 30% of Booroola ewes ovulated up to 3 ovules and the remainder ovulated up to 9 ovules, with an average number of 4.65. In the control group, Australian Merino ewes in 98.6% of them ovulated up to 2 eggs with an average number of 1.62. Maguer et al. (1999) found of 21 Booroola rams imported from New Zealand to Hungary that one ram lacked the FecB gene for high fecundity, 8 were homozygous (BB), 10 were heterozygous (B+), and 2 have not been identified. According to Davis et al. (1998) the animals were considered carriers of the gene, in which at least once an ovulation rate of 3 or more eggs was recorded. Davis and Hinch (1998) reported the successful transfer of the FecB gene in various semi-fine fleece breeds - Dorset, Romney-marsh, Border Leicester etc.). The authors found that the presence of the FecB gene increased the fecundity of the mothers by 0.7 lambs. This suggests that using a homozygous Booroola (BB) ram, all would receive the FecB gene and the mean fecundity of the daughters would increase by 0.7 lambs. If the father is heterozygous for the gene (B+) it is transmitted to half the offspring and the increase in fecundity of the daughters is on average 0.35 lambs. If the ram is homozygous for the gene for normal fecundity (++) there will be no changes in the fecundity of his daughters. The authors recommend the appropriate phenotypic criteria for judging the genotype of rams with respect to the FecB gene. Davis & Hinch (1981) recommended breeding crosses with rams carrying the F gene in order to increase the fecundity of various sheep breeds, including stock flocks. The productive direction of these breeds can be maintained if the Booroola blood is reduced by backcrossing to 12.5% or 6.25%. When applying the selection schemes, care must be taken to preserve the FecB gene in the crosses. The creation of an intrabreed type Awassi "+", with high fecundity in Israel, is an example of the transfer of the FecB gene from the multifertile merino Booroola in a very different productive direction, exterior and interior Awassi breed (Mihailova, 1998). In this case, homozygous Booroola rams (BB) are used only in the first cross. In the following generations, a reverse cross is made with Awassi and after 11 years, as a result of the applied selection schemes, the flock has 90% Awassi-blooded and only 10% Booroola-blooded. Heterozygous carriers of the F gene (B+) predominate, but there are also homozygous rams and sheep (BB) in the nucleus. Fecundity from 1.1-1.2 increases to 2 lambs born and 1.7 lambs weaned per ewe. The average milk yield of 500 liters in the starting flock of Awassi in F1 is almost halved. It is likely that the reduction in milk yield is the result of the influence of other Booroola merino genes. After backcrossing the final form with 90% Awassi, she reaches 400 liters per lactation, with almost double the fecundity. The most successful transfer of the FecB gene from the Booroola breed is obtained in merino and fine fleece breeds. Selection problems and negative effects are insignificant in sheep from one productive direction. A slight decrease in live weight and wool yield of the crosses was reported.

Martyniuk et al. (2009) summarized the results of the import and use of 121 Booroola embryos, homozygous carriers of FecB (BB) in Poland. The average ovulation rate for 5 years was 4.25 /ranging from 2 to 8/, average fecundity - 228% and up to 5 lambs per lambing were obtained. Despite the increased fecundity of crosses with Polish Merino and Suffolk in the second stage, the introduction of the FecB gene did not gain popularity in Poland, due to the lower level of the traits birth and weaning live weight, growth intensity, average daily gain and carcass conformation compared to parent breeds. Gootwine (2009) published results using homozygous (BB) Booroola Merino rams in Israel, where lamb production is the main source of income from non-dairy flocks and contributes about half of the gross income of dairy flocks. Crosses with Awassi and Assaf are named Afec - Awassi and Afec - Assaf. Fecundity of heterozygous B+ and homozygous BB ewes was 1.90 and 1.92 lambs per ewe, respectively, in Afec - Awassi and 2.40 and 2.55 lambs per ewe, respectively, in Afec-Assaf. Compared to the baseline fecundity of Awassi and Assaf, which is about 1.3 and 1.7 lambs, respectively, the increase is significant. Introduction of the FecB gene, however, had some adverse effects on lamb birth weight and survival, as well as on body weight and milk production. Van der Werf (2009) investigated the genetic aspects of the introduction of the FecB gene from Booroola into a commercial Australian sheep breed and found that the results obtained depended on the effect of the gene, the genetic distances between the two breeds and the specific interaction of the two genotypes. In developing breeding improvement strategies, the author recommends the use of gene markers to achieve rapid genetic progress. Walkden-Brown et al. (2009) reported that the use of the direct DNA test for FecB genotyping since 2001 created great opportunities for research deployment in Australia. The scientific interest of the authors is also focused on the negative effects observed in homozygous variants carrying FecB, which are expressed in low lamb survival and are associated with increased ovulation frequency. Further studies of genetic mechanisms and other factors modulating the effect of FecB are needed.

Fogarty (2009) published a large-scale study on the topic of the effects of the introduction of the FecB gene in different breeds, geographies and production systems. The author summarizes 45 reports with information on crosses with different types of breeds and studies of the different genotypes (homozygous BB, heterozygous B+ and non-carriers ++) and their reproductive outcomes. A positive effect of FecB (B+) on Ovulation Rate (HO - number of corpora lutea) +1.3 (range +0.8 to +2.0) and + 0.7 (range +0.4 to +1.3) for number of lambs born was found. According to Fogarty (2009), the effect of FecB (BB) homozygous genotypes is manifested by a greater increase in the Ovulation Rate, but without a significant change in the number of lambs born. His summarized data show that heterozygous variants are characterized by better growth intensity, number of lambs weaned and total weight of the animals weaned. The majority of studies have shown lower birth weight and less growth intensity in crosses with Booroola Merino and their progeny compared to parent forms. Fogarty (2009) argued that it is difficult to differentiate to what extent this is due to the effects of the introduction of the FecB gene or the general potential of Booroola Merino for these traits, as well as correlations with lamb size and dependencies with feeding and rearing conditions. Regarding other selection traits with economic significance, Fogarty (2009) found no significant and reliable effects. The introduction of the F (FecB) gene did not significantly affect seasonal estrogenic activity, carcass and meat quality, and breeding productivity.

With the idea of elucidating the mechanism of action of FecB, Abraham & Thomas (2012) investigated and found increased plasma levels of follicle-stimulating hormone (FSH) during the oestrous cycle and a higher concentration of FSH in the urine of Booroola Merino sheep of reproductive age. The authors found some differences in the structure of the ovaries and follicles compared to the Australian Merino control group. Of interest is the comparison of the reproductive characteristics of male Booroola and Australian Merino breeders. Abraham & Thomas (2012) found no significant differences in terms of testis size, quantitative and qualitative characteristics of the semen in the two Merino breeds. The results for hormonal status are also similar, which gives the authors reason to conclude that the phenotypic expression of the F (FecB) gene is limited by sex (Abraham & Thomas, 2012). Adkinson et al. (2013) analyzed the realities of sheep production in Turkey and the opportunities presented by the use of the FecB gene in breeding schemes to meet the increasing market demand for sheep meat. The authors report the absence of this gene in genetic studies of 6 local breeds, but consider that its introduction into the genotype of adapted breeds, through appropriate cross schemes, is a better option for increasing fecundity than importing homozygous introduced animals. The production of milk and meat from sheep has been significantly reduced in the last 20 years in Turkey, and the demand is increasing, and the use of the FecB gene has great potential and prospects for increasing productivity and economic efficiency in sheep farming (Adkinson et al., 2013).

Qi et al. (2020) found that between homozygous, heterozygous carriers of the FecB allele and those without it, there was no significant difference in frequency and duration of oestrus. The results show that carriers of this allele in both variants have a larger litter size, after oestrus synchronization and artificial insemination. Lamb survival rates at weaning did not differ between groups, but higher fecundity in ewes carrying the B allele was associated with lower offspring body weight at birth and weaning. Wang et al. (2021) investigated the effects of FecB gene on oestrus, ovulation and endocrine characteristics in Small Tail Han Sheep in China. The authors recommend that FecB allele heterozygous animals (B+) showing moderate ovulation and lamb size and a shorter oestrous cycle be widely used in sheep crossbreeding systems for commercial lamb production.

INTRODUCTION OF THE F GENE (FecB) FROM BOOROOLA IN BULGARIA

Economic analyzes of management in sheep farming in our country show that the main share of income, between 50% and 80%, depending on the breed, comes from the sale of lambs for meat. In this aspect, an important factor for achieving profitable production in the modern sheep farm is obtaining a greater number of born and weaned lambs, respectively the level of the fecundity characteristic. The fecundity of the Ovis species varies widely - from 1 to 6-7 lambs at one birth. Reproductive traits are characterized by a low coefficient of heritability (h^2). For most reproductive performance traits, heritability values are low and variable, around 0.1. This is due to the relatively small part of the additive genetic diversity in terms of reproductive traits. For this reason, it is considered that selection for these characters is not sufficiently effective. A certain increase in fecundity is achieved when cross-breeding methods are applied, as a result of the heterosis effect, and this is used in appropriate selection schemes, with the aim of consolidating the increased level of the trait in subsequent generations. The greatest successes in terms of increasing fecundity are achieved through genetic improvement. The number of lambs born at one birth was until recently considered a quantitative trait determined by a large number of genes. For most breeds and lines, this explanation is considered correct even now, but for some of them, such as the Booroola breed in Australia, some Icelandic, Pacific and new breeds, in recent years new hypotheses have been built and theoretical clarifications have been made (Venev & Stoykov, 2002). The most significant progress in genetic improvement of fecundity has been achieved in Australia, where the Booroola breed was created. Studies on the gene for high fecundity in sheep of this breed are of great interest, motivated by the idea of a relatively quick and effective way to increase fecundity.

In Bulgaria in 1988, 1351 doses of deep-frozen seminal fluid from two male breeders of the Booroola breed - No. 61 and No. 377, originating from the "Booroola" - Haldon flock in New Zealand (Boykovski et al., 2018) were imported. In the progeny evaluation carried out at the Heldon station, it was found that 80% of the daughters of breeder No. 61 had an ovulation rate of 3 and more eggs, and for ram No. 377 - 85% of the female offspring.

The process of creating inbred structures (lines) with high fecundity begins with the introduction of the FecB gene from Booroola in sheep from the North East Bulgarian Merino breed (NEBM) - Shumen type, bred at the Research Center for Agriculture in Targovishte. The idea of the breeders is that the predicted genetic progress on this trait will subsequently be transferred to the entire population of the NEBM breed. For the successful introduction of genetic variability for high fecundity, intrauterine insemination with cryopreserved sperm was performed by laparoscopy after pre-synchronization of oestrus. Inseminated were 274 sheep according to the scheme "♀ NEBM x ♂ Booroola" and 35 sheep according to "♀F1 (♀NEBM x ♂ Bo) x ♂Bo". The realized biological fecundity of the sheep was 152.21 lambs from 100 ewes obtained according to the scheme "♀ NEBM x ♂ Bo" and 220.00 from "♀F1 x ♂Bo" (Boykovski et al., 2002). Different levels of trait and line studies have been reported in the offspring of individual breeders. The ewes inseminated with ram No. 377 on both maternal bases (♀ NEBM) and ♀ (NEBM x Bo) realized higher fecundity 164.8 and 240.00, and those inseminated with material from ram No. 61 respectively 134.80 and 213, 33 lambs from 100 ewes. As a result of the introduction of the FecB gene from the multi-fruited Booroola breed into the genetic structure of the flock in Research Center for Agriculture - Targovishte, two breeding lines were formed, originating from the homozygous for this gene (BB) breeders No. 61 and No. 377. By using appropriate methods of breeding is aimed at increasing the fecundity of sheep and preserving the FecB gene, reducing the adverse impact of the introduction of the Booroola gene on the live weight and wool production of the animals in the following generations. The rearing of lambs from multiparous ewes creates technological problems, due to lower birth weight and reduced vitality, but this does not reduce the importance of high fecundity, as the main productive trait with great economic weight (Boykovski et al., 2018).

The exterior of the Booroola rams and ewes does not differ from the typical Australian Merino, of the "Medium" type, with a medium fine fleece (19-23 μ), an average staple length of 9 cm, an average live weight of 50-55 kg and a shear of unwashed wool 5.5 kg (Boykovski et al., 2009). Desired fecundity, according to Boykovski et al. (2009) is 2-3 lambs. Intensive rearing systems in the US and Israel produce more than 3 lambs. In New Zealand, they apply a scheme to combine high fecundity with good precociousness and meat-producing qualities in the Dorset breed. 1/8 blood ewes are obtained from homozygous rams (BB) and a backcross is used (Boykovski et al., 2009). An important condition for successful work in this direction is the discovery of animals that carry the gene for multiple fecundity. To a large extent, the number of lambs born from an ewe is determined by the number of ovulated eggs. These can practically be established by enumeration of the corpora lutea after slaughter or in vivo by laparoscopy.

Dimitrov (1997) studied the effect of the implemented schemes and presented results for the fecundity of the sheep from the flock in the Research Center for Agriculture - Targovishte (Table 1). The highest fecundity is the ½ Bo crosses at 2.5, 3.5 and 4.5 years of age - 202% on average, and the lowest - purebreds from the NEBM breed - 114%. The increase in fecundity is 88%. Animals with ¼ Bo occupy an intermediate position in this regard.

Table 1. Fecundity of different Booroola-blooded ewes (Dimitrov, 1997)

Groups	Age of ewes, years		
	2.5	3.5	4.5
	x	X	x
NEBM-purebred	1.125	1.303	1.000
½ Bo-blooded	2.000	1.932	2.125
¼ Bo-blooded	1.622	1.690	1.825

Data on the live weight of the studied animals are shown in Table 2. At weaning, the half-breeds with the lowest live weight were 26.74 kg, and the purebreds with the highest - 29.89 kg. At 18 months, half-breeds have 10.56 kg lower live weight than purebreds. This trend in live weight differences was maintained at 2.5 and 3.5 years of age. Animals ¼ Bo-blooded occupy an intermediate position, but at all ages studied, they have a significantly lower live weight than purebreds. Kumar et al. (2008) found that genotypes with the FecB allele had a significant genetic effect ($P < 0.01$) on live weight of lambs from birth to 12 months of age, in crossbred Garole x Malpura sheep.

Table 2. Live weight (kg) of different-blooded ewes at different ages (Dimitrov, 1997)

Groups	Age at			
	Weaning	18 months	2.5 years	3.5 years
	x	x	x	x
NEBM-purebred	29.89	61.95	67.60	71.32
½ Bo-blooded	26.74	51.39	55.97	61.55
¼ Bo-blooded	27.84	54.04	62.67	64.54

As a result of the crossbreeding, Dimitrov (1997) found changes in the levels of the wool production trait (Table 3). Half-breed crossbreds at 1.5 years of age have 2.127 kg lower wool production compared to purebreds from the NEBM breed. This difference is also confirmed at later ages. At 2.5 years of age, the wool production of half-breed crosses was 1.656 kg lower, and at 3.5 years of age by 1.008 kg, compared to that of purebred sheep of the NEBM breed. Quarter blood crosses occupy an intermediate position in this respect at the three studied ages. The clean wool yield of half-breeds is 3.31% higher than that of purebreds. In terms of clean fiber, the results were opposite, purebreds outperforming half-breeds by 0.635 kg. This is a logical consequence of the decrease in wool yield with the introduction of FecB.

Table 3. Influence of Booroola blood on wool production, clean wool yield and clean fiber (Dimitrov,1997)

Groups	Wool production at, kg			Clean wool yield, % at 2.5	Clean fiber, kg at 2.5
	1.5 years	2.5 years	3.5 years		
	x	x	x	X	x
NEBM-purebred	8.436	8.007	7.587	49.86	3.978
½ Bo-blooded	6.309	6.351	6.579	53.17	3.343
¼ Bo-blooded	7.430	6.849	7.177	51.67	3.439

Detailed results on the effects of crossing North-Eastern Bulgarian Thin Fleece sheep with Booroola rams have been published by Dimitrov (2001). He found that with an increase in the percentage of blood from the Booroola breed, the level of the biological fecundity trait increased significantly, with a slight negative effect on wool yield and the amount of pure fiber. The conclusions drawn by the author are in line with the findings of Young et al. (1991), that sheep obtained from a cross with Booroola realized about 5% lower live weight, produced on average 6% less clean fiber, but gave with 20% more weaned lambs compared to the starting forms.

Dimitrov (2001) considers that not only purebred breeders of the Booroola breed can be included in schemes for increasing biological fecundity, but also heterozygous variants (B+), judged by offspring and carriers of this gene. In the period 1989-1997, a number of studies were carried out in the Research Center for Agriculture - Targovishte, with the aim of making a comparative analysis of the crossbreeds ½ Booroola and ¼ Booroola with the purebred sheep of the NEBM breed. The obtained results regarding productive traits with greater economic weight and the degree of undesirable effects in some schemes outline the direction of breeding work with the Booroola breed. Analogous results regarding the experience with the introduction of the FecB gene in Hungary were reported by Laszlo et al. (1987). They use 13 Booroola rams to cross with the local Merino sheep. F1 crosses have a lower live weight at 100 days of age by 6 to 28%, at one year of age by 4 to 10% and at wool cut by 1 to 8% compared to the parent breed. At 22 months of age, 501 daughters of 3 imported rams realized 131.5% fecundity, 20% higher than that of the source breed - Hungarian Merino. Cenkova (1990) reported similar results in our country. These studies confirm that a substantial and sustained increase in the biological fecundity trait can be achieved through genetic improvement with the introduction of FecB from Booroola. According to most researchers of this process, the high fecundity rates give a significant negative effect on live weight, wool production and clean fiber in animals, the product of cross compared to purebred sheep.

Dimitrov (2001) investigated the effect of the inclusion of FecB for high fecundity in the genetic structures of the NEBM breed on the growth intensity of lambs and their fattening abilities (Table 4). He found that the average daily gain of purebred lambs of the Shumen inbred type of NEBM, fattened up to 40 kg of live weight was 2.55% higher than that of ½ blooded crosses with the Booroola breed. The age at final live weight of the ½ blooded crosses was 1.19% higher. The same tendency was found in terms of consumption of nutrients in feed units and digestible protein per 1 kg of growth. In crossbred animals, it was greater by 4.44% and 1.18% compared to purebreds. In ¼ Bo-blooded crossbreeds, the levels of the studied traits were close to purebreds and occupy an intermediate position.

Table 4. Influence of the FecB gene on growth intensity and fattening capacity (Dimitrov, 2001)

Groups	n	Average daily gain, g	Age up to 40 kg live weight, day	Consumption for 1 kg gain	
				Feed units	Digestible protein, g
NEBM	16	241	173.1	3.38	399
NEBM x Bo	16	235	176.4	3.53	417
NEBM x ½ Bo	16	240	174.0	3.42	403

Boykovski et al. (2002) provided data on the average increase in the number of offspring obtained when applying each individual breeding scheme (Table 5). The authors make a comparison between the theoretically expected effects and the actually obtained results, differentiated by schemes.

Rams No. 61 and No. 377 are homozygous for the FecB gene (BB) with 80.00% and 85.00% of their breeding value daughters respectively having an ovulation rate of 3 or more eggs, or an average of 82.50%. Theoretically, in a cross with sheep of the Shumen inbred type of the North East Bulgarian Merino breed, without this gene (++), all the offspring obtained (½ Bo) are carriers of one copy of FecB (B+) and the average increase in biological fecundity is hypothetically expected in them to be 82.50%.

Table 5. Average increase in number of offspring obtained from the used mating schemes (Boykovski et al., 2002)

№	Mating scheme ♀ ♂	Lambings	Biological fecundity, n	Increase in the number of offspring, n	Increase in biological fecundity	
					Theoretically, %	Practically, %
1	(NEBM x NEBM)	Average from 4	1.177	-	-	-
		including 1st lambing	1.138	-	-	-
2	(NEBM x Bo) (½ of Bo)	Average from 4	2.045	0.868	82.50	86.80
		including 1st lambing	1.962	0.824	82.50	82.24
3	(NEBM x ½ Bo) x (NEBM x ½ Bo) (½ of Bo)	Average from 4	1.863	0.686	74.25	68.60
		including 1st lambing	1.838	0.700	7425	70.00
4	(NEBM x ½ Bo) (¼ of Bo)	Average from 4	1.721	0.544	41.25	54.40
		including 1st lambing	1.648	0.510	41.25	51.00
5	¼ Bo x ½ Bo (⅜ of Bo)	Average from 4	1.938	0.761	61.87	76.10
		including 1st lambing	1.750	0.612	61.87	61.20
6	(½Bo x Bo) (¾ of Bo)	Average from 4	1.864	0.687	123.75	68.70
		including 1st lambing	1.942	0.804	123.75	80.40

The results obtained show a real increase of 86.80% on average from four years, incl. on the 1st - with 82.24%. Using heterozygous Booroola rams (B+) on the same foundation (NEBM) or (++), theoretically only half of the progeny are heterozygous carriers of FecB (B+), and average productivity should increase by 41.25%, but in practice, an increase of 54.40% was realized.

After about 3 generational intervals, Dimitrov (2006) carried out a new study on the influence of the Booroola bloodline in the genetic structure of the North East Bulgarian Merino sheep breed - Shumen type, raised in the Research Center for Agriculture - Targovishte. The object of the study was the weight development of the animals from weaning to 2.5 years and the biological fecundity of the 1st and 2nd lambings. Live weight measured at different ages was higher in purebred ewes by 3.04% to 10.05% compared to crosses carrying FecB. A tendency for a gradual reduction of differences in live weight with increasing age of the animals was established, and at 2.5 years the advantage in favor of purebreds was only 3.04% (Table 6).

Table 6. Influence of Booroola blood on live weight (kg) (Dimitrov, 2006a)

Groups	Age at							
	100 days		9 months		1.5 years		2.5 years	
	n	x	n	x	n	x	n	x
NEBM-purebred	453	28.68	406	39.97	345	55.47	206	58.26
NEBM x Bo	271	26.06	251	37.05	200	53.72	134	56.54

The fecundity of purebred sheep at 2.5 and 3.5 years was 117.1% and 111.4%, respectively, and for animals with Booroola blood - 141.5% and 137.5% (Table 7). In another study, Dimitrov (2006) investigated the influence of the Booroola-blooded breed on the wool production and natural wool length at 1.5 and 2.5 years of age of sheep of the Shumen inbred type of the NEBM breed. Similar to the previous studies, purebred sheep were found to have a higher amount of unwashed wool and clean fiber than those with Booroola blood at 1.5 and 2.5 years of age (Tables 8, 9). Clean wool yield was higher by 3.82% in Booroola crosses compared to purebreds (Table 9).

Table 7. Influence of Booroola blood on fecundity (Dimitrov, 2006a)

Groups	Age at			
	2,5 years		3,5 years	
	n	x	n	x
NEBM-purebred	228	1.171	158	1.114
NEBM x Bo	142	1.415	96	1.375

Table 8. Influence of Booroola blood on wool productivity and natural wool length (Dimitrov, 2006b)

Groups	Wool productivity at, kg				Natural wool length at, cm			
	1,5 years		2,5 years		1,5 years		2,5 years	
	n	x	n	x	n	x	n	x
NEBM	389	8.846	288	7.741	392	10.29	309	9.61
NEBM x Bo	231	7.647	152	6.624	233	10.41	180	9.59

Table 9. Influence of Booroola blood on clean wool yield and clean fiber (Dimitrov, 2006b)

Groups	Clean wool yield at, %				Clean fiber at, kg			
	1,5 years		2,5 years		1,5 years		2,5 years	
	n	x	n	x	n	x	n	x
NEBM	237	48.32	233	49.1	237	4.286	233	3.798
NEBM x Bo	130	52.01	125	52.9	130	3.889	125	3.462

In practice on farms, it is difficult to calculate the ovulation rate in sheep, so it is recommended to use the data on the number of lambs born in the lambing to judge breeders by progeny. The number of offspring is a more widely used and accessible method, but as a criterion it has a significantly lower reliability for proving the bearing of FecB for fecundity compared to the ovulation rate. It is characteristic of the action of the FecB gene that with respect to the trait ovulation rate there is an additive, and for the trait number of offspring born - a dominant action (Laleva et al., 2000). Laleva et al. (2000) found the presence of FecB in ewes of the Thracian thin-wool breed, daughters of rams crossed with Booroola. The rate of ovulation and number of lambs born at 1.5 and 2.5 years of age of the studied sample was determined. The ovulation rate at 1.5 years in crossbreds is significantly higher - 2.875 than in purebreds (1.500), with statistically significant differences. These differences are also reliably confirmed at 2.5 years of age. The data show that the ovulation rate (NO - number of corpora lutea) is 1.3 higher in sheep, the product of a cross of the two ages studied. Laleva et al. (2000) found no significant differences in live weight at birth between crossbred and purebred ewes. In a similar study involving live birth weight and growth intensity of Booroola × Merinos d̓Arles crosses, Abella et al. (2005) concluded that these traits were not affected by genotype effects, respectively by the presence of the FecB gene. Similar data were published by Fogarty et al. (1995) in Poll Dorset × Booroola crosses.

Slavov et al. (2008) found an increase in genetic variance when including genetic components from the Booroola, Australian Merino and Ile de France breeds in the NEBM-Dobrudzha type population. Laleva et al. (2014) published the results of a comparative study of the traits ovulation rate, fecundity and live weight at birth in ewes of the Thracian fine-fleece breed and its crosses with Booroola. Daughters of FecB (B+) heterozygous rams No. 4012 and No. 509 were found to outperform purebreds in terms of ovulation rate. At 1.5 years, the ovulation rate in the former is 2.875 and 2.560, respectively, and in purebred animals of the Thracian fine-fleece breed it is 1.526. The results obtained for the other studied age are similar. The sheep, a product of the cross, also realized a greater number of lambs born, compared to their purebred counterparts, except for the daughters of ram 509 at 4.5 years. High variation is characteristic of this trait. Laleva et al. (2014) reported C = 46.51% variation in fecundity of

Thracian fine-fleece sheep at 4.5 years. The results obtained and the fact that the authors did not find a significant negative effect on the live weight at birth feature allowed them to confirm the positive effect of the introduction of FecB from Booroola to increase the biological fecundity of sheep from the same productive line. Stancheva et al. (2020a) found that the genetic components of the Booroola breed had a negative effect on live weight at weaning, at 9 months and at 18 months in sheep of the North east Bulgarian Merino breed. The main part of the genetic variation of the traits related to wool productivity is due to the individual characteristics of the individuals (Stancheva et al., 2020b). The same authors claim that the variability of traits caused by the combination of genes from another breed in the genotype and the heterosis effect were very low.

CONCLUSIONS

The Australian Booroola Merino (BoM) is a valuable genetic resource for increasing the biological fecundity trait in fine-wool and other sheep breeds worldwide. The economic effect of the introduction of the FecB gene for high fecundity in sheep has been confirmed by a number of authors over a period of over 30 years. It has been established that crosses with Booroola achieve up to 30% higher ovulation rates and 20% more lambs after weaning compared to the purebred animals.

The positive effect of the introduction of genetic variability from Booroola in the Bulgarian fine-fleece breeds of sheep was established in terms of increasing the ovulation rate and the number of lambs born, with insignificant negative effects on live weight and wool production.

The analysis of the genetic variability of the intrabreed linear structures, carriers of the FecB gene in the only nucleus flock of sheep from the North East Bulgarian Merino breed - Shumen type, bred in the Research Center for Agriculture - Targovishte shows that the real increase in the biological fecundity of the F1 crosses NEBM x Bo is with 86.80%, with a theoretically expected 82.50%. Optimal reproductive performance in the nucleus of the breed is a basic condition for the extension of genetic progress for the fecundity trait throughout the population.

Achieving a balance between benefit and risk in the introduction of the FecB gene from Booroola is an important condition for increasing the economic effect of animal husbandry and creating sustainable production in sheep farms.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Agricultural Institute - Shumen and Institute of Agriculture - Karnobat and also was financed from Project Zh No. 158 of Agricultural Academy - Sofia.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

INNOVATIVE TECHNOLOGIES FOR FISH BREEDING WITH MINIMAL IMPACT ON THE ENVIRONMENT

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Abstract

This study refers to the common carp (Cyprinus carpio), which is an adaptable species that enriches the variability of quantitative and qualitative characteristics and increases genetic diversity. Local aquaculture populations of common carp, called "landraces," have developed due to different environmental conditions and breeding efforts. However, the introduction of carp in some areas has led to negative impacts on natural aquatic ecosystems. To improve the quality of economically important fish species, the variation in morphological, physiological, and biochemical characteristics is utilized. In this study, a patent application for a system for reproduction, selection, and growth of fish fry with the simulation of natural conditions is described. The article explains the method used to replicate the natural aquatic environment and create viable products with high genetic adaptability to its conditions. The process falls into the category of extensive aquaculture, promoting sustainable aquaculture by increasing the percentage of ecological and environmentally friendly productions. The study concludes with the results and the development of a set-up of the station for laboratory use.

Key words: *aquaculture, artificial inteligence, future, technologies.*

INTRODUCTION

One of the most adaptable species in the wild and aquaculture conditions, the common carp enriches the variability of quantitative and qualitative characteristics and increases its genetic diversity.

Local aquaculture populations of common carp have developed within the species due to different environmental conditions, the efforts of fish farmers to breed them, and the relatively small size of the breeding population due to systems of strictly closed breeding grounds. Different genotypes were specifically developed after the middle of the last century and are referred to as "landraces" (Bakos, 1979). These populations are adapted to the local environment and have a high level of genetic diversity.

The introduction of *Cyprinus carpio* in many areas has led to a significant development of aquaculture, and carp farming now plays an important role in the economies of many countries. On the other hand, in some developed countries, such as the United States and Australia, where the species is not consumed outside the poorer segments of society, it is considered a pest (Dowal, 1996) and significant efforts have been made to eradicate it.

The negative impact of *Cyprinus carpio* on natural aquatic ecosystems is clearly observed in their behavior, such as uprooting and destroying aquatic plants as a result of their feeding habits (Laird, 1996). Carps also increase water turbidity by digging and mixing the top layer of the bottom, which decreases light penetration and destroys macrophyte populations in spawning grounds of photophilic species (Star, 2011; kerutokoi.com).

The main purpose of fish breeding is to improve existing breeds and hybrids and to develop new breeds, thereby increasing their productivity. To improve the quality of economically important fish species, their variation in many morphological, physiological, and biochemical characteristics is utilized. A significant proportion of this variation is heritable, and its value is very high in fish populations, which helps in the application of fish selection methods.

Compared to domestic animal husbandry, aquaculture is a relatively young science in China and India, having been in vogue for a long time, but the domestication of fish and the creation of breeds that differ from their wild parents in terms of high productivity traits

actually began only a few centuries ago. With the notable exception of the golden crucian carp, ornamental carp, and perhaps the common carp, few fish can be considered domesticated, even though some strains of trout, for example, are much more adapted to hatchery conditions than their wild counterparts (FAO, 1985).
Among the representatives of *Cyprinus carpio* suitable for aquaculture activity, only the common carp has been bred for a sufficiently long period, and distinct breeds of this species have been developed through selection. In the USSR, these include the Ukrainian carp, Ropsha carp, first-generation hybrids of domestic carp and Amur Wild carp, Nivchan carp, Central Russian carp, Kazakh carp, Kasnodar carp, Belarusian breed, and Parra breed (Kirpichnikov, 1981). In Israel, there is the carp "Dor-70" (Wohlfarth, 1980), and in Hungary, the Hungarian strain (Bakos, 1979).

MATERIALS AND METHODS

In order to carry out this work, the data that formed the basis of the patent application titled "System for Reproduction, Selection, and Growth of Fish Fry with the Simulation of Natural Conditions" was centralized. The patent is registered with the Romanian State Office for Inventions and Trademarks.

Materials

The block diagram of the installation for fish breeding and selection, as presented in Figure 1, describes the setup used inside the laboratory.
The tanks are made of glass and filled with water from the breeders` aquatic environments.

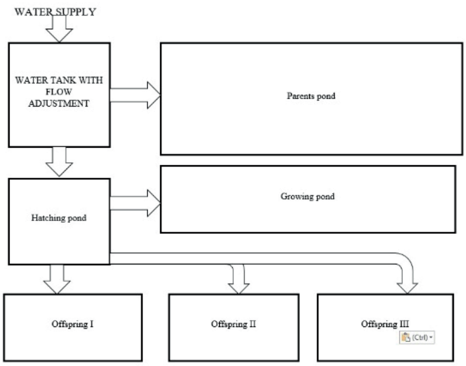

Figure 1. Block diagram of the installation for fish breeding and selection

A hydrological station is used to measure and monitor the qualitative parameters of the reference aquatic environment in order to replicate it in the reproduction station (Figure 2).

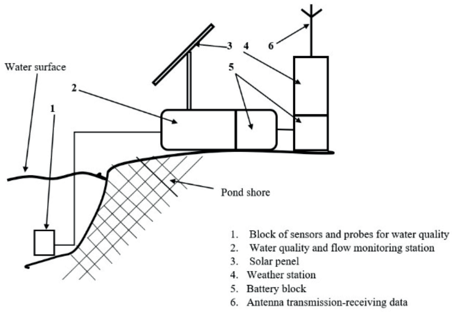

Figure 2. Installation for monitoring parameters of aquatic environments

Three specimens of *Cyprinus carpio* that have reached sexual maturity are represented by the biological material.

Method

Portions of the aquatic environments from which the breeders come and where the offspring will be released are mapped out to be created inside the reproduction station (Figures 3, 4).

Figure 3. Overall map of fish farm for catch and release fishing (Google maps)

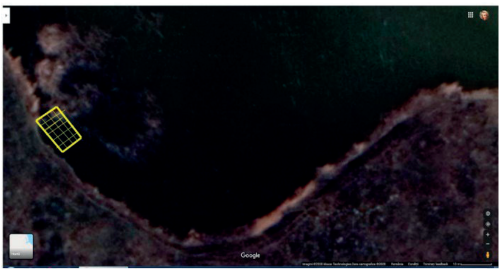

Figure 4. Map focusing on the area of interest with the exact area to be replicated clearly marked (Google maps)

All the materials used to replicate the natural aquatic environment are purchased from the reference aquatic environment, aiming to create viable products with high genetic adaptability to its conditions.
By applying these techniques, the process falls into the category of extensive aquaculture, at most semi-intensive, promoting sustainable aquaculture by increasing the percentage of ecological and environmentally friendly productions.
It is especially important that the food is ecologically sound, with the possibility of carrying out a sustainable and efficient activity for the conservation of resources.
The installation for monitoring parameters of the aquatic environment has been installed on the shore of the lake that provides for the breeders.
By virtue of the application of new technology, namely the ability to multiply carp at any time of the year and to apply genetic analysis on the parents and offspring, electroanesthesia was used to anesthetize the adult specimens, both the female and the three males (Șerban, 2020).
The applied method proved to be of real help, allowing the individuals to not be injured during manipulation, but it also led to the elimination of the semen without interfering with the pituitary gland. The observed semen was collected on a smear and analyzed in the physio-pathological analysis laboratory of USV Iași, with the result being the eligibility for reproduction, ascertaining the maturity and the ability to reproduce.

RESULTS AND DISCUSSIONS

After putting all the information together, we developed a set-up of the station shown in Figure 5 for laboratory use.

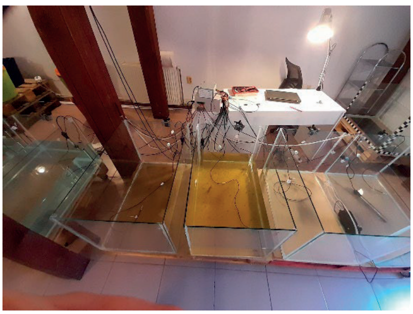

Figure 5. Laboratory set-up for hatching (original)

The selected biological material is represented by three *Cyprinus carpio* individuals: a female ornamental carp variety Koi Doitsu Kin Matsuba (Figure 6) and three male common carp varieties with scales, mirror, and topless (Figure 7).

Figure 6. Koi Doitsu Kin Matsuba under laboratory conditions (original)

Figure 7. Aquaculture carp, var. mirror under laboratory conditions (origanl)

The ornamental carp variety Koi Doitsu Kin Matsuba comes from a controlled environment, with special feed being administered during the growth process to maintain the color and exceed the standard growth parameters, an important aspect in aquaculture (Bhaskar, 2015).
Feeding was carried out four times a day during the periods when the water temperature was between 18-28°C, and gradually, depending on the evolution of the temperature, a single ration per week was applied with the commercial feed, which falls qualitatively into the premium class for ornamental fish. The qualities that recommended this feed were the presentation in floating extruded form, with 4mm granulation, and the nutritional composition of 37% protein and 8.5% fat.
The female Koi Doitsu Kin Matsuba was 13 months old, 25 cm, and 900 g at the time of purchase and transfer to the laboratory. It was in perfect health. For the last 5 months, it has been kept in a glass aquarium with 130 l of water from its place of origin. It has been fed the same food as before acquisition, and only aeration and

filtration operations have been applied to the aquatic environment. Filtration was applied during the summer, and now, with the aquatic environment temperature at 8-9°C, filtration is no longer necessary and feeding is applied once every 7 days. After 5 months in the laboratory, the specimen's metric indices are 31 cm and 1600 g, which falls within the limits of growth rates of ornamental carp (Table 1).

Table 1 Growth rate of ornamental carp*

Age (years)	Total length (m)	Body mass (kg)
0.5	0.12	0.05
1	0.23	0.3
1.5	0.32	1.1
2	0.39	1.4
2.5	0.45	2.25
3	0.51	3.15
3.5	0.55	4
4	0.59	4.95
4.5	0.62	5.7
5	0.64	6.55
5.5	0.67	7.3
6	0.69	7.86
6.5	0.71	8.48
7	0.72	9.03
8	0.73	9.5
9	0.75	10
10	0.76	10.4

*Adapted after koi owner`s notes (original).

The specimen purchased for the present study comes from a group of fifty ornamental carp of various varieties, all of the same age, purchased in Hungary. During its development, from the age of three months, it was kept in an outdoor pool on EPDM sheeting (Ethylene-Propylene-Diene-Monomer mixed with carbon black, oils, vulcanizing agents, and other auxiliaries). Great importance was given to transparency in order to observe the reactions of the carp and to identify any possible dysfunctions in the new aquatic environment.

Regarding stress, the female did not show extreme forms of manifestation, like the specimens of common carp that were brought to the laboratory. Used to the high transparency of the aquatic environment, it had no specific reactions to stress after transport or during handling during the study (e.g. no jumps when cleaning the filter).

The males selected for the present study come from an extensive rearing system, wherein feeding is almost non-existent, and food is provided by the natural productivity of the accumulation into which they are released in autumn, after being harvested from the pre-development ponds. This system of growth lends itself to accumulations with very large surface areas of water, where it is not profitable to invest in feed or incentives for the natural productivity of the pond.

Under these conditions, the fish are closer to the category of wild ones, exhibiting some characteristics in this regard. Carp raised in an extensive system differ from the others by the orange color of the lower part of the body, high tonicity, and vigorous appearance. At the time of harvesting, they are manifested by strong jumps and kicks that have a prolonged duration and a high intensity compared to common carp intensively raised, for example. Thus, due to this behavior and their vigor, they only lasted a maximum of 72 hours in the laboratory. Due to the strong impact on the walls of the aquarium, they presented parameters incompatible with life within a maximum of 3 days.

The three males selected to be transported to the laboratory - the scaled, mirrored, and topless varieties - were bred in June 2021 and are currently "parked" in a rearing pond at the collaborating farm. To ensure that there is no risk of losing the parents, they are kept in the conditions of the farm. They will only be brought to the laboratory for the application of reproductive procedures and the collection of samples for genetic analysis.

Carp length and body mass are parameters of interest in aquaculture, and in the extensive rearing system, the nutritional quality of the final product tends to be as close as possible to that of wild carp. As a result, Tables 2 and 3 highlight the standards encountered in wild carp and the limits of the recommended parameters in aquaculture, respectively.

Table 2 Growth rate of wild carp*

Age (years)	Total length (m)	Body mass (kg)0,
1	0.15	0.088
2	0.22	0.286
3	0.29	0.640
4	0.37	1.190
5	0.43	1.893
6	0.48	2.495
7	0.52	3.192
8	0.57	4.108
9	0.61	5.156
10	0.65	6.042

*After Papadopol, cited by Kaszoni, 1974.

Table 3 Development periods and body mass of aquaculture common carp*

Period	Duration	Body mass (g)
Larval	3–7 days	0.025-0.05
Juvenile	15-30 days	0.2-1.0
Fingerlings	45-85 days	25-50
Youth	120-170 days	250-500
Adult	120-170 days	>1 000
Breeder	2-4 years	>4000
	1-2 years	>3000
	0,75-1 years	>2000

*According to FAO, 2022 and Bud & Diaconescu, 2010 – adapted.

By comparison, it is easily seen that wild specimens weigh more than 1,000 g at the age of four years, while in aquaculture systems this weight is reached much earlier, after the second summer, at 16-17 months.

Specimens considered eligible for the present study fall within the standard measurements recommended in aquaculture: 27 cm and 1500 g (with scales), 27 cm and 1450 g (mirror), and 28 cm and 1500 g (topless). The three males are part of the same batch, and the same development technologies were applied to them; the differences are only registered as an aspect of their own evolution in the extensive system.

The semen obtained from the parents is divided into three equal parts and evenly distributed in the three aquatic environments that are the subject of the study: the aquatic environment of males, females, and bottled water.

These environments are in three aquariums, with each aquarium being introduced to 150 liters of water from each environment.

To create the necessary conditions in nature, *Pinus sylvestris* branches are placed as evenly as possible to cover as much of the available surface as possible, providing support for the adhesion of the fertilized eggs so that they do not pass underneath (Figure 8.).

Gauze "baskets" were created inside each aquarium and attached to the edges of the aquarium with special glass hooks in order to achieve aeration, swirling, and filtration inside the aquatic environment without disturbing the spawn attached to the pine branches.

Aeration (Figure 9), filtering, and heating (Figure 10) operations were applied to each environment.

As seen in Figure 11, the aerators used had adjustable flow and direction, making it possible to swirl the water under the gauze "basket".

Figure 8. Set-up for fertilized spawn (original)

On the opposite side, the filters were mounted, which also had the aeration function. Thus, the aquatic environment was swirled in both directions, ensuring the necessary conditions for the development of the larvae. On the same side as the filter was positioned the thermometer, which had an adjustment for the temperature value. By swirling the water on the opposite walls of the aquarium, the temperature was kept constant throughout the mass of the aquatic environment, avoiding large temperature fluctuations that are very dangerous for the development of future offspring.

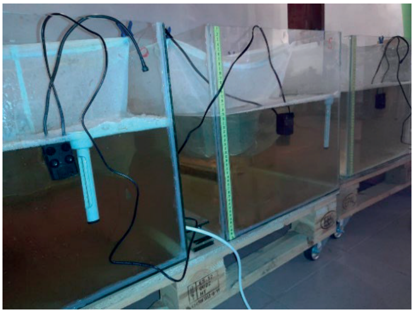

Figure 9. Aeration method applied to aquariums (original)

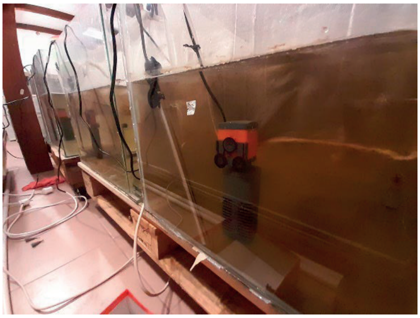

Figure 10. Method of filtering and heating aquariums (original)

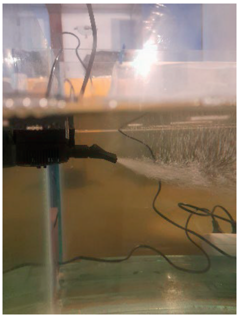

Figure 11. Aerator in operation (original)

To collect the data and create the database, a program specially developed for the used installation was used. It allows the user to set the time period during which the recordings are made, as well as the minimum and maximum limits of the monitored parameters, and it issues warnings regarding the recorded fluctuations (Figure 12.).

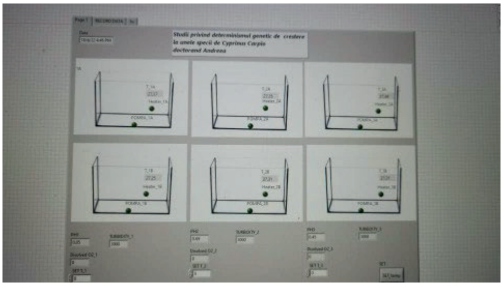

Figure 12. Software used in data collection (original)

After collecting the data, a sufficient database was created to conclude the effects of the techniques and technologies applied in the study at the aquaculture laboratory level.

CONCLUSIONS

The study describes the development of an installation for the reproduction, selection, and growth of fish fry with the simulation of natural conditions. The installation uses a hydrological station to monitor and replicate the qualitative parameters of the reference aquatic environment and biological material from three *Cyprinus carpio* individuals. The female is an ornamental carp variety Koi Doitsu Kin Matsuba, and the three males are common carp varieties. The feeding is carried out with a special feed, with high nutritional value, and is applied gradually, depending on the temperature of the water. Electroanesthesia was used to anesthetize the adult specimens, both the female and males, and the observed semen was collected on a smear and analyzed for eligibility for reproduction. The results showed that the setup can successfully replicate natural conditions for fish breeding, selection, and growth, promoting sustainable and environmentally friendly productions. The study's implication is that the findings can contribute to sustainable aquaculture and the conservation of resources. Further research is needed to explore the potential of the installation and the possibilities of genetic analysis.

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NUTRITION

Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

PEAS (*Pisum sativum* L.) AND LUCERNE (*Medicago sativa* L.) A VALUABLE PROTEIN SOURCES AS ALTERNATIVES TO SOYBEAN MEAL IN SWINE NUTRITION - A REVIEW

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Abstract

This review aims to evaluate partially substitution of soybean meal with peas (Pisum sativum) and lucerne (Medicago sativa) in pigs' feeding. Peas and lucerne offer favourable nutritional profiles, containing substantial levels of protein, essential amino acids, and dietary fibre. The two ingredients can be economically viable alternatives to soybean meal due to their relatively lower cost and availability in certain regions, reducing feeding costs, thus benefiting pig producers. Partially substituting soybean meal with peas and lucerne can alleviate the environmental burden associated with soybean production, because it is required less land and water resources, and their cultivation has a lower environmental footprint, making them sustainable choices for pig feed formulation. Although peas and lucerne may contain certain anti-nutritional factors, such as trypsin inhibitors and phytates, which can interfere with nutrient utilization and digestion in pigs, a proper processing technique, such as heat treatment or enzyme supplementation, are necessary to mitigate the negative effects of these compounds and improve nutrient availability. The palatability of the diet may be influenced by factors such as flavour, texture, and aroma, therefore a careful formulation and gradual introduction of these alternative protein sources can help ensure adequate feed intake and prevent any potential negative effects. The digestibility of protein and other nutrients of peas and lucerne can be lower compared to soybean meal, which may impact nutrient utilization and growth performance. However, optimization of formulation, feed processing, and enzyme supplementation can help overcome these challenges and improve nutrient digestibility.

***Key words**: alfalfa, alternatives, peas, pigs, protein, soybean meal.*

INTRODUCTION

According to FAOSTAT (2021), the first five global soybean crop producers are Brazil, United States of America (U.S.A.), Argentina, India and China. The European Union (EU) demands for plant proteins, both for food and feed purposes is increasing and its self-sufficiency rate in terms of soya crude proteins is relatively low, at approximately 5%. As a result, the EU heavily relies on imports, primarily sourced from South America, to meet its demand for soya (Debaeke et al., 2022). Overall, the EU's initiatives are to reduce soya imports and promote its own production of protein-rich crops trying to enhance self-sufficiency (Rauw et al., 2023). On the other hand, a more appropriate solution will be to replace partially if not totally soybean meal (SBM) with other protein options sources for food and feed, sort of "protein transition", to reduce negative environmental impacts, and to promote sustainable agriculture practices and animal welfare (Duluins et al., 2022). As of 2021, the European Union (EU) had a livestock population consisting of 142 million pigs, 76 million bovine heads utilized for meat and dairy production, 62 million sheep, and 12 million goats. Following pig meat, poultry is the second most widely consumed meat in the EU. In 2013, the EU raised approximately 891.4 million broilers, and as of 2021, there were 376 million laying hens (EU feed, 2023). The impact of Avian Influenza and COVID-19 caused Romania a decline of 5.9% compound feed production, and the presence of African Swine Fever (ASF) affected pig feed production in 2020 Romania by a significant decline (-4.5%) while other European countries overcome these obstacles and achieve substantial growth in pig feed demand (https://fefac.eu).

In 2020, Italy, Serbia, France, and Romania emerged as the primary soybean producers within the expanded European Union (EU), but when considering continental Europe as a whole the main soybean-producing nations were Russia and Ukraine (Debaeke et al., 2022). The primary utilization of soybeans involves their extraction into oil and soybean meal. Almost 75% of the global soybean meal production is designated for pigs or poultry feeding (Singh & Krishnaswamy, 2022). The soy protein is notable for its essential amino acids content: lysine, threonine, and tryptophan with high digestibility for pigs compared to other protein sources. Also pig diets, soy products serve as a significant source of energy, soybean meal containing digestible and metabolizable energy levels comparable to corn (Stein et al., 2013). According to National Research Council (NRC, 2012), the content of crude protein (CP), gross energy (GE), digestible energy (DE), metabolizable energy (ME), net energy (NE) of soybean meal and other soybean products present different values: full-fat soybeans (37.56% CP, 5227 kcal/kg GE, 4193 kcal/kg DE, 3938 kcal/kg ME, 2874 kcal/kg NE), dehulled SBM (47.73% CP, 4256 kcal/kg GE, 3619 kcal/kg DE, 3294 kcal/kg ME, 2087 kcal/kg NE), nondehulled SBM (43.90% CP, 4257 kcal/kg GE, 3681 kcal/kg DE, 3382 kcal/kg ME, 2148 kcal/kg NE), extruded expelled SBM (44.56% CP, 4692 kcal/kg GE, 3876 kcal/kg DE, 3573 kcal/kg ME, 2344 kcal/kg NE), enzyme treated SBM (55.62% CP, 4451 kcal/kg GE, 3914 kcal/kg DE, 3536 kcal/kg ME), fermented SBM (54.07% CP, 4533 kcal/kg GE, 3975 kcal/kg DE, 3607 kcal/kg ME), soy protein concentrate (65.20% CP, 4605 kcal/kg GE, 4260 kcal/kg DE, 3817 kcal/kg ME, 2376 kcal/kg NE), soy protein isolate 84.78% CP, 5386 kcal/kg GE, 4150 kcal/kg DE, 3573 kcal/kg ME, 2187 kcal/kg NE). Hence, while soy remains an important protein source for pigs, it is crucial to explore alternatives that can effectively lower feeding costs and support the pork industry and farmers.

Tilman et al. (2002) consider that the challenging is to maintain the sustainability, in terms of both pollution and resource depletion, if we take into consideration that protein holds vital nutritional importance as nitrogen is an essential component of DNA, RNA, and protein (Smil, 2002a).

The objective of this review was to identify and compare experimental studies realized on pigs in which soybean meal was partially or totally introduced by different inclusion dietary percentages of peas and lucerne.

MATERIALS AND METHODS

To fulfil the review's objective a number of 37 bibliographic studies from literature were consulted. Relevant articles were identified from scientific databases with keywords: protein, alternatives, soybean meal, peas, alfalfa, pigs. As searching strategy, we used to identify relevant studies by accessing information from web engines, selection-based search engines, and web portals, such as: Google Scholar, CiteULike, BASE, Microsoft Bing, MetaCrawler, Scopus, Web of Science - Core Collection, UOW Library SEARCH, Library Databases.

RESULTS AND DISCUSSIONS

PEAS (*PISUM SATIVUM*) POSSIBLE ALTERNATIVE TO REPLACE PARTIALLY SOYBEAN MEAL IN SWINE DIETS

Field pea (*Pisum sativum* L.) is an important source of dietary starch (approximately 300 g/kg) and protein (approximately 200 g/kg), with lower levels of crude protein (CP) and lysine (Lys) compared to SBM, but higher levels than cereal grains, more fiber than SBM, corn, or wheat (Woyengo et al., 2014). This is accompanied by the highest net energy (NE) value, likely due to its digestible starch and fermentable fiber content, according to NRC (2012). Stein et al. (2010) noticed that young pigs fed with 600 g/kg of raw field pea, replacing corn and SBM, had a constant feed intake, but their growth was reduced during the first two weeks and throughout the entire 28-day trial. Petersen et al. (2014) suggest that peas can be used as a substitute for soybean meal (SBM) in swine diets, leading to reduced feed costs. To enhance its nutritional properties, it is recommended to process field pea by grinding it and subjecting it to cold-pelleting (70-75°C), steam-pelleting (80-85°C), or extrusion (115°C), followed by re-grinding (Hugman et al., 2020). Heat treatment can effectively eliminate heat-labile protease inhibitors and lectins, thus improving nutrient digestibility of field pea and enhancing the growth performance of pigs. Leguminous seeds have the potential to contain trypsin inhibitors. Pigs can tolerate trypsin inhibitor levels of up to 3.0 trypsin inhibitor units/mg or 4.7 mg trypsin inhibitor/g in their diet (Woyengo et al., 2017). Trypsin inhibitor activity causes negative effects such as decreasing growth and feed efficiency (Chubb, 1982), causing pancreatic hypertrophy (Green & Lyman, 1972), and increasing endogenous protein loss with decreased exogenous protein absorption (Barth et al., 1993) therefore trypsin inhibitor must be denatured by heat and catalytic decomposition.

Figure 1. Enhance pea's nutritional properties by cold-pelleting, steam-pelleting and extrusion

Hugman et al. (2020) noticed that the substitution of 300 g soybean meal/kg and 100 g wheat/kg with 400 g field pea/kg in the diet of weaned pigs resulted in decreased apparent total tract digestibility coefficients for GE and CP, as well as the predicted NE value, an increase in average daily feed intake (ADFI), but did not show a corresponding increase in average daily gain (ADG), a reduced feed efficiency. Therefore, the heat processing is not necessary for optimal growth in nursery pigs (10-21 kg). Field pea can be a source of both starch and amino acids while maintaining the growth performance of weaned pigs. Landero et al. (2014) studied on 260 pigs (average 8.5 kg live weight) one week after weaning (19 days old) up to 300 g of soybean meal (SBM) per kilogram and 100 g of wheat per kilogram with 100, 200, 300, or 400 g of field pea/kg (*Pisum sativum* L., subsp. *Hortense*) in two phases: phase 1 - two weeks (1 to 14 days), to phase 2 - three weeks (15 to 35 days). The study findings indicate that up to 400 g per kilogram field pea can be used as a complete replacement for soybean meal (SBM) in nursery diets, provided that the diets were formulated to have equal net energy (NE) value and standardized ileal digestible (SID) amino acid content.

LUCERNE (*MEDICAGO SATIVA* L.) POSSIBLE ALTERNATIVE TO REPLACE PARTIALLY SOYBEAN MEAL IN SWINE DIETS

Alfalfa (*Medicago sativa* L.) is a perennial forage characterized by an abundant biomass production, favourable nutritional composition, and adaptability, utilized in animal feed as hay, meal, and silage. Alfalfa (lucerne) is highly valued for its rich protein content, which possesses a well-balanced amino acid profile. Additionally, alfalfa contains essential vitamins, carotenoids, and certain growth and reproduction factors, further enhancing its nutritional value for animals (Shi et al., 2014). Alfalfa exhibits the highest CP yield per hectare (2,000 to 3,000 kg), surpassing soybean, peas, and wheat. However, due to its composition rich in cellulose, hemicellulose, and lignin, which are fibrous components that are difficult to digest by monogastric animals, dried lucerne is unsuitable as a feed ingredient for certain young "high-performance" animals such as hens, chickens, calves, and piglets. Zijlstra & Beltranena (2007) stated that alternative protein sources can be distinguished based on their origin: feedstuffs derived from crop production and raw materials obtained through crop fractionation, including by-products and co-products The first group suitable for swine feeding include pea bean (*Pisum sativum*), lupin (*Lupinus* spp.), common bean (*Phaseolus vulgaris*), sunflower (*Helianthus annuus*), faba bean (*Vicia faba*), grass pea (*Lathyrus sativus*), and dehydrated alfalfa meal (*Medicago sativa*). The second group, crop fractionation, include corn and wheat gluten meal, concentrated potato protein, and wheat and corn distiller's dried grain. By combining both groups of alternative protein sources are reduced the need for additional dietary energy and amino acid supplementation.

The crude protein level is a crucial indicator of the nutritional quality of forage, and alfalfa stands out with a relatively high content of crude protein (18% to 20%) and a balanced composition ratio of amino acids, including lysine, tryptophan, with high digestibility. Notably, alfalfa is rich in lysine (0.80%) and methionine (0.23%) (Blume et al., 2021). Several studies were realized by supplementing alfalfa in piglet's diets, Adams et al. (2019) administrated 12% alfalfa and noticed positive effects concerning ADG and ADFI, piglets diarrhea incidence decreased; alfalfa meal 5% decreased the piglet diarrhea and mortality incidence (Liu et al., 2018), the positive effects of 5% fiber alfalfa was registered by an improved ADG, FCR, short-chain fatty acids (SCFAs) and abundance of cellobiolytic and anti-inflammatory bacteria which reduced the diarrhea (Sun et al., 2021).

Freire et al. (2000) supplemented 20% alfalfa meal in weaned piglet's diets and registered a decreasing digestive transit time and NDF and ADF digestibility, a dry matter apparent digestibility, nitrogen and energy.

Other authors found that alfalfa inclusion in pigs' diets had positive effects on growth, reproductive performance, health condition, and meat quality (Lindberg et al., 1995). This is attributed to several mechanisms. Firstly, the dietary fibre present in alfalfa can stimulate intestinal peristalsis, increase the activity of digestive enzymes, and promote the growth of beneficial bacteria in the intestines. This fermentation process produces short-chain fatty acids, which improve intestinal health. Furthermore, the high-quality protein and amino acids found in alfalfa contribute to improved animal health conditions. The presence of vitamins and mineral elements in alfalfa also plays a vital role in various physiological functions within the pig's body. Additionally, bioactive molecules present in alfalfa have been shown to enhance antioxidant and anti-inflammatory levels. Overall, supplementation of pig diets with alfalfa has been found to enhance reproductive performance in sows, improve growth performance in piglets, and enhance production performance and pork quality in growing-fattening pigs.

Since its introduction in France, a specific livestock concentrates known as PX 1 (leaf lucerne concentrate) has been utilized for particular animal species. This concentrate is nutritionally advantageous for monogastric animals like pigs, primarily due to its low indigestible fibre content and high levels of protein, vitamins, and pigments (Zanin,1998). Additionally, poultry benefits greatly from PX 1 due to its pigmenting properties, which enhance the coloration of chicken meat and egg yolks. When pigs are fed a daily dosage of 6-8 grams per kilogram of body weight of leaf Lucerne concentrate, they experienced an enhanced growth rates due to its low fibre content which contributes to its high protein digestibility (84%). A reduced faecal pollution, as the animal absorbs a relatively lower amount of nitrogen for an equivalent protein intake was noticed

Bourdon et al. (1980) demonstrated the effectiveness of PX 1 as a protein supplement for pig growth in a wheat-based diet, when incorporated at 10-20% levels, a digestible energy value of 3,735 kcal/kg DM (compared to 4,000 kcal/kg DM for soybean) and a metabolizable energy value of 3,322 kcal/kg DM (compared to 3,750 kcal/kg DM for soybean). The apparent utilization coefficient of digestible energy, at 72.7%, slightly lower (by 2%) compared to soybean oil cake. Consequently, when combined with wheat, lucerne LC serves as an ideal protein supplement for pig growth, partially or entirely replacing soybean cake.

Saponins, which are glycosides, possess a bitter taste, and their concentration in lucerne leaves is double that of the stems. As the plant matures, the saponin content diminishes. Poultry are more vulnerable to the effects of saponins compared to pigs. When saponin levels reach 0.4-0.5% in poultry feed, it leads to reduced feed consumption, decreased egg production, and weight loss in birds. This is because saponins interact with the physicochemical properties in the gut, inhibiting the uptake of specific nutrients such as monosaccharides and cholesterol (Cheeke & Shull, 1985).

Symptoms associated with the consumption of lucerne meal in non-ruminant animals, such as chicks and pigs, include anorexia, listlessness, gastroenteritis, and weight loss. The primary concern in these animals is the retardation of growth rate, primarily attributed to reduced feed intake. Lucerne meal contains antinutritional factors, including tannins, which are present in concentrations ranging from 0.1% to 3.0%. The high tannin content in lucerne meal negatively affects cellulose activity and consequently impairs the digestion of crude fibre. Additionally, tannins decrease the digestibility of dry matter, protein, and other nutrients (Ramteke et al., 2019).

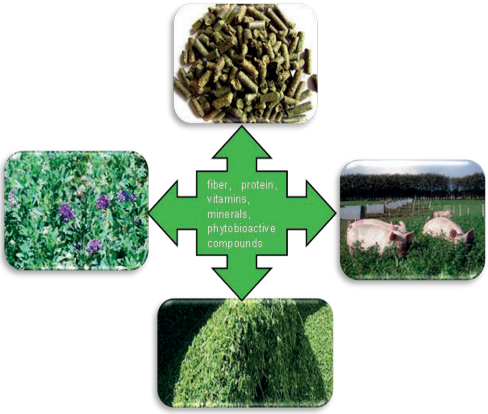

Figure 2. Alfalfa nutritional value in pigs' diet

Relying heavily on a single protein source like soybean meal can influence negatively the lack of diversity in the pig's diet. This can limit the range of nutrients available to the animals and increase the risk of nutritional imbalances. Anti-nutritional factors in soybean can interfere with nutrient absorption and digestion. Introducing new protein alternatives such as peas and alfalfa provides a broader array of nutrients, promoting a more balanced diet for the pigs. Peas and alfalfa offer different nutritional profiles compared to soybean meal (Laudadio et al., 2012). They are rich in protein, amino acids, and other essential nutrients, making them suitable alternatives for meeting the nutritional requirements of pigs. Therefore, by diversifying protein sources, we can potentially enhance the nutritional value of the pigs' diet.

CONCLUSIONS

Exploring new protein alternatives like peas and alfalfa can help overcome these challenges and provide a safer and more digestible protein source for pigs. The cost of soybean meal can vary due to factors such as market conditions, trade tariffs, and transportation costs. Finding alternative protein sources can provide more options for pig farmers, allowing them to choose cost-effective protein alternatives based on regional availability and pricing.

In conclusion, finding new protein alternatives to replace soybean meal in pigs' diets promotes dietary diversity, reduces environmental impact, improves nutritional value, addresses allergenicity and anti-nutritional factors, and offers potential cost savings for pig farmers.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Ministry of Research, Innovation and Digitization and also was financed from Project PN 23-20.01.01.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

THE INFLUENCE OF WALNUT KERNEL CAKE ON THE DIGESTIBILITY OF NUTRIENT SUBSTANCES FROM THE COMBINED FODDER INTENDED FOR YOUNG SOWS

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Abstract

The work presents the results of the study of the chemical composition of walnut kernel cake and its influence on productive performance, digestibility and the exchange of nutrients in young piglets. When using 4%/t of walnut cake in the feed of the sows, the cost price of 1kg of combined fodder decreased by 1,7 euro cents, an average daily increase of 613g was obtained, with the digestibility of the dry substance - 86.8%, crude protein -79.2%, crude fat - 62.3%, crude cellulose - 62.0%, organic substance - 88.4%, and when using 8%/t the cost price of 1kg of combined fodder decreased with 3,5 euro cents and the indices were 608 g - average daily gain, digestibility of dry matter - 85.4%, crude protein - 78.5%, crude fat - 69.1%, crude cellulose - 37.3%, organic matter - 87.2%.

Key words: *chemical composition, digestibility, specific consumption, walnut kernel cake, young piglets.*

INTRODUCTION

In the Republic of Moldova, is highlighted the need for efficiency of animal nutrition by using non-traditional feed resources with a pronounced nutritional effect and which can certainly reduce the cost of pork and ensure a greater benefit to pig farmers. The main challenge in intensive pork production is high productivity and efficient use of fodder (Makhaev et al., 2016). With the aim of increasing the productivity of pigs and the efficiency of fodder conversion, new ways and products are sought that would optimize the digestive processes and metabolic changes in the pigs' body (Kaisyn, 2010; Coshman et al., 2011). The organization of animal feeding must ensure the conditions for the physiological and morphological adaptation of the digestive system to the efficient use of fodder and the optimization of the microbiological processes of digestion. It is very clear the importance of identifying the requirements of the animals in nutrients and avoiding excess excretion of nutrients. Finding new solutions to increase the ecological productivity of pigs is one of the main tasks of nutrition specialists (Dinu et al., 2002). At the same time, the correct management of many wastes would allow not only to protect the environment, but also to reduce the cost price of nutrition rations, at the same time it could lead to obtaining an economic income and partially solving the ecological problem. The practical application of new fodder sources for feeding animals requires an in-depth study of the chemical composition and nutritional value, conducting digestibility studies, studying the impact on production, blood and economic indices (Danilov & Donica, 2017; 2020). In the Republic of Moldova, in order to provide animals with protein of plant and animal origin, large quantities of soybean meal, fish meal, skimmed milk, meat and bone meal and others are imported from abroad, at high prices. Taking into account all this, the diversification and increase of the assortment of protein fodder sources for the animal husbandry sector is an ever-present problem in the Republic of Moldova. In this context, it is necessary to find new effective alternative nutritional solutions for partial, total or complementary substitution of these raw materials with new local protein sources.

Nut growing occupies a significant place in the agriculture of the Republic of Moldova because statistical data show that the annual production of nuts is on average over 4.0-4.5 thousand tons. Currently, there are several small and medium enterprises operating in the republic specialized in the production of walnut kernel oil. After extracting the oil from the kernel, using the pressing method, a walnut cake is obtained, which is not further processed, and this product is a true storehouse of vitamins, minerals, amino acids and other valuable components for animals. As with any new ingredient, there are many questions about the nutritional benefits, limitations, and utilization of nut cake in animal feed for maximum economic value.

Regrettably, in the scientific and specialized literature, the information about the use of walnut kernel cake in the structure of combined fodder recipes intended for pigs is very limited or completely missing.

Starting from the premises described, the aim of the research was: to evaluate the nutritional potential of the walnut kernel cake and to study the impact of its use in the diet of young piglets, on the digestibility of nutrients, production performance, blood and economic indices.

MATERIALS AND METHODS

The experimental part of the investigations was carried out in the conditions of the physiological laboratory of the State Enterprise for Research in the Selection and Hybridization of Pigs "Moldsuinhybrid", Orhei district, and the chemical analyzes in the laboratories of the Scientific-Practical Institute of Biotechnologies in Zootechny and Veterinary Medicine.

In the research, the following were studied: the chemical composition of walnut kernel cake, combined fodder, excrement, the digestibility of nutrients, the morphological and biochemical parameters of blood, productive and economic indices depending on the share of walnut kernel cake in the combined fodder recipes. Physiological experience of digestibility was performed according to classical methods (Ovsyannikov, 1976), for which 9 biracial sows (Yorkshire x Landrace) were selected and divided into 3 groups: a control group and two experimental groups. According to the developed scheme, the sows from the control group were fed with basic combined fodder and in the combined fodder recipe from the experimental group I, soybean meal was substituted with walnut kernel cake in a proportion of 4%/t of combined fodder for the sows from experimental group II, soybean meal was substituted with walnut cake in a proportion of 8%/t (Table 1).

Table 1. Scheme of experience

Lot	Animal number (n)	Average weight (kg/head)	The mode of nutrition
Control	3	61.2	BCF - (recipe 1)
Experimental I	3	60.4	ECF * - (recipe 2)
Experimental II	3	61.6	ECF * - (recipe 3)

Note: BCF = basic compound feed, ECF*= experimental compound feed

The sows benefited from identical climatic conditions and maintained in special individual boxes, with the possibility of collecting the excrement of the sows (feces, urine). The combined fodder used in the experiment was made up of native fodder raw materials, and the concentration of nutrients was in accordance with the fodder norms for young breeding pigs.

Scientific research was carried out within the project: 20.80009.5107.12 "Strengthening the food-animal-production chain by using new feed resources, innovative sanitation methods and schemes" using the following methods.

The nutritional value of the nut cake, the combined fodder, as well as the droppings from the digestibility experiment was assessed according to classical methods (Petukhova et al., 1989): total moisture - according to general methods by drying, crude ash - by calcination, crude protein - according to Kjeldahl, crude fat - according to Sohlet, crude cellulose - by applying the Kirchner and Ganec method, calcium by the Pamberton method and phosphorus using the method described by Perov.

The sows were selected according to classical methods (Ovsyannikov, 1976).

The development of recipes for combined fodder intended for pigs was carried out based on the nutrition norms (Kalashnisov, 2003) by using the "HYBRIMIN" computer program. The specific consumption (feed conversion) was calculated based on the fodder administered, relative to the absolute increase in weight obtained during the record period (Petukhova et al., 1989). The digestibility of nutrients was assessed, using the calculation method, based on the data obtained about the chemical composition of the combined fodder, the amount of nutrients ingested with food and eliminated with excrement. The determination of the digestibility coefficients of the nutrients of the administered feeds was carried out according to the following formula:

$$\boldsymbol{DK} = \frac{(\boldsymbol{a}-\boldsymbol{b})*\mathbf{100}}{\boldsymbol{a}}$$

DKd - digestibility coefficient of nutrients
a - the amount of nutrients ingested with food
b - the amount of nutrients eliminated with excrement.

The analysis of the hematological parameters of the blood was performed using the biochemical analyzer STAT FAX-3300.

The statistical processing of the experimental data and the testing of the significance of the differences was carried out using the computer program EXCEL, using the classical methods (Plokhinsky, 1978).

RESULTS AND DISCUSSIONS

Preliminary investigations have shown that almost all the initial components of the walnut kernel can be found in the walnut kernel cake and the nutritional value can basically be described as raw material quite rich or dense in crude proteins and fats together with some minerals. It has been established that, depending on the thermal regime and the processing technology, the walnut kernel cake has a color variation from gray to dark brown, it is made up of pieces or powder of different sizes, without the presence of impurities, foreign particles and mold, it has a characteristic smell of walnut kernel and a bittersweet taste. The analysis of the chemical composition of the walnut kernel cake obtained by cold pressing showed that it contains: dry substance - 90.11%; nitrogen - 5.51%; crude protein - 344.5 g/kg; digestible protein - 306.6 g/kg; nutritional units - 1.29; crude cellulose - 62.69 g/kg; fats - 133.9 g/kg; metabolizable energy - 15.30 Mj/kg; calcium - 0.27% and phosphorus - 0.71%. The analysis of the quality characteristics and the chemical composition of the walnut kernel cake and the soybean meal highlights qualitative and quantitative differences of the walnut kernel cake, especially regarding the majority content of fats 133.90 g/kg compared to 78.6 g/kg times higher by 55.3 g/kg and minority of raw cellulose 62.69 g/kg compared to 76.10 g/kg or less by 13.4 g/kg.

Under the conditions of the combined fodder production section of State Enterprise "Moldsuinhybrid", using local ingredients, the experimental batches of combined fodder were prepared for the entire period of the experience according to the recipes in Table 2.

Table 2. Structure of compound fodder recipes

Ingredients	Group		
	Control	Experimental I	Experimental II
Maize	22.3	22.3	22.3
Barley	38.7	38.7	38.7
Wheat	24.6	24.6	24.6
Soybean meal	12.0	8.0	4.0
Walnut kernel cake	-	4.0	8.0
Chalk	1.0	1.0	1.0
Salt	0.4	0.4	0.4
Premix	1.0	1.0	1.0
Total	100	100	100

The data of the analysis of the chemical composition of the combined fodder used in the experiment showed that its nutritional value was: 14.6; 14.05; 13.94% crude protein, 11.90; 11.86; 11.85 Mj/kg metabolizable energy, crude fat 3.82; 3.62; 3.59%, crude cellulose 4.54; 4.48; 4.17%, non-nitrogenous extractives 70.14; 70.26; 70.63%, calcium 0.64; 0.70; 0.61% in absolute dry substance, which is within the limits of nutrition norms (Kalashnisov et al., 2003).

During the physiological digestibility experiment, the cost of 1 kg of combined fodder used in the control group was 30.1, in experimental group I - 28.4, experimental group II - 26.6 euro cents.

The results of the physiological experiment demonstrated that the replacement of soybean meal with walnut kernel cake in different proportions did not essentially influence the appetite and the intake of combined fodder during the actual experimental period. The average daily intake recorded during the physiological experience of digestibility was: 2,019 kg, 2,210 kg and 2,011 kg corresponding to the groups (Table 3). Evidence of the amount of feed consumed and excreta showed that the sows whose feed was supplemented with walnut cake in a proportion

Table 3. Ingest and excrete (medium/head)

Indices	Group		
	Control	Experimental I	Experimental II
Total ingestion, g	16152±81.171	17680±811.832	16088±1001.141
Average intake, g/day	2019±10.271	2210±101.479	2011±137.642
Total excretion, g	8373±487.584	7755±230.639	8525±541.119
Average excretion, g/day	1047±60.948	969±28.829	1066 ± 67.639
Total urine, ml	16394±2282.499	14610±4502.832	9585±1344.337
Urine, ml/day	2049±285.312	1826±562.854	1210±168.042

of 4%/t had a higher intake of combined fodder with 191 g and the proportion of 8%/t, provided an average daily intake of 8 g lower than that of the animals in the control group. At the same time, it was observed that the excreta was in larger quantities in the control and experimental group II, being respectively 1,047 kg/day and 1,066 kg/day. During the experimental period, a decrease in the amount of urine eliminated was observed at the sows from the experimental groups compared to the sows from the control group. At the beginning of the leveling period, the selected sows had very similar body masses, being on average: 60.4-61.6 kg and at the beginning of the record period of 63-64 kg. After processing the data of the digestibility test (Table 4) we found that the average values recorded for the evolution of body weight, achieved by the sows from the two experimental groups, indicate a superiority compared to those from the control group.

The data of the absolute and average daily increase obtained during the record period showed that the animals whose combined fodder was supplemented with walnut cake at the level of 4%/t of combined fodder (recipe 2) achieved an increase daily average amounting to 613 g being with 21 g higher, while the proportion of 8%/t (recipe 3) of 608 g times with 16 g higher than the sows in the control group. Sows from the experimental groups exceeded the control group according to this index by 3.55% and 2.70%, respectively. The best feed conversion index (kg combined fodder/kg weight gain) had the sows of the experimental group II which was lower than in the control group by 2.93%.

Table 4. Livestock dynamics and weight gain

Indices		Group		
		Control	Experimental I	Experimental II
Llive weight	at the beginning of the preceding period, kg	61.2±0.648	60.4±0.869	61.6±0.552
	at the beginning of the record period, kg	63.20±0.648	63.53±0.511	64.17±0.715
	at the end of the experience, kg	67.93±0.804	68.43±0.502	69.03±0.708
Spore	absolute, kg	4.733±0.163	4.900±0.308	4.867±0.108
	daily average, g	592±20.412	613±38.527	608±13.501
Feed conversion, kg fodder/kg weight gain, kg		3.41	3.61	3.31

The data on the evidence of ingestion and excretion, as well as the results of their chemical analyzes served as the basis for calculating the digestibility coefficients of the nutrients in the administered rations (Table 5; Figure 1). From the statistical processing of the data, a general trend of insignificant increase of these indices in the experimental groups is observed. The obtained results confirm that the animals of the experimental group I showed a higher dry matter digestibility than those of the control group by 1.98% and those of the experimental group II by 0.57%. A better protein digestibility was established at the sows from experimental group I, where it was by 2.09% higher than in the control group. At the same time, a lower fat digestibility was established in this group by 3.75% and in experimental group II this index was by 3.53% higher than in the control group. The digestibility test showed that the supplementation of the combined fodder with walnut kernel cake at the level of 4%/t favored a better digestibility of the raw cellulose, at a level of 62% or by 8.6% higher than at the sows from the control group.

Table 5. Digestibility of nutritional substances in experience, %

Indices	Group		
	Control	Experimental I	Experimental II
Dry substance	84.82±1.320	86.80±0.572	85.39±0.214
Azote	77.14±1.141	79.23±1.189	78.52±0.891
Crude protein	77.09±1.470	79.33±1.276	78.52±0.855
Crude fat	66.08±6.363	62.33±3.744	69.61±1.388
Crude cellulose	53.42±4.450	62.04±3.744	37.27±4.231
Ash	42.38±3.872	52.62±2.048	47.72±0.802
NNES	91.72±0.743	*93.15±0.210	92.58±0.150
Organic substance	86.76±1.204	88.40±0.510	87.24±0.185

Note: NNES = non-nitrogen extractive substances; *P<0.01

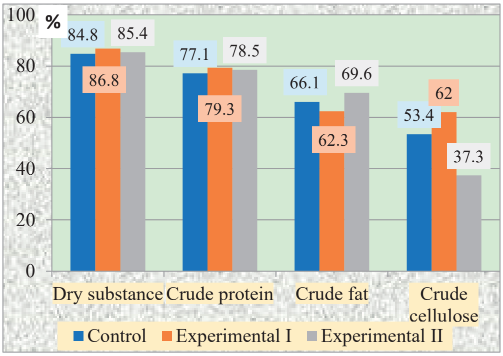

Figure 1. Digestibility of nutrients

From the group of organic substances, pigs make good use of non-nitrogenous extractive substances and in our research their digestibility was at a fairly high level with indices of: 91.72% in the control group and 93.15% the one that was with 1.43% (p<0.01) and respectively 92.58% with 0.86% more than the control group.

The digestibility of the organic substance in the experimental groups was 1.64% and 0.48% higher than at the sows from the control group.

The data on the evolution of the daily nitrogen balance prove that the sows in the experimental groups showed the best use of nitrogen both from the intake and from the digest. More nitrogen was ingested daily in experimental group I, being at the level of 43.32g, or by 6.75% more than in the control group.

The nitrogen balance was positive in all groups of animals, at the same time, by the sows from the experimental groups, more nitrogen was deposited from the ingested one, respectively by 3.64% and 7.01% (P<0.05) compared to this index at the sows from the control group (Table 6).

Table 6. Daily nitrogen balance in digestibility experience

Indices	Group		
	Control	Experimental I	Experimental II
Fed up with the fodder, g	40.58±0.206	43.32±1.989	39.21±2.683
Eliminated in faeces, g	9.27±0.544	9.02±0.856	8.39±0.254
Digested, g	31.31±0.732	34.29±1.307	30.82±2.442
Eliminated in the urine, g	5.04±0.724	4.68±1.298	2.65±0.609
Stored in the body, g	26.26±0.893	29.62±2.201	28.17±2.445
Stored in the body, %			
From the fed up amount	64.72±0.290	68.36±3.886	*71.73±2.115
From the digested amount	83.95±1.906	86.24±4.001	**91.34±2.088

Note: *P<0.05; **P<0.10

It was determined that the sows from the experimental groups showed the best use of nitrogen from the digested, being higher by 2.29% and 7.39% (P<0.10) compared to the control group. The percentage of nitrogen utilization from the ingested as well as the digested in the group fed with recipe 3 indicated statistically guaranteed values ($P<0.05$; $P<0.10$). The mineral balance data (Table 7; Figure 2) show us that the calcium intake at animals from experimental group I was higher by 2.60 g ($p<0.01$) and experimental group II by 2.34 g compared to the control group. The assimilation of calcium from the digested one was by 1.79% higher in the experimental group I and 3.82% in the experimental group II than at the sows from the control group.

Table 7. Calcium utilization in the physiological experience of digestibility

Indices	Group		
	Control	Experimental I	Experimental II
Fed up with the fodder, g	13.53±0.069	*16.13±0.741	15.87±0.881
Eliminated in faeces, g	5.61±0.404	6.23±1.806	8.33±0.760
Digested , g	7.92±0.466	9.90±2.068	7.54±0.044
Eliminated in the urine, g	4.42±0.732	5.55±1.867	3.92±0.777
Stored in the body, g	3.50±1.166	4.36±0.203	3.62±0.596
Stored in the body, g, %			
From the fed up amount	25.87±1.191	27.03±1.362	22.81±1.439
From the digested amount	44.19±8.461	45.98±7.226	48.01±3.938

Note:*P<0.01

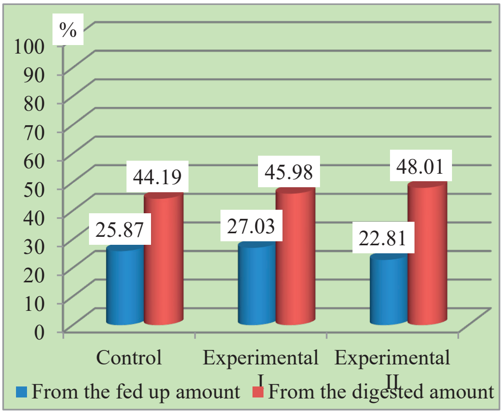

Figure 2. Calcium balance in experience

In the experimental groups, phosphorus, as a component of mineral metabolism, was eliminated from the body with fecal masses by 0.39 g and 0.66 g ($P<0.005$) more than by the sows from the control group. The use of walnut kernel cake in different proportions of the combined fodder recipes intended for sows did not have a negative impact on the blood morphological and biochemical profile. The results of the analyzes of the blood biochemical indices at the beginning and end of the experimental period demonstrated that all the animals were healthy and the blood indices did not show essential changes between the groups and were characterized by average values of the limits of the physiological norms for this category of animals (Table 8). According to the results obtained at the end of the experiment, improvements in protein and mineral metabolism were observed at animals from all groups. An increase in the content of proteins and albumins in the blood was reported at animals from all groups compared to the indices obtained at the beginning of the experiment (Figure 3).

Table 8. Average values of hematological indices at the end of the experiment

Indices	Unit of measure	Norm	Lot		
			Control	Experimental I	Experimental II
Hemoglobin	g/l	90-130	132.51±13.611	139.97±26.345	132.46±14.162
Erythrocytes	$x10^{12}$ g/l	5-7	7.2±1.435	8.233±1.096	7.667±0.852
Leucocyte	$x10^{9}$ g/l	11-22	8.4±0.787	8.70±1.812	10.10±1.412
ESR*	mm/oră	1.0-9.0	5.00±0.707	7.00±0.707	6.00±1.414
Lymphocytes	$x10^{9}$ g/l	35-75	63.67±3.894	71.33±2.483	68.00±5.612
Eosinophils	%	0-15	14.0±2.549	9.0±2.121	8.67±3.559
Unsegmented neutrophils	%	20-70	11.67±2.549	11.66±0.816	12.0±1.414
Segmented neutrophils	%	4-8	10.67±3.189	8.00±0.707	11.33±2.041

Note: * ESR- erythrocyte sedimentation rate.

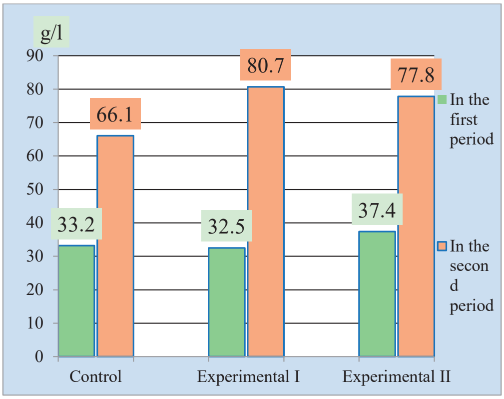

Figure 3. The amount of protein blood serum

The results of the biochemical analyzes of the blood serum showed that, at the end of the digestibility experiment, the total protein content was by 22.1% ($P< 0.10$) and 17.7% higher than in the control group. It was found that the albumin content in the blood serum at animals from all groups had close values, being at the level of 35.1; 28.5 and 35.5 g/l, respect-tively.

We find that the amount of blood creatinine at the end of the experiment was maintained in the range of 212; 193; 168 mmol/l correspond-ding to the groups, which fell within the limits of the reference values (70-208 mmol/l).

In our investigations, the level of uric acid was important, an important marker of the efficiency of the use of protein from food, which was in the range of 3.83 mol/l, 6.67 mol/l ($P < 0.10$) and 5.20 mol/l, this fell within the limits of admissible norms (2.8-8.8 mmol/l).

The investigated dynamics of serum alkaline phosphatase revealed average values of 144.85 u/l; 130.83 u/l and 148.20 u/l, which fell within the limits of physiological norms (41-176 u/l).

We consider that, due to the organoleptic qualities and the rich content of natural proteins, fats, mineral substances and the effect on growth and development, the walnut kernel cake can represent a viable solution to partially replace soybean meal in combined fodder recipes intended for sows.

CONCLUSIONS

It was found that the walnut kernel cake (obtained by cold pressing), has a color variation from gray to dark brown, is made up of pieces or powder of different sizes, without the presence of impurities, foreign particles and mold, has a specific smell of walnut kernels and a bittersweet taste. The nutritive value of the walnut cake used in this study was: dry matter - 90.11%; nitrogen - 5.51%; crude protein - 344.5 g/kg; digestible protein - 306.6 g/kg; nutritional units - 1.29; crude cellulose - 62.69 g/kg; fats -133.9 g/kg; metabolizable energy - 15.30 Mj/kg; calcium - 0.27% and phosphorus - 0.71%, and due to the organoleptic qualities and the rich content of natural proteins, fats, minerals and the low content of crude cellulose, can represent a viable solution for partial replacement of soybean meal in recipes of combined fodder intended for sows.

The results of the hematological investigations of the blood at the beginning and end of the digestibility test showed that the blood indices were within the limits of the reference norms. It was determined that the average daily growth rate was consistent with the digestibility of protein, cellulose and the balance of nitrogen, calcium and phosphorus in the ration.

The use in the feed of sows of walnut cake in the amount of 4%/t reduces the cost price of 1kg of combined fodder by 1.7 euro cents, ensures an average daily gain of 613 g, with digestibility of dry matter - 86.8%, crude protein - 79.3%, crude fat 62.3%, crude cellulose - 62.0%, organic substance - 88.4%, and the use of 8%/t reduces the cost price of 1kg of combined fodder with 3.5 euro cents, provides an average daily gain of 608 g, with digestibility of dry matter - 85.4%, crude protein -78.5%, crude fat - 69.1%, crude cellulose - 37.3%, organic matter - 87.2%.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

OPTIMIZATION OF THE FERMENTATION CONDITIONS AND SURVIVAL OF *Bacillus licheniformis* AS FREEZE-DRIED POWDER FOR ANIMAL PROBIOTIC APPLICATIONS

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Abstract

Preparations containing probiotic bacteria have a beneficial effect on animal health. The probiotics benefits translate into an increased interest in techniques for the preservation of microorganisms. In this study, the viability of Bacillus licheniformis (BL) ATCC 21424 strain, was evaluated in shake flask culture (Erlenmeyer 100 mL on shaking incubator) and batch 7-L stirred bioreactor under submerged fermentation (SMF), respectively. The inoculum was grown in a nutrient medium (37°C, 24 h±2 h, 200 rpm) and the viability was evaluated by 10-fold dilutions. The fermentation process in the bioreactor was examined at 37°C for 24 h under constant agitation (200 rpm). During SMF under controlled pH and oxygen availability, the cell growth rate was measured by optical density (OD 600 nm) at different interval times (6, 12, 18, 22 and 24 h). The maximum specific rate of BL in the exponential phase was calculated 0.524 h-1. When the stationary phase was reached, the OD in SMF increased, which was 2.01 times higher than that in flask culture. Without any cryoprotectant, the cell suspension was subjected to cold shock first and then freeze-dried. The proven survival rate of cells after freeze-drying was 90.65%. The viability of BL powder decreased only by 1.09 log (CFU/mL) vs. SMF, this resistance being also due to Bacillus spp. ability to sporulate. These results convincingly demonstrated that freeze-drying could be used in the preparation of BL ATCC 21424 strain as a lyophilized probiotic product with applicability in animal nutrition.

Key words: *animal nutrition, Bacillus, bioreactor, freeze-drying, probiotics.*

INTRODUCTION

Defined as “live microorganisms”, probiotics confer health benefits to the host when consumed in adequate quantities (FAO/WHO, 2007). Before probiotics utilization (Pandey & Vakil, 2017), culture bacteria must have the capacity to resist the harvest processing conditions (Dumitru et al., 2021). Moreover, the bacterial strains must retain functionality and viability during storage and transference as the lyophilized product (frozen or freeze-dried technique) with suitability for applications (Pandey & Vakil, 2017). Most probiotics sources are microorganisms from Gram-positive bacteria such as *Lactobacillus, Lactococcus, Streptococcus, Enterococcus, Bifidobacterium*, and *Bacillus* species (Pradipta et al., 2019).

It is known that loss of probiotic cell viability (CFIA, 2009) for long-term storage represents a major limitation factor (Weinbreck et al., 2010). Therefore, at the point of consumption, the viability of probiotic bacteria should contain a minimum level of 10^6 CFU/g (Mahmoud et al., 2020), respectively between 10^7-10^9 CFU/g at the time of ingesting to confer beneficial efficacy (Vidhyalakshmi et al., 2009). Further, probiotics must resist during gastrointestinal tract (GIT) passage, especially at low pH and aggressive intestinal fluids (bile salts and pancreatic juice), storage conditions (oxygen, high temperature, pH variations, relative humidity) and antimicrobial substances, which could determine the loss of cells viability (Cha et al., 2012; Dumitru et al., 2019; Dumitru et al., 2023). Instead, the above-mentioned criteria suggest that the selected strains are essential to be safe, viable and metabolically active within the GIT to involve beneficial results on the host. Moreover, these desirable characteristics facilitate the probiotic transition through the gut and enable bacteria proliferation and colonization (Divisekera et al., 2019).

As a good strategy to improve the viability of probiotics bacteria during processing, the encapsulation process represents an excellent substitute. Conditions optimization to achieve a dehydrated bacterial product with the possibility to restore its viability after rehydration represents the first step to extending the shelf life without changing the composition and undesirable properties that may appear during storage (Bolla et al., 2010). Several encapsulating techniques are used for the lyophilization of probiotics (Guo et al., 2022), but the most relevant are freeze-drying and spray-drying methods (Mahdi et al., 2020). Freeze drying or lyophilization is a drying process that trusts the sublimation of water in samples (Chantorn et al., 2022). It has been affirmed that is one of the most used procedures for the preservation of bacteria and concentrated starter cultures (Bolla et al., 2010). It is known that, during the lyophilization process, bacterial cells must face certain unfavorable conditions such as low temperature and low water activity, which could lead to decreased bacterial viability due to the damage of cell membranes and proteins (Chantorn et al., 2022). Moreover, the effectiveness of this bacteria preservation technique is up to 10 years (Harrison & Pelczar, 1963). Thus, in the drier form, the candidates' bacteria can be more easily utilized. In this context, the authors examined the viability of the *B. licheniformis* strain during the fermentation process and its subsequent exposure to the freeze-drying with the prior verification of the survival rate, in order to administer it as a source of probiotic product in animal feed.

MATERIALS AND METHODS

Bacterial strain, reagents and materials used

Bacillus licheniformis was delivered by American Tissue Culture Collection (ATCC 21424). The culture bacteria was reactivated in Nutrient broth medium (g/L: tryptone 10; meat extract 5.0; sodium chloride 5.0; pH medium 7.2 ± 0.2 before autoclaving), respectively agar medium (Merck) for cultural traits evaluation, followed by incubation in a shaker-incubator, 200 rpm, 37°C for 24 h. The inoculum was analysed by serial dilution (1:10 *v/v*) in 0.85% sterile physiological serum (SPS) for estimated the counts number (CFU/mL) viability (10^{12}-fold dilutions). From selected dilutions (10^8, 10^{10}, 10^{12}), 1 mL was well homogenized and spread on the nutrient agar plate. For each dilution, three replicates were done. The strain was preserved at -80°C with 20% sterile glycerol (*v/v*) and can be found in the Collection of National Research Development Institute for Biology and Animal Nutrition Balotești (INCDBNA), Romania, under the code IBNA 80.

Bioreactor Batch and Fermentation Process

The strain was fermented in a bench-top LAMBDA MINIFOR laboratory bioreactor. This model type is easy to handle and all-important cultural conditions can be measured and controlled. The minimum working volume was 2 L of the 7 L capacity of the bioreactor vessel. The inoculum (200 mL with a concentration of 10^{10} CFU/mL) was used as starter culture and expose to submerged fermentation (SMF) at 200 rpm, 37°C for 22 ± 2 h. The fermentation process was fitted with a temperature sensor, a rotation speed control and a pH sensor which maintained the medium constant at 6.5 ± 0.2 by two automatically peristaltic pomp [20% NaOH (*w/v*) and 1 N HCl (*v/v*)]. A peristaltic pump automatically adjusted the pH value by adding 20% NaOH, respectively 1N HCl. From time to time, as an antifoaming Antifoam 204 agent sterilized silicone oil (Sigma-Aldrich) was added as required (0.01%, *v/v*).

Freeze-drying process

The strain was subject to lyophilization procedure included the following steps: strain characterization (cultural, morphological, biochemical), bacterial biomass obtained after cultivation in nutrient broth (37°C, 18-24 h, 200 rpm) which was recovered and washed twice with PBS buffer (centrifugation 5000 rpm/10 min/4°C), freezing the sediment overnight at -20°C. As equipment was used a 4 L bench scale freeze dryer (Alpha 1-4 LSC basic, Martin Christ, Osterode am Harz, Germany). The process was performed at a pressure lower than 1.030 mbar (i.e., 0.110

mbar), with a condenser temperature of - 50°C for 18±2 h.

Determination of viable cell number

B. licheniformis cells was freeze-dried without protective agent. After freeze-drying, the powder strain was resuspended to the volume before freeze-dried (1:10, *w/v*) and rehydrating with PBS buffer solution. The viable cell number was determined immediately. The counts were enumerated as CFU per gram of powder (Log CFU/g).

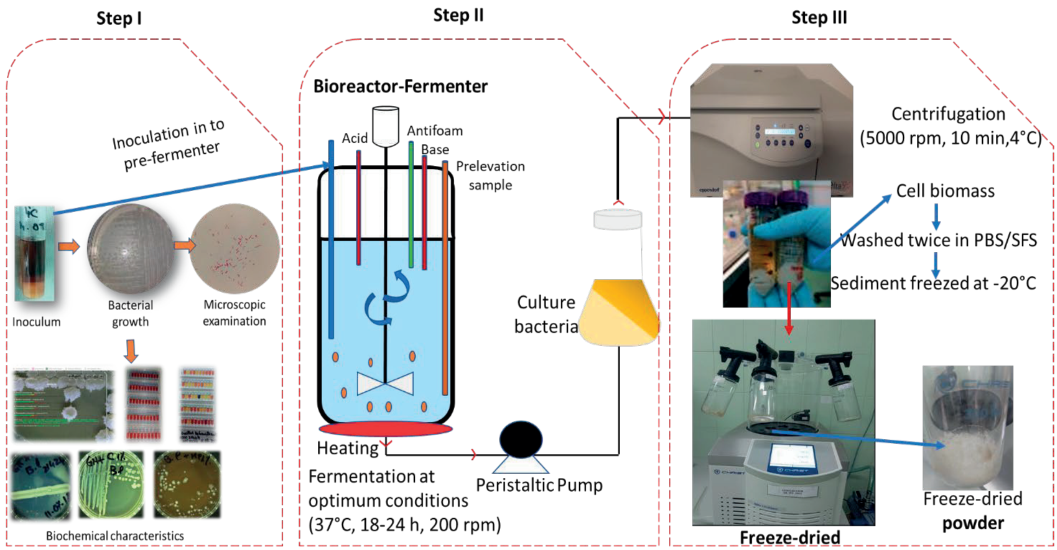

Figure 1. Bioprocess fermentation and lyophilization by freeze-drying

The survival rate was calculated as fallow: Survivability (%) = Log number of viable cells survived after freeze drying (CFU/mL)/Log number of viable cells before freeze-drying (CFU/mL) x 100.

Statistical Analysis

Variance analysis (one-way ANOVA) was used for statistical analysis of the data. All experiments were conducted in triplicate, with three independent measurements. Results are stated as mean values and standard deviation of the mean (SD). The graphics were generated using GraphPad Prism software V. 9.1.2 (Inc., La Jolla, CA, USA).

RESULTS AND DISCUSSIONS

Bacterial strain, reagents and materials used

The taxonomic characterization of *B. licheniformis* strain was detailed in other study (Dumitru et al., 2019a). According to literature data, a considerable group of bacterial probiotics is based on *Bacillus* spp. (*B. licheniformis, B. subtilis, B. cuagulans, B. amyloliquefaciens* etc.). These species are a field of rising scientific interest (Łubkowska et al., 2023). Further, when are added in animal feed, these bacteria provide numerous benefits, facilitate the digestibility, promotion the gut health (He et al., 2020), immune modulation, growth performance, and animal productivity index (Bernardeau et al., 2017; Qiu et al., 2021). Instead, due to the sporulation ability, *Bacillus* spp. form one oval endospore per cell making them to survive to the environmental stress and harsh conditions (Łubkowska et al., 2023). Furthermore, the results presented by Dumitru et al. (2019b) confirmed that the *B. licheniformis* spores presente tolerance and significant survivability in extreme simulated *in vitro* conditions (pH, bile salts, temperature, preservation, and storage). Moreover, the *Bacillus* group are a perfect model of microorganisms (Łubkowska et al., 2023) able to survive stabilization methods used in powder product generation such as freeze-drying (lyophilization), the method that was also used in the present study, which involves cell dehydration (Goderska, 2012; Kiepś & Dembczyński, 2022).

Bioreactor Batch and Fermentation Process

The fermentation process in a 7 L bioreactor was examined at 37°C for 24 h under constant agitation (200 rpm). During SMF under controlled pH and oxygen availability, the cell growth rate was measured by optical density (OD 600 nm) at different interval times (6, 12, 18, 22 and 24 h). The maximum specific rate of BL in the exponential phase was calculated 0.524 h^{-1}. When the stationary phase was reached, the OD in SMF increased, which was 2.01 times higher than that in flask culture (Figure 2).

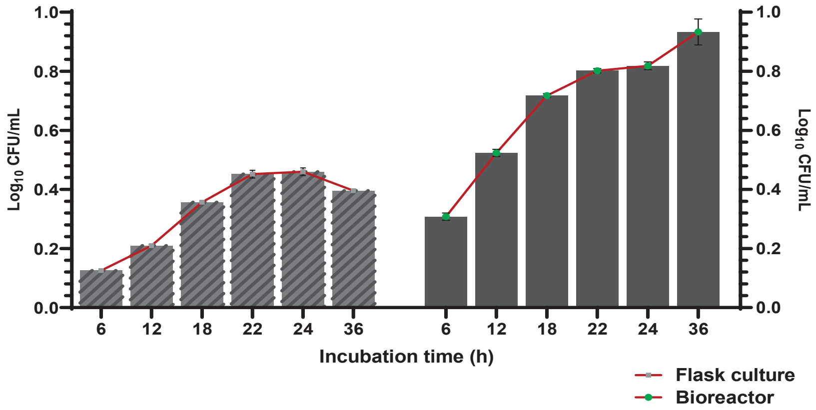

Figure 2. Optical density (OD 600 nm) of *B. licheniformis* strain during different conditions fermentation

This results indicates that SMF fermentation conditions prompted the strain viability *vs*. flask culture during 24 h of incubation.

The harvesting point was reached after 22 h in the bioreactor but with a double growth *vs*. flask cultivation where the pH conditions was non-regulated. Moreover, even from the beginning of the fermentations, the turbidity measurements in both cultivation models were different. At 6 h, the strain registered a point of 0.308 in SMF compared with flask where OD 600 nm was noted 0.126. After 36 h of incubation in the same conditions (37°C, 200 rpm), the cell cultivated without pH regulation established 11.00 Log_{10} vs. 11.70 Log_{10} in SMF, where the pH was 6.8.

Similar to Haindi et al. (2020), our culture strain under controlling acidification conditions began to present a smaller increase in turbidity as compared with the flask culture.

The controller pH, speed agitation (200 rpm), temperature (37°C) hold constant during entire fermentation in the bioreactor. In addition, it can be affirmed that, the pH control during SMF cultivation has a significant influence on strain growth rate.

Freeze-drying process

The experiment was designed to gain information on the cell survivability of a spore strain to produce viable probiotic powder. Without no protectant, the cell suspension was subjected to cold shock first (-20°C) and then freeze-dried for the viable cell number. The proven survival rate of cells after freeze-drying was 90.65%. The viability of *B. licheniformis* powder decreased only by 1.09 log (CFU/mL) *vs*. SMF, this resistance being also due to *Bacillus* spp. ability to sporulate. Further, as can be observed in Figure 3, *B. licheniformis* powder registered a decrease in survivability with 9.35% comparted to the fresh culture where cell number was 5 x 10^{11} CFU/mL.

According to literature data, a product containing probiotic organisms is more efficiently if it contains a number of viable cells higher than 10^6-10^8 CFU/g (Champagne et al., 2011; Dumitru et al., 2023). Insteed, more frequently, the probiotic bacteria used in animal nutrition are included in the form of dried biomass (Kieps & Dembczyński, 2022). Probiotic preparation in solid form, as powder, involve a strong stability and can be stored for a long period time comparatively than liquide suspensions (Kieps & Dembczyński, 2022). Regarding the drying method, freeze drying or lyophilization presents a multitude of advantages due to the maximisation and extend the viability of probiotic cultures. However, the method is very sensitive and damaging factors in drying the microorganisms must be considered. There are many studies which reported losses of strains viability during freezing technique (Chen et al., 2020; Silva-Carvalho et al., 2020; Luangthongkam et al., 2021). Comparatively with other genus, *Bacillus* group had the capacity to sporulate and the spore-ability involved high resistance to harvest environmental conditions, making this genus a good and strong candidate for developing efficent and stable probiotics products (Mari et al., 2014; Gotor-Villa et al., 2017).

The strain viability

The results on *B. licheniformis* strain growth after SMF fermentation and freeze-drying process was presented in Figure 3. As can be observed, the freeze-drying process decline the strain viability by 1.25% *vs*. SMF.

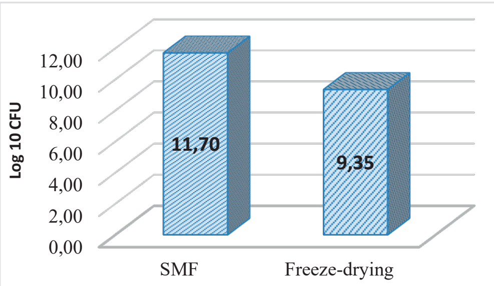

Figure 3. Effect of freeze-drying on *B. licheniformis* strain viability

In this study, satisfactory results were obtained regarding the lyophilization of the bacterial culture without the addition of cryoprotectant. If a decrease in cell viability was observed after freezing compared to SMF fermentation, the differences were not significant and the number of cells recorded was satisfactory.

Obtaining poor results in viability was probably caused by certain parameters such as the time of the drying process, which if it is too fast, the internal water can migrate outside the cell, and the frozen water inside the cell, ultimately leading to the loss of viability (Savedboworn et al., 2019).

Formulations can be completed through different methods including liquid and dry preparations (Gotor-Villa et al., 2017). Compared to liquid forms, generally, dried products, in our case obtained by freeze-drying are more feasible due to their ability to maintain the viability for a long period time of storage. In general, storage at 4°C determined the highest degree of cell viability for bacteria formulated as liquide cultures, but the shelf-life can be short at ambient or higher temperatures (Gotor-Villa et al., 2017).

Frequently, dried products involve lower viability rates because of thermal and dehydration stress found during the drying process (Melin et al., 2007). Besides, genus *Bacillus* is considered very amenable to drying because of its ability of spore production, which provides heat resistance (Yánez-Mendizábal et al. 2012). According to our results, supernatant medium without protectants can be used as medium for preserving *B. licheniformis* ATCC 21424 strain due to the number of living cells registered in the powder form after the freeze-drying method was applied. In addition, it can be stated that the selective culture medium retains, as much as possible, the nutrients necessary for the metabolism of the microorganism and the lyophilization process applied, without determining significant changes in the survival rate of the strain.

CONCLUSIONS

In conclusion, we could affirm that the *B. licheniformis* strain could be satisfactorily formulated with the freeze-drying technique. Nevertheless, this work presented and discussed the fact that the freeze-drying technique could be used in the preparation of the present strain, ensuring efficacy and stability as a lyophilized probiotic product with applicability in animal nutrition.

ACKNOWLEDGEMENTS

This study was funded by the Romanian Ministry of Agriculture and Rural Development through project ADER 8.1.7, Ministry of

Research, Innovation, and Digitalization through project PN 23-20.04.01 and supported by the program National Research Development Project to Finance Excellence (PFE)-8/2021.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

SILKWORMS PUPAE AS PROTEIN SOURCE FOR PIGS - A REVIEW

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Abstract

This study aimed to identify whether the silkworm pupae (SWP) added to pigs' diets can be considered a valuable protein-rich alternative source. The silkworm (Bombyx mori L.) and its by-products are highly valuable in terms of nutrition, medicine, and commerce. The SWP is the main by-product of the sericulture, considered a well-balanced source of nutrients in terms of proteins, lipids, minerals, vitamins and various bioactive substances such as fatty acids, peptides, and polyphenols with antioxidant, anticancer, cardiovascular, hepato-protective properties. The mulberry silkworm's potential for use in human food has been previously extensively documented, whereas the preponderance of literature data on SWP used in the livestock industry specific focus on poultry. There is limited pig-related data. In this study, we reviewed the most recently published papers on PubMed, Elsevier, MDPI, and Research Gate, using the keywords "silkworm pupae", "composition", "protein", and "pigs". We did not find any negative consequence on pigs' growth and health parameters in the reviewed published data.

***Key words**: feed, pigs, protein, pupae, silkworms.*

INTRODUCTION

Soybean meal, as the main feed plant protein source used in monogastric animal diets, has a considerable impact on the environment (Wiedemann et al., 2016; DiGiacomo & Leury, 2019). Furthermore, the availability of soybean meal for animal feed may be constrained because it is a component of the human diet and is not a highly productive crop.

According to the statistics (OEC; https://oec.world/), in 2020, Romania became the 32nd largest importer of soybean meal in the world (about $204M). Soybean meal was the 111th most imported product in Romania during the same year.

The protein level of soybean meal range between 40-49%, while lipid content varies between 0.5-3% (Banaszkiewicz, 2011).

Another important protein-rich source is fish meal (54-64% proteins, 8-14% lipids, and omega-3 fatty acids). The protein digestibility is comparable to silkworm pupae (SWP), and young pigs can easily digest it. The fish meal typically has no antinutritional factors. There is an increasing need for new, sustainable sources of protein due to the challenges that occurred when fish and soy meal consumption increased. Insects have been recognized as one of the possible options and a suitable method for developing a circular food system, along with other alternative protein sources (FAO, 2013; Aarts, 2020). In industrialized and developing nations, insects present a great opportunity to combine traditional knowledge and cutting-edge research (Meng et al., 2017).

An important monophagous, lepidopteran insect for the industry is the silkworm (*Bombyx mori*) due to the silk produced and its potential for human and animal food, pharmaceutical usage, biogas from substrates of mulberry silkworm (Zhang et al., 2012; Łochyńska & Frankowski, 2018; Wu et al., 2020; Karnjanapratum et al., 2022).

Furthermore, silkworms gained attention as a model in research, having characteristics such as low cost of reproduction, the large size of offspring, short generation times etc. (Zhang et al., 2012; Meng et al., 2017).

The silkworm (*Bombyx mori* L.) plays an important role in economic development, having a significant quantity of by-products that are highly valuable in terms of nutrition, medicine, and commerce (Trivedy et al., 2008; Karthick et al., 2019; Sharma et al., 2022). The silk reeling process generates waste such as pupae and leftover unreliable silk.

The SWP, as the main by-product of sericulture, could be considered a well-balanced source of nutrients in terms of proteins, lipids, minerals, vitamins (Herman et al., 2022; Tassoni et al., 2022) and various bioactive substances such as fatty acids, peptides, and polyphenols with antioxidant, anticancer, cardiovascular, hepato-protective properties (Sadat et al., 2022).

According to Valerie et al. (2015), the SWPs are usually referred to by a variety of names, including silkworm pupae meal, silkworm meal, defatted silkworm pupae meal, spent silkworm pupae, de-oiled silkworm pupae meal, non-defatted silkworm pupae meal, Eri silkworm pupae meal, and Muga silkworm pupae meal in various locations.

Due to its high protein content, SWP meal has been used to feed animals, especially for monogastric species such as poultry, pigs, and fish, as well as for ruminants (Sheikh et al., 2018).

With an emphasis on pigs, this review provides an overview of the current situation regarding SWP as a sustainable source of animal feed. It was also taken into consideration the mulberry leaves and pupae composition.

MATERIALS AND METHODS

We screened the most relevant and recently published papers available on PubMed, Elsevier, MDPI, and Research Gate, using the keywords "silkworm pupae", "composition", "protein", and "pigs". We also used the bibliographic material regardless of the years of publication when recent papers were not available. In this review, we tried to provide the scientific information reported about nutritional characteristics and the potential of using silkworm (*Bombyx mori* L.) pupae in animal feeding with a focus on pigs.

RESULTS AND DISCUSSIONS

Mulberry leaves nutrient composition

Mulberry (*Morus alba*) plants are adapted to temperate, tropical, and subtropical climates.

In Romania, sericulture developed firstly in Transylvania and Banat in the 14^{th} century (1348). The Turks introduced the practice of rearing silkworms in Moldova and Muntenia later in the 18^{th} century.

To support sericulture, 60,000 mulberry saplings were distributed for free in Moldova in 1845, which were to be planted in the southern areas. The Banat is a region where the mulberry tree is a symbol. Mulberries were introduced to this area by the Habsburg government in the 18^{th} century so that farmers could feed silkworms. The adoption of the trees was so widespread that one of the region's primary exports was silk. Dolis, (2008) specified that the old mulberries were still growing along Banat's roads. According to Tanase (2007), the Romanian vegetal sericulture patrimony has had 64 mulberry varieties, with increased productive potential (up to 30 leaf tonnes/ha) depending on the maintenance and exploitation technologies. Pau et al. (2008) cited by Pop et al. (2018) stated that Romania has had a diversity of mulberry species, about 10 local breeds and 49 foreign breeds and hybrids from Japan, China, Russia, Bulgaria and India. But in recent years in Romania, as far as we know, there are no clear evidence regarding the area cultivated with these mulberries, neither variety. Moise et al. (2018) and Dezmirean et al. (2018) reported the set-up of a mulberry plantation from Kokuso 21 variety (Japanese origin), at the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca in the Global Centre of Excellence for Advance Research in Sericulture and Promotion of Silk Production which is recognised since 2014 by the International Sericulture Commission. However, according to Sarkar et al. (2017), Rohela et al. (2020). mulberry trees are widely distributed across the continents and in a variety of environments, which suggests that it is more tolerant of changing environmental circumstances, being adapted to a variety of climates, soil types, and altitudes up to 4000 m above sea level.

The mulberry leaves become crucial to the sericulture sector since mulberry silkworms (*Bombyx mori* L.) exclusively eat mulberry leaves (Sanchez, 2000; Thaipitakwonga et al., 2018). However, due to compounds in mulberry leaves that may be sequestered by silkworms, such as essential oils, flavonoids, and terpenoids, the decomposition of mulberry-fed SWP results in an unpleasant odour. Palatability issues have been attributed to this disagreeable odour (Finke, 2002; www.feedpedia.com). However, mulberry leaves are highly effective in developing high-quality cocoons. Pupae represent approximately

60% of the dried cocoon's weight (Tassoni et al., 2022).

The main nutrients found in mulberry leaves are proteins, carbs, vitamins, sterols, phagostimulants, and minerals (Raghuvanshi & Bukhari, 2019). The dietary nutritional needs of silkworms have an immediate impact on all genetic characteristics, including cocoon weight, silk production, pupation, and reproductive features (Ramesha et al., 2010; Raghuvanshi & Bukhari, 2019).

According to Srivastava et al. (2006), the moisture content of dried mulberry leaf powder ranged from 5.11 to 7.24%, the crude protein (CP) content from 15.31 to 30.91%, the total ash concentration from 14.59 to 17.24%, the neutral detergent fibre (NDF) content from 27.60 to 36.66%, the crude fat (CF) content from 2.09 to 4.93%, the carbohydrate content from 9.70 to 29.64%, as well as the energy content from 113 to 224 kcal/100 g. Generally, CP levels are comparable to those of the majority of legume forages. Sanchez (2000) and Raghuvansh et al. (2019) report that the CP content of leaves varies from 15 to 28%, while Sanchez-Salcedo et al. (2017) found a level of 13.4-19.4%, Iqbal et al. (2012) noticed a content of 18.41-24.63% and Adeduntan & Oyerinde (2010) reported 21.24-21.66%. The composition depends on the variety, leaf age, and growing environment. In comparison to other vegetation, mulberry leaves have a low fibre proportion. The typical Ca, and P concentrations are 0.14 to 0.24% and 1.8 to 2.4%, respectively (Sanchez, 2000).

Moreover, according to Srivastava et al. (2006), mulberry leaves contain a significant source of beta-carotene (14,688 mg/100 g) compared to other frequently consumed green leafy vegetables, including spinach, amaranth, and fenugreek leaves. Furthermore, mulberry leaves are characterized by a high Fe and Ca level and are a source of other antioxidant elements, including Zn and ascorbic acid as well.

Such as Liu et al. (2000) specified spring leaves have significantly more nutritional value than autumn leaves. The silkworm possesses considerable variations in its ability to digest, absorb, and convert to body matter when fed the same quantity of mulberry leaves under various environmental, feeding, and dietary conditions (Rahmathulla & Suresh, 2011). It is generally established that nutritional parameters have a close relationship with feed intake and silk production. The conversion of mulberry leaves into silk is greatly influenced by the silkworms' ability to digest their diet. A more accurate economic indicator to produce cocoons is the efficiency of conversion of ingested mulberry leaves into silk or the rate of leaf silk conversion (Rahmathulla & Suresh, 2011). Only 620.70 kg of the 2472.80 kg of mulberry leaves that are produced by a hectare of mulberry fields are digested by silkworm larvae and turned into 211.20 kg of silk (Priyadharshini et al., 2017).

The haemolymph composition of larvae fed on *Morus alb*a and *Morus laevigata* mulberry leaves consists of 24-30 mg/mL proteins and more than 1500 mg/mL lipids and pupae weight varied between 705-885 mg (Mahmoud, 2017).

Silkworm pupae composition

The primary by-product of the sericulture sector is thought to be SWP which is left over after reeling silk fibre and can be used as animal feed due to its highly nutrient-dense composition (Dong et al., 2017; Lamberti et al., 2019). Literature sources highlighted that SWPs receive all their nutrients from mulberry leaves and convert plant proteins into silk.

Several authors evaluated the chemical composition of silkworm and their by-products. The SWPs nutritional value may change depending on the treatment technique. There were large variations of nutritional composition (Table 1). Thus, protein level ranging from 48 to 94.98% DM, lipid values between 6.2 and 37.1% DM, and fibre variation is between 2.5 to 5.8% DM. The content of mulberry leaves, feed consumption, digestibility coefficients, the bioavailability of nutrients, analytical procedures, sampling, etc. are some potential explanations for these variations.

On a similar note, Kumar & Rajesh (2015) found a variety of bioactive compounds, including 55 g of protein, 8.5 g of fat, 6 g of fibre, 25.43 g of carbohydrates, and 389.60 (Kcal/100 g) of energy which was found in each 100 g of SWP. On the other hand, Kotake-Nara (2002) pointed out the difference in lipid content between males and females, being 46% greater in females (9%) compared to males (4.8%).

Table 1. Proximate composition of SWP

Specification	%, DM bases	%, fresh	References
Dry matter (DM)	-	23.2-25.9	Lamberty et al. (2019)
		81-97.5	Feedpedia
Protein	48-60	-	Herman et al. (2022)
	49-54	21.5	Wu et al. (2021)
	59.52–94.98	13.81-16.83	Lamberty et al. (2019)
	60.7	-	Sheikh et al. (2018)
	51.6-70.6	-	Feedpedia
Lipid	30	-	Herman et al. (2022)
	-	13	Wu et al. (2021)
	6.2-37.1	-	Feedpedia
	25.7	-	Sheikh et al. (2018)
Fiber	2.5-5.8	-	Feedpedia
	3.9	-	Sheikh et al. (2018)
Ash	5.8	-	Sheikh et al. (2018)
	-	1.16-1.33	Lamberty et al. (2019)
	-	3.3-10.6	Feedpedia
Chitin	3-4	-	Herman et al. (2022)
Gross Energy - (MJ/kg)	-	5.09-6.82	Lamberty et al. (2019)
- (MJ/kg DM)	25.8	-	Feedpedia

Apart from the main nutrient in all the analyzed bibliographic sources, SWP has a high quality of protein due to the concentration of essential amino acids (Tomotake et al., 2010; Rafiullah & Khan, 2016; Wu et al., 2020). According to a study led by Zhou & Han (2006), silkworm pupae contain all 18 recognized amino acids, including the essential amino acids, mainly methionine, which is a limiting amino acid, especially for poultry. As seen in Table 2, a detailed composition of amino acids of the defatted and non-defatted SWP was provided by Mahata et al. (1994), quoted by Raja et al. (2019) and Lin et al. (1983), and by Longvah (2011) cited by Jeyaprakashsabari & Aanand (2021). Table 2 also shown the amino acids composition of SWP, expressed as g/100 g protein, reported by Roa et al. (1994), Zhou et al. (2006) and Longvah (2011), cited by Kumar & Rajesh (2015).

Table 2. Amino acids composition of SWP

Specification	g/16 g N[1]		g/100 g protein[2]
	Defatted	Non-defatted	
Alanine	4.4	5.6-5.8	5.5
Arginine	5.1	5.8-5.6	6.8
Aspartic acid	7.8	10.4	10.9
Glutamic acid	8.3	13.9	14.9
Cystine	0.8	1.0	1.4
Methionine	3.0	3.5	4.6
Lysine	6.1	7.0	7.5
Isoleucine	3.9	5.1	5.7
Leucine	5.8	7.5	8.3
Phenylalanine	4.4	5.1	5.1
Threonine	4.8	5.2-5.1	5.4
Tryptophan	1.4	0.9	-
Histidine	2.6	2.6	2.5
Proline	5.2	5.2	4.0
Serine	4.5	5.0	4.7
Glycine	3.7	4.8	4.6
Tyrosine	5.5	5.9	5.4
Valine	4.9	5.5	5.6

References: [1]Lin et al. (1983); Mahata et al. (1994); Raja et al. (2019); Longvah (2011) quoted by Jeyaprakashsabari, and Aanand (2021); [2]Roa et al. (1994); Zhou et al. (2006), and Longvah (2011) cited by Kumar & Rajesh (2015).

With regards to the fatty acids profile of SWP oil, Nakasone et al., (1967) and Kotake-Nara (2002) and presented composition differentiated by sex as is described in Table 3.

Table 3. Fatty acids composition of SWP[1]

Fatty acids (% of total lipids)	Male	Female
C16:0 (palmitic)	28.60	22.80
	24.90	19.50
C16:1n-7 (palmitoleic)	3.10	1.80
	0.80	0.60
C18:0 (stearic)	2.60	4.30
	5.40	6.30
C18:1n-9 (oleic)	29.0	27.20
	24.30	22.60
C18:2n-6 (linoleic)	7.30	8.50
	6.30	7.70
C18:3n-3 (alpha-linolenic)	29.20	34.90
	36.00	40.70

[1]References: Nakasone & Ito (1967); Kotake-Nara et al. (2002).

Comparative to SWP oil, the same authors described soybean oil and linseed oil fatty acids composition (wt % of total lipids) as follows: soybean oil C6:0 10.6, C18:0 3.5, C16:1n-7 0.1, C18:1n-9 20.5, C18:2n-6 54.4, C18:3n-3 6.5, C20:3n-3 0; linseed oil C6:0 5.5, C18:0 3.1, C16:1n-7 0.1, C18:1n-9 14.8, C18:2n-6 14.2, C18:3n-3 56.2, C20:3n-3 0.1. A significant concentration of omega-3 (including α-linolenic), omega-6 (including linoleic fatty acids), docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids, was found as well by Kumar and Rajesh (2015) in the lipid structure. This statement was also supported by Payne et al. (2016), who reported low levels of saturated fat and significant levels of monounsaturated and polyunsaturated fatty acids.

According to Kumar et al. (2015), another important aspect is the SWP's excellent minerals and vitamins composition (Table 4).

On the other hand, Priyadharshini et al. (2017) noticed that approximately 8% of dried SWP are nitrogen, 0.29% are calcium, and 0.58% are phosphorus, whereas wet pupae contain 0.65% calcium, 1.22% phosphorus, 0.30% natrium, 0.80% potassium, 0.325% magnesium, 230 mg/kg iron and 285 mg/ kg zinc.

Table 4. Minerals and vitamins composition of SWP[1]

Minerals	Concentration	Vitamins	Concentration
Calcium (mg/100 g)	102.31	Vitamin A (µg)	273.99
Potassium (mg/100 g)	1826.59	Vitamin E (IU/kg)	51.45
Magnesium (mg/100 g)	287.96	Vitamin C (mg)	<5.78
Phosphorus (mg/100 g)	1369.94	Vitamin B1 (mg)	1.91
Natrium (mg/100 g)	274.57	Vitamin B2 (mg)	5.43
Iron (mg/100 g)	9.54	Vitamin B3 (mg)	15.20
Zinc (mg/100 g)	17.75	Vitamin B5 (mg)	12.49
Manganese (mg/100 g)	2.08	Vitamin B7 (µg)	144.51
Copper (mg/100 g)	2.08	Vitamin B12 (mg/100 g)	0.50
Selenium (mg/100 g)	0.08		

[1]Reference: Kumar et al. (2015).

Silkworm pupae for animal feeding

Similar to other insects, silkworms are cold-blooded, quick-growing, and an essential component of the natural diets of many species. Their varying protein contents and protein digestibility (76%-98%), which highlight their importance in contemporary trends in food science and related fields, led to an increased interest in terms of potential application as animal feed.

Sheikh et al. (2018) mentioned that animals, particularly monogastric (poultry, pigs, and fish), but also ruminants, can be fed with SWP meal. As stated by Asimi et al. (2017) and Herman et al. (2022), only 25-30% of waste pupae have been used for livestock feeding.

Despite this, a significant amount of SWP waste is discarded every year. A series of trials were running to determine the impact of the dietary addition of SWP, as a replacement of soybean meal, on growth parameters and carcass characteristics, apparent nutrients and energy digestibility, haematological profile in the broiler (Rafiullah and Khan, 2016) and laying hens (Saikia et al., 1971 and Aruga, 1994 quoted by Priyadharshini et al., 2017). Although it is slightly lower quality than fish meal, Sheikh et al. (2018) confirmed that SWP meal is a valuable and less expensive alternative protein source that can be utilized in feeding chickens. Numerous studies carried out around the world have demonstrated that it is safe to partially replace (50%) the main protein source (fish meal) in most experiments, albeit it may be necessary to supplement with minerals. While total replacement is sometimes achievable, the performance is more likely to be affected in broilers. Inclusion percentages often range between 5 to 10% (Valerie et al., 2015). Hence, as Fagoonee (1983a; 1983b) and Purushothaman and Thirumala (1995) mentioned, growth performance and feed efficiency were not negatively impacted when SWP replaced 50% of the fish meal but was negatively affected by 100% replacement. In 2020, Miah et al. conducted a trial to determine the impact on growing chickens' performance and meat traits of the partial replacement of soybean meal and oil with full-fat silkworm (*Bombyx mori* L.) meal. The findings demonstrated that full-fat silkworm meal could partially replace soybean meal/oil in the chickens' diet, maintaining appropriate performance and meat qualities and meat with a healthier n-6/n-3 ratio.

When utilized SWP as a protein supplement, hens' egg characteristics were improved, yolk's colour as well (Saikia et al., 1971; Priyadharshini et al., 2017). Layer chicken body weight, feed intake, hen day production (%), egg weight, feed conversion ratio, blood profile, and egg quality parameters did not change significantly when soybean meal was substituted with SWP meal (0, 25, 50, or 100%) (Ullah, 2017). Similarly, Khatun et al. (2005) found that layer chicks of the Rhode Island Red pure line grew more rapidly, produced more eggs, and were more profitable if fed one of three isonitrogenous and isoenergetic diets: D1 (6% protein concentrate+0% SWP), D2 (0% protein concentrate+6% SWP), and D3 (0% protein concentrate+8% SWP). These authors demonstrated that SWP is a less costly option to protein concentrate, which increased profitability. On the other hand, lysine and methionine amino acids have a high digestibility (94% and 95%, respectively) in geese (Penkov et al., 2002, quoted by Sheikh et al., 2018).

In Japan, SWP meals are fed to cattle, pigs, and poultry. Despite having a high percentage of indigestible protein and a favourable amino acid composition, feeding SWP meals to calves is restricted because of its high oil content (Sheikh et al., 2018). SWP meals *in situ* nitrogen degradability is not very effective. According to numerous researchers (Chandrasekharaiah et al., 2002; Sheikh et al., 2018), the effective degradability values (5%/h outflow rate) for undefatted SWP were 29% and 25%, and only 20% for the defatted meal FCR (Chandrasekharaiah et al., 2004). As a result, a significant amount of protein was not degradable, especially from the defatted meal, which was higher in protein.

The rabbits fed SWP recorded a noticeably higher rate of fat deposition and fur growth (Priyadharshini et al., 2017).

In aquafeed, the SWP can replace partially or totally fish meal without adverse effects on fish. Shakoori et al. (2013) replaced the fish meal with SWP in rainbow trout considering for their trial 4 diets groups: T1 with 100 % fish meal, T2 with 5 % SWP + 95 % fish meal, T3 with 10 % SWP + 95 % fish meal, and T4 with 15 % SWP + 95 % fish meal and one control group. The findings showed that SWP can induce some anaemia-related symptoms while stimulating the immune system in rainbow trout.

Silkworm pupae for pigs feeding

The volume of knowledge on using SWP in pig diets is modest. The majority of pigs farmers complete soybean meal in growing phases 1, 2, and 3 with fish meal due to the high protein requirement of pigs. The issues arise when it is necessary to replace classically fed ingredients with more sustainable sources of protein and oil. Interestingly, pupae's protein is superior to that of soy and fish. Due to its high protein content, the pupal powder is recommended to be used as food for pigs (Trivedy et al., 2008), although the higher oil content can determine a negative response.

Table 5. Effects of dietary addition of SWP in pigs' diet compared to classical fish meal (FM) protein rich source

References	SWP level	Pigs' category and breed	Period	Performances
Ramamoorthi and Mercy (2003)	0, 3% and 4% and 6.7% (substitute for FM protein 0, 50 and 100%)	Growing-finishing Large White Yorkshire ♀	90 days	- weight gain (kg): 45.9 and 44.6 vs. 43.3 kg; - average daily gain (g/day): 510.1 and 495.7 vs. 481.4 g; - feed conversion ratio (kg feed to kg gain): 3.63 and 3.62 vs. 3.78.
Medhi et al. (2018)	0, 3% and 7% (0, 50 and 100% replacement of FM, with or without enzymes supplementation at the rate of 65 g/100 kg feed).	Growing crossbred (Hampshire Ã - Assam local pigs)	70 days	No significant differences were observed for average daily gain, dry matter intake, digestibility of nutrients as well as balance of energy and nitrogen, except that the digestibility of ether extract increased by supplementation of enzyme in the diet.
Choudhury et al. (2021)	SWP muga, 0, 2% and 4% as substitute for FM	Grower pigs Large White Yorkshire	45 days	- average daily gain (g/day): 127 and 146 vs 125; - feed conversion ratio (kg feed to kg gain): 3.16 and 2.87 vs 3.45.

Coll et al. (1992), cited by Sheikh et al. (2018), mentioned that a non-defatted SWP meal could partially replace soy oil meal in growing and finishing pigs' diets without significantly affecting growth performance or carcass traits. However, there was noticed a negative effect on intake when the substitution rate exceeded 50%. The lower intake was compensated, though, by a higher feed conversion rate. Medhi (2011) carried out an investigation on finishing cross-breed pigs to assess the alteration of certain blood parameters as reference markers for health status. No significant differences were observed. According to a study conducted by Choudhury et al. (2021), Table 5, feeding Large White Yorkshire grower pigs 2% and 4% of Muga SWP enhanced overall production performance while lowering production costs.

CONCLUSIONS

This review revealed the various applications of one of the main silkworm by-products for the livestock industry, emphasising pig feeding. While looking through more than 60 papers online, there are few comprehensive scientific studies on using SWP in pig diets. Most of them target fish and poultry, but they also target cattle. Mulberry leaves, the single component of the diet of silkworms *Bombyx mori*, have a strong correlation with the SWP composition. All authors agree that SWP is a remarkable source of nutrients for animal feeding, with protein constituting the predominant compound which provides a valuable amino acid composition. Additionally, the structure of the lipids is advantageous to health due to their high content of essential fatty acids, particularly n-3. The vitamins, minerals, and other bioactive compounds complete this nutritional value of SWP. Due to the SWP composition, this could be an important ingredient for animal feed. There were no detrimental effects on environmental pollution, meat and egg quality, animal health, or livestock productivity. However, to complete the informational package, the subsequent pig research should be performed.

ACKNOWLEDGEMENTS

This study was funded by the Ministry of Agriculture and Rural Development and supported by the Academy of Agricultural and Forestry Sciences, Romania.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

NUTRITIONAL BENEFIT OF COMPOSITE FLOUR BASED ON GERMINATED SORGHUM AND CATERPILLAR FOR CHILD MALNUTRITION ERADICATION

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Abstract

The COVID-19 epidemic and the Russian-Ukrainian crisis have aggravated an already catastrophic food security and nutrition situation in the majority of low-income nations, with 12.9% of the population undernourished and over 45% of children under five dying. As a result, edible insects may be a good source of alternative protein as a dietary supplement. The goal of this study was to first assess the in vivo nutritional impact of composite flours made from sorghum and caterpillar and then choose the best flour for biochemical characterization, amino acid, fatty acid, and vitamin quantification. A statistical study demonstrated a significant difference in weight growth (7.57 ± 0.53 g/d and 10.82 ± 2.56 g/d), but no difference in the biological value (0.80-0.94) of the composite flours. F2 composite flour has a protein level of 22.31 ± 0.44% and the presence of important amino acids, a low fat content of 6.56 ± 0.07%, and the presence of vitamins A, B1, B2, B9, B12, C, and E. The nutritional potentialities F2 composite flour imply that this flour might be used in newborn feeding to prevent infant malnutrition in the current COVID epidemic and Russian-Ukrainian crisis.

Key words*: child malnutrition, complementary foods, germinated sorghum, protein, Shea caterpillars.*

INTRODUCTION

A balanced diet is essential to provide the body with the energy and nutrients it needs to function properly. In children, it helps optimize health, development, and school performance (CAPOP, 2012). However, this adequate nutrition is threatened by a number of factors, including climate change, rapid population expansion, natural resource degradation, and pressure on agricultural production (FAO 2019). Most recently, the COVID-19 pandemic and the Russian-Ukrainian crisis have exacerbated this already alarming situation (OECD, 2020). According to forecasts by the heads of the Food and Agriculture Organization of the United Nations, the International Monetary Fund, the World Bank Group, the World Food Programme, and the World Trade Organization, the global food and nutrition crisis will exceed 840 million people by 2030 if nothing is done (FAO, IFAD, UNICEF, WFP, and WHO in 2022). The consequences of this food crisis for children living in rural areas and in poor households, and whose mothers have not benefited from school education, are stunted growth and wasting (FAO, IFAD, WHO, WFP, and UNICEF, 2022). In addition, in Côte d'Ivoire, economic inflation leads women with limited financial resources to buy cheaper infant formula of very poor nutritional quality on local markets to feed their children (Kouadio et al., 2023). Faced with this crucial problem, the FAO, IFAD, WHO, WFP, and UNICEF, in their 2022 declarations, recommend the participation of public and private actors through the implementation of local and sectoral solutions that will make it possible to resolve the problem of undernourishment and its corollary of malnutrition. In Côte d'Ivoire, a local solution could come from the use of sorghum (*Sorghum bicolore*) and caterpillars (*Cirina butyrospermi*) to combat child malnutrition, the prevalence of which was estimated to be rising by 4.7% during the period 2018–2020 (FAOSTAT, 2021a). Sorghum is a food rich in starch, minerals, and vitamins, whose annual production in 2021 was 70,000 tons (FAOSTAT, 2021b). As for the caterpillar, it is a real source of protein (55.49%), essential amino acids, fat (23.10%), essential fatty acids, and nutrients (vitamins, polyphenols, etc.) (Foua Bi et al., 2015). Their use through food formulation technology would allow the production of complementary foods with high nutritional potential, whose consumption would solve the problems of child malnutrition in Côte d'Ivoire. In vivo nutritional assessment technology is a method that reports on the effectiveness of a food formula and allows for consideration of its impact on consumer well-being (FAO, 2013). The use of this method, coupled with food formulation technology, will allow the development of diets capable of combating malnutrition. Thus, this study aims to evaluate the in vivo nutritional impact of flours composed of sorghum and caterpillars and then select the best flour for its biochemical characterization and its quantification in amino acids, fatty acids, and vitamins.

MATERIALS AND METHODS

The material consisted of sorghum grain (*Sorghum bicolor*) purchased at the Gouro market in Adjamé, Abidjan, and shea caterpillars (*Cirina butyrospermi*) collected in the town of Ferkessédougou in Côte d'Ivoire.

1. Formulation of the composite flour of germinated sorghum and caterpillar

1.1. Preparation of the sprouted sorghum flour

The grains were threshed three times, sorted, and washed with water that included 1% bleach to produce the germinated sorghum flour. The cleaned grains were given 72 hours at laboratory room temperature (25 ± 2°C) to germinate. The germinated grains were then stripped of their seedlings, rinsed, and dried for 24 hours in an oven set at 45°C. The dried grains were blended in a blender (Binatome, China), and the resultant powder was sieved through a sieve with a 100 µm diameter. The finished flour was placed in glass jars and preserved in the freezer at -6°C.

1.2. Preparation of the shea caterpillar flour

The caterpillar meal was obtained following a series of unit operations that included sorting them, washing them with 1% bleach water, and drying them in an oven (Venticell, Fisher Bioblock Scientific) at 45°C for 72 hours. The dried caterpillars were crushed into flour using a blender (Binatome, China). The flour was then sieved through a 100 µm sieve and kept in a glass box in the refrigerator at - 6°C.

1.3. Formulation of the composite flour from germinated sorghum and caterpillar flour

The composite flour was produced by mixing previously manufactured sprouted sorghum and caterpillar flours. As a result, three formulations (F1, F2, and F3) were produced. The F1 recipe contains 95% germinated sorghum flour and 5% caterpillar flour. The F2 recipe contains 90% sprouted sorghum flour and 10% caterpillar flour. F3 is composed of 85% germinated sorghum flour and 15% caterpillar flour. All of the resulting composite flours are homogenized with a spatula until a homogenous mixture is achieved, then kept in glass jars at room temperature.

2. Nutritional characteristics of the composite flour of germinated sorghum and caterpillar

The animal experiments were carried out in accordance with Adrian et al. (1991) method. Twenty-five young weanling albino rats, aged 35 to 45 days and weighing 50 to 60 g on average, were purchased from the École Normale Supérieure (ENS) animal house in Abidjan, Côte d'Ivoire. The juvenile rats were separated into five groups of five rats each and kept in individual metabolic cages in at 25 ± 2°C room with urine and feces collecting facilities. After five days of acclimation, rats were fed five experimental diets for 15 days, comprising two control diets (with and without protein) and three composite meal diets. Throughout the trial, water was available at any time. Food consumption was assessed every day, and weight was checked every three days. Data on food intake were gathered by recording the amount of food ingested by each rat at baseline and the amount remained after feeding. The rats' weight increase or decrease was also tracked. Rat feces were collected daily, dried to a consistent weight at 85 ± 2.0°C, then pulverized for fecal nitrogen measurement. To avoid ammonia loss, urine samples were collected in vials containing 0.1 N HCl and maintained in a freezer until urinary nitrogen analysis. The Kjeldhal technique was used to determine rat fecal and urine nitrogen (AOAC, 2005). According to FAO/WHO (1989) and AOAC (2000) guidelines, protein efficiency ratio (PER), net protein utilization (NPU), biological value (BV), real digestibility (RD), and net protein retention (NPR) were assessed. Three rats from each batch were starved for 16 hours at the end of the animal experiment. After this period, the rats were sedated with ether and slaughtered for blood collection in red and purple tubes, respectively, to determine blood biochemical and haematological parameters.

3. Study of the biochemical and nutritional properties of the selected composite flour of germinated sorghum and caterpillar

3.1. Study of biochemical properties

The biochemical characteristics of the selected composite flour were investigated using the AOAC techniques (2005). Consequently, the AOAC (2005) technique was used to assess protein (Kjeldahl method, N x 6.25), fat (hexane Soxhlet extraction method), ash (muffle furnace method at 550°C), and fiber contents. Carbohydrate content was calculated by subtracting 100 from the sum of moisture, fat, protein, crude fiber, and ash content as defined by the AOAC (2005). Sample energy values were derived by multiplying the energy ingredients (protein, fat, and total carbo-hydrate) by their respective Atwater specific energy conversion coefficients (FAO, 2003).

3.2. Study of nutritional properties

The profiles of vitamins, amino acids, and fatty acids were measured by HPLC chromatography using an instrument of type SHIMADZU SPD 20 A, according to the technique of Abidi (2000). About the phenolic compounds, they were extracted in methanol using the technique of Singleton et al. (1999), and the phenolic profile was quantified using HPLC chromatography employing an equipment of type SHIMADZU SPD 20 A, according to the method of Abidi (2000).

3.3. Statistical investigation

The dietary assessment was carried out in quintuplicate, whilst the biochemical blood, hematological, and nutritional analyses were carried out in triplicate. On the figures, means, standard deviations, and error bars were computed using Microsoft Windows 10 Excel program. Duncan's test at the 5% threshold was used to compare the means of the different samples using XLSTAT software (version 2019, XLSTAT, USA). To locate samples with similar features, Principal Component Analysis (PCA) and Hierarchical Ascending Classification (HAC) were used.

RESULTS AND DISCUSSIONS

Table 1 illustrates the weight gain, food consumption, efficiency coefficients, and protein retention of young rats fed composite flours. The juvenile rats fed with composite flours appeared to have more harmonious growth and development than the rats fed the control diets. The nutritional measures, namely the amount of composite flour consumed, weight growth, feed and protein efficiency coefficients, and the amount of protein retained, indicated a significant difference (P<0.05) in the three batches of rats.

Table 1. Weight gain, consumption, and efficiency coefficients of composite flours

Parameters / Diets	Weight gain (g/d)	Feed consumption (g/d)	Feed efficiency ratio	Protein efficiency ratio	Protein retention (g)
Control batch	8.21 ±0.37^{b}	8.66 ±1.58^{b}	0.98 ±0.20^{a}	6.77 ±1.37^{a}	5.57 ±1.08^{b}
F1	3.94 ±0.04^{d}	7.57 ±0.53^{b}	0.52 ±0.03^{b}	2.05 ±0.19^{c}	2.02 ±0.62^{c}
F2	6.34 ±0.20^{c}	9.51 ±0.50^{ab}	0.67 ±0.04^{b}	3.01 ±0.18^{b}	5.32 ±0.56^{c}
F3	9.07 ±1.17^{a}	10.82 ±2.56^{a}	0.88 ±0.24^{a}	3.50 ±0.90^{b}	7.34 ±1.68^{a}
PP	-1.78 ±0.09^{e}	4.82 ±0.54^{c}	-0.37 ±0.04^{c}	-4.56 ±0.55^{d}	

The values are the mean ± standard deviation of tests performed in quintuplicate. Values with different exponents are significantly different from each other at the 5% level (P<0.05) on the same line.

Duncan's test shows that the potentialities indicated at the parameter level are a function of the amount of caterpillar flour included in the sprouted sorghum meal. Similar findings in terms of feed intake and weight increase were reported by Kouadio et al. (2015) at the enriched Dockounou level. Rats fed the F3 diet showed maximum potential in all measures except the protein efficiency coefficient, where they did not differ significantly from those fed the F2 diet. Nonetheless, the values obtained for the feed efficiency coefficient are greater than 2.3 according to the PAG (Protein Advisory Group) guidelines and 2.7 according to FAO/WHO (1989) standards for rats given the F2 and F3 diets. Its conformity to the specified requirements shows that the F2 and F3 diets are of high nutritional quality, and their use may have a favorable influence on body development as well as maintenance.

Figure 1 depicts the protein digestibility of the young rats' composite diet. The lack of a significant difference in apparent digestibility between the control and F2 diet rats implies that the proteins in these feeds may have the same degrees of hydrolysis (Mazorra-Manzano, 2018). The digestibility rates of the F2 and F3 diets exceed 70%, which is the FAO/WHO minimal threshold (1989). In terms of actual digestibility, net protein use, and biological value, statistical analysis showed no significant difference (P<0.05) between rats fed F2 and F3 diets. This lack of difference shows that these two diets, while not including the same amounts of shea butter, have the same nutritional potential. Actual digestibility rates of 0.82 ±0.01 and 0.85±0.02 for the F2 and F3 diets, respectively, are higher than FAO/WHO recommendations (1989). These digestibility rates are equivalent to the 84.92%-84.50% achieved by Chrenkova et al. (2002) in rats given transgenic maize meal. Furthermore, at the level of 7F3A and 5F5A formulations, the extremely high biological value of these diets is equivalent to that of Vazquez-Rodrguez et al. (2013). These high numbers imply that eating the meal may have a favorable influence by promoting development and aiding in illness prevention.

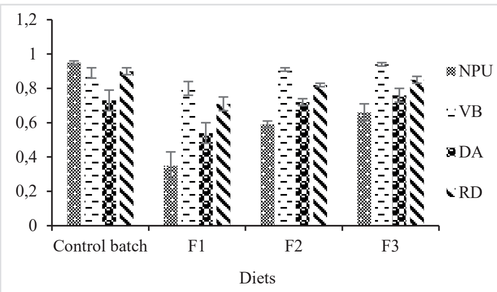

Figure 1. Protein digestibility of composite flour consumed by young rats

The content of biochemical components in the serum of rats fed the composite meal and the control batch is shown in Table 2. Statistical analysis showed that rats fed the control diet had a significant (P<0.05) influence on their blood glucose, blood uremia, and blood phosphorus levels compared to those fed the low-content composite flours.

Table 2. Metabolic parameters of rats fed with the composite meal

Parameters / Diets	Control batch	F1	F2	F3
Glucose (g/L)	0.79 ±0.04^{a}	0.73 ±0.09^{ab}	0.71 ±0.04^{ab}	0.75 ±0.04^{ab}
Total protein (g/L)	75.60 ±9.96^{a}	77.80 ±10.87^{a}	74.80 ±7.19^{a}	76.00 ±10.02^{a}
Creatinine (mg/L)	10.40 ±2.70^{a}	8.20 ±1.64^{ab}	7.20 ±0.84^{b}	7.20 ±1.10^{b}
Urea (g/L)	0.48 ±0.04^{a}	0.43 ±0.09^{ab}	0.41 ±0.04^{ab}	0.45 ±0.04^{ab}
Cholesterol (g/L)	0.52 ±0.09^{b}	0.58 ±0.04^{b}	0.69 ±0.05^{a}	0.62 ±0.11^{ab}
Triglycerides (g/L)	0.86 ±0.16^{b}	0.80 ±0.07^{b}	1.04 ±0.36^{b}	1.64 ±0.21^{a}
HDL (g/L)	0.33 ±0.08^{b}	0.45 ±0.07^{a}	0.48 ±0.11^{a}	0.39 ±0.04^{ab}
Ca (mg/dL)	160.66 ±46.02^{a}	141.83 ±14.58^{b}	135.00 ±10.7^{b}	133.83 ±14.50^{b}
Phosphorus (mmol/L)	24.97 ±9.21^{a}	21.62 ±4.58^{ab}	19.14 ±2.93^{ab}	20.89 ±6.44^{ab}
Ca/P ratio	2.11 ±3.64^{a}	2.27 ±1.11^{a}	2.06 ±3.29^{a}	1.84 ±5.46^{a}

Values are the mean ± standard deviation of trials conducted in quintuplicate. Values with different exponents are significantly different from each other at the 5% level (P < 0.05) on the same line.

The blood glucose levels of rats fed the composite flours are equivalent to those reported by Akapo et al. (2017) for fermented cassava and sun-dried cassava diets (0.65 and 0.79 g/L). Nevertheless, the results in this study are much lower than those reported by Laleg et al. (2019) for the FVHT and FLT diets (1.04 and 1.06 g/L). Consequently, the comparatively low serum levels of the rats given the composite flour show that blood glucose levels are well maintained by natural homeostasis (Kuo et al., 2015), because an abnormal glucose level is symptomatic of diabetes, according to Kim et al. (2013). Statistical analysis showed no significant change in total serum protein levels between the control rat batch and those given the composite flours. These values, however, are greater than the 0.40 to 0.65 g/L reference limit indicated by Kong et al. (2016). Its high total protein concentration in the blood shows caterpillars' good influence on rat growth, development,

and well-being as a protein source. Statistical analysis showed a significant difference (P<0.05) in creatinine, cholesterol, triglycerides, HDL cholesterol, and calcium contents. Whereas the control rats had greater creatinine and calcium levels, the rats given the composite flours had higher cholesterol, triglyceride, and HDL-cholesterol levels. Calcium and phosphorus are vital elements for bones, neurons, and muscles. Also, they are the most plentiful in the body (Penido & Alon, 2012). The quantity of phosphate in the blood impacts the amount of calcium, and the two are inversely connected in humans (Levine et al., 2014). As a result, any deviations in the signs might indicate organ malfunction or inflammation within the body.
The immunological characteristics of young rats fed the composite meal are shown in Table 3.

Table 3. Immunological parameters of rats fed with the composite meal

Diets \ Parameters	Control batch	F1	F2	F3
Hemoglobin g/100 mL	12.22 ±0.87^a	12.26 ±0.83^a	12.86 ±0.77^a	11.96 ±0.96^a
Hematocrit (%)	36.40 ±2.74^a	36.54 ±2.51^a	38.04 ±2.26^a	35.50 ±2.93^a
Neutrophils (%)	59.00 ±4.30^a	60.40 ±4.21^a	58.40 ±2.41^a	59.00 ±2.28^a
Basophils (%)	0^a	0^a	0^a	0^a
Eosinophils (%)	1.40 ±0.55^a	1.20 ±0.55^a	1.00 ±0.55^a	1.40 ±0.55^a
Monocytes (%)	8.40 ±1.34^a	8.00 ±1.64^a	8.00 ±1.58^a	7.60 ±1.58^a
Lymphocytes (%)	31.20 ±4.32^a	30.40 ±4.03^a	32.60 ±1.64^a	32.00 ±1.92^a

Values are the mean ± standard deviation of trials conducted in quintuplicate. Values with different exponents are significantly different from each other at the 5% level (P < 0.05) on the same line.

According to the statistical analysis, the rats' intake of the enhanced sorghum composite meal had no negative (P>0.05) effect on hemoglobin, hematocrit, neutrophil, basophil, eosinophil, monocyte, and lymphocyte contents compared to the rats given the control batch. The hemoglobin concentration of blood from rats fed the F2 composite meal was within the reference range advised by Melo et al. (2012), which is 12.8-15.9 g/100 mL. Its conformity to the specified requirements shows that hemoglobin content may support the body's smooth functioning via reversible oxygen fixation (Wajcman, 2005). Leukocytes are made up of neutrophils, basophils, eosinophils, monocytes, and lymphocytes that work together to protect the body against pathogenic germs and external chemicals (Wynn & Vannella, 2016). Hence, low blood levels of lymphocytes, eosinophils, and basophils might indicate the lack of any form of immunological response (inflammation, allergies, etc.). (Karasuyama et al., 2018; Arock, 2021). Yet, a higher than normal monocyte and neutrophil count implies an enhanced mobilization of these blood elements for primary defense of the body against primary viral and bacterial infections (Kumar et al., 2018).

1. Selection of the best composite flour from principal component analysis (PCA) and hierarchical ascending classification (HAC)

In order to determine the best composite flour, PCA was used to link the different composite flours to the nutritional factors investigated (Figures 2, 3, and 4).

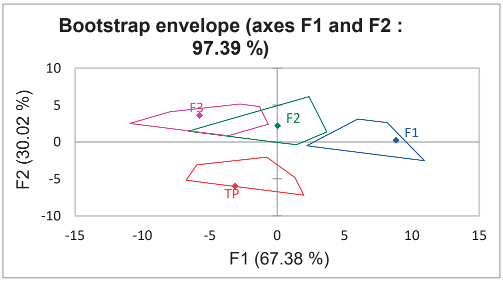

Figure 2. Bootstrap envelope showing points of similarity between composite flours

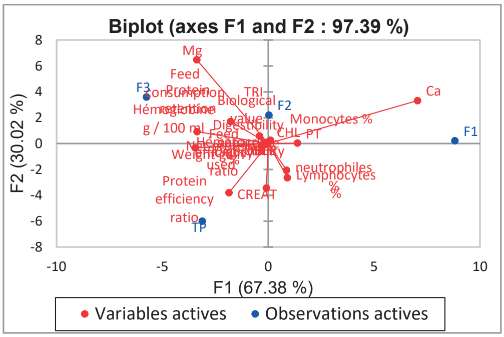

Figure 3. Principal component analysis showing the relationship between composite flours and nutritional parameters

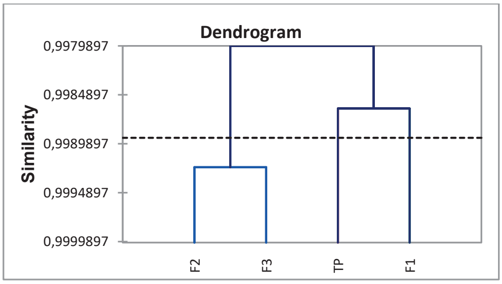

Figure 4. Hierarchical ascending classification of composite flours

The projection of the nutritional metrics and composite flours on the T1 and T2 axes in the biplot plane indicates a total of 97.39%. The F1 composite flour correlates favorably with the T1 and T2 axes, whereas the F2 and F3 composite flours correlate negatively with the T1 axis and positively with the T2 axis. The control batch has no correlation to the T1 or T2 axis. Figure 2 depicts the bootstrap analysis, which demonstrates a substantial difference between the composite flours and the control diet. Nonetheless, the nutritional properties of the composite flours F2 and F3 are similar. The plane distribution of the active variables (nutritional parameters) and active observations (composite flours) shows that the nutritional parameters weight gain, feed intake, feed efficiency ratio, protein efficiency ratio, apparent digestibility, digestibility, protein retention, amount of protein used, biological value, and creatinine are strongly shared by the composite flours F2 and F3. Based on the similarity of the active factors, the hierarchical analysis indicates that the F2 and F3 composite flours are similar but statistically distinct from the F1 composite flour and the control batch. On this basis, the composite flour F2 was chosen as the best flour since it contains a significant amount of caterpillar, which has intriguing nutritional properties.

2. Biochemical properties of the composite flour F2

Table 4 shows the biochemical content of the F2 composite flour. The ash, protein, carbohydrate, and calorie contents are in line with FAO/WHO (1991) recommendations for supplemental meals for children aged 3 to 36 months. Trumbo et al. (2002) estimate the daily protein requirement for a kid of this age group to be 0.87 g/kg /day. Consequently, for a 3-year-old child weighing 15 kg, consuming at least 100 g of F2 composite flour daily should give the body with 13.05 g of protein. This is significantly less than the protein content of 100 g of F2 composite flour. The energy content is greater than that determined by Kouadio et al. (2022) for a ready-to-eat plantain dockounou infant flour. The combination of carbohydrate, fat, protein, and fiber content results in a highly essential quantity known as energy value. It helps youngsters aged 3 to 36 months meet their body's development and maintenance demands (Butte, 2000). The ash levels are within suggested limits, implying that consuming F2 composite wheat might fulfil the body's mineral requirements.

Table 4. Biochemical composition of the composite flour F2

Parameters (mg/100 g DM)	Content	References*
Fiber (%)	7.00 ±0.50	<5
Ash (%)	0.93 ±0.23	<3
Titratable acidity (meq g/100 g)	6.15 ±0.26	ND
Fat (%)	6.56 ±0.07	10-25
Protein (%)	22.31 ±0.44	>15
Carbohydrates (%)	67.92 ±1.19	64
Starch (%)	58.88 ±1.07	ND
Energy (kcal/100 g)	420.01 ±3.45	400-425

Values are the mean ± standard deviation of trials performed in triplicate. Values with different exponents are significantly different from each other at the 5% level (P<0.05) on the same line; *FAO/WHO (1991).

3. Nutritional properties of F2 composite flour

The amino acid and fatty acid composition of F2 composite flour is shown in Table 5. This balanced profile is consistent with the F2 composite flour's relatively high protein level (22.31±0.44%). The essential amino acid concentration is lower than that found by Solomon et al. (2020) in beetle larvae, but greater than the IOM (Institute of Medicine's) guideline (2005). When ingested by youngsters, the F2 composite flour may thus be regarded as an excellent source of protein with high biological value due to its comprehensive profile of necessary and non-essential amino acids. High-performance liquid chromatography measurement of essential fatty acid concentrations indicates significant levels of palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, and arachidic acid. Notwithstanding the low fat content, the high quantities of essential fatty acids indicate that the F2 composite flour is of extremely high nutritional quality. Actually, essential fatty acids must be present in meals since the human body cannot generate them (Solomon et al., 2020). Moreover, these important fatty acids serve as precursors for the production of various vitamins and hormones required for optimal human body function.

Table 5. Amino acid and fatty acid content of the F2 composite flour

Amino acids (mg/100 g protein)		Fatty Acid (mg/100 g)	
Types	Content	Types	Content
Leucine	451.30 ± 0.01	Palmitic acid	705.50 ± 0.10
Methionine	163.60 ± 0.24	Stearic acid	692.70 ± 0.05
Phenylalanine	382.40 ± 0.01	Oleic acid	594.67 ± 0.57
Isoleucine	365.70 ± 0.01	Linoleic acid	468.20 ± 0.10
Valine	202.40 ± 0.21	Linolenic acid	459.80 ± 0.05
Histidine	423.67 ± 0.01	Arachidic acid	372.40 ± 0.30
Threonine	379.33 ± 0.51		
Tryptophan	368.40 ± 0.32		
Lysine	483.70 ± 0.22		
Arginine	332.33 ± 0.06		
Alanine	623.30 ± 0.11		
Glutamic acid	213.11 ± 0.01		
Glycine	523.70 ± 0.14		
Proline	373.60 ± 0.08		
Tyrosine	232.05 ± 0.33		

The values representing the contents are averages from trials carried out in triplicate.

4. Vitamins and phenolic content of F2 composite flour

The vitamin and phenolic content of F2 composite flour is shown in Table 6.

Table 6. Vitamins and phenolics content of composite flour

Vitamins (mg/100 g)		**Phenolic compounds (mg/100 g)**	
Types	Content	Types	Content
Vitamin A	534.93 ± 0.05	Caffeine	129.10 ± 0.08
Vitamin B1	589.97 ± 0.05	Catechin	804.81 ± 0.01
Vitamin B2	553.67 ± 0.57	Arbutin	511.21 ± 0.00
Vitamin B9	700.90 ± 0.01	Rutin	654.72 ± 0.02
Vitamin B12	275.53 ± 0.50	Naringenin	265.08 ± 0.07
Vitamin C	695.00 ± 0.00	Proto-catechin acid	391.03 ± 0.01
Vitamin E	235.40 ± 0.01	Gallic acid	574.50 ± 0.17
		Ellagic acid	166.93 ± 0.00
		Coumaric acid	114.52 ± 0.31
		Cinnamic acid	118.70 ± 0.07

Values are the mean ± standard deviation of trials performed in triplicate. Values with different exponents are significantly different from each other at the 5% level ($P < 0.05$) on the same line.

HPLC examination showed that various vitamins, particularly B vitamins, are present in appropriate levels. The concentrations in mg per 100 g of DM are substantially greater than those reported by Parker et al. (2020). Furthermore, the F2 composite meal satisfies the Trumbo et al. (2020) recommended daily requirement of vitamins for babies aged 6 to 12 months and children aged 1 to 3 years. In terms of phenolic compounds, HPLC quantification confirms the presence of various phenolic chemicals in the F2 composite flour, including caffeine, catechin, arbutin, rutin, naringenin, proto-catechin acid, gallic acid, ellagic acid, coumaric acid, and cinnamic acid. The presence of these phenolic compounds protects the body against the damaging effects of free radical reactions.

CONCLUSIONS

The purpose of this study was to assess the nutritional value of composite flours made from sorghum and Shea caterpillar. The statistical treatments linked with the zootechnical data demonstrate that the F2 composite flour has nutritional potentials that warrant its inclusion in newborn feeding. The conformity of the constants to the current norms at the level of biochemical, blood, and immunological parameters demonstrates that the intake of F2 composite flour has no abnormalities on the well-being of children aged 3 to 36 months. Because of its balanced profile in essential amino acids, fatty acids, vitamins, and phenolic components, the biochemical and nutritional characteristics of F2 composite flour reveal properties that validate its use as a post-weaning supplemental meal. The F2 composite flour might be a true answer to the problem of child malnutrition that exists in Côte d'Ivoire because of the Russo-Ukrainian crisis and COVID 19.

ACKNOWLEDGEMENTS

We are grateful to Dr. Gnahoué Goueh for granting us access to the *vivarium* of the École Normale Supérieure of Abidjan, Côte d’Ivoire.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

USE OF *Petroselinum crispum* AND VITAMIN E TO PROTECT AGAINST CARMOISINE CHANGES IN RATS

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Abstract

Carmoisine is a food coloring found in many foods, with several restrictions. The aim of this work was to evaluate the hematological and biochemical parameters of blood in Wistar albino laboratory rats after oral administration of carmoisine every day for 6 weeks by dissolving the additive in water. The effect of temperature on parsley was also studied, drying it at different temperatures, then observing at what temperature the amount of ascorbic acid was maintained in the greatest proportion. At most haematological parameters, higher values were observed in rats in the 100 mg carmoisine group compared to the control and parsley groups. Similarly, the biochemical parameters analyzed showed higher values in rats in the group receiving 100 mg carmoisine compared to the control group, and parsley administered to rats was able to bring mean values closer to those obtained in the control group.

Key words: *biochemical, blood, carmoisine, FT-IR, hematological parameters, petroselinum crispum, vitamin E.*

INTRODUCTION

Carmoisine is a synthetic food coloring, it is also called azorubin, encoded with E 122, has a brown-red color and belongs to the category of azo dyes (Coroian, 2019). HPLC and HPLC-DAD shall be used for the determination of carmoisine in foods, flavouring substances, alcoholic beverages, fruit drinks, jams, confectionery (Minioti et al., 2007). Carmoisine is a synthetic food coloring specifically used in foods to be heat treated after the fermentation process. Similar to other azo dyes, carmoisine can cause allergies, especially for those with aspirin intolerance. Since it is a histamine releaser, it is not recommended for people suffering from asthma, as it can intensify this disease and adverse effects. Children are not recommended products containing carmiozine and other additives from the category of benzoates, as they can cause hyperactivity syndrome and lack of concentration (EFSA, 2009; Coroian, 2013; 2019). Nouioura et al. (2023) made a complex based on various plants with high antioxidant capacity, including *Petroselinum crispum*. Recent studies by Peshkova et al., 2023, characterize the translocation of copper and gold nanoparticles in *Petroselinum crispum*. Evaluates the influence of hydroalcoholic extract of (*Petroselinum crispum*) for anxiety in rats that were treated with lead acetate (Fatemeh et al., 2023). Studies conducted on *Petroselinum crispum* show that it slows down the aging process of the skin, helps people with low immune systems, is very useful in the diet of people suffering from indigestion, constipation and in case of pancreas problems (Jassim, 2013). Parsley has a very high content of vitamin C, which helps strengthen the immune system, is beneficial for physically exhausted people (IFNB, 2000). Due to the content of vitamins and provitamins it is extremely beneficial for a balanced diet (Heinonen et al., 1989; Coroian, 2019). Exhibits high antioxidant capacity (Jassim, 2013). The effect of antioxidant activity of (*Petroselinum crispum)* and vitamin C, used against oxidative stress, has been reported by (Meister, 1992; Podmode et al., 1998, Nielsen et al., 1999; Zhang et al., 2006). Characterisation of food additives, risk assessment and interaction with food are provided by (Basu & Gopinatha, 2014; Scotter, 2015; Tofană, 2006; Tomaska & Brooke-Taylor, 2013). The purpose of this paper is to evaluate changes in biochemical and hematological parameters in rats and to evaluate weight oscillations after carmoisine administration and protective use of parsley and vitamin E.

MATERIALS AND METHODS

The experiment was conducted over a period of 6 weeks during which the substances were administered to rats daily. The carmoisine used in the study is presented as a reddish-brown powder with no other substances that may influence the test results. It was administered orally to the animals in the experiment. Carmoisine being water soluble, it was dissolved in water and then administered to rats. The maximum allowable dose for the human body is 4 mg/kg body weight. Since carmoisine toxicity is weakly expressed in amounts slightly higher than the maximum allowable dose, over a relatively short period of time, we administered 60 and 100 mg carmosine per day in this study. Parsley was used in this experiment due to the high amount of ascorbic acid it holds. This, together with vitamin E, has antioxidant action, thereby reducing the effects of oxidative stress caused by carmoisine. The parsley was dried at room temperature at 40ºC, 70ºC and 90ºC respectively to avoid oxidation of vitamin C in parsley. After the parsley was dried, it was shredded, then the rats were fed. Vitamin E was stored in the form of gelatin capsules at 21ºC in a dark space. From inside these capsules, vitamin E was extracted using disposable syringes and, after a short time, administered to rats. There were 4 groups in the study: 1 – control group; 2- the group to which I administered 60 mg carmoisine; 3 – the group to which I administered 100 mg carmoisine + parsley and vitamin E; 4 – the group to which I administered 100 mg carmoisine.

Animals. Rats in the experimental groups were weighed at the beginning and end of the experiment to observe any changes in weight for each individual. A total of 5 rats were used for each batch in the experiment. Before the experiment began, the rats were acclimatized in the laboratory animal unit at the University of Agricultural Sciences and Veterinary Medicine in Cluj-Napoca. The research project was approved by the Institutional Committee for Research Ethics, nr. 110, and was authorized by the sanitary veterinary authority, Cluj-Napoca, under no. 8187.

Hematological and biochemical analysis of blood. For the analysis of hematological parameters, the automatic hematology analyzer Abacus Junior was used, which uses 25 µl of blood for analysis. To determine the biochemical parameters, immediately after collection the blood was subjected to centrifugation in order to separate the serum. For biochemical analysis, the semi-automatic screen point analyzer with reagents was used (model: STAT-FAX 1904 Plus, GMI, Inc. 6511, Minnesota, 55303 USA).

FT-IR Analysis. The FT-IR/FT-Raman 4100 Jasco spectrometer (the resolution of the obtained spectra was set to 4 cm^{-1}), was used for parsley analysis. The beam divider in KBr was used for analysis and the method of pastylating the sample in powder form (5 mg) with potassium bromide (300 mg) by pressing at 10 t/cm^2 and the MIR probe for non-destructive testing on the spectral range 350-4500 cm^{-1} was used. With OPUS software, version 6.0 spectra were processed. The parsley samples were subjected to a heat treatment at different temperatures, namely: 40^0C, 70^0C, 90^0C, but also dried parsley at room temperature, to observe changes depending on temperature.

RESULTS AND DISCUSSIONS

By weighing at the beginning and end of the experiment, differences in weight can be observed by comparing the control group with the other groups receiving carmoisine. Batch 2, which received carmoisine, parsley and vitamin E, did not undergo major weight loss. In contrast, the groups receiving 60 mg carmoisine and 100 mg carmoisine, respectively, experienced significant weight loss. Also, at the end of the experiment, they showed quieter behavior and lacked energy. From the data obtained by weighing at the beginning and end of the experiment, differences in weight are observed by comparing the control group with the other groups receiving carmoisine. The group that received carmoisine, parsley and vitamin E did not undergo major weight loss. In contrast, groups receiving 60 mg carmoisine and 100 mg carmoisine experienced significant weight loss. Also, at the end of the experiment, they showed quieter behavior and lacked energy.

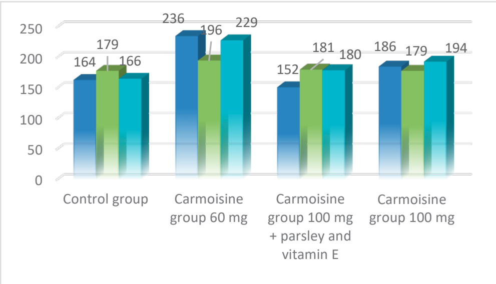

Figure 1. The weight of rats at the beginning of the experiment (g)

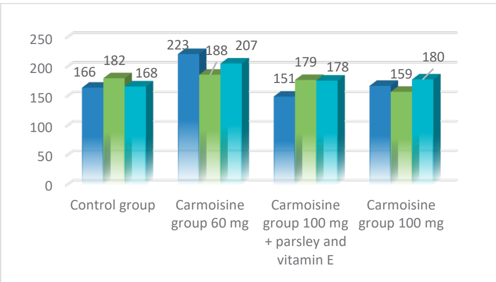

Figure 2. Weight of rats at end of experiment (g)

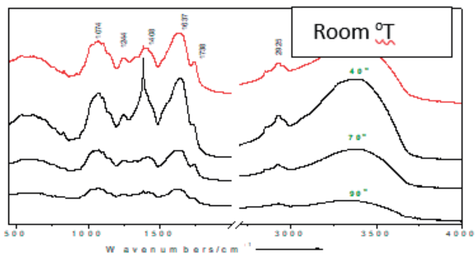

Figure 3. FT-IR spectra for parsley under the influence of temperature

Analyzing the FT-IR spectrum obtained from dried parsley, shown in figure 3, we can see a high pick intensity from 1637 cm^{-1} of the spectrum obtained from dried parsley at room temp and dry parsley at 40°C. This intensity may be due to the bond C=C. It can be seen that this band decreases in intensity with increasing temperature. We can also observe a decrease in intensity of the strip from 1075 cm^{-1} to the C=O bond. Also, the bands from 1244 and 1403 that are quite obvious in the case of dried parsley lose their intensity depending on the temperature to which the plant has been subjected. It can be noted that the band from 2925 cm^{-1} attributed vibrationally to the CH group is almost non-existent in the spectrum obtained from dried parsley at a temperature of 90^0C.

Evaluation of haematological parameters in rats after carmoisine administration

Tables 1 to 4 show the mean values for haematological indices in the blood analysed from the rats in the experiment.

Table 1. Mean values and variability of haematological parameters in control rats

Parameter	Unit	Control group	
		$X \pm S_x$	V%
WBC	10^9/l	5.042±2.15	19.35
LYM	10^9/l	3.916±1.62	22.58
MID	10^9/l	0.64±0.08	29.25
GRA	10^9/l	1.6±0.66	21.04
LY	%	60.40±4.21	4.47
MI	%	5.20±0.10	4.51
GR	%	21.80±0.92	9.47
RBC	10^12/l	7.636±0.17	5.04
HGB	g/l	133.40±0.75	1.25
HCT	%	38.01±0.29	1.70
MCV	fl	42.60±1.60	8.40
MCH	pg	14.380±0.25	3.95
MCHC	g/l	332±11.62	7.83
RDWc	%	21.32±1.06	11.09
PLT	10^9/l	716.60±8.45	2.64
PCT	%	0.59±0	1.2
MPV	fl	6.5±0.07	2.43
PDWc	%	32.62±0.26	1.81

X-value average; Sx-standard deviation; v- variability; n-5 copies/lot.

Table 2. Mean values and variability of haematological parameters in rats in the carmoisine 60 mg group

Parameter	Unit	Carmoisine group 60 mg	
		$X \pm S_x$	V%
WBC	10^9/l	5.99±1.03	38.60
LYM	10^9/l	4.08±0.54	29.32
MID	10^9/l	0.70±0.18	57.38
GRA	10^9/l	1.90±0.42	49.55
LY	%	62.60±1.54	5.49
MI	%	6.26±0.15	5.49
GR	%	28.26±2.02	15.98
RBC	10^12/l	7.99±0.28	7.94
HGB	g/l	130.60±2.66	4.55
HCT	%	39.79±0.56	3.17
MCV	fl	43.40±1.17	6.01
MCH	pg	14.6±0.73	11.11
MCHC	g/l	330.80±2.22	1.50
RDWc	%	21.74±0.21	2.17
PLT	10^9/l	759.80±13.27	3.90
PCT	%	0.66±0.02	5.59
MPV	fl	6.60±0.12	4.15
PDWc	%	32.62±0.14	0.98

X-value average; Sx-standard deviation; v- variability; n-5 copies/lot.

Table 3. Mean values and variability of hematological parameters in rats of the carmoisine group 100 mg + parsley and vitamin E

Parameter	Unit	Carmoisine group 100 mg + parsley and vitamin E	
		X±S_x	V%
WBC	10^9/l	6.49±0.61	20.96
LYM	10^9/l	3.99±0.52	29.18
MID	10^9/l	0.65±0.16	57.02
GRA	10^9/l	1.37±0.28	45.49
LY	%	64.87±2.11	7.26
MI	%	6.72±0.15	4.87
GR	%	29.84±0.87	6.51
RBC	10^12/l	8.89±0.19	4.91
HGB	g/l	139.60±1.60	2.56
HCT	%	42.87±0.50	2.62
MCV	fl	47.8±1.24	5.81
MCH	pg	15.66±0.28	3.98
MCHC	g/l	334.80±6.61	4.42
RDWc	%	22.8±0.21	2.06
PLT	10^9/l	768.40±10.75	3.13
PCT	%	0.66±0.02	6.95
MPV	fl	6.66±0.12	3.92
PDWc	%	33.46±0.32	2.11

X-value average; Sx-standard deviation; v- variability; n-5 copies/lot.

Table 4. Mean values and variability of haematological parameters in rats of the carmoisine group, 100 mg

Parameter	Unit	Carmoisine group 100 mg	
		X±S_x	V%
WBC	10^9/l	12.02±0.54	10.11
LYM	10^9/l	4.59±0.26	12.58
MID	10^9/l	1.07±0.21	44.16
GRA	10^9/l	1.96±0.11	12.93
LY	%	70.26±4.70	14.96
MI	%	7.10±0.10	3.30
GR	%	33.42±0.57	3.83
RBC	10^12/l	9.22±0.27	6.44
HGB	g/l	139.4±1.08	1.73
HCT	%	43.17±0.14	0.72
MCV	fl	53.60±2.01	8.41
MCH	pg	17.26±0.45	5.79
MCHC	g/l	325.2±3.72	2.56
RDWc	%	23.72±0.84	7.92
PLT	10^9/l	781.40±7.70	2.20
PCT	%	0.698±0.09	29.02
MPV	fl	7±0.07	2.26
PDWc	%	33.740±0.24	1.62

X-value average; Sx-standard deviation; v- variability; n-5 copies/lot.

The WBC showed highest values in group 3, which received 100 mg carmoisine, with a value of 12.02±0.54. The WBC showed highest values in group 3, which received 100 mg carmoisine, with a value of 12.02±0.54. Parsley along with vitamin E were able to keep the value of white blood cells at an optimal value. LYM shows similar values in the control group compared to the group which, in addition to carmoisine, was given parsley together with vitamin E, but the group given only 100 mg carmoisine showed higher values 4.59±0.26. The same applies to the MID parameter, where the control group and group 2 show low differences (0.64±0.08, 0.65±0.16) and group 3 show high values (1.07±0.21) exceeding the normal value of this parameter of 0.98. Although there is a difference between granulocyte values in the four groups of rats, they do not exceed normal values, as well as for LY, where group 3 (70.26±4.70) shows the highest values not exceeding the limit of 97.

As for hematocrit, the values between the four groups are close, none of them exceeds the normal limit of this parameter. For MI mean value for control group was 5.20±0.10, for group 60 mg carmoisine mean value was 6.26±0.15, for group 2 with carmoisine and vitamins was 6.72±0.15 and for group 100 milligram carmoisine was 7.10±0.10. Haemoglobin (HGB) and mean red blood cell haemoglobin (MCHC) concentration were not affected by the food additive administered to the rats in the experiment, the values of these parameters not changing with the amount of carmoisine ingested by the rats.

Haematological parameters showed higher values in rats in the carmoisine group compared to the control group and the one given parsley and vitamin E, it can be concluded that the food additive can alter haematological parameters even for a short period of time.

Evaluation of biochemical parameters in rats after carmoisine administration.

Biochemical profile in the blood is carried out to assess the health of an organism. By analyzing biochemical constituents, various pathophysiological states and metabolic disorders in animals can be diagnosed (Green et al., 1992). Tables 5 to 8 show mean values of biochemical parameters of blood collected from rats in the experiment (control group, group 1 with carmoisine 60 mg, group 2 with 100 mg carmoisine+parsley and vitamin E, group 3 with 100 mg carmoisine). Following analysis of biochemical parameters, it can be seen that mean AST (U/l) increased in group 3, which ingested 100 mg carmoisine (131.6±0.75), compared to the control group, where the mean value was (115.6±2.56). Regarding the ALT parameter (U/l) it can be seen that there are no large differences between the batches, but, as in the case of AST, the highest values are presented by the group with 100 mg carmoisine (64±1.30). In the case of glucose, it can be seen that there are no differences between the control group (224.6±2.50) and group 2 (224.6±2.50), despite the fact that the second group received 100 mg carmoisine and parsley and the control group did not receive this additive.

The carmoisine 60 mg group had a decrease in this parameter (206.4±3.08) compared to the control group, and group 3 had an increase in this value (242±2.51), thus outperforming all other groups.

Related to cholesterol (mg/dl), triglycerides (mg/dl) and creatinine (mg/dl) it can be said that the differences between the groups are insignificant.

Cholesterol was little influenced by administration of the additive to rats. In contrast, triglycerides increased slightly comparing the control group (57.8±1.39) with group 3 (59.4±1.36). However, administration of parsley and vitamin E seems to decrease the value in group 3, to 59±1.3.

Table 5. Average values and variability biochemical parameters in control rats

Parameter	Unit	Control group	
		$X \pm s_x$	V%
AST	U/l	115.6±2.56	4.95
ALT	U/l	59.80±1.39	5.21
Glucose	mg/dl	224.6±2.50	2.49
Cholesterol	mg/dl	68.6±1.03	3.36
Triglycerides	mg/dl	57.8±1.39	5.39
Creatinine	mg/dl	0.48±0.01	5.27

X-value average; Sx-standard deviation; v-variability; n-5 copies/lot.

Creatinine also underwent slight oscillations, but the differences between the batches are insignificant. In a study conducted by Amin & Hameid (2010) was tested the influence of carmoisine on parameters such as ALT, AST, ALP, creatinine, glucose. After administration of food coloring over a period of 30 days, they noticed a significant increase in the mean value for these parameters (Amin & Hameid, 2010). It should be noted that by administering carmoisine to experimental rats, the value of the GR parameter analyzed increased significantly from the value of 21.80±0.92 of the control group to the value of 33.42±0.57 of the rats of group 3. Groups 1 and 2 also showed close values despite the different amounts of carmoisine administered to each group.

Table 6. Mean values and variability of biochemical parameters in rats in the carmoisine 60 mg group

Parameter	**Unit**	**Carmoisine group 60 mg**	
		$X \pm s_x$	**V%**
AST	U/l	119.2±4.07	7.63
ALT	U/l	60±1.48	5.53
Glucose	mg/dl	206.4±3.08	3.33
Cholesterol	mg/dl	67±0.71	2.36
Triglycerides	mg/dl	51±0.71	3.10
Creatinine	mg/dl	0.47±0.01	2.45

X-value average; Sx-standard deviation; v-variability; n-5 copies/lot.

Table 7. Mean values and variability of biochemical parameters in rats of the carmoisine group (100 mg + parsley and vitamin E)

Parameter	Unit	Carmoisine group 100 mg + parsley and vitamin E	
		$X \pm s_x$	V%
AST	U/l	128.2±0.66	1.16
ALT	U/l	62±0.84	3.02
Glucose	mg/dl	224.6±2.50	2.49
Cholesterol	mg/dl	69.8±0.86	2.76
Triglycerides	mg/dl	59±1.3	4.94
Creatinine	mg/dl	0.48±0.01	5.19

X-value average; Sx-standard deviation; v-variability; n-5 copies/lot.

Table 8. Mean values and variability of biochemical parameters in rats of the carmoisine group

Parameter	Unit	Carmoisine group 100 mg	
		$X \pm s_x$	V%
AST	U/l	131.6±0.75	1.27
ALT	U/l	64±1.30	4.56
Glucose	mg/dl	242±2.51	2.32
Cholesterol	mg/dl	70.2±0.37	1.19
Triglycerides	mg/dl	59.4±1.36	5.13
Creatinine	mg/dl	0.5±0.01	2.98

X-value average; Sx-standard deviation; v-variability; n-5 copies/lot.

Amin & Hameid (2010) observe an increase for AST, ALT, alkaline phosphatase, urea, creatinine and albumin, for the group treated with carmoisine in low quantity and at the same time these values increasing significantly in those treated with high dose carmoisine. Gaunt et al. (1967) note that carmoisine negatively affects and alters biochemical markers in vital organs such as liver and kidneys at both lower and high doses. Studies on the effect of carmoisine on the biochemical profile, evaluation of oxidative stress in laboratory animals and in the womb, as well as acute and chronic toxicity studies, have been conducted by (Mason et al., 1974; Holmes et al., 1974; Ford et al., 1987; Amin & Hameid, 2010; Lamia et al., 2016; Coroian, 2019).

CONCLUSIONS

Most of the parameters analysed showed higher values in rats in the carmoisine group compared to the control and groups that also received parsley and vitamin E. The way and temperature of drying can influence the chemical structure of parsley and implicitly its properties.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

EFFECTS OF A PECKING STONE-BASED FEED SUPPLEMENT ON ZOOTECHNICAL PERFORMANCE AND SOME BIOCHEMICAL PARAMETERS IN COBB 500 BROILERS

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Abstract

In order to reduce problems linked to mineral deficiency during broilers production, as well as to improve digestive capacity of nutrients, the present study was conducted to evaluate the effects of pecking stone on broilers production. The study was conducted from October 2022 to February 2023 on 207 chicks of COBB 500 strain. The chicks were divided from the first day into three (3) batches of 69 birds and each batch was split into 3 sub-batches serving as repetitions. A batch T used as a control did not receive any mineral block, while, batch A and B were respectively supplemented with pecking stones A (peck A) and B (peck A). The results obtained showed that the feed conversion ratio of the animals fed the diet supplemented with pecking stones decreased significantly (2.73 and 2.70 for the Peck A and Peck B batches respectively) compared with the control batch (2.97). Body weight gain carcass weight and the feed cost of producing one kilogram of body weight of chicken was significantly ($p<0.05$) ameliorated with the use of pecking stones. In general, the feed supplement based on pecking stones improved the zootechnical and biochemical parameters of the chickens.

***Key words**: biochemical parameters, broiler, COBB 500, growth performance, pecking stone.*

INTRODUCTION

The number of people suffering from undernourishment worldwide reached 1.02 billion in 2009, with sub-Saharan Africa accounting for 30% of this number (FAO, 2009). Effectively combating this problem requires an increase in the production of both plant and animal resources. The population is rising sharply and is set to increase from 6.8 billion to 9.1 billion by 2050, i.e. a third more people to feed than today (FAO, 2013). Despite the selection and intensification of animal production, animal protein intake is still insufficient. Given this situation, there is an urgent need to find alternatives for quantitative and qualitative production at lower cost.

In Cameroon, according to statistics reported by GIZ (2018), the poultry sector accounts for 42% of national meat production, and has now become an important sector. This has been helped by the introduction of policies to increase meat products, such as the promotion of short-cycle species such as poultry (Ohouko, 2017).

Under these conditions, feed is the highest cost item in breeding and can therefore contribute to nearly 70% of the production cost of broilers. To achieve good performance, it is necessary to formulate balanced diet (energy, proteins, amino acids, vitamins, essential fatty acids and minerals). As a result, farmers' limited purchasing power means that they cannot afford to buy them on a regular basis. However, animals selected for their rapid growth and high feed conversion efficiency, such as broilers, suffer from numerous skeletal disorders (rickets and osteomalacia) due to mineral and vitamin D deficiency. In addition, phosphorus in the form of phytic acid (contained in plant seeds) is often poorly utilized, and this also reduces the bioavailability of calcium. As a result, practical diets fed to broilers are in most cases deficient in calcium and phosphorus unless supplemented with inorganic salts (Bao & Choct, 2009). To solve this problem locally, the population uses natural mineral resources and the uncontrolled use of which would lead to poor production performance, hence the importance of this study. In addition, growth promoters are widely used in poultry farming and there is growing interest in finding alternative solutions (Kiki et al., 2013). Feed supplements such as pecking stones made from natural by-products (oyster shells, calcium carbonate, bone meal, etc.) can be used as an alternative to chemical additives in order to compensate for deficiencies that occur during the various stages of poultry development.

The mineral block or pecking stone is a feed supplement made available to poultry with the aim of playing a number of roles, namely providing additional minerals as part of the feed through its composition, improving the degradability of feed as well as the digestive capacity of nutrients, animal welfare by guaranteeing natural pecking behaviour, and finally its usefulness in combating boredom. In addition, chemical mineral additives are increasingly expensive on the market and do not always guarantee poultry welfare and product quality (Chaouchi & Benattia, 2017). It is in this context that this study was initiated with the general objective of contributing to further improving knowledge of feed supplements for chickens based on pecking stone formulated from local by-products.

MATERIALS AND METHODS

Study site and period

This study was conducted between October 2022 and February 2023 on an experimental farm in the Bamyanga district. This district is located in the municipality of Ngaoundere 1st, Vina department, in the Adamawa region of Cameroon. The climate of the Ngaoundere municipality is humid tropical. It is characterised by an average annual rainfall of 1,500 millimeters. Peak rainfall is usually recorded in August. During the rainy season, it is affected by the monsoon, responsible for torrential rains sometimes accompanied by tornadoes. The average temperature is 22°C and diurnal temperature variations are very wide during the dry season. Total monthly evaporation is very high between December and March. It can reach 1,982.4 mm. Between June and September, however, it decreases very rapidly and stabilizes at between 34.6 and 40.4 mm, before rising again rapidly to reach a maximum in February.

Experimental birds

A total of 207 one-day-old Cobb 500 chicks with an average weight of 42 g were used in this study over a period of 49 days. They were randomly assigned following a completely randomized design to 3 treatments replicated 3 times with 23 chicks each and reared under the same conditions on litter made of white wood shavings at a density of 20 chicks/m^2 at the starter phase (1-21 days) and 10 chickens/m^2 at the growth and finishing phase (22-49 days). Water and feed were provided to the animals according to the method proposed by Rossilet (2004).

Prophylaxis

Birds were vaccinated against Newcastle disease (Hitchner B1®) and infectious bronchitis (H120®) on the 4th day with a booster dose on the 18th day. The vaccine against Gumboro disease (IBA Gumboro®) was administered on the 11th day. From the second week, the anticoccidial (Vetacox®) was administered three days a week in the drinking water. An anti-stress was administered to birds via drinking water for the first three days upon entry into the henhouse and each time before and after vaccination, weighing and transfer of birds to the finisher house.

Pecking stones

The pecking stone formulation process was carried out manually. The stones were made from bone meal; limestone powder; oyster shell; cooking salt; blood meal (from blood collected from Ngaoundere II slaughterhouse, then boiled for 5 hours to reduce potential pathogens and dried for 24 hours in the sun) and plant sap from the thorny *Acacia raddiana* tree, which served as a binder. This plant sap was dissolved in water at a ratio of 0.4 kg to 1 liter of water. Thus, for the formulation of the pecking blocks, two formulas were applied with different proportions as presented in Table 1.

Table 1. Proportion of ingredients for the two pecking stones

Components \ Pecking stones	Peck A	Peck B
	Proportion (%)	
Bone meal	15	15
Limestone powder	20	10
Oyster shell	30	40
Cooking salt	15	15
Blood powder	20	20

The molded blocks were exposed to the sun for 48 h to stabilize before being fed to the animals. The frequency of feed distribution depended on the growth phase of the animals (Ndiaye, 2006). During the start-up phase, feed was given *ad libitum*. From the growth to the finishing phase, diets were served twice a day (7 a.m. and 16 p.m.).

Chemical composition of pecking stones

The composition of the Peck A and Peck B pecking stones was determined by the complexometric titration method. It was carried out at the Soil Analysis and Environmental Chemistry Research Unit (SAECRU) at the Faculty of Agronomy and Agricultural Sciences, University of Dschang. Table 2 shows the mineral composition of the Peck A and Peck B pecking stones.

Table 2. Mineral composition of Peck A and Peck B pecking stones

Mineral composition	PECK A	PECK B
Calcium (mg/100 g)	1570.00	1810.00
Magnesium (mg/100 g)	437.40	413.10
Potassium (mg/100 g)	39.23	60.70
Sodium (mg/100 g)	479.25	589.06
Total Phosphorus (mg/100 g)	532.35	471.95

Experimental Rations

Three experimental rations were used in this study, depending on the development stage of the birds (start-up - days 1 to 21, growth - days 22 to 35 and finishing - days 36 to 49). These feeds were supplied by feed mill Company of Cameroon and their chemical composition is presented in Table 3.

Table 3. Chemical composition of diets depending on growth stage

Composition	Start-up	Growth	Finishing
Crude protein (%)	22	21	19
Moisture (%)	12	12	12
Fat (%)	6	6	8
Crude cellulose (%)	3.5	5	5
Calcium (%)	0.9	1	1
Phosphorus (%)	0.7	0.6	0.6
Crude ash (%)	5	5	5
Methionine (%)	0.6	0.58	0.55
Lysine (%)	1.3	1.2	1.15
Metabolizable energy (Kcal/kg)	2870	2950	3000

Experimental Design

At the start of the study, birds were divided into three batches: a control batch (T), two experimental batches (batch A and batch B), and each batch was subdivided into 3 sub-batches to allow repetition of each treatment:
- Control batch: made up of 69 birds receiving standard minerals (OLIGOPHOS®) in drinking water as positive controls, with the day of administration depending on prophylaxis,
- Batch A: Consisting of 69 birds not receiving standard minerals, but supplemented with Peck A pecking stone served ad-libitum throughout the cycle;
- Batch B: Consisting of 69 birds not receiving standard minerals but supplemented with Peck B, served ad-libitum throughout the cycle.

Each sub-lot was evenly distributed throughout the henhouse.

Data Collection and Studied Parameters

Growth performance

Data were collected every 7 days on feed intake, live weight, weight gain and feed conversion ratio. Feed was weighed and distributed to the animals daily and at the end of each week, the left overs were collected then weighed. Feed intake was calculated as the difference between the quantity served and the left over in each experimental unit. At the beginning of the trial and every 7 days thereafter, birds in each experimental unit were weighed and weekly weight gain was calculated as the difference between two consecutive week's weights. Feed conversion ratio (FCR) was calculated as the ratio of the amount of feed intake during the week and the weight gain of the same week.

Carcass characteristics

At 49 days old, 12 chickens (6 males and 6 females) per treatment were randomly selected and submitted to a 24-hour fasting, then weighed, sacrificed, plucked and eviscerated for carcass evaluation (Kana et al., 2015). The relative weight of each organ (gizzard, liver, heart, legs, etc.) was respectively calculated by dividing the weight of carcass or that of corresponding organs by the live body weight of the bird.

Evaluating the quantity of mineral block pecked

The amount of mineral block pecked by the subjects was compared between Batch A exposed to the Peck A pecking stone and Batch B exposed to the Peck B pecking stone. This amount was determined by taking the difference between the weight of the starting block (Week 4) and that at the end of the trial (Week 7). The different pecking stones were weighed per week and per sub-batch.

Biochemical determination of minerals: Ca, K and Na

Blood sampling was carried out at the end of the experimental cycle, in the 7th week (49 days old), on 36 birds at the time of slaughter, including 4 birds per sub-batch taken at random. These determinations were carried out as describe by Abdel-Fattah et al. (2008)

Evaluation of feed production costs

The evaluation of the feed production cost per kg live weight considered the average feed conversion ratio for each treatment during the growth and finishing periods (4^{th} and 7^{th} week) and the price per kg feed. The feed cost per kg live weight of chicken was estimated by multiplying the price per kg feed by the feed conversion ratio (FCR) defined as the ratio of feed consumed to weight gain for the same period.

$Cx\ (Fcfa) = \mathrm{FCR}x * Px\ (Fcfa)$

With: Cx: cost per kg live weight (Fcfa); FCRx: feed conversion ratio; Px: Price per kg diet (Fcfa); x: period considered.

Data analysis

Data on feed intake, live weight, feed conversion ratio, biochemical parameters and carcass characteristics were submitted to one-way analysis of variance (ANOVA). When there was a significant difference between treatments, Duncan's multiple range test at a 5% threshold was used to separate means. SPSS 20.0 (Statistical Package of Social Sciences) software was used for the analyses.

RESULTS AND DISCUSSIONS

Feed consumption

Individual feed consumption (Table 4) did not differ significantly (p>0.05) between the different batches from Week 4 to Week 7, although the highest values were recorded with batch B (Peck B).

These observations are similar to those reported by Pizzolante et al. (2007), on the different levels of calcium in Japanese quail aged 54, 39 and 45 to 57 weeks, respectively, who observed no influence on food consumption. This is because the ingredients used to make these blocks do not significantly improve the taste of the diets.

Table 4. Individual feed consumption according to treatments (IFC)

Age (In weeks)	Individual food consumption (means ± standard deviation)			
	Batch Control	Batch A	Batch B	***p*-value**
4^{th} Week	677.67^{a}±18.60	677.99^{a}±21.25	681.67^{a}±19.95	0.964
5^{th} Week	726.91^{a}±19.36	726.91^{a}±19.36	738.09^{a}±0.00	0.630
6^{th} Week	923.88^{a}±24.61	923.88^{a}±24.61	938.09^{a}±0.00	0.630
7^{th} Week	797.25^{a}±21.24	797.25^{a}±21.24	809.52^{a}±0.00	0.630
IFC Average	**781.43^{a}±20.95**	**781.51^{a}±21.62**	**791.84^{a}±4.98**	**0.720**

a, b: Means followed by different letters within the same line are significantly different

Weight growth

Growth rate and live weights

The evolution of the Average Daily Gain (ADG) according to the treatment (Figure 1) shows that the ADG was not significantly influenced (p>0.05) by the use of the pecking blocks. However, although no significant difference was observed, the mean ADG of batch A (41.25±2.97g) and batch B (42.56±1.79g) were higher than that of the control batch.

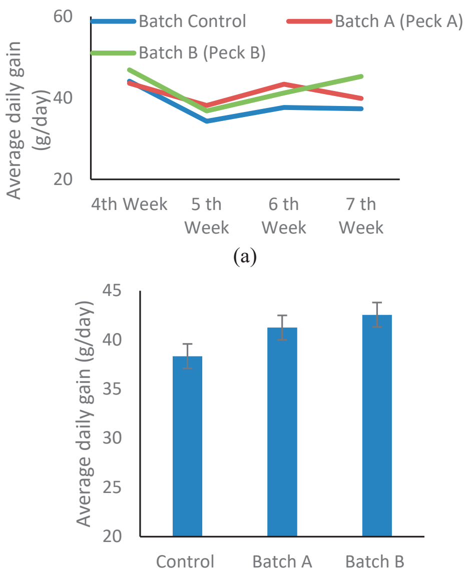

Figure 1. Average daily gain according to treatments

The data on the evolution of the average live weight of the subjects in relation to age and treatment (Table 5) show that no significant difference ($p>0.05$) was recorded between the batches during growth (4th and 5th week). On the other hand, the mineral supplements (Peck A and Peck B) significantly ($p<0.05$) influenced the live weight of the animals at week 7. The live weights of batches A and B remained comparable and significantly ($p<0.05$) higher than that of the control batch.
The live weight values recorded are higher than those of Gbodo (2021) in Benin, who obtained live weights of 1407.5 g and 1592.75 g at 8 weeks of age by incorporating oyster shells and eggshells respectively into the broiler ration. This difference would be due to an imbalance of ingredients in the feed formulation caused by the incorporation of calcium sources such as oyster shells and eggshells directly into the basic broiler ration. Indeed, it has been reported by Bao & Choct (2009) that phosphorus in the form of phytic acid (contained in large quantity in minerals of plant origin) is poorly utilized, and phytic acid also reduces the bioavailability of calcium (Ca). Consequently, practical diets fed to broilers are always deficient in calcium and phosphorus (P) unless supplemented with inorganic salts. This is why it is necessary to formulate a fully-fledged source of mineral intake that can be used as a feed supplement.

Table 5. Effect of pecking stone on live weight of chickens

Age (weeks)	Live weights in grams (mean ± standard deviation)			
	Batch Control	Batch A	Batch B	P
4th	$825.22^{a}\pm37.85$	$811.52^{a}\pm13.24$	$848.16^{a}\pm53.89$	0.540
5 th	$1065.16^{a}\pm36.04$	$1078.62^{a}\pm31.26$	$1105.87^{a}\pm33.79$	0.383
6 th	$1328.85^{a}\pm40.28$	$1415.63^{a}\pm57.19$	$1405.26^{a}\pm81.30$	0.251
7 th	$1680.47^{a}\pm50.58$	$1694.58^{a,b}\pm16.29$	$1755.77^{b}\pm31.64$	0.048

a, b: Means followed by different letters within the same line are significantly different.

Feed conversion ratio

The feed conversion ratio (FCR) of broilers in the different batches as a function of age (Figure 1a) did not vary significantly ($p>0.05$) from week 4 to week 7. However, the average feed conversion of batches A and B (Figure 1b) remained comparable and significantly ($p<0.05$) lower than that of the control batch.

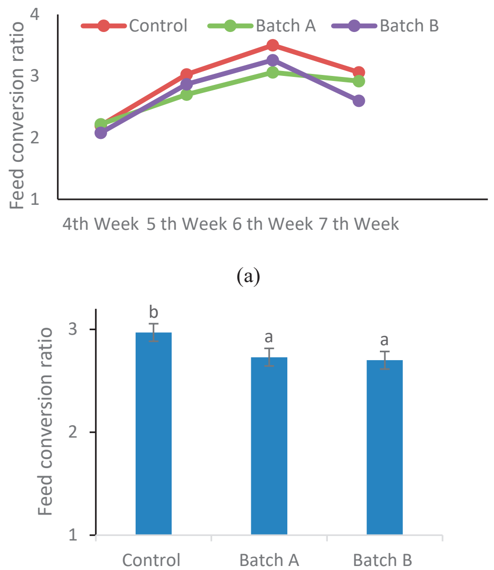

Figure 2. Feed conversion ratio according to treatments

These results do not corroborate those of Sultana et al (2007), who investigated the effects of calcium sources and levels, but found no significant difference in the feed conversion ratio. The lower feed conversion rate recorded in these studies with the use of pecking stones would be linked to their composition and texture, which would improve diet degradation and digestive utilization capacity of nutrients.

Aviagen (2012) also reports that if live weight gain correlates with feed intake, then higher feed intake improves feed conversion rate because the required slaughter weight is reached more quickly.

Carcass weight, yield and relative organ weights

The results for carcass weight and yield (Table 6) show that the carcass weight of the chickens increased significantly with the use of mineral supplements (Peck A and Peck B). The carcass weights of batch A (1443.41±29.05) and batch B (1466.66±25.81) remained similar and significantly ($p<0.05$) higher than those of the control batch. However, carcass yield was not significantly ($p>0.05$) influenced by the use of mineral supplements.

Table 6. Effect of pecking stone on carcass yield

	Carcass weight and yield (averages ± standard deviation)			
Parameters	Batch Control	Batch A	Batch B	*P*
Carcass weight (g)	1379.83^{a}± 40	1443.41^{a,b}± 29	1466.66 ^{b}±25.81	0.03
Yield (%)	81.00^{a} ±2	80.33^{a} ±1.52	79.66^{a} ±3.21	0.79

a, b: Means followed by different letters within the same line are significantly different

Various organs weighed at slaughter according to treatment (Table 7) show that, apart from liver weight, which increased significantly ($p<0.05$) with the use of mineral supplement pecking blocks, no significant difference ($p>0.05$) was recorded between the relative weight of different organs irrespective of treatment.

These results are not in line with those recorded by Makinde et al. (2013); Attia et al. (2013). The block proportions used in this work would not influence the relative proportions of the organs.

Table 7. Relative weight of organs according to treatment

Organs	Traitments	Means±SD	*P*
Heart	Control batch	9.08^{a}±0.87	0.645
	Batch A (Peck A)	9.08^{a}±0.52	
	Batch B (Peck B)	9.50^{a}±0.25	
Liver	Control batch	30.25^{a}±0.75	0.001
	Batch A (Peck A)	33.08^{b}±0.62	
	Batch B (Peck B)	36.25^{c}±1.50	
Gizzard	Control batch	40.00^{a}±1.08	0.492
	Batch A (Peck A)	38.91^{a}±2.50	
	Batch B (Peck B)	40.58^{a}±0.76	
Abdominal fat	Control batch	22.58^{a}±2.12	0.068
	Batch A (Peck A)	18.08^{a}±2.32	
	Batch B (Peck B)	21.41^{a}±1.18	
Intestinal mass	Control batch	221.58^{a}±10.75	0.817
	Batch A (Peck A)	221.83^{a}±4.01	
	Batch B (Peck B)	226.50^{a}±14.05	
Legs	Control batch	84.58^{a}±3.75	0.205
	Batch A (Peck A)	90.50^{a}±7.39	
	Batch B (Peck B)	92.75^{a}±2.81	
Head	Control batch	43.00^{a}±0.75	0.390
	Batch A (Peck A)	44.08^{a}±1.62	
	Batch B (Peck B)	45.16^{a}±2.51	

a, b: Means followed by different letters within the same column for the same organ are significantly different ($p<0.05$).

Effects of the pecking stone on some biochemical parameters

The plasma concentration of the three minerals measured (Table 8) shows a significant increase ($p<0.05$) in plasma concentration in batches receiving the pecking block compared to the control. Plasma concentrations of sodium and potassium, on the other hand, remained comparable irrespective of treatment, although the highest values were obtained with the batch receiving Peck B mineral supplement (127.35±22.01 mEq/L and 7.27±1.94 mEq/L for sodium and potassium respectively).

Table 8. Plasma mineral concentrations in chickens

Plasma Concentrations	Treatments	Mean ± SD	Minimum	Maximum	*P*
Sodium (mEq/L)	Control batch	106.60^{a}±21.53	72.72	144.79	0.097
	Batch A	116.59^{a}±24.43	82.00	159.92	
	Batch B	127.35^{a}±22.01	94.20	164,64	
Potassium (mEq/L)	Control batch	6.82^{a}± 1.98	3.53	11.02	0.844
	Batch A	7.07^{a}± 1.74	3.83	9.30	
	Batch B	7.27^{a}± 1.94	4.53	11.51	
Calcium (mg/L)	Control batch	75.40^{a}± 4.47	67.8	83.5	**0.011**
	Batch A	80.19^{b}± 4.61	72.4	89.5	
	Batch B	80.90^{b}± 4.62	73.3	87.7	

a, b: Means followed by different letters within the same column for the same parameter are significantly different ($p<0.05$).

Plasma calcium concentration increased significantly ($p<0.05$) in chickens fed mineral supplements (Peck A and Peck B), and fell within the ranges recommended by Fontaine (1992) and Campbell (2004), which are 88-240 mEq/L and 80-110 mEq/L respectively. It is also comparable to those reported by Gbodo (2021), which are 81.78-91.91 mEq/L. Thus, this increase in blood calcium levels in Batch A and B compared with the Control Batch would be due to the ingredients making up the pecking blocks. Bone meal, oyster shells and calcium carbonate are the by-products that are essentially rich in calcium. Eggshell contains 95.6% of the mineral calcium carbonate, while bone meal contains 85.5% of the mineral (Pion, 1970). In addition, given the extraction and statistical distribution of variables according to the treatment elucidated by Principal component analysis (PCA), calcium (Ca), sodium (Na) and potassium (K) measured in this trial are strongly associated with Batch A and B, unlike the Control Batch. This strong association may be due to the ad-libitum supplementation of Batch A and B subjects, which would have favored the progressive assimilation of these minerals, which constitute the Peck A and B pecking stones.

In addition, although no significant differences ($p>0.05$) were recorded in sodium and potassium concentrations. Chickens supplemented with Peck A and Peck B pecking stones nevertheless showed higher blood sodium and potassium levels than chickens in the control batch. Thus, the Sodium value is lower than that proposed by authors such as Fontaine (1992) and Campbell (2004). This could be explained on the one hand by the low proportion (15%) of salt (NaCl) incorporated at the time of formulation of the pecking stones and on the other hand it has been reported by Nedjoua (2013) that vegetable feed is largely deficient in sodium, but rich in potassium. Furthermore, Bao & Choct (2009) report that there is little to worry about in terms of practical Na and Cl deficiency in chickens. Hence, there is no need to incorporate a high proportion of salt in their feed.

Effects of pecking stone on feed production costs

Evaluation of the production feed cost per kilogram live weight of broilers as a function of treatment during growth and finishing (Table 9) shows that Peck A and Peck B feed supplements significantly ($p<0.05$) influenced the production feed cost of broilers in the finishing phase.

Results concerning the production feed cost per kilogram live weight of chickens supplemented or not with pecking stone show that the lowest feed costs were recorded with the batches receiving mineral supplements (Peck A and Peck B).

Table 9. Production feed costs according to treatments

PFC (CFAF) per kg of live weight	LOTS			*p*
	Control batch	Batch A (Peck A)	Batch B (Peck B)	
Growth	1098.63[a]	1040[a]	1041.5[a]	0.148
Finishing	1186.46[b]	1142.10[a]	1121.19[a]	0.024

a, b: Means followed by different letters within the same line for the same growth phase are significantly different ($p<0.05$). PFC: Production feed cost.

This could be explained by the fact that the supplements had a significant effect ($p<0.05$) between treatments on the feed conversion ratio, which in turn influenced the food cost of producing one kg of chicken live weight. In fact, the similarity of the feed conversion ratio of batches A and B, combined with its strong representation on the control batch, probably reflects the improved feed conversion of chickens supplemented with Peck A and Peck B pecking stones.

CONCLUSIONS

At the end of this study on the effect of the pecking stone-based feed supplement on zootechnical performance and some blood minerals in COBB 500 broilers, it was found that live weight gain and feed conversion ratio were significantly improved by the use of pecking blocks in growing-finishing broilers. Feed consumption, average daily gain, carcass yield and relative organ weights were not significantly influenced by the use of pecking blocks. Carcass weight, plasma concentration of calcium and feed production cost of growing-finishing broilers were significantly improved by the use of pecking stones. Although the results of this study are satisfactory with peck A and peck B pecking stones, it would be desirable to carry out a similar study on other poultry strains, such as layers, in order to assess the impact of such a feed supplement on their production performance.

ACKNOWLEDGEMENTS

The authors are please to thank the University of Ngaoundere and Agricultural Research

Institute for Development of Wakwa where some analysis was carried out.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

THERMAL STRESS ANALYSIS OF DIETARY FATS

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Abstract

The aim of this paper is the assessment of thermal stress effects on dietary fats. For this purpose, oils and fats from those sold on the Romanian market, intended for home use and in public food establishments were selected (sunflower oil, palm oil, lard and vegetable fat). These oils and fats are some of the most used ones in cooking. Dietary fats were subjected to heat stress by gradually heating to different temperature levels as well as repeated exposure to high temperatures. Subsequently, the influence of temperature on the characteristic parameters of food fats was analysed: free acidity, indices of: acidity, iodine, peroxide, saponification, refraction, water content and volatile substances, unsaponifiable matter. The results of the present study provide useful information for choosing a dietary fat suitable for frying food, in order to minimize the undesirable effects for the health of the consumer and to obtain a quality fried product.

***Key words**: animal fat, dietary fats, oil, Romanian market, thermal stress.*

INTRODUCTION

Dietary fats are part of the macronutrients category, which, along with proteins and carbohydrates, are important nutrients that the body needs to function normally (Banu et al., 2002; Alais et al., 2020; Oteng & Kersten, 2020). In the daily diet, the intake of fats is ensured by the consumption of fats of animal and vegetable origin, as such (butter, lard, oils) or contained in food (meat and meat products, dairy products, fish, avocados, olives, oleaginous seeds) (Lee, 2017).

The classification of fats is based on several criteria: origin and properties, consistency at 20^0C, type of fatty acids that predominate in their composition. Another criterion for classifying dietary fats is the water content (Banu et al., 2002; Alais et al., 2020). Thus, they can be divided into three types: vegetable and animal oils (maximum 0.2% water content), animal fats (maximum 8% water), butter and margarine (maximum 16% water) (Malesza et al., 2021).

In the context of the globally accelerated increase of edible oils prices, the trend in choosing them for consumption, especially for the thermal preparation of food by frying, is directed towards the purchase of products at affordable prices, without emphasizing quality. For the same reason, when frying, oil is used more times than recommended, which is not beneficial, neither for health nor for the quality of the fried products (Radzikowska et al., 2019; Santos et al., 2018; Ding et al., 2022).

During the frying process, chemical reactions that occur cause irreversible changes in the properties of the oily product, during the entire thermal process (Choe & Min, 2007; Bhat et al., 2022; Choe & Min, 2007; Matthäus, 2010). These reactions are determined by the biochemical composition of the oil and also by the physico-chemical stress factors (temperature, light, presence of oxygen in the air) (Hassanzadazar et al., 2018).

Thermal stability evaluation is an important tool to determine the optimal utilization of the oil process in order to maximize profitability and satisfy consumer preferences and consumer safety requirements (Zhuang et al., 2022; Negishi et al., 2003).

The frequent consumption of foods in the preparation of which heated oil was used may trigger a postprandial pro-inflammatory status (Malesza et al., 2021; Lee, 2017).
At the same time, the consumption of products thermally prepared in burnt oil should be avoided, as there is a risk of atherosclerosis and rheumatoid arthritis (Radzikowska et al., 2019). This oil is more dangerous because it doesn't necessarily have a burning taste, so it's not easy to identify (Ioannou et al., 2023).
Compounds formed during frying can contribute to the imbalance of the microbiome, that can lead to inflammatory bowel disorders (Narula et al., 2021). At the same time, lower microbiome diversity as a result of fried food consumption is associated with lower immunity and digestive problems (Wastyk et al., 2021).

MATERIALS AND METHODS

The edible fats subjected to heat stress (oils and fats) were selected from those sold on the Romanian market, intended for domestic use and in public catering establishments: oils obtained from oilseeds (sunflower, rapeseed), oil fruits (palm), vegetable fat (margarine) and animal fat (lard).
The ingredients, as well as the recommendations regarding how to use each dietary fat can be found in Table 1 and are those written on the label/packaging of each product.

Table 1. Characteristics of the analysed dietary fats

Product name	Ingredients	Recommendations
Refined sunflower oil "Vita D'or"	refined sunflower oil rich in monounsaturated fatty acids (oleic acid)	Recommended for frying
"Yunus" palm oil	refined vegetable oil (palm oil and its fractions), antioxidant (butylhydroxytoluene (BHT) - E321), antifoam (dimethylpolysiloxane - E900a)	Recommended for frying
"Angst" lard	lard, unsalted sea salt	-
Spreadable vegetable fat 72%, "Vita D'or"	vegetable fats (palm, coconut), water, rapeseed oil, emulsifiers: lecithin, mono- and diglycerides of fatty acids 0.2% salt, flavour, acidity corrector: citric acid, vitamin A, vitamin D, dye: carotenes	Product for baking and cooking

Samples preparation

The thermal stress was applied in a temperature gradient regime (100^{0}C, 200^{0}C and 240^{0}C, 30 minutes for each temperature) (program 1), respectively constant temperature (cycles of 30 minutes at 175^{0}C) (program 2). The work scheme is presented in Figure 1.

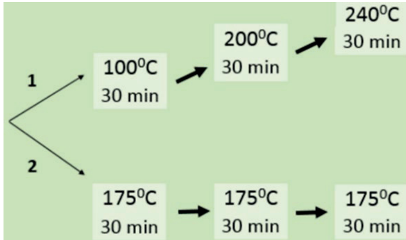

Figure 1. Scheme of thermal stress applied to food fats

Analysis methods used for sample characterization

Both the raw (before thermal stress) and the heat-treated samples were analysed according to the standardized analysis methods mentioned in Table 2.

Table 2. Standardized methods used for physico-chemical characterization of samples

No.	Analysed parameter	Unit	Standardized method
1.	Free acidity	% oleic acid	SR EN ISO 660-2020
2.	Acidity index	mg KOH / g fat	
3.	Iodine index	g I / 100 g	SR EN ISO 3961:2013
4.	Peroxide index	meq O_2 / kg	SR EN ISO 3960-2017
5.	Saponification index	mg KOH / g fat	SR EN ISO 3657:2020
6.	Unsaponifiable	%	SR EN ISO 18609:2002
7.	Refractive index	-	SR EN ISO 6320:2017
8.	Water and volatile substances	%	SR EN ISO 662:2002

Free acidity is the percentage of free fatty acids from the total fatty acids contained in the fat sample, which is determined by dissolving a quantity of the sample in an alcohol-ether mixture, followed by titration with KOH solution in the presence of phenolphthalein.
The **acidity index** depends on the amount of free fatty acids resulting from basic hydrolysis and autoxidation of fats. Therefore, this index allows to appreciate the degree of fats freshness. Thus, the

smaller it is, the fresher the fat is, which means that the rancidity process has not started.

The **iodine index** indicates the unsaturation degree of the fat, varies with storage time and represents the amount of iodine, expressed in grams, which is added to the double bonds of unsaturated fatty acids in 100 g of triacylglycerol and titrated with sodium thiosulphate solution, in the presence of starch as an indicator.

The **peroxide index** is a parameter that reflects the content of oxygen in the form of peroxide (hydroperoxide) in a fat, indicating the degree of fat freshness. The peroxides formed from the oxidation of polyunsaturated fatty acids from fats in the presence of atmospheric oxygen react with potassium iodide, releasing iodine which is titrated with sodium thiosulphate in the presence of starch.

The **saponification index** represents the quantity of potassium hydroxide in mg, required for the saponification, respectively the transformation into soap, of one gram of oil. This index characterizes the molecular weight of the fatty acids that make up the oils.

Unsaponifiables are the organic substances in oils and fats that do not react with alkalis and therefore do not form soap. This category includes higher aliphatic alcohols, pigments, aromatic and volatile compounds. They are soluble in common solvents.

The **refractive index** is also a parameter used in assessing the quality of food fats and represents the ratio of the sine of the angle of incidence to the sine of the angle of refraction. Its determination was made using an Abbe refractometer. For oils, the determinations were made at 20^0C, and for fats which are solid at room temperature, at 40^0C.

The determination of the **water content and volatile substances** was carried out by oven drying at 105°C until constant mass, followed by cooling the sample and weighing to the nearest 0.001 g.

RESULTS AND DISCUSSIONS

Dietary fats were analysed before being subjected to thermal treatments, the analysis methods being those presented in the previous Table. Each sample being analysed in triplicate. The results obtained fall within the limits provided in the quality standards (Table 3).

Table 3. Physico-chemical characterization of food fat samples, before the application of thermal stress

S	Free acidity, % oleic acid	Acidity index, mg KOH/g fat	Iodine index, g I/100 g	Peroxide index, meq O2/kg	Refractive index	Water and volatile substances, %	Saponification index, mg KOH / g fat	Unsaponifiable, %
1	0.21	0.5	128	2.12	1.47	0.02	189	0.98
2	0.12	0.3	51	2.85	1.46	0.02	198	1.15
3	0.5	3.4	65.2	6.67	1.47	9.4	194	0.77
4	0.45	4.95	34	2.45	1.45	15.9	200	1.88

*S-Sample

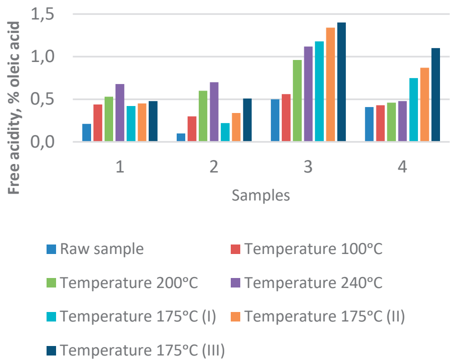

Figure 2. Comparison of free acidity values for both heat stress programs

According to the graph shown in Figure 2, the increase in free acidity values of all samples with temperature increase shows an accumulation of free fatty acids, which is in accordance with the fact that fat degradation is driven by increasing temperature.

As can be seen in Figure 2, sample 1 has a better stability during program 2 compared to program 1, while in sample 3 there is an almost threefold increase in the amount of free fatty acids.

Table 4. Acidity index values (mg KOH/g fat) of the analysed samples

S	Raw sample	Temperature					
		100^{0}C	200^{0}C	240^{0}C	175^{0}C (I)	175^{0}C (II)	175^{0}C (III)
1	0.5	0.56	0.62	1.35	0.58	0.6	0.62
2	0.3	0.98	1.62	2.56	0.45	0.7	0.83
3	2.4	2.6	2.8	3.1	2.7	3.1	4.5
4	4.8	4.9	5.2	6.3	5.1	6.4	6.8

*S-Sample

The increase in the acidity indices values (Table 4) is the consequence of the accumulation of free fatty acids at each temperature step, which results in fats degradation.

In the graphic representation from Figure 3, the degree of unsaturation is reflected by the obtained values. Thus, the samples with the highest content of saturated fatty acids are the margarine samples (4), the palm oil (2) and the lard sample (3) and, finally, the refined oil (1).

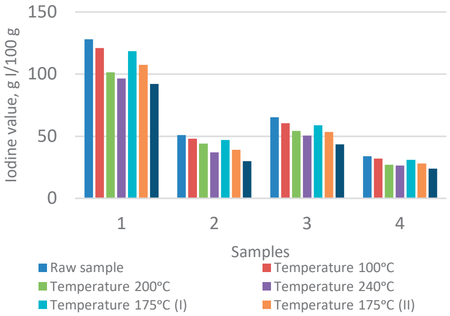

Figure 3. Comparison of iodine index values for both heat stress programs

For all samples, the degree of unsaturation decreases with increasing temperature, as a result of fat degradation reactions.

According to Figure 3, it can be concluded that the variation of the unsaturation degree of the four samples is the same, regardless of the applied program.

Regarding the variation of the peroxide indices (Figure 4), the obtained values show that, at temperatures higher than 200^0C, fat degradation occurs with the formation of considerable amounts of free radicals and, implicitly, peroxides, best example being sample 4, where the values of the peroxide increased by about 100%.

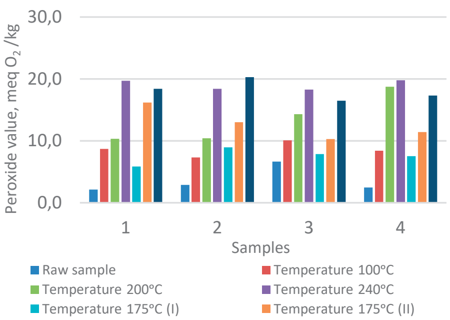

Figure 4. Comparison of peroxide index values for both heat stress programs

These values continue to increase at 240^0C, which shows that the thermal degradation increases as the temperature goes high. At the end of both programs, comparable peroxide index results are obtained for all samples, meaning that they have the same level of degradation.

The formation of free fatty acids causes an increase in the amount of saponifiable substances, as can be seen in Figure 5.

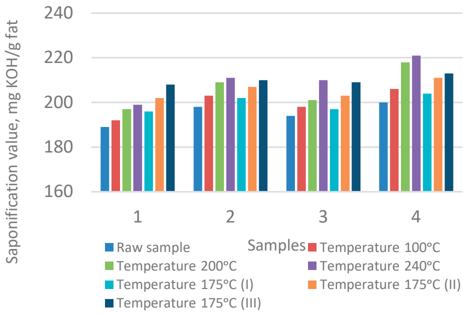

Figure 5. Comparison of saponification index values for both heat stress programs

The increase in saponification index occurs with increasing temperature and keeps the same trend for all studied dietary fats.

Regarding the influence of the temperature programs, the saponification index values are comparable for all samples, so the optimal program cannot be chosen.

The thermal degradation of food fats also results in the formation of unsaponifiable matter, thus causing their quantity to increase as the temperature increases. According to the graph in Figure 6, a significant difference can be observed in the variation of the amounts of unsaponifiables for sample 2. In the case of program 2, the amount of unsaponifiable matter increases with increasing temperature, the variation being the same for all samples. The lowest amount of unsaponifiables was determined for the lard sample, while vegetable fat had the highest values.

Thermal stress influences the values of the refractive indices of fats, as a result of the increase in the concentration of fatty acids resulting from thermal degradation. Thus, there is an increase in the value of the refractive index as the products resulting from the autoxidation reaction accumulate.

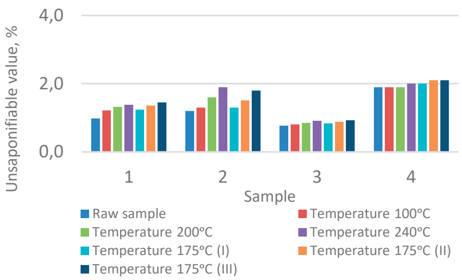

Figure 6. Comparison of unsaponifiables values for both heat stress programs

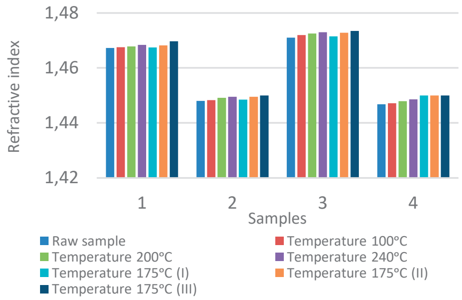

Figure 7. Comparison of refractive index values for both heat stress programs

As can be seen in Figure 7, in the case of the studied samples, the refractive index values are directly proportional to the increase in temperature, in both thermal stress regimes. Furthermore, the variations in values for both heat stress programs are comparable, the difference between the final value of each program and the initial value being around 0.002.

During heat treatment, evaporation of water from fats, as well as volatile substances, takes place. The decrease in the values of this parameter is more pronounced in the case of program 1 (Table 5).

Table 5. Comparison of water content values for both heat stress programs

S	Raw sample	Temperature					
		100°C	200°C	240°C	175°C (I)	175°C (II)	175°C (III)
1	0.018	0.015	0.013	0.008	0.016	0.013	0.010
2	0.019	0.016	0.014	0.009	0.017	0.015	0.012
3	9.400	8.800	7.900	7.300	8.700	8.100	7.500
4	0.450	0.120	0.070	0.020	0.090	0.070	0.060

*S-Sample

CONCLUSIONS

Analysing the results obtained following the application of the two temperature programs on food fats, the following can be concluded:

- sample 1 (Refined sunflower oil "Vita D'or") has a better stability during program 2 compared to program 1, while in sample 3 ("Angst" lard) there is an almost threefold increase in the amount of free fatty acids;
- the samples with the highest content of saturated fatty acids are the margarine samples (4), the palm oil (2) and the lard sample (3) and, finally, the refined oil (1);
- referring to the variation of the peroxide indices, the obtained values show that, at temperatures higher than 200^{0}C, fat degradation occurs with the formation of considerable amounts of free radicals and, implicitly, peroxides, best example being sample 4, where the values of the peroxide increased by about 100%;
- regarding the influence of the temperature programs, the saponification index values are comparable for all samples, so the optimal program cannot be chosen;
- the lowest amount of unsaponifiables was determined for the lard sample, while vegetable fat had the highest values;
- the variations of refractive index values for both heat stress programs are comparable, the difference between the final value of each program and the initial value being around 0.002;
- the decrease in the values of water content and volatile substances is more pronounced in the case of program 1.

The final conclusion is that, for all food fats analysed from the point of view of thermal stability, heating to 100^{0}C is safe, and for repeated heating to 175^{0}C it is recommended to do it twice at most, to avoid their sharp degradation and, implicitly, the occurrence of negative effects on the health and quality of products prepared by frying.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

CURRENT ANALYSIS OF THE "ŢARA DORNELOR" GEOGRAPHICAL AREA AND THE DEVELOPMENT OF TRADITIONAL AGRICULTURAL POTENTIAL

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Abstract

The Dornelor Depression occupies a mountainous geographical area with subalpine specifics, known as "Ţara Dornelor". The geological, pedo-climatic, and floristic structure of this area represents the basis of the traditional agriculture development, which is mainly centered on the growth of some autochthonous cattle breeds (Transylvanian Pinzgau, Black Pinzgau/ Dorna Cow and Brown cow of Maramureș). This study aimed to analyse the current level of geological, pedological, climatic, and biodiversity conditions, as well as the need to preserve Pinzgau cow herds and the procedures for preparing traditional dairy products. The obtained results confirmed the location of Suceava county among the leading areas in domestic cattle breeding, Romanian Spotted Cattle being predominant. The analysis of the specific climate has revealed that it is directly influenced by the relief which is structured in altitude steps, the Dornelor land being a representative geographic area for the national and community heritage, through the protected natural areas of the "NATURA 2000" European Ecological Network.

Key words*: mountain area, pedo-climatic factors, traditional foods.*

INTRODUCTION

The Carpathian Mountains rarely exceed an altitude of 2500 m and are characterized by a temperate-continental climate, rich in forests and natural meadows, which cover almost 74.000 km^2. This climate confers shorter periods of vegetation and lower temperatures, compared to those specific to similar areas in the Alps or the Pyrenees (Rey, 1997). More precisely, according to the latest criteria for the delimitation of mountain areas (HG no. 949 of 2002;https://www.cdep.ro/pls/legis/legis_pck.htp_act?ida=39247), the mountainous area of Romania represents 32.7% of the national territory, and the Carpathian Mountains outline one of the largest European mountain ranges located in a single country (Rey, 1997). The provisions of the MADR (Ministry of Agriculture and Rural Development) (Order 97/1332/2019;https://www.cdep.ro/pls/legis/legis_pck.htp_act?ida=156772) are also worth noting, showing that in this area, the climatic and biological conditions are less favorable and limit the level of agricultural production.

However, the research on the evolution of anthropology in the mountainous area of Bucovina records the existence of man on the territory of the Dornea Basin since ancient times, dating back to the Paleolithic and Neolithic eras (Andronic, 2009). As a geographical unit, the Dornelor Depression is located in the northwest of the Bistrița basin, being located entirely in the mountainous area, on the border between the Nordic Group and the Central Group of the Eastern Carpathians.

The altitude reaches 750 m in the depression area, gradually increasing to 1900 m, on the crests of the Călimani Mountains, at significant heights, where the Bârgău Mountains are located in the northern and northwestern areas. This area has a rich hydrographic network, circumscribed by several rivers with medium or low flows (Dorna, Dornișoara, Teșna, Neagra, Coșna). In the Romanian geographical literature, the depression is also called "Ţara Dornelor", suggesting that it includes the depression hearth and the surrounding mountain range, along the entire economic domain (Mihalca, 2014).

The practice of traditional household-type agriculture, based on grazing, the use of traditional hay, and natural fertilizers contributed to the preservation of this area and the maintenance of its biodiversity. As mentioned by many researchers in the field, traditional agriculture has a decisive role in the conservation of mountain biodiversity in general, which was also noted in the case of this area (Plieninger, 2007, Senf et al., 2015). The practice of intensive agriculture in mountain areas is limited by pedo-climatic conditions and other specific factors. In this context, the basic agricultural culture in the Dornelor area is limited to the exploitation of potatoes. An important share also belongs to the natural meadows, which are mostly forested and provide special conditions for grazing during the summer and the utilization of hay for obtaining dry fodder. The conservation of these meadows in natural conditions is a priority for supporting the botanical and faunal diversity, necessary to ensure the biological and nutritional value of the traditional food products specific to these areas (Boesi, 2014). It should also be remembered that forests, which occupy the largest part of the surface (53.72%), pastures, hayfields, and reserves of ferrous and non-ferrous minerals, peat, as well as mineral waters, participate in the creation of the local resources. Comparatively, the share of arable areas is very low (10-12%), most of the agricultural land being intended to support livestock production.

Regarding the valorization of traditional dairy products and mineral water resources, tourism in the area, including balneal tourism, has a particular economic impact (Pop, 2000). Over the centuries, the population in the mountainous areas has adapted to the conditions and possibilities offered by these secular areas, learning to protect nature and live healthily in harmony with it, which explains why the households in these areas are a true "archive of resources and knowledge", which can be a guarantee for supporting future projects regarding the development of sustainable agriculture (Knowles, 2011).

The purpose of this research was to analyze the current knowledge in the spirit of the sustainable development of mountain agriculture in "Ţara Dornelor", mainly focused on the support of biodiversity, breeding of domestic cows, and valorization of traditional dairy products.

MATERIALS AND METHODS

The research was carried out in the period 2019-2022 and consisted of the collection and analysis of some data, which were later used in the geographical, geological, pedo-climatic, and socio-economic characterization of the Dornelor area and implicitly in the evaluation of its biodiversity and productive potential. The geographical identification and delimitation began with the research of this area from a bibliographic point of view, consisting of the collection of data, their processing, and interpretation. Thus, for the identification and characterization of the geographical area, the available bibliographic documentation was first analyzed, and later completed with the field evaluation of the territorial surfaces and the main cow-breeding households, and some milk processing units, respectively.

The visits that were made later allowed the direct evaluation of the surfaces necessary for the contouring of the pedological and biodiversity studies. The investigated and analyzed variables were focused on the geological structure, the morphological and physicochemical profiles of the soil, the composition of the flora and fauna, as well as the specifics of the agro-zootechnical activity in the Dornelor area. The present study also resorted to the processing of some data provided by the Faculty of Geography of the "Alexandru Ioan Cuza" University in Iași, which mainly included the vector data used in the creation of cartographic and raster materials (used as background/support of geological and pedological data). Also, files from the ESRI (Esri: GIS Mapping Software) geodatabase (ArcGIS; https://www.arcgis.com/index.html) were used in making the maps.

In order to evaluate the land use, the mapping method based on the classification system proposed in the Corine Land Cover (CLC) project was used. According to this project, the land use classes were established by the classical method, keeping the inventory direction of the project (CLC 2006 Technical Guidelines). The software used to complete the cartographic materials was ArcMap 10.8, a component of the ArcGIS program, provided by ESRI. The data relating to the evolution of livestock, during the period 2018-2022, were verified by the county services ANSVSA (The National Veterinary Sanitary and Food Safety Authority), after being collected from current official sources (Statistical Report of Suceava Sanitary Veterinary and Food Safety Directorate). The data that were used to characterize the evolution of climatic factors had been collected from two meteorological stations in the Dornelor region (Poiana Stampei and Călimani), which monitor the evolution of standardized climatic parameters in our country daily. In addition, some data for the year 2022 were collected and processed from the Bucharest National Meteorological Administration (ANM) (https://www.meteoromania.ro/). In the statistical analysis of the data, the application of the XL STAT program, version 2022, was used, in which $p < 0.05$ values were considered statistically significant.

RESULTS AND DISCUSSIONS

In order to present and analyze the obtained results coherently, it is opportune to correlate them with the set of current data related to the Dorna depression, addressing the geographical layout, the pedological dominants, the evolution of biodiversity, the climatic conditions, the trends of agritourism and traditional agriculture, mainly focused on obtaining and utilization of dairy products.

Geographical layout. Geographically, this lowland area is surrounded by mountainous massifs of different altitudes, such as Suhard with the Oușorul peak (1369 m) to the north; Giumalau (1857 m) to the northeast; The Bistriței Mountains with Budacu peak (1864 m) to the east and southeast; The Călimani Mountains with Pietrosul peak (2100 m), Tămădău peak (1863 m) and Gura Haitii (1620 m) to the south; The Bârgău Mountains with Heniul Mare Peak (1611 m) to the northwest. Moreover, this area also presents the following natural communications: Mestecăniș Pass (to the northeast, 1100 m), Bistrița Aurie valley with Prislop Pass (to the northwest, 1413 m), Zugrenilor Gorge (to the east, 740 m), Tihuța Pass (to east, 1413 m), Păltiniș Pass (to the southeast, 1327 m) (Figure 1). The subdivision of the lands in the area was marked by the multitude of relief forms, the gradual increase in altitude generating a specific layering, correlated with the topoclimatic aspect. Thus, the easy transition from swamps and wet meadows to hayfields was favored, and on the upper floors to meadows and mountain hayfields to subalpine meadows (Achim, 2015).

The Dornelor basin is rich in flowing surface waters, such as the Bistrița Aurie river, with the Dorna and Neagra rivers as its main tributaries; their confluence downstream continues the Bistrița river, which is one of the most important fluvial systems in the Eastern Carpathians, crossing various geological forms that contribute to the accumulation of different sediments (Oncescu, 1965).

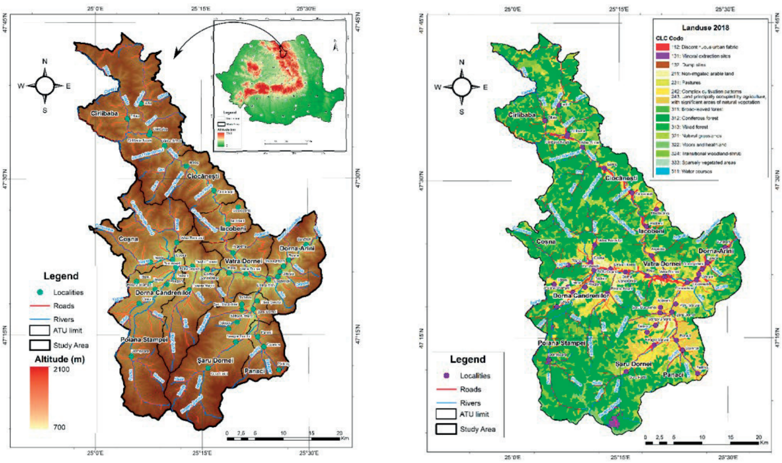
Geopedological aspects. From a geological perspective, the dominance of Proterozoic rocks is reflected in the northeastern compartment of the depression. On the southwestern side, igneous, eruptive rocks predominate (Neogene volcanic forms). In the north of the area, Triassic limestones are found. The depression area is dominated by the disaggregation rocks (alluvium) of the Quaternary age, built on an older foundation, of metamorphic rocks. The geological substratum of the territory, from which the Dorna gathers its waters, is particularly complex, being represented by a great petrographic variety. It is the result of the fact that the Dornelor Basin overlaps the boundary zone of three large geological formations: eruptive (Călimani Mountains), Transcarpathian flysch (Bârgăului Mountains), and crystalline (Suhardului Mountains) (Figure 2a) (Roșu, 1980). There are geostructural arguments, which confirm the crystalline-Mesozoic nature of this depression. The Dornelor area is a mineralogical complex (Cu-Pb, Cu-Mo, Bi-As, Zr-Ti, Zi-Rb, Cu-Zr, Ti-Cu, Cu-Rb, Zi-Bi, Zn-As, Pb-Zr, Pb-Rb Sr-Ti, Mo-Rb, Ag-Ca, Ti-Rb), in which manganese deposits predominate, usually combined with Fe and Cu (Leniuc, 2015).

The influence of geological and geo-chemical factors on the migration of many elements in the soil has led to the formation of landscape features that reflect the variability of the concentrations of existing elements in the soil, with effects in the enrichment of plant biodiversity (Makhinova et al., 2014). Regarding the valorization of pyrite and chalcopyrite manganese deposits, the Dornelor area represented an important national supplier, which is currently maintained only in three localities: Ulm (Dorna Arini), Oița (Ciocănești) and Arșița (Iacobeni). Another major wealth of the Dornelor region is represented by mineral and carbonated waters, which are associated with the post-volcanic activity of the eastern area of the Călimani Mountains.

The volcanic rocks date back over 10 million years, and at great depth, there is a mixture of meteoric water with CO_2, followed by a long-term natural filtration that takes place deep in the Eastern Carpathians (Băncilă, 1958).

The distribution and potential of soils were studied for a long time and several types of soils were identified that correspond to 9 classes, with different degrees of fertility that had a significant role in agricultural practices as well as in the way of exploitation and capitalization of the lands.

The most important and most predominant for the Dornelor area is the "Cambisols" class (44.70%), followed by the "Spodosols" class (43.98%), classes that fall into the category of those with low fertility, corresponding to mixed forest vegetation and conifers as well as pastures and hayfields. They are followed by the classes: "Andosols/Umbrisols" (4.37%), "Protisols/Antrisols" (3.59%), "Luvisols" (1.18%), "Cernisols" (0.78%), "Hidrisols" (0.76%), "Histosols" (0.42%) and "Salsodisols" (0.22%) (personal calculation according to the database provided by ArcGIS Online https://www.arcgis.com/index.html) (Figures 2b and 3).



a b

Figure 1. Detailed geographical map of the Dornelor area (a) and land use (b)
(Data source: ArcGIS Online/CLC 2018-https://land.copernicus.eu/pan-european/corine-land-cover/clc2018 / https://www.arcgis.com/index.html- personal processing)

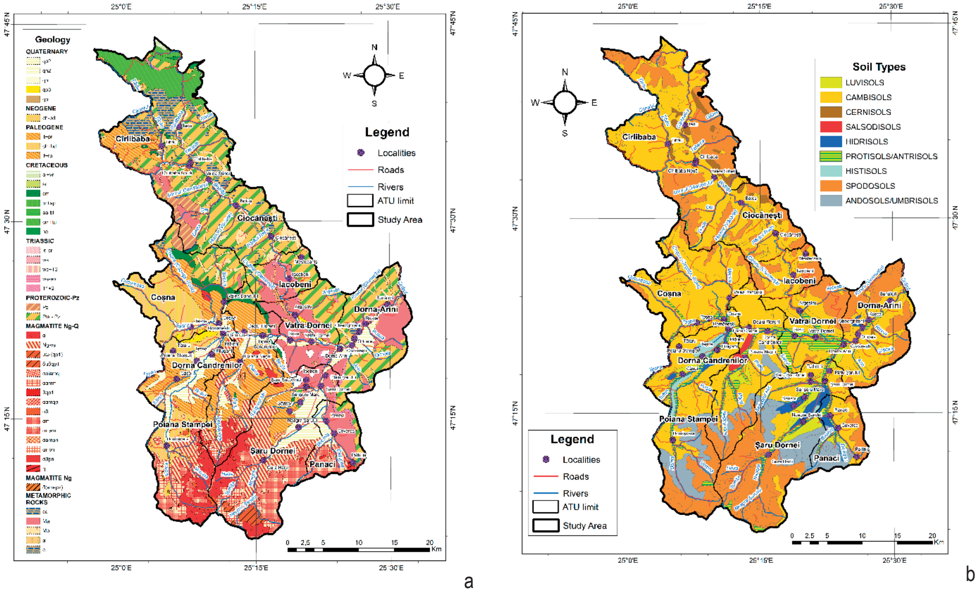


Figure. 2. Geological (a) and pedological cartographic representation of the Dorna Basin (b) (Data source ArcGIS Online; https://www.arcgis.com/index.html- personal processing)

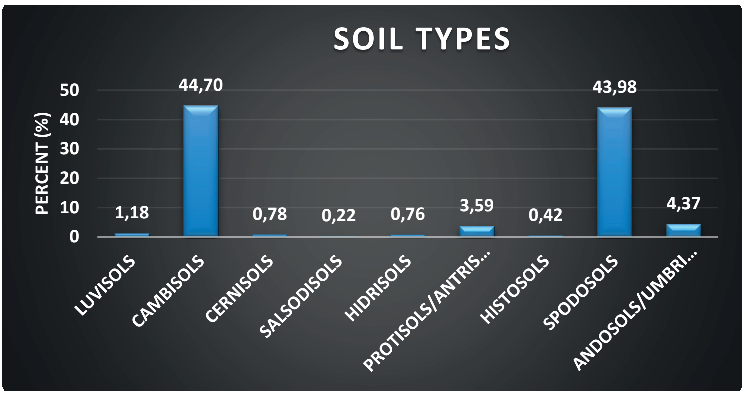


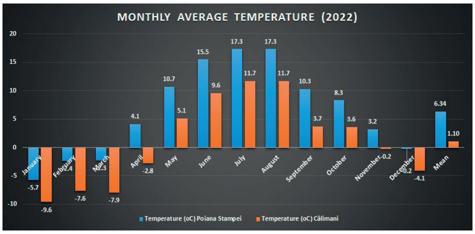
Figure 3. The share of pedological classes in the Dornelor area (Data source: ArcGIS Online - https://www.arcgis.com/index.html - processing in Microsoft Office Excel)

The evolution of climatic factors. In the Dornelor Depression, the temperate-continental climate prevails. In this regard, the obtained results highlighted some differences between the values that were recorded at the two meteorological stations during the year 2022 (Figure 4). In this regard, it appears that the trend of seasonal dynamics is manifested by emphasizing the differences between the evolution of the average values of the climatic parameters in the depression and alpine areas. These revealed important variations in the average values of temperature (6.3°C and 1.1°C, respectively) (Figure 4a), air humidity (85.08% and 84.58%, respectively) (Figure 4b), average monthly precipitation (64.63 mm and 103.38 mm, respectively) (Figure 4c), air pressure (909.32 and 796.73, respectively) (Figure 4d).

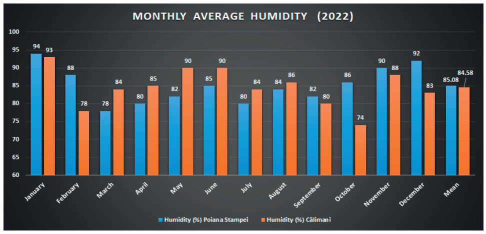
The evolution of the average monthly values revealed the highest temperature values during July and August, both for the depression area (17.3^{0}C) and for the alpine area (11.7^{0}C), and the lowest temperatures during January for both areas (-5.7 and -9.6°C, respectively) (Figure 4a).

Regarding air humidity, higher average values were noted in January, both in the depression area (94%) and in the alpine area (93%), respectively lower average values of humidity in March (78%) and October (74%) (Fig.4b). The evolution of the precipitation regime was characterized, as expected, by the recording of higher monthly averages in the July-September period (September: 152.2 - 245.2 mm) compared to the cold period (March: 11.5 mm for Poiana Stampei station; February: 39.6 mm for the Călimani station) (Figure 4c).

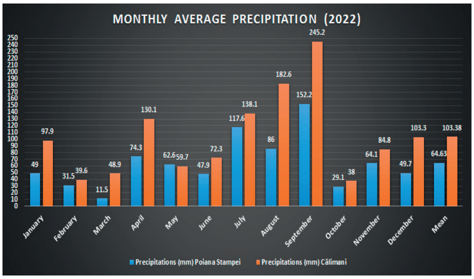
It is important to mention that the total amount of precipitation was 775 mm in the case of the Poiana Stampei station and 1240 mm in the Călimani station. The atmospheric pressure showed important oscillations related to the differences in altitude, with higher average values recorded in August for both areas (912.5 mb and 801.6 mb, respectively, with a maximum of 803.7 mb in June and 914.3 mb in September), and lower average values in January, respectively (902.5-790.5 mb) (Figure 4d).



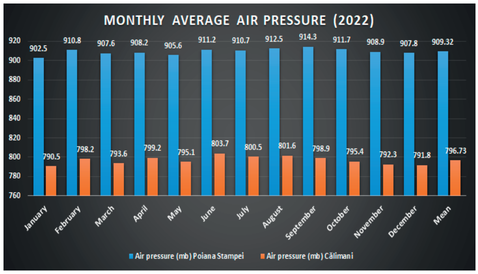
a



b



c



d

Figure 4. The evolution of the average monthly values of temperature (a), humidity (b), precipitation (c), and air pressure (d)

Biodiversity characterization. The vegetation in the Dornelor land is mostly forest, occupying more than half of the total area (71%). The rest (29%) belongs to pastures, hayfields, and a small percentage of crops (especially potatoes), small vegetable gardens, and anthropogenic land https://land.copernicus.eu/paneuropean/corine-land-cover; CLC 2018) (Figure 5); of the entire agricultural area, pastures represent only 15% (26650 ha) and hayfields, 8.8% (15630 ha) (Negrea et al., 2022) (Figure 5); the remaining 6.6% (11020 ha) include arable land and land with natural meadows (Figure 5). As evidenced by the data presented in Figure 5, natural meadows and hayfields hold the largest share. They provide grazing for the animals during the summer and fibrous fodder for the winter season, respectively. Within the area under study, the forests have a relatively well-defined stratification depending on the altitudinal level. Thus, in the depression area and up to approximately 950 m, resinous trees mixed with deciduous trees (beech and mountain ash rowan tree, and fir and spruce, respectively) predominate, and above the altitude of 1000 m, almost pure spruce trees predominate. The predominant species are represented by spruce and fir (65% and 25%, respectively), followed by beech, sycamore, birch, gray alder, black alder, and willow trees (Negrea et al., 2022). As for the specific flora, in this area, there is a diversity of plant taxa, which includes approximately 970 species and subspecies, with an allochthonous character, representing approximately 27% of the total species present in the national flora of Romania (approx. 3700 taxa and subtaxa) (Mititelu et al., 1988). Among these, the most representative are the following families: *Asteraceae, Fabaceae, Ranunculaceae, Apiaceae, Brassicaceae, Cyperaceae, Caryophyllaceae, Scrophulariaceae, Orchidaceae,* and *Polygonaceae* (Popovici et al., 1996). Analyzing the phytogeographic origin of the species, the following spectrum has been obtained: Circumpolar (13%), Eurasian (36%), European (14%), Central European (10%), Alpine-Carpathian (4%), Endemic (3%), Carpatho-Balkan (3%), Pontic (1%), Ponto-Mediterranean (3%), Continental (5%), Adventive (3%) and Cosmopolitan (5%). Therefore, due to the geographical position (about 48° lat. N) and the mountainous relief, the northern and mountain-alpine species make up the majority (Eurasian + European + European-central + Alpine-Carpathian = 77%), while species of southern Eastern origin (Carpathian-Balkan + Pontic + Pontic-Mediterranean + Continental) constitutes 12%; only 9% are cosmopolitan and adventive species, and 2% are Dacian endemics (Pricop et al., 2021).

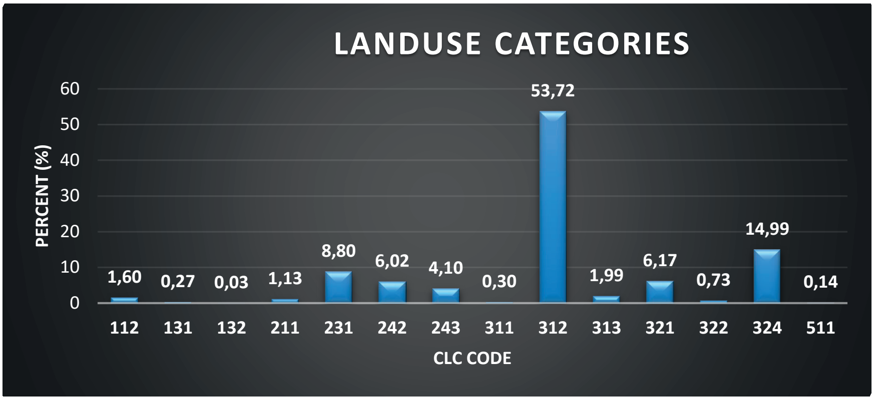


Figure 5. Land use categories in Dorna Basin (Source of data: ArcGIS Online/CLC 2018 - https://land.copernicus.eu/pan-european/corine-land-cover/clc2018/https://www.arcgis.com/index.html, personal processing in Microsoft Office Excel)

Trends in traditional agriculture. Climatic variations associated with other mountain conditions favor biodiversity, generating support for the growth of large and small ruminants and implicitly the development of households and micro-farms specific to mountain areas (Khan et al., 2013; Zhang et al., 2013; Necula et al., 2022a; Török et al., 2014). Cattle breeding has a major impact on the country's animal economy and is essential for mountain communities. Suceava County has an appreciable land fund, which is divided into a plateau and a mountain region. Animal breeding in this county has a traditional character and a major economic impact, having large herds of cattle and sheep. At the end of 2008, the livestock of Suceava county included 123147 cattle, predominantly from indigenous breeds (Pinzgau, Brown Cow of Maramureş, and Simmental), which produced 3455000 hl of milk/year; 234833 sheep, predominantly from the Ţurcană and Ţigaie breeds; 16533 goats; 19618 pigs. Comparatively, at the end of 2022, cattle and pig herds decreased significantly (to 111366 and 14554, respectively), whilst sheep and goats herds decreased moderately (223059 and 11337, respectively) (Table 1). Moreover, the dynamics revealed important oscillations. Thus, at the end of 2022, the number of cattle decreased by 9.56%, and of pigs, by 25.81%. The decreases were less important in the case of sheep (by 5%) and goats (by 31.42%) (Statistical Report of Suceava Sanitary Veterinary and Food Safety Directorate-personal processing).

The main activities of "Ţara Dornelor" are forestry and agriculture, the two branches being adapted to the area and local potential. Traditional foods in the Dornelor area are mainly products of animal origin, obtained to a large extent from cattle and sheep breeding, and to a lesser extent, from the breeding of goats and pigs. In this context, it could be said that "Ţara Dornelor" with its mountainous landscape, with its meadows, and hayfields, include and offer, through centuries-old traditions, the breeding of cattle and sheep.

In the "Ţara Dornelor" region, livestock numbers evolved differently compared to the county's statistics (Table 1). In 2018, cattle populations were 16288, remaining approximately constant with small fluctuations until 2020 (16516 heads), with even a small increase in 2021 (16780 heads), followed by a significant decrease ($p<0.05$) in 2022 (15806 heads). Regarding sheep, in 2018, there were 3154 animals with a significant increase ($p<0.05$) in 2019 (3544 animals), then a decrease ($p<0.05$) was recorded in 2020 (3312 animals), a new increase in 2021 (3632 animals), followed by a significant decrease ($p<0.05$) in 2022 (2937 animals). The total number of goats in 2018 was 497, followed by a constant decrease until 2021 (342 animals), and followed next, by a significant increase ($p<0.05$) in 2022 (747 animals). Concerning pigs, in 2018, the herd consisted of 548 animals, followed by a constant increase until 2021 (824 animals), and then, a significant decrease ($p < 0.05$) being registered in 2022, by halving the herd (434 animals) (Statistical Report of Suceava Sanitary Veterinary and Food Safety Directorate). Moreover, of the total cattle herd (in 2022), 77.61% (3538) appeared in the "Official Control of Production Performance" (Order 188/2011 on the amendment of the norms for the assessment of breeding cattle (https://www.cdep.ro/pls/legis/legis_pck.htp_act?ida=105257). At the end of 2022, the old autochthonous cattle breeds were still well represented in the Dornelor area; therefore, Transylvanian Pinzgau and Dorna Cow, totaling 1080 animals, while Brown cow of Maramureș was represented by 668 animals. In addition, the Romanian Spotted Cattle (1124 animals) and Romanian Black Spotted cattle (666 animals) were added to the aforementioned data (Statistical Report on the number of animals by breed, 2022). At the same time, the monitored specimens are valuable from the point of view of genetics and milk production, and their number as genuine breeds can be greatly increased by the inclusion of half-breeds and related youth. The domestic bull breeds, Transylvanian Pinzgau and Brown of Maramureș, are genetically and historically adapted to the natural conditions of high mountain areas with wild meadows, making very good use of cellulosic fodder (Mang, 2011). Furthermore, they show particular resistance to adverse climatic conditions, weather, and pathologies (Rey, 1979; Popa et al., 2021; Necula et al., 2022b). Mountain climatic factors also have positive effects on milk quality, directly influencing the physical-chemical and hygienic-sanitary parameters (Someșan et al., 2015; Necula et al, 2021; Necula et al, 2022a). It is also known that the relationship between the nature of the lands, the functioning of ecosystems, and animal production is supported by the biodiversity of mountain ecosystems (Dumont et al., 2013; Souza et al., 2015). The quality of the milk from the Dornelor region is well known, and this has already been appreciated on a national scale. Dorna dairy products are also highly appreciated, being centered around the Dorna Swiss cheese, which is the most representative dairy product (brand) in the area. Currently, this assortment of cheese is produced according to traditional standards, as an authorized product specific to the Dornelor area, and labeled as "Mountain Product" (http://www.madr.ro/docs/ind-alimentara/Ghid-produs-montan.pdf).

Swiss cheese is a traditional Emmental cheese, which is part of the category of fine cheeses, having a specific manufacturing technique, with a long maturation time, at least 90 days, which requires special skill from those who carry out this technological process. The success of obtaining this assortment implies appropriate climatic conditions, which are specific to this area since this cheese is obtained only in mountainous regions. Geo-climatic conditions in mountainous areas have a major impact on the health and well-being of animals, both through direct action on them and indirectly on the biodiversity of fodder resources (Machiko et al., 2014; Someșan et al., 2015).

Table 1. Evolution of livestock in the Dornelor area (in the period 2018-2022)
(Statistical report on the number of live animals in holdings during 2018-2022 - personal processing)

No. crt.	UAT	CATTLE					SHEEP					GOATS					PIGS				
		2018	**2019**	**2020**	**2021**	**2022**	**2018**	**2019**	**2020**	**2021**	**2022**	**2018**	**2019**	**2020**	**2021**	**2022**	**2018**	**2019**	**2020**	**2021**	**2022**
1.	Cîrlibaba	671^{iE}	701^{iD}	743^{iA}	729^{iB}	717^{hC}	27^{iH}	25^{jH}	25^{iH}	25^{jH}	150^{hG}	50^{eN}	18^{gP}	19^{fP}	22^{fO}	229^{bM}	9^{jU}	6^{jV}	32^{iT}	55^{fS}	2^{jW}
2.	Ciocănești	825^{hC}	860^{hB}	875^{hA}	876^{hA}	820^{gD}	330^{eG}	305^{eH}	274^{eK}	281^{fI}	278^{eI}	12^{hN}	15^{hN}	19^{fM}	18^{gM}	20^{hM}	35^{eV}	44^{fU}	57^{fT}	56^{eT}	67^{bS}
3.	Iacobeni	260^{jD}	265^{jB}	263^{jC}	270^{jA}	248^{iE}	98^{hG}	90^{iH}	83^{hI}	80^{iK}	84^{jI}	4^{iM}	4^{iM}	3^{hM}	4^{iM}	3^{iM}	10^{iT}	11^{iT}	10^{jT}	11^{jT}	23^{hS}
4.	Vatra Dornei	953^{gC}	931^{gD}	970^{gB}	972^{gA}	824^{gE}	152^{gK}	284^{fI}	347^{dG}	340^{eH}	280^{dJ}	57^{dN}	61^{cM}	63^{cM}	60^{cM}	40^{fO}	48^{dT}	23^{hV}	51^{gS}	31^{hU}	9^{iW}
5.	Dorna Arini	1809^{eC}	1785^{eD}	1905^{eB}	1923^{eA}	1669^{eE}	410^{dI}	379^{dJ}	578^{bG}	527^{dH}	370^{eK}	45^{fN}	45^{eN}	36^{eP}	40^{dO}	250^{aM}	17^{hV}	69^{dS}	38^{hT}	16^{iV}	33^{fU}
6.	Panaci	2111^{cA}	1974^{cC}	2047^{cB}	1966^{cD}	1914^{dE}	152^{gI}	143^{hJ}	220^{fH}	230^{gG}	135^{iK}	116^{aM}	94^{aN}	92^{aN}	79^{aO}	79^{cO}	159^{aS}	152^{aT}	108^{cV}	113^{dU}	61^{cW}
7.	Șarul Dornei	3394^{aB}	3205^{aD}	3421^{aA}	3388^{aC}	3186^{aE}	496^{cK}	873^{aG}	791^{aH}	774^{aI}	623^{aJ}	81^{cM}	76^{bN}	68^{bO}	67^{bO}	56^{dP}	89^{cV}	144^{bU}	231^{aS}	159^{bT}	52^{dW}
8.	Dorna Candrenilor	2886^{bC}	2894^{bB}	2869^{bD}	3087^{bA}	2867^{bE}	583^{bH}	482^{cJ}	487^{cI}	648^{bG}	276^{fK}	43^{gM}	41^{fM}	18^{gN}	13^{hO}	43^{eM}	20^{gW}	66^{eU}	98^{dT}	172^{aS}	40^{eV}
9.	Coșna	1462^{fD}	1457^{fE}	1465^{fC}	1503^{fA}	1489^{fB}	234^{fG}	160^{gJ}	159^{gJ}	179^{hH}	169^{gI}	0^{jN}	3^{jM}	3^{hM}	3^{jM}	0^{jN}	30^{fU}	30^{gU}	62^{eS}	54^{gT}	26^{gV}
10.	Poiana Stampei	1917^{dE}	1940^{dD}	1958^{dC}	2066^{dB}	2072^{cA}	658^{aH}	803^{bG}	348^{dK}	548^{cJ}	572^{bI}	89^{bM}	49^{dO}	52^{dN}	36^{eP}	27^{gQ}	131^{bT}	103^{cV}	133^{bT}	157^{cS}	121^{aU}
	TOTAL	16288^{C}	16012^{D}	16516^{B}	16780^{A}	15806^{E}	3140^{J}	3544^{H}	3312^{I}	3632^{G}	2937^{K}	497^{N}	406^{O}	373^{P}	342^{Q}	747^{M}	548^{V}	648^{U}	820^{T}	824^{S}	434^{W}

Legend: Values followed by distinct lowercase letters in the same column indicate significant differences between localities ($p < 0.05$), values followed by distinct capital letters in the same row (A-E for cattle, G-K for sheep, M-Q for goats, S-W for pigs) indicate significant differences between years ($p < 0.05$); UAT- territorial administrative units.

The agritourism potential. Rural tourism is in continuous development in the Dornelor area, which has a picturesque, unpolluted natural setting with a multitude of recreational options and valuable gastronomic, cultural, and historical potential. More and more domestic and foreign tourists prefer this rural environment, being attracted by the possibility of discovering the environment, changing the way of life, as well as by the agricultural activities specific to mountain areas. The number of guesthouses has considerably grown, especially in recent years. In addition, the mountainous area of the Dornelor region meets all the necessary conditions for the development of this form of tourism. Since the 1990s, the area has effectively established itself in the practice of this type of tourism, gradually becoming more and more sought after by tourists.

The main favorable factors that have given a continuous development to this form of tourism in the Dornelor region are the beauty of the area, the air quality, the density of houses and guesthouses, the special quality of the ecological products obtained in this area, with national and international value, as well as the existence of a rich biodiversity (http://politici.weebly.com/turism/turismul-in-suceava).

The tourist endowment of the Dornelor land can be highlighted by the beauty of natural landscapes, the multitude of natural monuments, the wealth, diversity and originality of folk and ethnographic art; the presence of balneo-climatic resorts, and the abundance of biodiversity. Thanks to the special tourist potential, the Dornelor area is at the forefront of national tourism, under the name of "Northern Bucovina" (http://politici.weebly.com/turism/turismul-in-suceava).

CONCLUSIONS

The Dornelor area is a geographical area rich in natural resources, generated by the interaction of specific factors, such as the petrographic and tectonic structure, the pedo-geoclimatic profile, the forest fund, the meadows, and mountain meadows favorable for raising animals and the local anthropogenic factor. These factors give value and preserve the natural framework specific for obtaining genuine traditional products. The climate of the area is influenced by the structuring of the relief on altitude steps, distinguishing depression and mountain variations in temperature, humidity, precipitation, and atmospheric pressure. The special level of biodiversity is ensured by a valuable floristic and faunal diversity, with a great diversity of taxa (forest fruits, edible mushrooms, medicinal plants) and a rich hunting and fishing fund. The specific natural conditions favor the development of forestry, animal breeding (mainly cattle and sheep), and implicitly the production of traditional foods, which can constitute a solution in the current food crisis.

ACKNOWLEDGEMENTS

This work was supported partly by the project PROINVENT in the framework of Human Resources Development Operational programme 2014-2020, financed from the European Social Fund under contract number 62487/03.06.2022 POCU 993/6/13/ – Code SMIS: 153299.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

EFFECT OF DIETARY SUPPLEMENTATION OF CHROMIUM AND ZINC ON PERFORMANCE, SERUM BIOCHEMICAL PARAMETERS, CARCASS DEVELOPMENT, AND INTESTINAL MICROFLORA BALANCE IN BROILER CHICKENS REARED UNDER HEAT STRESS

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Abstract

The present study evaluates the effect of dietary chromium and zinc (Cr-Zn) supplementation on performance, serum biochemical parameters, carcass development and intestinal microflora of broilers reared under high heat stress (HS). An experiment was carried out on 60 Ross 308 broiler chickens, assigned to two experimental groups (C and Cr-Zn) with 30 chickens/group and maintained under high heat stress (32°C). Compared to the control diet (C), the experimental diet included the addition of a premix with 20 mg chromium picolinate +2.5 g Zn/kg premix (Cr-Zn). Dietary Cr-Zn did not affect the performance and serum biochemical parameters of broiler chickens reared under HS conditions. The use of Cr-Zn in broiler diet led to a significant reduction of Enterobacteriaceae, E. coli, staphylococci in the caecal and intestinal content. Both in the caecum and in the intestinal contents of Cr-Zn broiler chickens, the number of lactobacilli was significantly higher than in the C broilers. The combination of Zn and Cr in broiler feeding has a positive impact on maintaining the balance of intestinal microflora during heat stress

Key words*: broiler, chromium, heat stress, intestinal microflora balance, zinc.*

INTRODUCTION

Heat stress has adverse effects on general health, growth performance, physiology, etc. (Xu et al., 2018). High ambient temperatures compromise performance and productivity by reducing feed intake and nutrient utilization, growth rate and meat quality, resulting in economic losses (Freeman, 1987). Given the negative consequences of heat stress, the problem of heat stress quickly became a point of particular interest in the livestock sector (Lara & Rostagno, 2013). That is why the need for well-developed strategies to mitigate the negative effects of thermal stress was imposed. During heat stress, a reduction in plasma and tissue levels of vitamins (vitamin C, E, folic acid) and minerals (Zinc) has been observed, which may be associated with reduced food consumption and high-water consumption in broilers (Abudabos et al., 2018). The decrease in the concentration of vitamins and minerals leads to cell membrane damage (Sahin et al., 2001), as well as the generation of free radicals that can affect homeostasis mechanisms leading to pathological changes (Teeter et al., 2005), tissue damage, as well as adverse effects on erythrocytes (Adenkola & Ayo, 2009). In addition, intestinal balance is impaired associated with intestinal diseases (Saracila et al., 2021). Minerals play the central role of all metabolic functions in broiler chickens (Alagawany et al., 2021). Many studies have shown the positive effects of mineral supplementation (e.g., selenium, chromium, zinc, etc.) in the diet of chickens under heat stress conditions (Saracila et al., 2021; Untea et al., 2021). Mineral supplementation is important to reduce heat stress and maintain performance and production in the poultry sector. Studies have shown the significant effects of minerals on improving performance parameters, reducing heat shock proteins and lipid peroxidation (Rao, 2016). Improved performance, anti-oxidant and immune responses were reported in chicken fed higher concentrations of Cr and Zn (Burrell et al., 2004). Chromium (Cr) has been used to increase feed efficiency, improve body weight and organs in broilers; therefore, Cr is considered a popular mineral supplement as a result of acting a dynamic role in improving the retention of other essential elements in the blood and decreasing their excretion (Sahin et al., 2017). Zinc (Zn) is one of the essential nutrients that is a cofactor for 200 enzymes. Thus, it has a central role in antioxidant capacity, growth, immunity and nutrient digestion through interaction with other minerals in the gut (Naz et al., 2016). The present study evaluates the effect of dietary supplementation with chromium and zinc (Cr-Zn) on the performance, serum biochemical parameters, carcass development and intestinal microflora of broilers raised under high heat stress (HS).

MATERIALS AND METHODS

The experiment was carried out in accordance with the animal welfare principles set out by Directive 2010/63/EU. The experimental protocol was approved by the Ethics Commission of INCDBNA-IBNA Balotesti (no. 4775/02.08.2019). The experiment was carried out on a number of 60 chickens from the Ross 308 hybrid for six weeks (1-42 days). At the age of 1 day, the chicks were divided according to the average body weight and divided into two groups (C and Cr-Zn), with 30 chicks/group. They were housed in experimental halls equipped with a three-level Zucammi battery, in digestibility cages (cage dimensions 65 x 75 x 45 cm). Chicks were reared under heat stress with 32°C constant temperature. Microclimate parameters (ventilation, %; humidity, %; temperature, °C; carbon dioxide, ppm and ammonia, ppm) were controlled using the Viper Touch computer (Big Dutchman, Germany). The light program was ensured according to the growth age of the chicks (23 h light/1 h dark). Water and feed were given ad libitum. The diet structure was in accordance with the nutritional requirements (NRC, 1994) and the nutritional requirements of the hybrid Ross 308. Compared with the control diet (C group), the experimental diet (Cr-Zn group) included 200 µg chromium picolinate (Cr)/kg diet+0.025 g Zn/kg diet (Cr-Zn). The diet structure is presented in Table 1. Chromium supplement was used in the premix in the form of chromium picolinate ($Cr(C_6H_4NO_2)_3$) (Santa Cruz Biotechnology, CA, USA) and Zinc was purchased from DSM Nutritional Products Romania SRL.

At the end of the experiment (42 days), 6 chicks were randomly selected from each group. From each group, blood samples were collected aseptically from the sub axial region, respectively the brachial vein, in vacutainers with anticoagulant (Li-heparin) and prepared as described by Saracila et al. (2018). The obtained supernatant (serum) was used to determine biochemical parameters (glucose, cholesterol, triglycerides, urea, albumin, phosphorus, phosphorus, iron, alanine aminotransferase and aspartate aminotransferase, gamma GT). Analyses were performed using kits according to the manufacturer's instructions.

After blood sampling, the broiler chickens were slaughtered by cervical dislocation. The carcasses were eviscerated, and then the organs (gizzard, liver, heart, and spleen) and the breast and thigh samples were collected. Both carcasses and organs were weighed using a Sartorius balance in order to calculate the relative weight of the carcass and organs, according to the following calculation formula:

$$\text{Relative weight of organ/carcass (\%)} = \frac{\text{Live bodyweight x organ/carcass weight}}{100}$$

Consequently, the entire gut has been removed from the oesophagus to the cloaca. Samples of intestinal and caecal contents were collected in aseptic medium in sterilised plastic tubes and stored at -20ºC until the bacteriological assays (*Enterobacteriaceae, Escherichia coli*, staphylococci, lactobacilli, *Salmonella* spp.).

The bacteriological analyses were performed according to the assays described by Saracila et al. (2020; 2021). For counting bacterial colonies was used Scan 300, Interscience (France). The results were reported as log base 10 colony-forming units (CFU) per gram of caecal/intestinal contents.

Table 1. Diet formulation

Ingredient	Starter (1-14 days)		Grower (15-28 days)		Finisher (29-42 days)	
	C	Cr-Zn	C	Cr-Zn	C	Cr-Zn
	%					
Corn	32.73	32.73	36.63	36.63	40.64	40.64
Wheat	20	20	20	20	20	20
Corn gluten	2	2	4	4	6	6
Soybean meal	36.17	36.17	30.2	30.2	23.95	23.95
Sunflower oil	3.85	3.85	4.3	4.3	4.72	4.72
Monocalcium phosphate	1.68	1.68	1.52	1.52	1.43	1.43
Calcium carbonate	1.5	1.5	1.38	1.38	1.31	1.31
Salt	0.39	0.39	0.38	0.38	0.33	0.33
Methionine	0.33	0.33	0.25	0.25	0.21	0.21
Lysine	0.3	0.3	0.29	0.29	0.36	0.36
Choline	0.05	0.05	0.05	0.05	0.05	0.05
Vitamin-mineral premix*	1	1**	1	1**	1	1**
TOTAL	**100**	**100**	**100**	**100**	**100**	**100**
Calculated Metabolisable energy, kcal/kg	3039.79		3128.99		3217.72	
Chemical composition- calculated (%)						
Crude protein	23.00		21.50		20.00	
Ether extractives	5.48		6.01		6.49	
Crude fibre	3.77		3.57		3.36	
Calcium	0.96		0.87		0.81	
Phosphorus	0.77		0.70		0.65	

*1 kg premix contains: = 1100000 IU/kg vit. A; 200000 IU/kg vit. D3; 2700 IU/kg vit. E; 300 mg/kg vit. K; 200 mg/kg Vit. B1; 400 mg/kg vit. B2; 1485 mg/kg pantothenic acid; 2700 mg/kg nicotinic acid; 300 mg/kg vit. B6; 4 mg/kg vit. B7; 100 mg/kg vit. B9; 1.8 mg/kg vit. B12; 2000 mg/kg vit. C; 8000 mg/kg manganese; 8000 mg/kg iron; 500 mg/kg copper; 6000 mg/kg zinc; 37 mg/kg cobalt; 152 mg/kg iodine; 18 mg/kg selenium.
**Vitamin-mineral premix + 2.5 g Zn/ kg premix+ 20 mg Cr picolinate/kg premix
Where: C- conventional diet; Cr-Zn- conventional diet + 2.5 g Zn / kg premix + 20 mg Cr picolinate/kg premix.

Statistical analysis

The statistical analyses were performed using Prism-GraphPad software v. 9.03 (San Diego, CA, USA).

The effect of dietary treatments on tested parameters was analysed using one-way analysis of variance (ANOVA). Significant differences among treatment means were determined at $p<0.05$ by Tukey's multiple-range test.

RESULTS AND DISCUSSIONS

Table 2 presents the results of the determination of the primary chemical composition and the bacterial and mycological analysis of the compound feeds.

The results of the determinations regarding the chemical composition of the feeds showed that they were balanced from an energy-protein point of view.

With regard to the bacteriological and mycological analysis of the combined feeds, the values fall within the stipulated maximum limits, regulated, published in the Official Gazette of Romania no. 362/2003.

In all feed compounds, *Salmonella* spp. was absent (Table 2).

In Table 3 is presented the effect of tested compound feeds on the productive performance of chickens raised under heat stress. It is observed that none of the tested compound feeds had a significant influence on the production parameters.

Consistent with this, many studies suggest that Cr in various forms (chromium picolinate, chromium chloride) behaves better in combination with minerals such as Zn in terms of body weight gain (Sahin & Sahin, 2002; Perai et al., 2013; Attia et al., 2015) especially under stress conditions such as high or low temperature and humidity.

Some studies support the synergistic action of Cr and other antioxidants under stress conditions; by mutually amplifying their actions, they lead to enhanced performance in birds (Sahin et al., 2001; Haq et al., 2017; Al-Sultan et al., 2019).

Table 2. Mineral composition and bacteriological and mycological analysis of compound feeds

Item	Starter (1-14 days)		Grower (15-28 days)		Finisher (29-42 days)	
	C	Cr-Zn	C	Cr-Zn	C	Cr-Zn
	%					
Mineral composition						
Ash, %	6.27	6.50	6.29	5.66	7.02	6.15
Calcium, %	0.89	0.88	0.89	0.90	0.89	0.90
Phosphorus, %	0.84	0.95	0.94	0.95	0.81	0.82
Cu, mg/kg	8.06	7.79	7.13	6.46	7.44	7.18
Fe, mg/kg	354.7	358.2	343.2	302.3	301.6	333.4
Mn, mg/kg	105.1	104.4	89.0	94.3	115.9	102.6
Zn, mg/kg	110.3	124.0	106.1	114.6	109.0	118.2
TAC, mM equivalent ascorbic acid	42.7	43.5	43.3	44.0	42.8	44.5
TAC, mM equivalent vit. E	43.3	44.8	44.5	46.6	45.3	47.2
Bacteriological and mycological analysis						
TNG, Col/g	57×10^3	37.6×10^4	49×10^3	39×10^4	32.5×10^3	32.4×10^4
Total coliforms /g	40	1.4	2	0.9	0.3	1.4
E. coli/g	11.5	0	0.9	0	0	0
Salmonella Col/g	-	-	-	-	-	-
TNF, Col/g	7750	3500	1725	3500	2570	2500

Maximum permissible level (MPL): (MO 362 bis/2003): TNG (Total number of germs): max. 15x 106 col/g (SR 13178-1); total coliforms: max. 3000 col/g (SR 13178-2); *E. coli*: max. 100 col/g (SR 13178-2); Salmonella sp.: 0 col/g (SR EN 12824); TNF (total number of fungi): max. 5 x 104 col/g (STAS 6953-81). Where: SR= Romanian Standard; STAS= State Standards; SR EN= European Standards TAC= Total antioxidant capacity.

Table 3. Effect of the tested compound feeds (1-42 days) on productive performance (average values± SD)

Parameter	Days of age	C	Cr-Zn	General effects of diets
		$\bar{X} \pm S_{\bar{X}}$		
Body weight (g)	1	46.4±2.9	46.4±3.5	ns
	14	446.1±43.3	450.2±44.2	ns
	28	1213.0±152.3	1216.0±152.0	ns
	42	1988.0±347.0	1935.0±399.1	ns
Average daily feed intake (g/day)	1-14	34.9±16.9	34.5±17.1	ns
	15-28	77.4±9.8	78.7±10.5	ns
	29-42	102.0±3.6	99.3±2.1	ns
	1-42	71.4±29.8	70.8±29.7	ns
Body weight gain (g/broiler/day)	1-14	28.6±3.1	28.8±3.1	ns
	15-28	54.7±9.9	54.7±10.4	ns
	29-42	55.5±28.6	51.4±28.9	ns
	1-42	46.2±8.3	44.9±9.5	ns
Feed conversion ratio (g feed/g gain)	1-14	1.22±0.04	1.20±0.03	ns
	15-28	1.42±0.02	1.45±0.04	ns
	29-42	1.83±0.1	1.90±03	ns
	1-42	1.53±0.05	1.56±0.1	ns

ns: non-significant.

Among other things, differences between the results of the studies may be due to the source, the level of inclusion, the bioavailability of Zn and chromium in the chicken diet.

Sahin et al. (2003) showed that Cr (400 mg Cr/kg diet) added to the diet of chickens (21-42 days old) raised in heat stress led to obtaining a higher final weight, average weight gain, average daily consumption and better specific consumption.

Other authors showed that 1.50 mg/kg Cr-nicotinic increased FCR of 4% in broilers exposed to heat stress (Toghyani et al., 2012).

Table 4 presents the effect of dietary treatments on serum biochemical parameters.

Table 4. Serum biochemical parameters (average values ± SD)

Parameter	C	Cr-Zn	General effects of diets
Energy profile			
Glycaemia, mg/dL	247.1±33.35	213.2±17.49	ns
Cholesterol, mg/dL	144.8±22.8	132.9±6.79	ns
Triglycerides, mg/dL	34.50±8.53	50.53±14.21	ns
Renal metabolism			
Albumin (mg/dL)	1.00	1.00	ns
Urea (mg/dL)	4.87±0.89	4.63±0.44	ns
Mineral profile			
Phosphorus (mg/dL)	5.69±0.51	5.59±0.21	ns
Iron (µg/dL)	80.78±11.57	77.31±3.90	ns
Hepatic parameters			
ALT (TGP), U/L	3.41±0.62	3.59±0.94	ns
AST (TGO), U/L	453.0±112.8	309.2±60.65	ns
Gama GT, U/L	15.23±4.74	16.36±6.14	ns

Where: ALT- Alanine aminotransferase; AST- aspartate aminotransferase; ns: non-significant.

Results showed that dietary Cr-Zn did not affect serum biochemical parameters of broilers reared under HS condition. Contrary, Sahin et al. (2002) showed that dietary chromium and zinc supplementation decreased serum glucose and cholesterol concentrations and increased protein concentrations in laying hens reared under low ambient temperature.

Table 5. Effect of the tested compound feeds (1-42 days) on carcass development and relative organ weight[1]

Parameter	C	Cr-Zn	General effects of diets
$\bar{X}\pm S_{\bar{X}}$ (g)			
Live body weight	2357.50±0.02^{a}	2155.83±1.20^{b}	*
Carcass weight	83.34±20.01	82.20±1.52	ns
Breast weight	16.58±8.16	19.87±1.63	ns
Thigh weight	19.36±2.63	20.37±0.96	ns
Gizzard	1.61±0.18	1.67±0.33	ns
Liver	1.51±0.26	0.09±0.09	ns
Heart	0.32±0.02^{a}	0.42±0.07^{b}	*
Spleen	0.04±0.01	0.06±0.03	ns

[1]Percent from Live body weight; * $^{a\text{–}b}$ Means within a column with no common superscript differ ($p<0.05$).

Table 5 shows that under conditions of heat stress, supplementing the diet of chickens with Cr+Zn led to a significantly lower live body weight compared to those fed with C. Also, within the same group, a higher relative weight of the heart was recorded compared to group C. Otherwise, the dietary treatments did not influence the weight of the carcass, the relative weight of the breast, thigh, liver and spleen.

Table 6 shows the effect of the dietary treatments on the caecal microbiota of chickens raised under heat stress. The data obtained in this study showed that the inclusion of Cr+Zn had a significant effect against the tested pathogens (*Enterobacteriaceae*, *E. coli*, staphylococci) in the cecum of chickens raised under heat stress (Table 6). Thus, the number of *Enterobacteriaceae* colony-forming units was significantly lower in Cr-Zn compared to C. Also, the chickens that included Cr+Zn in the diet had a significantly lower number of *E. coli* and staphylococci compared to those who were given the conventional diet. At the same time, the number of lactobacilli was significantly higher in chickens from Cr-Zn compared to group C.

Table 6. Effect of the tested compound feeds on caecal microbiota (log_{10} CFU*/g caecal content)

Parameter	C	Cr-Zn	General effects of diets
$\bar{X}\pm S_{\bar{X}}$			
Enterobacteriaceae	11.39 ± 0.009^{a}	11.37 ± 0.005^{b}	****
E. coli	10.16 ± 0.019^{a}	10.10 ± 0.008^{b}	****
Staphylococci	8.91 ± 0.009^{a}	8.66±0.015b	****
Lactobacili	10.99 ± 0.007^{a}	11.13 ± 0.010^{b}	****
E. coli:lactobacilli	0.87 ± 0.001^{a}	0.85 ± 0.001^{b}	****

$^{a-b}$Means within a column with no common superscript differ (p<0.05).

In Table 7 is presented the effect of diets tested on the intestinal microbiota. The number of colony-forming units of *Enterobacteriaceae*, *E. coli* and staphylococci was significantly lower in the intestinal contents of the chickens from the Cr-Zn group compared to C.
The addition of Cr+Zn to the chicken diet led to the detection of a higher number of lactobacilli in the intestinal contents compared to those fed the C diet. Regarding the *E. coli* : lactobacilli ratio on the cecal content, it was significantly lower in the groups whose diet was supplemented with Cr+Zn compared to the C group.

These observations are important because heat stress is known to favour the growth of pathogenic bacteria over beneficial ones (Yadav & Jha, 2019). The previous results might be due to the antioxidant mechanism of Cr and Zn supplements, through which attenuates these adverse effects on gut microflora. Some authors showed that Zn can be used as anti-oxidative stress agent, down-regulating ROS production and accumulation through several mechanisms including inhibition of oxidation of macro-molecules such as (DNA)/ribonucleic acid (RNA) and proteins as well as inhibition of inflammatory response (Prasad & Bao, 2019).

Table 7. Effect of the tested compound feeds on intestinal microbiota (log_{10} CFU*/g intestinal content)

Parameter	C	Cr-Zn	General effects of diets
$\bar{X}\pm S_{\bar{X}}$			
Enterobacteriaceae	7.46 ± 0.001^{a}	7.43 ± 0.004^{b}	****
E. coli	6.14 ± 0.004^{a}	6.11 ± 0.004^{b}	****
Staphylococci	5.85 ± 0.006^{a}	5.78 ± 0.019^{b}	****
Lactobacili	7.37 ± 0.005^{a}	7.40 ± 0.004^{b}	****
E. coli:lactobacilli	0.84 ± 0.001^{a}	0.82 ± 0.001^{b}	****

$^{a-b}$Means within a column with no common superscript differ (p<0.05).

Chromium can play a secondary antioxidant role (Farag et al., 2017; Krol et al., 2017) and also improves the immune system of chicks raised under heat stress (Dalólio et al., 2018).
There is a strong relation between immune system and the intestinal health in chickens (Adedokun & Olojede, 2019), so improving the immune system also maintains the balance of intestinal microflora and prevents the adhesion of pathogenic bacteria to the mucosa.
In addition, Cr can interact with gut microbiota. Feng et al. (2019) showed that the use of Cr and micronutrients as dietary supplements can provide significant protection for intestinal microflora.

CONCLUSIONS

The diet supplementation with Cr and Zn did not significantly affect the production parameters of heat-stressed broilers and biochemical parameters of the serum. The inclusion of Cr and Zn supplements in broiler chickens' diet reduced the number of tested pathogens (*Enterobacteriaceae*, *E. coli*, staphylococci) and increased the abundance of beneficial bacteria (lactobacilli).

ACKNOWLEDGEMENTS

This research work was funded by Romanian Ministry of Research and Digitalization, grant number PN 19 09 0102 and National Research

Development Project to Finance Excellence (PFE)-8/2021.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

THE COMPOSITIONAL AND HYGIENIC-SANITARY ANALYSIS OF JENNET MILK

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Abstract

Nowadays, the interest in jennet milk has considerably increased worldwide due to its nutritional characteristics. This study aimed to analyse the main physicochemical and hygienic-sanitary parameters of some jennet milk samples, by using current methods of quality monitoring. The following physicochemical (dry matter - DM, non-fat dry matter - NDM, fat, total protein, β-casein, lactose, and cryoscopic point), metabolic (urea, acetone, and β-hydroxybutyrate - BHB) and hygienic parameters (total bacteria count - TBC, somatic cell count - SCC and differential somatic cell count - DSCC) were assessed. Additionally, electrical conductivity (EC), pH, temperature, and density were investigated. The results revealed variations of total DM (9.64-11.11%), which were mainly given by the NDM oscillations (8.62-9.54%) and to a lesser extent by the fat content (0.25-1.66%). The study also emphasized low values of EC (1.75 mS/cm), TBC (22.16-62.15x10^3 CFU/mL), and SCC (3-30x10^3 cells/mL). The good health status was also confirmed by other metabolic indices, such as urea (36.27 mmol/L), acetone (0.06 mmol/L), and BHB (0.14 mmol/L).

***Key words**: Fossomatic, jennet milk, Milkoscan, quality testing.*

INTRODUCTION

Jennet milk has been known since Antiquity, especially for its healing effects, being recommended even by Hippocrates in the treatment of joint pain, wounds, or intestinal obstruction. In this context, it is worth mentioning that in the 1880s, a Parisian nursery "Hospice des Enfants'' used to feed newborns, deprived of breast milk with jennet milk (Lauziers, 2011).
Currently, jennet milk is considered a rare and difficult-to-obtain product, and its sampling requires keeping the young near the mother and even resorting to stimulating milk ejection with oxytocin (Salimei & Fantuz, 2012). The jennet lactation lasts between 6-12 months, and the average milk production is 1.5-2.0 L/day; moreover, the quantity and quality of milk are influenced by the breed, the number of lactations, the lactation stage, feeding, season, and other intrinsic or extrinsic factors (Alabiso et al., 2009; Chiavari et al., 2005; Polidori et al., 2010). Regarding the jennet herd distribution, the 2014 statistics placed Ethiopia (7,428,037 animals) at the top of the countries, followed by China (6,033,500) and Pakistan (4,942,000) (Food and Agriculture Organization of the United Nations, 2012). In the absence of available data on jennet milk production in Ethiopia or Pakistan, China ranks first in the world, with about 40,000 tons that were reported annually in the northeastern part of the country (Guo et al., 2007). Regarding the processing capacity, the Chinese company YuKunLun (founded in 2007) stands out, processing annually 20 tons of jennet milk, in the form of freeze-dried powder and 500 tons of fresh milk, intended for human consumption, especially as a substitute for human milk (www.chinadaily.com.cn/cndy/2015-05/18/content_20743564.htm).
The biochemical composition of jennet milk is similar to woman's milk, which makes it the most suitable natural substitute for infants; moreover, jennet milk is very well tolerated,

even by infants suffering from cow milk protein allergies (CMA) or intolerance (Cosentino et al., 2012; Molodecky et al., 2012; Swar, 2011; Polidori & Vincenzetti, 2013a). Jennet milk also stands out for its antimicrobial potential, due to the high lysozyme content, which can reach up to 4 g/L (Šarić et al., 2014; Vincenzetti et al., 2008).
A study on milk physicochemical components in the first four lactations of a jennet revealed constant increases in fat content (from 0.78 to 2.38%) and lactose (from 6.68 to 6.76%), and slight oscillations of the protein content, with the lowest average (1.72%) in the second lactation (Marchiş et al., 2015), respectively. Jennet milk is, therefore, poor in nitrogenous substances, similar to the mare and woman's milk; nitrogenous matter in these species represents only 20% of the dry matter, compared to 80% in cow milk. It is worth mentioning that the rich content of jennet milk in water-soluble and fat-soluble vitamins provides good antioxidant potential and beneficial effects on human health (Tafaro et al., 2007). Thus, cobalamin, ascorbic acid, thiamine, and riboflavin are present in higher concentrations in jennet milk, compared to bovine and woman's milk (Cunsolo et al., 2011; Salimei & Fantuz, 2012). The mineral content of jennet milk is similar to that of human milk, but calcium and phosphorus levels are higher. The essential trace elements, such as Zn, Co, and I exhibit similar concentrations to human milk, while Fe, Cu, and Se are found in lower concentrations. After the first month of lactation, the mineral content of jennet milk decreases significantly, correlating with the reduction of the casein content, because minerals are mainly associated with casein micelles (Aspri et al., 2017; Fantuz et al., 2012).
Regarding the hygienic-sanitary parameters of jennet milk, there are still little data available. However, the study carried out by Marchiș et al. (2015) revealed lower values of TBC (58-75x10^3 CFU/mL) and similar values of SCC in jennet milk, compared to cow’s milk (255-340x10^3 cells/mL). Those decreased values of microbial load could be correlated with the increased resistance of the mammary gland to infection and the high antimicrobial potential of jennet milk (Aspri et al., 2017).

MATERIALS AND METHODS

The present research was performed on milk samples collected from a batch of indigenous jennets, raised in an individual household from the Apuseni Mountains, Romania. Regarding the geographical conditions, this area is located on the outskirts of Câmpeni and is rich in mountain pastures and coniferous forests. The animal batch consisted of 12 jennets (10 lactating and 2 pregnant), 3 donkeys, and 10 foals.
The household made the necessary arrangements for raising and maintaining the animals in enclosed shelters, providing common boxes and outdoor paddocks, and lush pastures, respectively. In the summer season, the feeding was predominantly based on natural grazing, supplemented with coarse fodder (2-4 kg/animal) or concentrate mixtures in lactating jennets (1-1.5 kg/animal). In the winter season, the feed ratio was predominantly (80-90%) based on hay and coarse feed, with the addition of feed concentrates, depending on the stage of lactation (10-20%). The milking procedure consisted of separating the lactating jennets from the foals during the night, while in the morning, the jennets were manually milked. Regarding hygiene and milking techniques, the household staff resorted to the usual sanitation measures, including the separate collection of the first 3-4 jets of milk and the washing, disinfection, and light massage of the mammary gland (Ognean et al., 2007).
The study began with a complete clinical examination, based on the general semiotic methods and specific investigations to assess the health status of the mammary gland, including an organoleptic examination of milk, by applying the Contrast test (Ognean et al., 2007). At a preliminary examination, no changes were found regarding the general condition and mammary gland, all the jennets being considered clinically healthy and therefore, subjected to testing. Thus, milk samples were collected from the lactating jennets batch (no=10), in compliance with the hygienic-sanitary requirements (Ognean et al., 2007). The milk samples were initially collected separately and then reunited into average sample/animal, resulting thus, the biological material being tested. The fresh milk

samples were stored at 4ºC during transportation, without additional preservatives; moreover, the samples were investigated within 8 hours.

The investigations were performed by using the Combifoss automatic system, consisting of Milkoscan (FT 6000) and Fossomatic devices that operate in compliance with European standards (ISO 9622/IDF 141:2013 and AOAC official method 972.16). From a technical point of view, MilkoScan FT 6000 is represented by a high-capacity automatic spectrophotometer, equipped with IDF and FTIR technologies for milk analysis. Thus, the Combifoss system resorted to the FTIR technique for determining the compositional parameters; furthermore, an automatic BactoScan™ FC equipment was used to determine the hygienic-sanitary parameters of the milk samples. The usage of this complex system allowed the testing of several compositional, metabolic, and hygienic parameters, such as dry matter (DM), non-fat dry matter (NDM), fat, total protein, β-casein, lactose, cryoscopic point (°C), urea (mmol/L), acetone (mmol/L), β-hydroxybutyrate (BHB) (mmol/L), total bacteria count (TBC; x10^3/mL), the somatic cell count (SCC; x10^3/mL) and differential somatic cell count (DSCC; %). Additionally, the electrical conductivity (EC; mS/cm), pH, temperature (°C), and milk density were determined, by using a pH meter/Conductometer (C532) and a lactodensimeter, respectively.

The obtained data were statistically analyzed by using the GraphPad Prism 6, InStat, and Microsoft Excel programs, which allowed the calculation of primary statistical parameters, such as average, standard deviation (St. dev), standard error (St.error), median, minimum (Min.) and maximum (Max.), Coefficient of variation (CV). The results were processed by using the Foss Integrator software platform, with a Process Hazard Analysis (PHA) diagram, which allowed a quick and accurate assessment of the investigated parameters.

RESULTS AND DISCUSSIONS

Evaluation of the physical parameters. The main physical parameters of jennet milk revealed important oscillations of the individual values, which may be included within physiological limits for cow milk and the main ruminant species (Table 1). As it is known, the determination of these parameters, relevant to the testing of milk quality and health in most mammalian species, has been seldom reported in the case of jennet milk (Aspri et al., 2017; Marchiş et al., 2015).

The values recorded when determining the density of jennet milk could be characterized by decreased individual variations (1.030-1.037 g/cm^3), around the average of 1.034 g/cm^3 (Table 1). Based on the obtained data, the jennet milk density varied in intervals close to those of bovine milk, except for a few cases, where values were slightly higher. The results obtained at the pH evaluation also indicated evolutions within the physiological intervals for raw milk in general, with average values of 6.77 and individual values between 6.68 and 6.72 (Table 1). The cryoscopic point highlighted an average value of -0.528°C, considered to be very close to the lower limit of the physiological range for milk in general (Bu et al., 2013). Moreover, the individual values of this parameter were also at the lower physiological limit, with several decreases below its level (-0.50°C and -0.51°C), which excluded, however, the possible addition of water to the milk sample (Table 1). Furthermore, the electrical conductivity pointed out minor differences between the investigated samples, even though they were collected from jennets in different stages of lactation. The distribution of the obtained data suggested an average value of 1.75 mS/cm, with tight oscillations in the range of 1.52-2.01 mS/cm (Table 1).

Evaluation of the biochemical parameters. The results obtained when evaluating the main biochemical indices of jennet milk (Table 1 and Figure 1) emphasized major differences between the composition of jennet and cow milk and other ruminant species. Thus, we recorded a decreased level of total dry matter content, the average value being 10.36%, while the individual values varied between 9.64% and 11.11%, as expected. Additionally, the non-fat dry matter content proved to be unimportant, with individual variations (8.62-9.54%) that suggested limited oscillations around the average value of 9.02%. Particular attention was given to the fat content analysis, as it is known that this index is lower in the case of jennet milk, with a major impact on milk's marketing and processing, in general. In this context, the evolution of the investigated fat content showed wide variations around the average of 0.95% (Table 1). The total protein content emphasized characteristic evolutions, recording an average value of 1.73% and less important individual variations, between 1.37 and 2.09% (Table 1; Figure 1). The lactose content also pointed out valuable features, thus completing the picture of the compositional parameters in jennet milk. Regarding this parameter's evolution, the average values were much higher than those recorded in the case of protein content and especially in lipids, the average level being 6.30%, with mild individual oscillations, between 6.01-6.57% (Table 1; Figure 1).

Table 1. Statistical data obtained of the main physicochemical and hygienic-sanitary parameters of jennet milk

	Parameters	Min.	Max.	Mean	Std. Dev.	Std. Err.	CV%
Physico-chemical parameters	Density (g/cm^3)	1.0300	1.0370	1.0340	0.0021	0.0007	0.20%
	pH	6.7200	6.8300	6.7790	0.0345	0.0109	0.51%
	Cryoscopic point (ºC)	-0.5530	-0.5060	-0.5275	0.0129	0.0041	2.44%
	Conductivity (mS/cm)	1.5200	2.0100	1.7500	0.1514	0.0479	8.65%
	DM (%)	9.6400	11.1100	10.3700	0.5947	0.1881	5.74%
	NDM (%)	8.6200	9.5400	9.0260	0.2499	0.0790	2.77%
	Fat (%)	0.2500	1.6600	0.9560	0.5190	0.1641	54.29%
	Protein (%)	1.3700	2.0900	1.7340	0.2670	0.0844	15.40%
	β-casein (%)	1.1900	1.7300	1.4470	0.1787	0.0565	12.35%
	Lactose (%)	6.0100	6.5700	6.3020	0.1716	0.0543	2.72%
Hygienic-sanitary parameters	TBC (x 10^{3}CFU/mL)	22.1600	62.1500	38.8400	13.9000	4.3950	35.79%
	SCC (x 10^{3}cells/mL)	3.0000	30.0000	8.3000	7.9450	2.5120	95.72%
	DSCC (%)	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Metabolic parameters	Urea (mmol/L)	20.5000	55.3000	36.2700	10.1800	3.2210	28.08%
	Acetone (mmol/L)	0.0000	0.2000	0.0560	0.0769	0.0243	137.34%
	BHB (mmol/L)	0.0000	0.3000	0.1400	0.0904	0.0286	64.59%

Legend: St. Dev. – Standard Deviation; St. Err. – Standard Error of Mean; Min. – Minimum; Max. – Maximum; CV% - Coefficient of Variation; Cryoscopic P – Cryoscopic point; EC – Electrical conductivity; DM – Dry matter; NDM – Non-fat dry matter; TBC – Total Bacteria Count; SCC – Somatic cell count; DSCC – Differential somatic cell count; BHB – β-hydroxybutyrate.

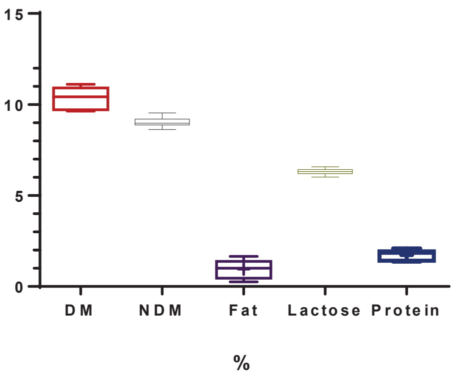

Figure 1. Distribution of the obtained values of the biochemical parameters

Evaluation of the hygienic-sanitary indices. The total bacteria count (TBC) registered much lower values than those provided by the standards required for cow milk (100,000 CFU/mL) (Ognean, 2019). In the animal batch used for testing, the average value of TBC was below this level, 38.83 CFU/mL. The distribution of individual values was within the range of 22.16-62.15x10^3 UFC/mL, being situated below the maximum limits required for cow milk (Figure 2).

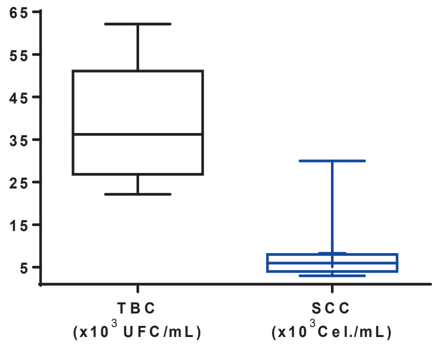

Figure 2. Distribution of the obtained values of the hygienic-sanitary parameters

The somatic cell count (SCC) revealed values at the lower limit of the standards that are required for cow milk, whose maximum level is set at 400.000 cells/mL (Ognean, 2019). Compared to these standards, the recorded data highlighted much lower individual and average values in the case of jennet milk, with decreased oscillations (3-30x10^3 cells/mL), around the average of 8.3x10^3 cells/mL. These values indicated a good milk quality, which was also confirmed by the results obtained in the differential somatic cell count (DSCC). Following the investigations, zero values of DSCC were obtained for all the tested samples, suggesting the lack of polymorphonuclear leukocytes and implicitly, a very good level of mammary gland health and milk quality.

Evaluation of the metabolic indices. Currently, a particular relevance is attributed to lactic indices in the evaluation of the metabolic profile of lactating females, which comes down to the detection of subclinical ketosis, a carbohydrate dysmetabolism found only in highly productive lactating cows (Ognean, 2019). The obtained results emphasized very low values of urea, acetone, and BHB (36.27 mmol/L, 0.06 mmol/L, and 0.14 mmol/L, respectively) (Table 1), indicating the absence of ketosis and other dysmetabolic syndromes in the jennet batch subjected to testing.

The analysis of the physical parameters of jennet milk revealed evolutionary trends more or less similar to those recorded by other researchers in the field. Thus, the density of jennet milk showed wide oscillations, within the physiological intervals for raw milk (Ognean et al., 2007), whereas unlike the findings of some researchers (Aspri et al., 2017), the level of alkalinity was not superior to that of cow milk. Regarding the electrical conductivity (EC), we only mention that this highly topical physical parameter in biomedical tests is given by the increase of the ionic content of milk (Na^+, K^+) to the detriment of the lactose concentration (Ognean, 2019). These components present important oscillations, which in turn generate variations in the milk's electrical conductivity. Among these, the most important ones are considered to be the EC increases, following physiological or pathological conditions of the mammary gland, such as the beginning and the end of lactation and the onset and evolution of mastitis, respectively (Lin et al., 2006; Ognean et al., 2007). In the case of lactating cows, these changes are regarded as being extremely accurate, which explains the special extension of the tests based on determining the milk's electrical conductivity to monitor the mammary gland health status and even to detect the oestrus period (Ognean et al., 2007). Moreover, the oscillations of the fat percentage confirm that lipids represent one of the most variable components in jennet milk. In this regard, available data reported a low average fat content in jennet milk (0.38%) and it was thought that this aspect was due to the increased individual variability (Salimei et al., 2004). Furthermore, these researchers analyzed the lipid fractions of jennet milk and detected high levels of linoleic and linolenic acids. It is also unanimously accepted the major impact of milk fat on the physical, organoleptic, and sensory characteristics of dairy products, these influences being less studied in the case of jennet milk (Bu et al., 2013). The available data on the protein content of jennet milk is also sporadic and controversial, leading to a considerable intensification of current concerns in this area (Hussain et al., 2012). Regarding the protein profile of jennet milk, the following distribution of protein fractions was reported:

lactoferrin (4.48%), serum albumin (6.18%), β-lactoglobulins (29.85%), lysozyme (21.03%) and α-lactalbumin (22.56%) (Salimei et al., 2004). Overall, the results were in accordance with another research, which confirmed a low content of dry matter (8.19%), low protein content (1.34%), a high amount of lactose (6.07%), and low-fat content (0.16%) (Malacarne et al., 2019).

Following the correlation of the data obtained in the present study with those synthesized from literature, a brief characterization of the nutritional and biologically active components of jennet milk could be done, as follows:

- Electrical conductivity (1.52-2.01 mS/cm) close to that of bovine milk and slightly higher alkalinity (pH 7.0-7.2);
- High and variable lactose content (6-7%), which gives it good palatability and it facilitates intestinal absorption of calcium and phosphorus, and the transfer of minerals to bones and helps to prevent osteoporosis, respectively (Heaney, 2012; Salimei & Fantuz, 2012);
- Very low-fat content (0.29-1.82%) and a lipid profile rich in saturated and polyunsaturated fatty acids, linolenic and linoleic acids (Alabiso et al., 2009; Chiofalo et al., 2011);
- Very low protein content (1.3-1.8%), the nitrogenous matter being reduced to 20% of DM (compared to 80% in bovine milk), with a soluble protein/casein ratio close to 1 (compared to 0.2 in bovine milk, and 2 in human milk, respectively). Jennet milk, therefore, has a lower proportion of caseins (dominated by β-casein) (40-50%) and a higher proportion of soluble proteins (α-lactalbumin, β-lactoglobulin, and lysozyme) compared to human and bovine milk (18-66%). This protein profile, characterized by decreased levels of caseins and very high levels of lysozyme (4 g/L or 15% of total protein, throughout the 150 days of lactation), confers an increased degree of digestibility and a particular antimicrobial potential to jennet milk (Carminati et al., 2017; Guo et al., 2007; Polidori and Vincenzetti, 2013b; Šarić et al., 2014);
- Richer content of water-soluble vitamins (cobalamin, ascorbic acid, thiamine, riboflavin, and other B-complex vitamins) and lower content of fat-soluble vitamins (A, E) compared to bovine and human milk (Claeys et al., 2014);
- Mineral content is close to that of human milk, with higher levels of calcium and phosphorus, similar concentrations of Zn, Co, and I, and lower levels of trace elements, such as Fe, Cu, and Se, respectively (Aspri et al., 2017; Fantuz et al., 2012).

Taking into account the results obtained at total bacteria count determination, it might be assumed that the predominantly low values of this parameter could be correlated with the good level of mammary gland health in the investigated jennets. Regarding the potential risks, it is known that milk is extremely exposed to microbial contamination, and various pathogens could reach the mammary gland in an ascending way or contaminate milk in an exogenous way. In addition to this, the natural risk of contamination of the mammary gland and implicitly, of jennet milk is considerably lower than in the case of bovine and other ruminant species milk, due to low production and the rich content of components with antibacterial action.

Regarding the impact of the hygienic-sanitary indices on the evaluation of mammary gland health and of the milk intended for public consumption, it is important to mention that the automatic TBC and SSC monitoring systems have gained remarkable attention in the production and processing field of cow milk, with real possibilities for expansion in terms of quality control in goat, buffalo or sheep milk. In the case of jennet milk, which is barely exploited, the cellular and microbial testing is sporadic and possesses a predominantly scientific character. In this regard, the obtained results highlighted the possibility of using automatic systems for determining TBC and SCC in jennet milk, as well as the need to outline physiological limits for this species. Thus, compared to the present study, other researchers obtained lower values for TBC (24-46x10^3 CFU/mL) (Cavallarin et al., 2015; Coppola et al., 2002; Malissiova et al., 2016; Pilla et al., 2010; Salimei et al., 2004) and higher values for SCC, respectively (254-340x10^3 cells/mL) (Marchiş et al., 2015). As it is already proven, this low microbial load is due to the high antimicrobial potential of jennet milk, conferred by the high content of lysozyme, immunoglobulins, lactoferrin, and lactoperoxidase (Aspri et al., 2017; Brock, 2002). On the other hand, the determination of TBC and SCC contribute to the diagnosis, prophylaxis, and combating of mastitis (Rotaru & Ognean, 1998). Thus, it could be stated that the risk of mastitis in jennets is considerably lower than in cows, goats, or sheep. Moreover, it has been observed that among the many pathogens involved in mastitis, strains of *B. cereus* and *Staphylococcus* spp. have been isolated from jennet milk (Cavallarin et al., 2015), but without any mammary gland complications being reported (Verraes et al., 2014). There are also data showing that *Enterococcus faecalis,* a potentially pathogenic, thermotolerant, lysozyme-resistant bacterial strain of public health interest, was isolated from raw jennet milk (Aspri et al., 2017).

Regarding public health regulations, it should be recalled that in accordance with European norms (EC Regulation 853/2004), the clause "other milk-producing species" applies for jennet milk, providing for TBC, values that are lower than 1,500,000 CFU/mL in the case of raw milk (at 30°C) and lower than 500,000 CFU/mL in the case of milk intended for processing, whilst for SCC, values that are below 500,000 cells/mL.

CONCLUSIONS

The physicochemical and hygienic-sanitary analysis of the investigated jennet milk samples allowed the characterization of the main compositional indices and of some markers with relevance in monitoring the mammary gland health and the biologically active potential of milk. Thus, the total content of DM revealed low values and less important individual variations, mainly given by NDM, as the fat content was poorly represented. Unlike in ruminant species milk, lactose reached the highest proportions, and the protein percentage, dominated by β-casein, was close to the fat content. The evolution of the indices with a major impact in monitoring the health of the mammary gland and milk was also relevant, materialized by low levels of EC, TBC, and SCC and by zero values of DSCC, respectively. A unique character might be attributed to the evolution of lactic indices with metabolic relevance (urea, acetone, BHB), indicating a good general health status, the lack of susceptibility to mastitis, and the absence of predisposition to ketosis in lactating jennets.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

THE QUALITY OF FORAGE FROM PERENNIAL RYEGRASS (*Lolium perenne*) AND TALL FESCUE (*Festuca arundinacea*) UNDER THE CONDITIONS OF MOLDOVA

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Abstract

The main objective of this research was to evaluate the quality of green mass, hay and silage prepared from perennial ryegrass Lolium perenne and tall fescue Festuca arundinacea, grown in monoculture in the experimental plot of the "Alexandru Ciubotaru" National Botanical Garden (Institute), Chisinau, Republic of Moldova. It was established that the concentration of nutrients and energy in whole-plant dry matter of studied grass species, harvested in pre-flowering stage was 10.74-14.10% CP, 3.10-3.58 % EE, 29.95-31.66% CF, 36.35-45.76% NFE, 6.20-19.24 % sugar, 1.54-2.99 % starch, 10.45-14.31% ash, 2.9 g/kg Ca, 2.5-2.7 g/kg P, 8.93-9.49 MJ/kg ME and 4.97-5.06 MJ/kg NEl. The biochemical composition of the prepared silages was: pH= 4.06-4.21, 27.9-28.9 g/kg lactic acid, 0-0.4 g/kg butyric acid, 1.5-5.0 g/kg acetic acid, 9.12-9.67% CP, 3.37-3.99% EE, 39.16-41.30% CF, 30.53-37.37% NFE, 0.33-1.40 % sugar, 0.50-0.60 % starch, 10.98-14.51% ash, 36.34-56.33 mg/kg carotene, 8.31-9.53MJ/kg ME and 4.48-5.34 MJ/kg NEl. The nutritive value of prepared hay: 9.95-10.84% CP, 1.87-2.55 % EE, 36.90-37.32% CF, 38.48-39.99% NFE, 10.82-11.28% ash, 2.5-3.5 g/kg Ca, 2.6-2.7 g/kg P, 8.31-8.85MJ/kg ME and 4.54-493 MJ/kg NEl. The studied grass species contain a lot of nutrients, which make them suitable to be used as multi-purpose feed for livestock.

Key words: *biochemical composition, Festuca arundinacea, green mass, hay, Lolium perenne, silage.*

INTRODUCTION

Currently, the interest in conserving the remaining permanent grasslands, as well as restoring and planting temporary grasslands on degraded and polluted agricultural land is topical in many states around the world.

The Plant List includes 646 accepted species names of the genus *Festuca* and 11 accepted species names of the genus *Lolium.* According to Marusca (2011), in Romania, there are 32 species of the genus *Festuca* and 4 species of the genus *Lolium.* In the spontaneous flora of the Republic of Moldova, there are 8 *Festuca* species and 2 *Lolium* species (Negru, 2007). *Festuca arundinacea* Schreber and *Lolium perenne* L. are among the perennial species with the highest frequency in the floristic composition of grasslands, buffer strips and lawns in green spaces. They are fast-growing grasses, establishing rapidly, adapted to a wide range of soils and climatic conditions, can be used for a range of purposes including pasture, hay, silage and turf, but also as feedstock for biorefineries and bioenergy production (Duke, 1983; Mahnert et al., 2005; Maruşca et al. 2011; Surmen et al., 2013; Akdeniz et al., 2019; Ţîţei & Roşca, 2021). In the Catalogue of Plant Varieties of the Republic of Moldova there are no registered grass cultivars, but in the Official Catalogue of Varieties of Agricultural Plants of Romania, 2022y., 10 cultivars of *Festuca arundinacea* and 12 cultivars of *Lolium perenne* are listed. The cultivars created in Romania have a productivity of 37-65 tons/ha of fresh mass or 9.3-17.0 tons of hay (Maruşca et al. 2011). The main objective of this research was to evaluate the quality of green mass, hay and silage prepared from perennial ryegrass *Lolium perenne* and tall fescue *Festuca arundinacea* and the prospects of its use as feed for livestock in Republic of Moldova.

MATERIALS AND METHODS

The local ecotype of perennial ryegrass *Lolium perenne* and the Romanian cultivar 'Valrom' tall fescue *Festuca arundinacea*, created at the University of Agricultural Sciences and

Veterinary Medicine Cluj-Napoca, Romania, cultivated in monoculture in the experimental plot of the National Botanical Garden (Institute) "Alexandru Ciubotaru", Chişinău, latitude 46°58′25.7″N and longitude N28°52′57.8″E, served as subjects of the research. The plant samples were collected in the pre-flowering stage, in the second growing season. The prepared hay was dried directly in the field. The harvested plants were chopped into 1.5-2.0 cm small pieces, with a laboratory forage chopper; the dry matter content was detected by drying samples up to constant weight at 105°C. The silage was prepared from chopped green mass, compressed in well-sealed glass containers, stored at ambient temperature (18-20°C). After 45 days, the containers were opened, and the sensorial and fermentation indices of the conserved forage were determined in accordance with standard laboratory procedures - the standard SM 108* of R. Moldova. The fresh mass and the fermented fodder samples were dehydrated in an oven with forced ventilation at a temperature of 60°C; at the end of the fixation, the biological material was finely ground in a laboratory ball mill. The evaluation of fodder quality: crude protein (CP), crude cellulose (CF), crude fat (EE), nitrogen-free extract (NFE), soluble sugars (SS), starch, ash, calcium (Ca), phosphorus (P), carotene, silage pH index, concentration of organic acids (lactic, acetic and butyric) in free and fixed state were carried out in the Laboratory of Nutrition and Forage Technology of the Scientific-Practical Institute of Biotechnology in Animal Husbandry and Veterinary Medicine, in accordance with the methodological indications. The gross energy (GE), metabolizable energy (ME), net energy for lactation (NEl) were calculated according to standard procedures:

GE=23.9xCP+39.8xEE+20.1xCF+17.5xNFE;

ME=14.07+0.0206xEE-0.0147xCF-0.0114 xCP+4.5%;

NEl=9.10+0.0098xEE-0.0109xCF-0.0073xCP.

RESULTS AND DISCUSSIONS

In the second year, the studied perennial grasses start active growth in early spring, when the average temperature is above +5-8°C. We would like to mention, for comparison, that the tall fescue, *Festuca arundinacea* at the time of first cut, reached 98.0 cm in height, but perennial ryegrass *Lolium perenne* 63.8 cm in height.

The biochemical composition and nutritive value of the harvested green mass of the studied *Poaceae* species, is presented in Table 1. The *Festuca arundinacea* fodder was characterized by a significantly higher content of crude proteins, crude fats, crude cellulose and ash. The *Lolium perenne* green mass was richer in nitrogen free extract, soluble sugar, starch and phosphorus, the concentration of calcium was at the same level as in tall fescue forage. The higher amount of organic matter and lower amount of crude cellulose had a positive effect on the energy supply of the *Lolium perenne* forage.

Table 1. The biochemical composition and the nutritive value of the green mass from the studied *Poaceae* species

Indices	*Festuca arundinacea*	*Lolium perenne*
Crude protein, % DM	14.10	10.74
Crude fats, % DM	3.58	3.10
Crude cellulose, % DM	31.66	29.95
Nitrogen free extract, % DM	36.35	45.76
Soluble sugars, % DM	6.20	19.24
Starch, % DM	1.54	2.99
Ash, % DM	14.31	10.45
Calcium, g/kg DM	2.9	2.9
Phosphorus, g/kg DM	2.5	2.7
Gross energy, MJ/kg DM	17.51	17.83
Metabolizable energy, MJ/kg DM	8.93	9.49
Net energy for lactation, MJ/kg DM	4.97	5.06

Different results regarding the biochemical composition and the nutritive value of the green mass from *Lolium perenne* and *Festuca arundinacea* whole plants are given in the specialized literature. According to Duke (1983) the *Lolium perenne* fresh mass contained 26.6% DM, including 3.0% CP, 1.3% fat, 6.7% fiber, 13.2% NFE, 2.4% ash, 0.12% Ca, P, 0.07% P, 0.51% K. Fairey (1985) mentioned that the quality characteristics of the studied cultivars of perennial ryegrass were 208 g/kg DM, 2.21% nitrogen, 66.9% DDM; hybrid ryegrass contained 191 g/kg DM, 2.07% nitrogen, 64.3% DDM; orchard grass – 206 g/kg DM, 2.16% nitrogen, 59.4% DDM and reed canary grass – 214 g/kg DM, 2.48% nitrogen and 63.2% DDM. Volchenkova (1994) found that the biomass of tall fescue, depending on the amount and type of applied fertilizers contained 4.37-9.19% CP, 1.67-2.74% EE, 28.33-40.42% CF, 7.11-13.40% ash. Burlacu et al. (2002) revealed that *Lolium perenne* green forage contained 170-250 g/kg DM, 8.6-17.8% CP, 2.8-5.0% EE, 21.2-32.7% CF, 7.2-11.4 % ash, 5.6-6.5 g/kg Ca, 2.8-4.1 g/kg P, 575-718 g/kg DDM, 18.00-18.25 MJ/kg GE, 10.77-14.05 MJ/kg DE, 8.85-11.41 MJ/kg ME. Lee et al. (2002) mentioned that *Lolium perenne* contained 172.6-173.1 g/kg DM with 92.32-92.36% OM, 2.49-3.11% N, 24.61-28.61% ADF, 45.09-54.03% NDF, 9.5-17.9% WSC, 64-68% IVDMD. Mahnert et al. (2005) found that the concentration of nutrients in fresh perennial ryegrass was 176-256 g/kg DM, 11.9-14.7% CP, 2.1-2.4% EE, 24.8-29.1% CF, 10.8-19.3% WSC, 9.4-9.9% ash. The research conducted in Australia by Hayes et al. (2010), revealed that herbage quality of tall fescue cultivars harvested in November was 8.2-9.5% CP, 35.0% ADF, 64.1-64.6% NDF, 7.0-8.0% ash, 58.6-59.6% DMD, 8.6 MJ/kg ME. Surmen et al. (2013) revealed that perennial ryegrass breeding lines were characterized by 9.43-12.09% CP, 55.31-58.52% NDF, 37.24-40.36%ADF, 49.24-53.27% TDN, RFV=92.19-98.57. Küchenmeister et al. (2014) remarked that *Lolium perenne* plants grown under moderate drought stress conditions contained 9.0% CP, 21.6% WSC, 52.7% NDF, 28.6% ADF, but under strong drought stress conditions – 11.5% CP, 12.3% WSC, 59.0% NDF and 33.1% ADF. McEniry & O'Kiely (2014), mentioned that, in Ireland, the biomass of *Festuca arundinacea*, harvested in the middle of May, contained 15.2% CP, 8.6% ash, 52.9% NDF, 26.7% ADF, 16.1% soluble carbohydrates, and the biomass harvested in the first days of June contained 11.2% CP, 9.0% ash, 62.3% NDF, 37.2% ADF, 9.2% soluble carbohydrates. Kshnikatkina et al. (2016) mentioned that the chemical composition and energy nutritional value of *Festuca arundinacea* first cut green mass was: 9.25% CP, 6.51% DP, 1.15 % EE, 29.57% CF, 52.36% NFE, 8.19% ash, 9.62 MJ/kg ME, 0.75 nutritive unit/kg DM, 86.9 g DP/nutritive unit, but in second cut green mass – 8.40% CP, 5.90% DP 1.32 % EE, 27.80% CF, 53.91% NFE, 8.42% ash, 9.74 MJ/kg ME, 0.76 nutritive unit/kg DM, 76.8 g DP/ nutritive unit, respectively. Nicolae (2016) reported that the nutritive quality of perennial ryegrass green mass was characterized by: 164-275 g/kg DM, 7.3-14.2% CP, 1.8-2.5 % EE, 23.9-32.9% CF, 8.5-10.2% ash, 47.4-50.0% NFE. Pocienė & Kadžiulienė (2016) found that the biomass of tall fescue, depending on the amount and type of applied fertilizers, contained 14-20% hemicellulose, 34-36% cellulose and 6-9% lignin. Flores et al. (2017) mentioned that, in the USA, tall fescue contained 56.5-67.8% NDF, 27.7-34.9% ADF, 28.8-34.0% hemicellulose, 25.0-28.1% cellulose, 3.61-10.05% lignin. Dronova et al (2018) mentioned that, on irrigated lands of the Lower Volga region, Russia, depending on the sowing methods and seeding rates, tall fescue fodder contained 10.3-14.1% CP, 3.12-4.15% EE, 23.8-27.2% CF, 38.2-39.9% NFE, 0.22-0.23% P, 8.49-9.38 MJ/kg ME, 0.47-0.51 nutritive unit/kg DM, 44.3-55.9 g /kg DP. Temel et al. (2018) mentioned that, in the *Festuca arundinacea* plants, cultivated on nonsaline halomorphic soil, the dry matter yield was 5.94 t/ha with 11.86% CP, 58.63% NDF, but in high saline soil – 4.59 t/ha with 10.86% CP, 55.38% NDF, respectively. Amaleviciute-Volunge et al. (2020) revealed that the chemical composition of *Lolium perenne* fresh mass was as follows: 10.28% CP, 6.67% ash, 54.75% NDF, 32.8% ADF, 3.97% ADL, 20.3% WSC, 28.9% Cel, 21.9% g HC, 57.9% DDM, but *Festuca arundinacea* green mass – 15.058% CP, 7.52% ash, 52.19% NDF, 31.3% ADF, 4.02% ADL, 16.4% WSC, 27.3% Cel, 20.9% g HC, 62.5% DDM. Coblentz et al (2020) found that the biochemical composition and nutritive value of tall fescue herbage were 75 g/kg CP, 82 g/kg ash, 107.3 g/kg WSC, 627 g/kg NDF, 356 g/kg ADF, 27.9 g/kg ADL, 1.40

Mcal/kg NEl, but meadow fescue herbage contained 71 g/kg CP, 90 g/kg ash, 98.3 g/kg WSC, 609 g/kg NDF, 364 g/kg ADF, 27.5 g/kg ADL, 1.40 Mcal/kg NEl, respectively. Karbivska et al (2020) mentioned that the chemical composition and energy nutritional value of *Lolium perenne* green mass was: 11.4% CP, 2.8% EE, 29.6% CF, 48.3% NFE, 58% DDM, 8.2 MJ/kg ME, 109 g DP/nutritive unit, but with applied mineral fertilizers – 11.5-15.6% CP, 2.8-2.9% EE, 29.6-30.0% CF, 43.7-48.3% NFE, 8.2-8.3 MJ/kg ME, 115-151 g DP/ nutritive unit, respectively. Wang et al. (2020) studied effects of cutting time and cultivar on chemical compositions on perennial ryegrass found the harvested herbages were characterized by 934-940 g/kg OM, 32.0-39.8 g/kg N, 112-217 g/kg WSC, 388-414 g/kg NDF, 78.4-83.0 % IVDMD. Dong et al. (2021) revealed that *Festuca arundinacea* forage contained 306 g/kg DM, 7.29% CP, 24.8% ADF, 54.7% NDF, 14.4% WSC, 9.08% ash, 6.97% EE. Olszewska (2021) reported that the studied perennial ryegrass cultivars contained 13.0-143.4 g/kg CP and 144.2-148.3 g/kg WSC. Rancāne et al. (2021) remarked that the nutritive value of the tested *Lolium perenne* genotypes was 6.99-10.68% CP, 38.79-46.74% NDF, 19.92-25.11% ADF, 69.34-73.38% DDM and 6.71-7.02 MJ/kg NEl. Sosnowski et al. (2022) The average net energy of lactation (NEL) was greater in *Lolium perenne* forage (5.98 MJ/kg DM)

Table 2. The biochemical composition and the nutritive value of the silage from the studied *Poaceae* species

Indices	*Festuca arundinacea*	*Lolium perenne*
pH index	4.06	3.81
Content of organic acids, g/kg DM	30.4	30.8
Free acetic acid, g/kg DM	2.6	0
Free butyric acid, g/kg DM	0	0
Free lactic acid, g/kg DM	7.3	5.5
Fixed acetic acid, g/kg DM	5.5	1.5
Fixed butyric acid, g/kg DM	0	0.4
Fixed lactic acid, g/kg DM	15.0	23.4
Total acetic acid, g/kg DM	6.1	1.5
Total butyric acid, g/kg DM	0	0.4
Total lactic acid, g/kg DM	22.3	28.9
Acetic acid, % of organic acids	24.64	4.87
Butyric acid, % of organic acids	0	1.30
Lactic acid, % of organic acids	73.36	93.83
Crude protein, % DM	9.67	9.12
Crude fats, % DM	3.64	3.30
Crude cellulose, % DM	31.30	39.16
Nitrogen free extract, % DM	40.88	37.37
Soluble sugars, % DM	0.33	1.40
Starch, % DM	0.55	0.60
Ash, % DM	14.51	10.08
Calcium, g/kg DM	0.29	2.90
Phosphorus, g/kg DM	0.23	2.60
Carotene mg/kg	56.53	36.34
Gross energy, MJ/kg DM	17.20	17.84
Metabolizable energy, MJ/kg DM	9.53	8.31
Net energy for lactation, MJ/kg DM	5.34	4.48

Fodder conservation is necessary in most parts of Earth to maintain feed supply, particularly during winter season. Silage is the main conserved green succulent roughage fodder for domestic herbivores and its quality is the key to a good animal health and productivity, lowering the need to feed animals with concentrated fodder and increasing profitability during the housing period. During the sensorial assessment, it was found that, in terms of colour, the ensiled mass from tall fescue had yellow homogeneous olive colour with pleasant smell, like pickled cucumbers; the ryegrass ensiled mass was light olive, with pleasant smell, specific to pickled apples. The results regarding the silage quality are shown in Table

2. It has been determined that the biochemical composition and the nutritive value of the ensiled forage depended on the grass species, thus, ryegrass silage had higher amount of organic acids in fixed form, butyric acid was detected in very small quantities (0.4 g/kg), but the level of acetic acid was very low in comparison with tall fescue silage. It was found that during the process of ensiling of tall fescue, the concentrations of crude protein decreased considerably, the concentrations of crude fats, crude cellulose and minerals are at the same level, but the amount of nitrogen free extract is increased. As compared with the initial fresh mass, the silage from perennial ryegrass had high concentration of crude cellulose, which had negative impact on energy concentrations.

Several studies have evaluated the quality of grass silages. According to Fisher et al. (1991), the *Festuca arundinacea* silage contained 449 g/kg DM, 154 g/kg CP, 337 g/kg ADF, 550 g/kg NDF, 6.3 g/kg Ca, 3.4 g/kg P, but – from *Dactylis glomerata* – 229 g/kg DM, 125 g/kg CP, 370 g/kg ADF, 595 g/kg NDF, 3.9 g/kg Ca, 3.3 g/kg P, respectively. Burlacu et al. (2002) reported that the quality of perennial ryegrass silage was characterized by: 205-235 g/kg DM, 11.9-13.1% CP, 3.8-4.9 % EE, 27.3-31.4% CF, 8.4-9.3% ash, 5.9-6.0 g/kg Ca, 3.4 g/kg P, 614-671 g/kg DDM, 18.85-19.12 MJ/kg GE, 12.07-13.38 MJ/kg DE, 9.84-10.93 MJ/kg ME. Pozdíńek et al. (2003) reported that nutritive value of *Festuca arundinacea* silage was: 118.5 g/kg CP, 33.8 g/kg EE, 261.9 g/kg CF, 477.4 g/kg NFE, 528.9 g/kg NDF, 308.6 g/kg ADF, 108.4 g/kg ash, 9.54 MJ/kg ME, 5.65 MJ/kg NEl. Mahnert et al. (2005) found that the silage from perennial ryegrass was characterized by 187 g/kg DM, pH 4.6, 17.0 % CP, 4.9 % EE, 31.3 % CF, 3.4 % WSC and 11.5 % ash, but from cocksfoot – 273 g/kg DM, pH 6.1, 18.4% CP, 4.6% EE, 30.3% CF, 3.1% WSC and 11.2% ash, respectively. Burke et al (2007) mentioned that the chemical composition and energy value of silage, prepared without any additives, from the first cut of a predominantly perennial ryegrass sward were 204 g/kg DM, pH=3.9, 39 g/kg lactic acid, 33 g/kg acetic acid, 2 g/kg butyric acid, 17.9% CP, 7.4% ash, 4.5% EE, 76.2% DMD, 76.1% OMD, 10.6 MJ/kg ME. Dewhurst et al. (2009) revealed that perennial ryegrass silage had 925 g/kg OM 14.0 % CP 52.6% NDF and 309% ADF. Jancik et al (2011) found that the chemical composition of dry matter silage prepared from *Festuca arundinacea* was: 17.80% CP 2.76% EE, 8.59% ash, 51.20% NDF, 31.10% ADF and 2.66% ADL, but from *Dactylis glomerata* – 14.90 CP%, 3.08% EE, 7.66% ash, 54.10% NDF, 33.30% ADF, 3.12% ADL and from the hybrid *Lolium multiflorum* × *Festuca arundinacea* – 11.90% CP, 2.92% EE, 8.78% ash, 59.5% NDF, 34.90% ADF and 2.51% ADL, respectively. Kupryś-Caruk & Kołodziejski (2016) reported that the dry matter content and chemical the composition of tall fescue silages was 214 g/kg DM, pH=5.2, 89.7 g/kg lactic acid, 2.3 g/kg acetic acid, 10.8% CP, 11.7% ash, 2.3% fats, 5.0% mono sugars, 3.0% ADL, 30.4% cellulose, 5.7% hemicellulose. Coblentz et al. (2020) compared the feed quality and energy value of grass silage found than tall fescue silage was characterized by pH=5.63, 16 g/kg lactic acid, 8.2 g/kg acetic acid, 2.4 g/kg butyric acid, 85 g/kg CP, 91 g/kg ash, 74.7 g/kg WSC, 649 g/kg NDF, 366 g/kg ADF, 26.9 g/kg ADL, 1.37 Mcal/kg; meadow fescue silage – pH=5.60, 21.7 g/kg lactic acid, 7.6 g/kg acetic acid, 3.0 g/kg butyric acid, 77 g/kg CP, 97 g/kg ash, 51.4 g/kg WSC, 644 g/kg NDF, 391g/kg ADF, 30.1 g/kg ADL, 1.34 Mcal/ kg NEl, but orchard grass silage – pH=5.50, 17.8 g/kg lactic acid, 7.8 g/kg acetic acid, 3.2 g/kg butyric acid. 104 g/kg CP, 115 g/kg ash, 34.3 g/kg WSC, 611 g/kg NDF, 357 g/kg ADF, 26.2 g/kg ADL, 1.34 Mcal/kg NEl. Richard et al. (2020) remarked than tall fescue silage contained 341 g/kg DM, 903 g/kg OM, 136 g/kg CP, 543 g/kg NDF, 353 g/kg ADF, 21.2 g/kg EE, 86.13% IVTD, 1.26 Mcal/kg NEl.

Haying is the most common method of storing forages. In some cases, hay is the major, if not the only source of essential fibre, energy, protein, vitamins and minerals for many classes of livestock during the winter season. Hay quality varies because of different factors such as forage species, fertilization, stage of maturity, harvesting/preserving practices and storage. Making better quality hay can significantly reduce the need for supplemental feed purchases and help keep adequate condition on animals. It is the best source of energy, protein, vitamins, minerals and, most importantly, fibre that is necessary for normal gut function.

The biochemical composition, nutritive and energy value of the prepared hays are presented in Table 2. The prepared hays contained 9.95-10.84% CP, 1.87-2.65 % EE, 33.38-36.90% CF, 39.99-40.54% NFE, 11.28-12.59% ash, 2.60-3.50 g/kg Ca, 2.30-2.70 g/kg P, with 17.44-17.84 MJ/kg GE, 8.31-8.85 MJ/kg ME, 4.4.54-4.93 MJ/kg NEl. The tall fescue hay is characterized by higher concentration of crude protein, crude fats and ash. The concentration of nitrogen free extract did not differ significantly in prepared hays. The energy concentrations were higher in tall fescue hay as compared with perennial ryegrass hay.

Some authors mentioned various findings about the yield and quality of the perennial ryegrass and tall fescue hays. Duke (1983) remarked the *Lolium perenne* hay contains 88% DM, 9.2% protein, 3.1% fat, 24.2% fibre, 43.4% NFE, 8.1% ash. Aitchison et al. (1986) mentioned that chemical composition of perennial ryegrass hay was 911 g/kg OM, 619 g/kg NDF, 332 g/kg ADF. Burlacu et al. (2002) reported that nutritive value of perennial ryegrass hay were: 11.5-17.0% CP, 2.7-4.0% EE, 22.0-29.0% CF, 8.5-12.0 % ash, 5.4-6.6 g/kg Ca, 3.0-4.4 g/kg P, 604-686 g/kg DDM, 17.95-18.10 MJ/kg GE, 11.40-13.28 MJ/kg DE, 9.33-10.79 MJ/kg ME. Angima & Kallenbach (2008) revealed the hay prepared from tall fescue cv. Kentucky 31 contained 6.37- 7.85% CP with RFV 96-98. Gallo et al (2013) reported that grass hays contained 825-920 g/kg DM, 62-14 g/kg ash, 51-130 g/kg CP, 21-32 g/kg EE, 540-730 g/kg NDF, 303-448 g/kg ADF, 19-116 g/kg ADL, 96-94 g/kg NFC, 0.85-1.52 Mcal/kg NEl. Bender et al. (2016) mentioned that tall fescue hay contained 143 g/kg CP, 107 g/kg ash, 30 g/kg fats, 644 g/kg NDF, 81 g/kg ADL and 16 g/kg starch. Nicolae (2016) reported that hay quality prepared from perennial ryegrass was: 6.9-11.5% CP, 1.2-1.4% EE, 28.7-34.7% CF, 8.7-9.1% ash, 47.4-49.7% NFE, 0.64-0.81 UFL/kg and 0.55-0.74 UFV /kg. Akdeniz et al. (2019) found that perennial ryegrass hay prepared in the second year contained 9.13% ash, 12.75% CP, 1.52% EE, 36.33% CF, 35.51% NDF, 24.82% ADF, 61.24% DDM, but tall fescue hay respectively 9.54% ash, 9.86% CP, 1.15% EE, 44.85% CF, 64.05% NDF, 47.64% ADF, and 51.79% DDM. Silva Déley et al. (2019) revealed that the hay prepared from *Lolium perenne* contained 163.4-183.3 g/kg CP, 274.7-320.9 g/kg CF, 71.2-115.3 g/kg ash, 612-637 g/kg NDF, 508-521 g/kg ADF, 41-43 g/kg ADL, 65.99-71.13% DDM, 66.73-73.70% ODM, but *Cenchrus clandestinum* hay contained 121.5-19.5 g/kg CP, 348.4-372.2 g/kg CF, 70.4-96.0 g/kg ash, 344-376 g/kg NDF, 263-297 g/kg ADF, 19-23 g/kg ADL, 65.13-81.71% DDM and 68.54-82.68% ODM, respectively. Xu et al. (2019) reported that the nutritive quality of wild-type *Lolium perenne* herbage dried under natural condition was 3.25% EE, 11.13% ash, 49.46% NDF, 26.79% ADF, 3.40% lignin, 9.45% sugars, 80.75% IVTDMD, but in generated *Lolium perenne* transgenic lines 3.09-3.75% EE, 10.36-11.16% ash, 45.50-51.20% NDF, 21.50-25.58% ADF, 3.83-4.51% lignin, 8.46-10.18% sugars, 78.83-85.49% IVTDMD, respectively.

Table 3. The biochemical composition and the nutritive value of the hay from the studied *Poaceae* species

Indices	*Festuca arundinacea*	*Lolium perenne*
Crude protein, % DM	10.84	9.95
Crude fats, % DM	2.65	1.87
Crude cellulose, % DM	33.38	36.90
Nitrogen free extract, % DM	40.54	39.99
Ash, % DM	12.59	11.28
Calcium, g/kg DM	2.60	3.50
Phosphorus, g/kg DM	2.30	2.70
Gross energy, MJ/kg DM	17.44	17.84
Metabolizable energy, MJ/kg DM	8.85	8.31
Net energy for lactation, MJ/kg DM	4.93	4.54

CONCLUSIONS

The local ecotype of perennial ryegrass *Lolium perenne* and the Romanian cultivar 'Valrom' of tall fescue *Festuca arundinacea* under the climatic conditions of the Republic of Moldova were characterized by optimal growth rates and productivity. The green mass and the prepared hay and silage contain a lot of nutrients, which make them suitable to be used as a part of diverse livestock diets.

ACKNOWLEDGEMENTS

The study has been carried out in the framework of the projects: 20.80009.5107.02 *“Mobilization of plant genetic resources, plant breeding and use as forage, melliferous and energy crops in bioeconomy”*.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

THE BIOCHEMICAL COMPOSITION AND THE FEED VALUE OF FODDERS FROM *Cicer arietinum* L. PLANTS

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Abstract

Chickpea, Cicer arietinum, is a multipurpose Fabaceae species, widely used around the world, notably as a source of protein, used to restore soil fertility, which can tolerate high temperatures and arid climate. This research was aimed at evaluating the nutritive value of fodder from the chickpea, local cultivar 'ICHEL', grown in monoculture in an experimental field of the National Botanical Garden (Institute), Chişinău, Republic of Moldova. The results revealed that the dry matter of the whole Cicer arietinum plant contained 19.7% CP, 20.5%CF, 12.6% ash, 24,0% ADF, 37.6% NDF, 4.6% ADL, 18.1% TSS, 19.4% Cel, 13.6% HC, with nutritive and energy value 815 g/kg DMD, 737 g/kg DOM, RFV=174, 11.25 MJ/kg ME and 7.26 MJ/kg NEl. The quality of the prepared hay was: 19.4% CP, 23.8% CF, 13.4% ash, 28.3% ADF, 43.8% NDF, 5.4% ADL, 8.9% TSS, 22.9% Cel, 15.5% HC, 748 g/kg DMD, 647 g/kg DOM, RFV=142, 10.76 MJ/kg ME and 6.77 MJ/kg NEl. The dry matter of the fermented fodder contained 22.2% CP, 15.0% CF, 14.6% ash, 18.1% ADF, 31.3% NDF, 2.0% ADL, 19.8% TSS, 16.1% Cel, 13.2% HC, 59.62 mg/kg carotene, 890 g/kg DMD, 810 g/kg DOM, RFV=222, 13.2 MJ/kg ME, 7.48 MJ/kg NEl. The harvested biomass of the chickpea cultivar 'Ichel' can be used as alternative fodder for farm animals.

Key words*: Cicer arietinum, green mass, hay, nutritive value, silage.*

INTRODUCTION

Fabaceae species are widespread in all climatic zones, growing under the most diverse ecological conditions. The importance of *Fabaceae* species in people's life cannot be underestimated, since they are used as food, fodder, technical, medicinal, honey and ornamental crops, play an essential economic and ecological role in the development of sustainable agriculture by fixing atmospheric nitrogen and mobilizing phosphorus, have positive impact on the physical and chemical properties of soil, help reducing soil erosion processes, besides, they are an important food source for wild and domestic animals, provide nectar and pollen for bees and other useful insects (Kulkarni et al., 2018).

The genus *Cicer* L. of the *Fabaceae* family includes about 10 annual species and 36 perennial species, occurring in the Mediterranean Basin and southwest Asia, among the annual species only *Cicer arietinum* L., is domesticated and widely cultivated in more than 50 countries from different regions of the Earth, being the second legume pulse crop in the world. In the flora of Bessarabia, only one species, *Cicer arietinum* L., known by the common name chickpea, occurs sporadically, having been cultivated as a food plant for grains for hundreds of years. Chickpea is an annual plant, with a solid, erect, 4-angled, glandular-pubescent, branched stem, 30-70 cm tall; with falsely imparipinnate, short-petiolate leaves, with 4-8 pairs of elliptic or oblong-ovate leaflets, with finely serrate-toothed margins, 6-15 cm long; with small, toothed stipels. The inflorescences are raceme with solitary flowers, 10-20 mm long, with white, yellow, pink or blue-purple corolla; vexil with red or brown veins. It blooms in June and July. Fruit – an elliptical pod, yellow or reddish-yellowish, swollen, glandular, 2-3 cm long and 1-1.3 cm wide, on a curved pedicel, with 1-3 globular, variously colored seeds (yellow to blackish), 5-13 mm long. There are two main types of chickpea, which differ in seed size, shape and color. The first type of chickpea called *'desi'* produces small, angular, dark colored seeds, grown in the semi-arid tropical areas, and the other type *'kabuli'* produces large, round, light colored seeds and is grown in areas with temperate climate. The root system consists of a thick taproot with several lateral roots, the epidermis is hairy, the exodermis is absent, and the endodermis is thin. The presence of nodules on the roots indicates a symbiotic relationship between chickpea and *Mesorhizobium ciceri* bacteria leading to biological nitrogen fixation of 120-150 kg/ha. The root system is so robust that it reaches more than 3 m deep into the soil, making it easier for the plant to survive under conditions of insufficient moisture. Of all the leguminous plants, the chickpea tolerates drought conditions the most easily. The minimum temperature for seed germination is 3-4°C, and at 6-8°C, and the plants emerge evenly at the soil surface within no more than 10 days after sowing. In the spring, chickpea seedlings withstand frost up to -6°C. It grows well in sandy soils and slightly salinized soils, but it is not recommended to plant chickpea in heavy, excessively wet, poorly aerated soils. (Maessen, 1972; Balashov et al., 2012; Izverscaia, 2020; Voshedsky et al., 2020). The cultivation of chickpea is a possible method of adaptation to climate change (Vargas-Blandino et al., 2021). In our country, chickpea has been researched and, as a result, cultivation technologies have been developed, plant resources have been mobilized and new high-productivity varieties have been created (Arseni, 1974; Celac & Machedon, 2010). Currently, 4 chickpea varieties are registered in the Catalog of Plant Varieties of the Republic of Moldova.

The main objective of this research was to evaluate the quality of green mass, hay and silage prepared from chickpea, *Cicer arietinum*.

MATERIALS AND METHODS

The local cultivar *'Ichel'* of chickpea, *Cicer arietinum* L., created at the Institute of Genetics, Physiology and Plant Protection by professor Valentin Celac, and grown in monoculture on the experimental land of National Botanical Garden (Institute) Chişinău, N 46°58′25.7″ latitude and E 28°52′57.8″ longitude, served as subject of the research, the common sainfoin, *Onobrychis viciifolia,* cultivar '*Anamaria*' and the low-cumarin local ecotype of yellow sweet clover, *Melilotus officinalis*, were used as control variants. The chickpea samples were collected in the flowering - early pod stage, yellow clover - in the flowering stage, common sainfoin - in the budding-flowering stage. The leaf/stem ratio was determined by separating the leaves from the stem, weighing them separately and establishing the ratios for these quantities (leaves/stems). The prepared hay was dried directly in the field. The chickpea and yellow clover silages were prepared from directly harvested green mass, but common sainfoin haylage was produced from wilted green mass, cut into small pieces and compressed in glass containers. The containers were stored for 45 days, and after that, they were opened and the organoleptic assessment and the determination of the organic acid composition of the persevered forage were done in accordance with the Moldavian standard SM 108. The dry matter content was detected by drying samples up to constant weight at 105°C. For biochemical analysis, the plant samples were dried in a forced air oven at 60°C, milled in a beater mill equipped with a sieve with diameter of openings of 1 mm and some assessments of the main biochemical parameters: crude protein (CP), ash, acid detergent fibre (ADF), neutral detergent fibre (NDF), acid detergent lignin (ADL), total soluble sugars (TSS), digestible dry matter (DDM), digestible organic matter (DOM) have been determined by near infrared spectroscopy (NIRS) technique PERTEN DA 7200. The concentration of hemicellulose (HC), cellulose (Cel), digestible energy (DE), metabolizable energy (ME), net energy for lactation (NEl) and relative feed value (RFV) were calculated according to standard procedures.

RESULTS AND DISCUSSIONS

Analysing the results of the assessment of biomorphological peculiarities of the local cultivar *'Ichel'* of chickpea, *Cicer arietinum*, it can be noted that seedlings emerged uniformly at the soil surface at the end of April, the development of shoots was observed in the middle of May, the budding-flowering stage - at the end of May, the flowering - early pod stage - in the middle of June. At the time when the green mass was harvested, the chickpea plants reached 49.7 cm in height, common sainfoin 99.2 cm and yellow clover - 111.6 cm. The chickpea yield was 2.42 kg/m^2 fresh mass or 0.71 kg/m^2 dry matter, yellow sweet clover - .3.78 kg/m^2 fresh mass or 1.17 kg/m^2 dry matter and common sainfoin-4.23 kg/m^2 fresh mass or 1.01 kg/m^2 dry matter According to Petrov (2004) the productivity of single-species chickpea was 2.8 t/ha dry matter, 290 kg/ha digestible protein, 23.8 GJ/ha metabolizable energy and 175 g digestible protein/fodder unit, but chickpea mixed with barley produced 2.2-2.6 t/ha DM, 200-230 kg/ha digestible protein, 18.1-21.2 GJ/ha ME and 160-168 g digestible protein/fodder unit, respectively. Makenova (2005) remarked that chickpea crops in the southern forest-steppe of the Omsk region, Russia, made it possible to obtain an average of 21.2 t/ha of green mass, 4.66 t/ha of fodder units and 0.91 t/ha of crude protein. Lingorski & Kertikov (2014) mentioned that under the conditions of Troyan, Bulgaria, the productivity of forage chickpea reached 12.82 t/ha green mass and 3.27 t/ha dry mass, the structural elements of the forage were 23.13% leaves, 28.53% inflorescences and 52.64% stems. Kertikov & Kertikova (2016) found that the chickpea plants contained 40.8% stems, 50.7% leaves and 6.7% inflorescences, the productivity was 21 t/ha fresh mass, 4.58 t/ha dry mass and 774 kg/ha crude protein.

The biochemical composition, nutritive and energy value of the harvested green mass from the studied *Fabaceae* species are presented in Table 1. Analysing the results of the biochemical composition of green mass, we found that the dry matter of the studied species differs essentially in the concentration of crude protein, structural carbohydrates and energy. The chickpea fodder is characterized by high amount of crude protein, minerals, total soluble sugars, but lower amount of crude fibre, hemicellulose and cellulose. The content of acid detergent lignin in chickpea fodder was lower than in common sainfoin fodder, but higher than in yellow sweet clover fodder. The chickpea fodder is characterized by very high digestibility, which has a positive effect on relative feed value and energy concentration as compared with the forage produced from yellow clover and common sainfoin.

Table 1. The biochemical composition and nutritive value of harvested mass of the studied *Fabaceae* species

Indices	Chickpea	Common sainfoin	Yellow sweet clover
Crude protein, % DM	19.7	17.7	17.9
Crude fibre, % DM	20.5	29.3	33.0
Minerals, % DM	12.6	9.6	11.8
Acid detergent fibre, % DM	24.0	30.9	33.1
Neutral detergent fibre, % DM	37.6	44.7	47.3
Acid detergent lignin, % DM	4.6	4.9	4.4
Total soluble sugars, % DM	18.1	11.4	7.2
Cellulose, % DM	19.4	26.0	28.7
Hemicellulose, % DM	13.6	13.8	14.2
Digestible dry matter, g/kg DM	815	669	651
Digestible organic matter, g/kg DM	737	615	543
Relative feed value	174	135	124
Digestible energy, MJ/ kg	13.70	12.73	12.42
Metabolizable energy, MJ/ kg	11.25	10.45	10.20
Net energy for lactation, MJ/ kg	7.26	6.48	6.22

Table 2. The biochemical composition and nutritive value of the prepared hay from the studied *Fabaceae* species

Indices	Chickpea	Common sainfoin	Yellow sweet clover
Crude protein, % DM	19.4	16.3	15.0
Crude fibre, % DM	23.8	33.8	37.4
Minerals, % DM	13.4	9.9	8.3
Acid detergent fibre, % DM	28.3	35.0	38.5
Neutral detergent fibre, % DM	43.8	49.6	49.3
Acid detergent lignin, % DM	5.4	5.2	5.6
Total soluble sugars, % DM	8.9	6.3	8.0
Cellulose, % DM	22.9	29.8	31.7
Hemicellulose, % DM	15.5	14.6	16.3
Digestible dry matter, g/kg DM	748	625	562

Digestible organic matter, g/kg DM	647	560	493
Relative feed value	142	115	103
Digestible energy, MJ/ kg	13.11	12.17	11.80
Metabolizable energy, MJ/ kg	10.76	9.99	9.69
Net energy for lactation, MJ/ kg	6.77	6.01	5.70

Table 3. The biochemical composition and the nutritive value of the fermented mass from the studied *Fabaceae* species

Indices	Chickpea	Common sainfoin	Yellow sweet clover
pH index	4.40	4.68	4.52
Organic acids, g/kg DM	44.8	23.40	40.50
Free acetic acid, g/kg DM	2.20	1.10	1.90
Free butyric acid, g/kg DM	0	0	0
Free lactic acid, g/kg DM	11.80	4.40	9.00
Fixed acetic acid, g/kg DM	2.80	2.20	3.20
Fixed butyric acid, g/kg DM	0.40	0	0
Fixed lactic acid, g/kg DM	27.60	15.70	26.40
Total acetic acid, g/kg DM	5.00	3.30	5.10
Total butyric acid, g/kg DM	0.40	0	0
Total lactic acid, g/kg DM	39.40	20.10	35.40
Acetic acid, % of organic acids	11.16	14.10	12.59
Butyric acid, % of organic acids	0.89	0	0
Lactic acid, % of organic acids	87.95	85.90	87.41
Crude protein, % DM	22.2	14.2	17.8
Crude fibre, % DM	15.0	31.2	34.8
Minerals, % DM	14.6	11.8	10.3
Acid detergent fibre, % DM	18.1	31.7	33.3
Neutral detergent fibre, % DM	31.3	47.0	46.2
Acid detergent lignin, % DM	2.0	4.0	3.8
Total soluble sugars, % DM	19.8	13.5	7.0
Cellulose, % DM	16.1	27.7	28.5
Hemicellulose, % DM	13.2	15.3	12.9
Digestible dry matter, g/kg DM	890	653	632
Digestible organic matter, g/kg DM	810	582	566
Relative feed value	222	127	129
Digestible energy, MJ/ kg	13.20	12.63	12.41
Metabolizable energy, MJ/ kg	10.84	10.37	10.19
Net energy for lactation, MJ/ kg	7.48	6.38	6.20

In the literature sources, there is little information regarding the chemical composition and nutritional value of whole plants of *Cicer* species. According to Larin et al. (1952), *Cicer macracanthum* green fodder contained in dry matter 14.8% CP, 3.5% EE, 21.6% CF and 53.8% NFE. Maessen (1972) remarked that *Cicer arietinum* forage contained 10.8-11.3% CP, 2.1-2.2% EE, 27.2-33.1% CF, 444.9-48.0% NFE, 9.1-11.4% ash. Kirilov et al. (2016) compared the quality of green mass of whole plants of perennial and annual legumes harvested in the flowering-pod formation stage, and reported that the chemical composition of *Cicer arietinum* was 14.06 % CP, 3.44% EE, 27.14 % CF, 44.04% NFE and 11.32% ash; *Onobrychis viciifolia,* in turn, contained 17.53% CP, 3.12% EE, 20.08% CF, 51.17% NFE and 8.1% ash; *Medicago sativa* 17.36% CP, 2.32% EE, 27.84% CF, 42.63% NFE and 9.85% ash; *Lotus corniculatus* 17.14% CP, 3.14% EE, 25.63% CF, 45.32% NFE and 8.77% ash; *Pisum sativum* 13.04% CP, 2.14% EE, 25.06% CF, 58.30% NFE and 8.01% ash; *Glycine max* 13.13% CP, 2.48% EE, 29.87% CF, 45.50% NFE and 9.02% ash. Tedeeva (2018) reported that chickpea leaves contained 2.16-3.48% N, 0.31-0.49% P_2O_5, 2.09-2.36% K_2O and chickpea stems respectively 1.57-2.40% N, 0.28-0.36% P_2O_5, 1.71-2.01% K_2O. Semina & Telic (2020) evaluating the quality of 15 collection samples of *Cicer arietinum* of various ecological and geographical origin, mentioned that the protein content in the green mass varied from 10.64% to 15.06%. Voshedsky et al. (2020) found that the chemical composition of *Cicer arietinum* plants harvested in the flowering period was 2.41-4.19% N, 0.84-1.24% P_2O_5, 3.22-4.12% K_2O.

The conservation of fodder and crop residues is a traditional way of reducing seasonal variations in feed availability. Hay is the oldest, and still the most important, conserved fodder, despite its dependence on suitable weather at harvest time. Hay is an essential part of livestock diet, providing them, during winter, with the necessary protein, fibres and other nutrients they need to maintain good health and be productive. We would like to mention that in the haymaking process, we noticed an increase in the concentration of structural carbohydrates, lignin and a decrease in the content of crude protein, total soluble sugars, digestibility, relative feed value and energy concentration as compared with the harvested green mass. The results regarding the forage quality of hay prepared from the studied *Fabaceae* species are shown in Table 2. The prepared hays contained 15.0-19.4% CP, 23.8-37.4% CF, 8.3-13.4% ash, 28.2-38.5% ADF, 43.8-49.6% NDF, 5.2-5.6% ADL, 6.3-8.9% TSS, 22.9-31.7% Cel and 14.6-16.3% HC. The digestibility, nutritive value and the energy concentration of prepared hays were 562-748 g/kg DMD, 493-647 g/kg DOM, RFV=103-142, 11.80-13.11 MJ/kg DE 9.69-10.76 MJ/kg ME and 5.70-6.77 MJ/kg NEl. The hay prepared from *Cicer arietinum* is characterized by very high content of crude protein and minerals, optimal content of soluble sugars and hemicellulose, but lower content of structural carbohydrates, which have a good effect on digestibility, nutritive and energy value. According to Maessen (1972) the chemical composition of chickpea hay was 12.9% CP, 1.5% EE, 36.3% CF, 38.1% NFE and 11.2% ash. Sainz-Ramírez et al. (2022) found that the dry matter content, the chemical composition and nutritive value sunflower-chickpea hay were 694.07 g/kg DM, 17.34% CP, 17.12% EE, 42.52% NDF, 27.03% ADF, 11.00% ash and 67.84% IVDOM, but alfalfa hay contained 880.14 g/kg DM, 18.05% CP, 2.25% EE, 36.06% NDF, 28.05% ADF, 10.00% ash and 67.04% IVDOM, respectively.

Ensiling, a fermentation process, is now a major conservation method for large-scale enterprises. The production of fermented fodder, silage and haylage, minimizes the risk associated with field losses, which can be incurred under rainy conditions during hay making. Besides, silage is an important source of nutrients for the dairy production sector in the autumn - middle spring period. When opening the glass containers with chickpea silage, there was no gas or juice leakage from the preserved mass. The chickpea fermented mass had homogeneous, agreeable olive colour with pleasant smell, similar to the smell of green pea, the texture was preserved, in comparison with the initial green mass, without mould and mucus. The yellow clover silage consisted of olive stems with dark green leaves and had a peculiar smell, similar to pickled apples, the sainfoin haylage had yellowish-green leaves and yellow-green stems with pleasant smell like pickled vegetables. The results regarding the quality of the fermented fodder from studied *Fabaceae* species are illustrated in Table 3. It was determined that the pH values of the fermented fodder depended on the species, thus, chickpea silage had pH=4.4, lower than sainfoin haylage and yellow clover silage. The concentration of organic acids in the chickpea silage is very high in comparison with sainfoin haylage. Most organic acids in the investigated fermented fodders were in fixed form. According to the Moldavian standard SM 108, the ratio of acetic acid and lactic acid of the studied fermented fodders corresponds to the first class quality. In chickpea silage butyric acid was detected in fixed form, in very small quantity (0.4 g/kg). Analysing the biochemical composition of fermented fodders, it has been determined that the concentrations of nutrients in the dry matter varied: 14.2-22.2% CP, 15.0- 34.8% CF, 18.1-33.3% ADF, 31.3-46.2% NDF, 2.0-4.0% ADL, 7.0-19.8% TSS, 16.1-28.5% Cel, 12.9-15.3% HC and 10.3-14.6% ash. The nutritive and energy values of the fermented fodders were 632-890 g/kg DMD, 566-810 g/kg DOM, RFV=127-222, 12.41-13.20 MJ/kg DE, 10.19-10.84 MJ/kg ME and 6.20-7.48 MJ/kg NEl. We would like to mention that chickpea silage was characterised by very high content of crude protein, minerals and total soluble sugars, but reduced concentration of cell wall fractions (NDF, ADF, ADL) which had a positive effect on the digestibility, nutritional value and energy supply of the feed.

CONCLUSIONS

The dry matter of the harvested in flowering - early pod stage of *Cicer arietinum* plants contained 19.7% CP, 20.5%CF, 12.6% ash, 24,0% ADF, 37.6%NDF, 4.6% ADL, 18.1% TSS, 19.4% Cel, 13.6% HC, with nutritive and energy value 815 g/kg DMD, 737 g/kg DOM, RFV=174, 11.25 MJ/kg ME and 7.26 MJ/kg NEl.

The quality of the chickpea hay was: 19.4% CP, 23.8% CF, 13.4 % ash, 28.3% ADF, 43.8% NDF, 5.4% ADL, 8.9% TSS, 22.9% Cel, 15.5% HC, 748 g/kg DMD, 647 g/kg DOM, RFV=142, 10.76 MJ/kg ME and 6.77 MJ/kg NEl.

The chickpea silage was characterized by pH = 4.40, 5.0 g/kg acetic acid, 0.4 g/kg butyric acid, 39.4 g/kg lactic acid, 22.2% CP, 15.0% CF, 14.6% ash, 18.1% ADF, 31.3% NDF, 2.0% ADL, 19.8% TSS, 16.1% Cel, 13.2% HC, 59.62 mg/kg carotene, 890 g/kg DMD, 810 g/kg DOM, RFV=222, 13.2 MJ/kg ME, 7.48 MJ/kg NEl. The harvested biomass of the chickpea cultivar *'Ichel'* can be used as alternative fodder for farm animals.

ACKNOWLEDGEMENTS

The study has been carried out in the framework of the project: 20.80009.5107.02 *"Mobilization of plant genetic resources, plant breeding and use as forage, melliferous and energy crops in bioeconomy"*.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

INFLUENCE OF DIFFERENT LEVELS OF VITAMIN AND MINERAL NUTRITION OF DAIRY COWS ON MORPHOLOGICAL AND BIOCHEMICAL INDICATORS OF BLOOD AND THEIR PRODUCTIVITY

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Abstract

Successful animal husbandry is impossible without the organization of a balanced feeding system, and in particular is providing rations with optimal level of mineral elements and vitamins, which serve as a guarantee of the intensity of metabolic processes in the body as well as assimilation of feed nutrients and their conversion into products. Multi-ingredient feed supplements are the best source of replenishing the rations of dairy cows with nutrients. The morphological and biochemical composition of blood is a symptomatic reflection of the metabolism intensivity. Feeding cattle an improved vitamin and mineral supplement (VMS), as part of the improved compound feed K 60-32-89, makes it possible to ensure a sufficient level of vitamin and mineral nutrition in the Pre-Carpathia zone. The balanced ration of dairy cows with biologically active substances (BAS) enhances the redox reactions of the body (probably a higher level of erythrocytes, hemoglobin, total protein, nitrogen fractions) while simultaneously accumulating energy in the cell (increasing total acid-soluble phosphorus and nucleic acids (NA)) and the growth of the level of milk productivity by 9.8%.

Key words*: blood, dairy cows, milk, premix, vitamin and mineral supplement.*

INTRODUCTION

The food security of the state and the improvement of the population's well-being are largely determined by the effective management of agriculture. Cattle breeding, as an extremely perspective branch of agriculture, is the most important indicator of the state of the livestock industry, as it provides mankind with irreplaceable food products, while the food and processing industry with valuable raw materials (Kukhar, 2013; Antoshchenkova, 2020). In particular, the population consumes with livestock products more than 2/3 of animal protein, while milk accounts for 50% (Bozhydarnik, 2010). According to FAO data, 16% of the human energy comes from food of animal origin, namely milk. Every Ukrainian on average consumes less than 200 kg of milk and dairy products during the year, while norm is 380 kg (Antoschenkova, 2020). Thus, the priority task of agricultural science is to increase the volume of production, which is achieved by increasing the intensity of metabolic processes in the body.

An important link in achieving the genetic maximum of productivity, reproductive capacity, preserving the health of animals and extending productive longevity, the birth of strong offspring, as well as the prevention of alimentary diseases is the organization of rational complete feeding of cows (Spears, 2011; Sawant et al., 2013; Keshri et al., 2021). The level of livestock nutrition is determined by the amount of energy, protein, essential amino acids, fats, carbohydrates and a wide range of biologically active substances, including mineral elements and vitamins (Farionik, 2020; Spears & Weiss, 2014). The optimal ratio of these substances determines the full functioning of the animal organism, the rational use of feed resources and the effective transformation of nutrients into products (Yanovych & Sologub, 2000; Vorobel & Pivtorak, 2011; Weiss, 2017).

Based on the analysis of scientific literature, it is known that mineral elements and vitamins play a leading role, both in the formation of milk and in building muscle mass. It is also known that the provision of the ration with these nutrients is reflected in the composition of the blood, since ontogenetic hematological changes are closely related to the feeding factor and the health of the organism (Kozlovskyi, 2013; Dovhii et al., 2019). According to the researches of a number of scientists, it was established that the lack of one or more nutrients contributes not only to a lower level of productivity corresponding to their deficiency, but also negatively affects the body's use of other feed elements and causes metabolic disorders in the animal's body (Kozlovskyi, 2013; Yatoo et al., 2013). To ensure of vitamin and mineral nutrition of ruminants, it is advisable to use complex feed supplements in the rations, which would have the optimal amount of vitamins and mineral elements in their composition, which act as a catalyst-regulating factor of this or that metabolic link in the body (Sawant et al., 2013; Dovhii et al., 2019). The use of feed supplements in livestock rations makes it possible to improve the digestibility of nutrients, which thereby leads to an increase in protein, carbohydrate and lipid metabolism, and as a result, an increase in the volume of production at a lower cost (Sawant et al., 2013; Kotets et al., 2020).

The analysis of literary sources shows that there are a number of geochemical zones on the territory of Ukraine, which are characterized by a deficiency of certain mineral elements in soils, fodder, as well as in animal rations (Kravtsiv et al., 2001; Doletskyi, 2010; Sachuk et al., 2019). Research by scientists confirms the impossibility of using a single recipe for a feed supplement without taking into account the zonal aspect of the region, since the needs of animals in each nutrient are not fully met (Mc Dowell, 1985; Sachuk et al., 2019; Farionik, 2020).

Taking into account the above, it should be noted that physiologically, the body of animals cannot fully function without providing an optimal amount of BAS, which is reflected in the composition of the blood, and therefore in the level of productivity. Thus, ensuring the livestock's need for vitamins, macro- and microelements through the use of multi-ingredient feed supplements, developed taking into account the geochemical zone and clarifying their effect on blood indicators and productivity, remains a promising direction of research.

The purpose of the research was to establish the effectiveness of the use of various variants of vitamin and mineral supplements in the feeding of dairy cows on the morphological and biochemical indicators of the blood, their productivity in the Pre-Carpathia region.

MATERIALS AND METHODS

In order to find out the influence of different levels of vitamin and mineral nutrition on the morphological and biochemical indicators of dairy cows and their productivity, an experiment was conducted at the PAC «Berezhnytsia» of the Lviv Region. The experimental part of the research lasted 120 days (equalization period - 30 days, main - 90 days) on a grass-concentrate ration in the summer-pasture period. The study was conducted on 20 dairy cows of the Simmental breed, from which two groups were formed: I - control, II - experimental, 10 cows in each. Selection of animals into groups was carried out according to the method of analogues, taking into account origin (Simmental breed), age (4.0-4.5 years, second lactation), live weight (515-530 kg), productivity (third month of lactation, average daily yield was at level of 19-20 kg).

Cows were fed in accordance with scientifically based norms (Bohdanov et al., 2012). The main ration of the experimental animals was represented by pasture grass, green mass of cereal-legume mixtures of the green conveyor (75% cereals, 25% legumes), cereal-various grass hay and molasses. The concentrate group of the cow's ration included control and experimental compound feed K 60-32-89, premix P 60-5M and an improved vitamin and mineral supplement. Grains - wheat, barley, oats and their processing products - wheat bran served as constituent components of both experimental and control feed. The macroelement composition of the combined feed was represented by monocalcium phosphate, magnesium oxide and common salt. Premix P 60-5M contains fat-soluble vitamins (A, D), trace elements (zinc, cobalt, iodine) and a filler - wheat bran. Experimental animals were fodder twice a day with 50% of the total nutritional value of the ration: in the morning and in the evening. Cows were milked twice by machine.

Experimental animals of both groups were fodder control combined feed K 60-32-89 and premix P 60-5M at the same time as the main ration feed during the equalization period.

During the main period, dairy cows of the control group received an identical ration. The animals of the experimental group were fed similar fodder with the only difference that the compound feed K 60-32-89 (improved in terms of phosphorus and sulfrur) to replace the premix P 60-5M included VMS in the amount of 1%. The supplement additionally included copper and selenium and is adjusted according to the level (according to need) of microelements (zinc, cobalt, iodine) that are limited in the Pre-Carpathia zone as well as the scientifically based level of fat-soluble vitamins: A, D. Wheat bran acts as a filler. The experimental compound feed, unlike the control feed, is characterized by a larger amount of monocalcium phosphate and the additional inclusion of glauber salt, which enriches the latter with phosphorus and sulfur and, accordingly, has a positive effect on the content of these macroelements in the ration of cows.

The material for research was fodder, blood and milk. In the course of the experiment, fodder samples were taken for full zootechnical analysis – nutrition and chemical composition. For studies of morphological and biochemical indicators, blood was collected in the morning before feeding from the jugular vein of 3 animals from each group. The following indicators were determined in the blood: the number of erythrocytes and the level of hemoglobin, the content of total protein in the blood serum, fractions of nitrogen (amine, total, protein and residual) and phosphorus (total acid-soluble, inorganic and organic, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA)), urea according to generally accepted methods (Vlizlo et al., 2012).

The digital material was processed by the methods of variational statistics using the standard package of *Microsoft* Excel та *AtteStat* application programs using Student's t-test. Arithmetic mean values (M) and arithmetic mean errors (m) were calculated. Differences between mean arithmetic values were considered statistically significant by: *P<0.05; **P<0.01; ***P<0.001.

RESULTS AND DISCUSSIONS

The analysis of the actually consumed amount of fodder by dairy cows shows a slight difference between the control and experimental groups only in the green mass of cereal-legume mixtures of the green conveyor, which in percentage is 0.7%. As for the consumption of other components of the grass-concentrate ration - hay, pasture grass, compound feed, and molasses - it was 100% in both groups of animals. In the experiment, fodder was used that met the requirements of the standard and was noted for its good taste, based on the percentage of its consumption, therefore, it was of high quality. The productive effect of the rations, that is, the level of metabolism in the body of dairy cows of both groups, is determined by the use in the structure of compound feed K 60-32-89 of premix P 60-5M and optimized VMS in the composition of (improved in terms of phosphorus and sulfur) compound feed K 60-32-89.

In the process of conducting the research, no difference between the groups was also found in a number of nutrients – exchangeable energy, dry matter, crude and digestible protein, crude fat, crude fiber, sugar. The optimal ratios were observed in ruminant's rations: sugar to protein - 0.9: 1.0, carbohydrates to protein - 2.0: 1.0, calcium to phosphorus - 1.8-2.0: 1.0, nitrogen to sulfur - 11.1-11.9: 1.0, potassium to sodium - 5.4-5.9: 1.0. Simultaneously with the optimum of the above mentioned nutritional indicators, the analysis of the feeding of dairy cows of the two groups shows the different supply of BAS level in rations.

As a result of the assessment of the chemical composition of the , that were included in the structure of the grass-concentrate ration of the experimental animals of the control group, it was established that the standard compound feed K 60-32-89 in a complex with the premix P 60-5M does not provide scientifically justified needs for a number of macro- (phosphorus, sulfur) and trace elements (copper, zinc, cobalt, iodine, selenium) and requires correction by the content of fat-soluble vitamins (A, D). It should be noted that, in contrast to the control group, improved VMS as a component of the experimental compound feed K 60-32-89 (improved in terms of phosphorus and sulfur) eliminates BAS deficiency, i.e. brings the level of vitamin and mineral nutrition of dairy cows to the recommended norm.

Correction of rations for deficient mineral elements and vitamins is one of the ways to improve hematopoietic processes in tissues, and in this way – to activate metabolism in the animal's body.

The obtained experimental data show that the consumption of experimental cows in the summer-pasture period of K 60-32-89 combined feed, improved in terms of phosphorus and sulfur, improved VMS, corrected for mineral elements and fat-soluble vitamins in the Pre-Carpathia zone, had a positive effect on the investigated morphological and biochemical indicators of blood (Table 1).

Table 1. Morphological and biochemical parameters of the blood of dairy cows (M±m, n=3)

The investigated indicator and unit of measurement	Groups of animals	
	I (control)	II (experimental)
Erythrocytes, T/l	6.61±0.13	6.91±0.10
Hemoglobin, g/l	95.87±2.98	114.7±2.52**
Total protein, g/l	80.00±0.50	82.90±0.43*
Nitrogen, mmol/l:		
amine	3.36±0.08	3.73±0.06*
total	1842.3±12,0	1894.0±6.62*
protein	1804.2±13,7	1856.0±6.50*
residual	38.10±1,70	38.00±0.58
Urea, mmol/l	4.14±0,07	3.72±0.07*
Phosphorus, mmol/l:		
total acid-soluble	2.61±0.04	2.92±0.05**
Inorganic	1.56±0.01	1.66±0.02**
Organic	1.05±0.04	1.26±0.04*
RNA	597.9±16.8	660.3±5.6*
DNA	275.7±11.4	317.2±7.9*

Note. The difference is probable comparing to the control: *P<0.05; **P<0.01; ***P<0.001.

According to the results of the research, it was found that on the background of improved VMS in the blood of ruminants of the research group, the intensity of redox processes in the body increases. This is confirmed by the increase in the number of erythrocytes in the blood of dairy cows of group II, compared to group I. In percentage terms, the difference between the groups is 4.5%. In this plane, in the animals of the experimental variant, relative to the control, a significantly higher degree of saturation of erythrocytes with hemoglobin is observed. The percentage advantage of the former over the latter is 19.6%. The intergroup difference is within the limits of high probability (P<0.01). Erythrocytes and hemoglobin participate in the metabolism, which is the basis of the vital activity of the body. So, in particular, red blood cells adsorb amino acids and distribute them with blood throughout the body, which ensures the creation of conditions for the intensification of synthesis processes. Free amino acids are used for the formation of proteins of organs and tissues, and their balance is replenished due to the desorption of amino acids bound by erythrocytes (Yanovych & Sologub, 2000; Luz et al., 2005). At the same time, it should be noted that a high level of free amino acids, i.e., amino nitrogen, is an indicator that determines the intensity of protein synthesis, which is observed in the blood of dairy cows of the research group of this experiment fed with optimized VMS. In particular, in the blood of experimental animals of the II group, a higher level of amino nitrogen is observed, compared to the control analogues by 11.0%. The probability criterion is P<0.05. Confirmation of the intensive course of synthetic processes, simultaneously with the growth of amino nitrogen, is an increase in the content of total protein. In particular, the advantage of this indicator in the blood of dairy cows of the experimental variant, in contrast to the control, in percentage terms is 6%. The intergroup difference is within the limits of probability (P<0.05). The analysis of the conducted studies shows that when feeding test animals of the II group with improved VMS there is an increase in total nitrogen in the blood of animals by 2.8%, compared to premix P 60-5M. According to the statistical calculation, the difference is within the limits of probability (P<0.05). As for the content of protein nitrogen in the blood of dairy cows of the experimental variant, the intergroup difference in percentage terms is 2.9%, compared to the control variant. The probability criterion is $P<0.05$. Such a studied indicator as the level of residual nitrogen in the blood of experimental animals of both groups is almost at the same level, however, in a statistical sense, the difference is improbable ($P>0.05$).

An indirect indicator of the intensity of protein metabolism in the body of ruminants in general is the level of urea in blood serum. In the course of research, it was established that the urea content in the blood of dairy cows on the background of the use of optimized VMS is lower, compared to the control analogue (premix P 60-5M). The percentage advantage in favor of the control is 10.1%. According to the data of statistical processing, the intergroup difference is probable ($P<0.05$). In the context of the above, a decrease in the level of urea in the blood of animals of the experimental variant, and probably in the rumen of ammonia, indicates a high level of anabolic processes in the pre-stomachs.

Correction of vitamin and mineral nutrition of experimental animals by feeding a new VMS results in significant changes in the fractions of phosphorus metabolism in the blood. An element like phosphorus plays a key role in energy processes. It is a component of the redox buffer system of blood and exists in two forms: acid-soluble (organic and inorganic fractions) and acid-insoluble (nucleoproteins and lipids) (Demydiuk et al., 2011). Analyzing the indicators of phosphorus metabolism in the blood of dairy cows, it should be noted that the difference in the content of total acid-soluble phosphorus in percentage terms is 11.9% in favor of the II group. According to the statistical calculation, the advantage is probable ($P<0.01$). Such a studied indicator as inorganic phosphorus in the blood of animals of the experimental variant in percentage terms increases by 6.4% in contrast to the control. The probability criterion is $P<0.01$. At the same time, the amount of such a fraction as organic phosphorus increases by 20.0% when consumed by dairy cows of the II group of improved VMS. The difference between the groups in statistical terms is within the limits of probability ($P<0.05$).

At the same time, the leading role in the anabolic chain belongs to nucleic acids. In the studies of many scientists, there is a direct correlation between the synthesis of protein molecules and the level of NA. Thus, the number of NAs in animal tissues can to some extent be used as a test of the intensive course of protein metabolism. In the blood of ruminants of the II group, with the use of optimized VMS, there is an increase in the level of NA Phosphorus. In particular, in the blood of dairy cows of the experimental variant, a higher concentration of RNA phosphorus was noted relative to the similar indicator of the control, which in percentage terms is 10.4%. The degree of probability is equal to $P<0.05$. The advantage in the content of DNA phosphorus in the blood of ruminants of the II group compared to the I group is 15.1%. According to the statistical calculation, the intergroup difference is probable ($P<0.05$). A higher level of NA phosphorus, as well as total protein in the blood of dairy cows of the experimental variant, indicates the intensification of synthesis processes in the body as a whole.

The above changes in the morphological and biochemical indicators of blood, which were noted in experimental animals of the II group on the background of improved VMS, are consistent with the results of similar studies (Dovhii et al., 2019; Kropyvka & Bomko, 2020; Farionik, 2020). Thus, the introduction of a complex of mineral elements (copper, cobalt, zinc, manganese, iodine) and the vitamin drug "Solvimin Selen" into the ration of lactating cows led to an increase in the level of productivity and an improvement in morphological indicators (number of erythrocytes, hemoglobin), which indicates the activation of hematopoesis. The researches of Farionik (2020) established the positive effect of using both sulfuric acid salts of trace elements (zinc, copper and manganese) and mixed ligand complexes in the rations of cows on hematological parameters, live weight, productivity and chemical composition of milk. Other scientists have also found a positive effect of different levels of mineral elements – zinc, manganese and cobalt in the feeding of highly productive Holstein cows on morphological and biochemical indicators of blood, their productivity and reproductive functions (Kropyvka & Bomko, 2020).

Taking into account the above, it should be noted that the correction of vitamin and mineral nutrition of dairy cows due to the inclusion in the structure of the improved compound feed K 60-32-89 of the improved VMS provides the rations with the optimal amount of mineral elements and vitamins, which thereby activates redox and synthetic processes in the blood, contributes to a higher level of productivity by 9.8% and improvement of the chemical composition of milk (fat, protein, milk sugar, calcium).

CONCLUSIONS

The perspective of using in the feeding of dairy cows in the summer-pasture period of maintenance in the structure of the improved compound feed of the optimized VMS has been experimentally established and scientifically substantiated. Feeding VMS to cattle stimulates hematopoietic processes. In particular, it helps to increase the level of erythrocytes, hemoglobin, total protein, fractions of nitrogen (amine, total, protein) and phosphorus (total acid-soluble, organic, NA) and conditions the growth of quantitative and qualitative indicators of milk.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

A CRITICAL REVIEW OF SCREENING METHODS TO DETERMINE THE ANTIOXIDANT CAPACITY IN LEGUME

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Abstract

Legumes are a rich source of bioactive compounds such as phenolic or polyphenolic compounds, particularly tocopherols. Legumes antioxidants are widespread for their radical scavenging proprieties as active biologic compounds belonging to various chemical classes. Polyphenols are the most studied molecules of both nutritional and pharmaceutical interest. Furthermore, an overview concerning the antioxidant capacity and determination is mandatory for the precise and accurate method selection, involving cost-effectiveness and time-saving, towards gathering networks between research and development fields. The current review aims to summarize the presence of the natural antioxidant, their multiple biological effects, and the various approaches to methods determinations.

***Key words**: antioxidant activity, legumes, phenolic compound, vitamin.*

INTRODUCTION

Legumes are a rich source of bioactive compounds such as phenolic acids, flavanols, flavones, flavanols, flavanones, isoflavones, anthocyanins, and tannins, (Nicolás-García et al., 2021).

Phenolic compounds are present in all anatomical parts of plants (Amarowicz et al., 2017) and exhibited mostly in the seed tegument, represented by anthocyanins, condensed tannins, kaempferol glucoside, and quercetin. Whereas phenolic acids such as ferulic, synaptic, chlorogenic, and other hydroxycinnamic acids are found primarily in the cotyledon (Nurzyńska-Wierdak et al., 2019). Several authors have emphasized the importance of phenolic compounds such as natural biocontrol agents (Dresch et al., 2014) implicated in the resistance of some grapevine cultivars to fungi, oomycetes, bacteria, phytoplasma, and viruses; one of the most well-known properties of these compounds is their antioxidative activity (Aouey et al., 2016), which allows them to scavenge free radicals and might have positively health outcomes (Waffo-Teguo et al., 2008). The antioxidant activity of phenolic compounds extracted from legume seeds has been previously studied using several in vitro chemical assays. Antioxidants can neutralize free radicals and reduce the risk of damage (Hayat et al., 2009). By competing with free radicals, antioxidants function as chain-breaking inhibitors, interrupting the chain process, with major role in apoptosis and cell-signalling mechanism (Halliwell, 1996).

The current review aims to summarize the presence of the natural antioxidant, their multiple biological effects, and the various approaches to methods determinations.

MATERIALS AND METHODS

The summarised and synthetised information of the current review were literature-based assessments (Figure 1).

The systematic review methodology was employed by using scientific database available, such as Google Scholar, Science direct, Web of Science, Scopus, Springer link, and MDPI. The identification protocol was based on keyword, year, and article type filtration (Figure 1).

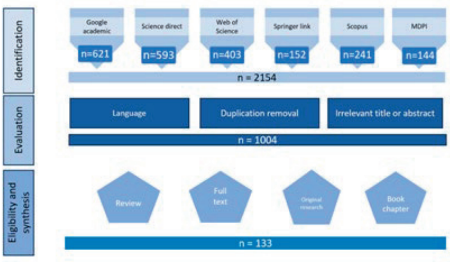

Figure 1. Identification of relevant information

1. Endogenous antioxidant

1.1. Enzymatic antioxidant

This chapter provides an overview of two protein antioxidants (with enzymatic activity), which are the first line of defence against oxidative stress on the body: superoxide dismutase (SOD) and catalase (CAT).

Superoxide dismutase (SODs) is a class of enzymes that operate as the first line of antioxidant defence by dismutation extremely reactive superoxide radicals into hydrogen peroxide and molecular oxygen. There are four isozymes of superoxide dismutase (Table 1).

Table 1. Identification of four isozymes of Superoxyde dismutase (adapted by Fink & Scandalios, 2002)

Isozymes of SOD	Metal cofactors	Localizations	References
SOD1	Cu / Zn	Cytosol Chloroplast	(Mccord et Fridovich, 1969); Laukkanen, 2016 ; Fink et Scandalios, 2002)
SOD2	Mn / Fe	Mitochondria	(Laukkanen, 2016 ; Weisiger et Fridovich, 1973)
SOD3	Cu / Zn	Extracellular	(Laukkanen, 2016; Marklund, 1982)
SOD4	Ni	Aerobic soil bacteria	(Wuerges et al., 2004 ; Anju et al., 2013)

Catalase is a tetrameric porphyrin-containing enzyme that is mainly found in peroxisomes. It catalyzes the conversion of H_2O_2 to water and molecular oxygen in two steps (Aslani et Ghobadi, 2016):

$$\text{Catalase-Fe (III)} + H2O2 \rightarrow \text{compound A}$$
$$\text{Compound A} + H2O2 \rightarrow \text{catalase-Fe (III)} + 2H2O + O2$$

CAT is one of the most active catalysts produced by nature, it plays a crucial role in systems that have evolved to allow organisms to live in aerobic environments. Because of its evolutionary conservation, wide distribution, and capacity to rapidly degrade hydrogen peroxide, CAT provides the cell with a highly efficient mechanism for removing hydrogen peroxide. Then when cells are challenged for energy and rapidly produce H_2O_2 via "emergency" catabolic processes, H_2O_2 is destroyed in an energy-efficient manner by CAT. So, there is a net gain in decreasing equivalents and, as a result, cellular energy (Scandalios, 2005).

2. Exogenous antioxidants

Over the decades, people's health prevention and diet were a preoccupation of worldwide scientists (Xu et al., 2017; Dominguez-Perles et al., 2020; Loizzo et al., 2021). Antioxidants were previously studied for their effects through anti-inflammatory, anti-aging, anti-atherosclerosis and anticancer (Manach et al., 2004; Xu et al., 2017; Menga et al., 2023). Moreover, exogenous antioxidants are found especially in food such as vegetables, fruits, medicinal plants, or cereals (Xu et al., 2017). The representative components of exogenous antioxidants found in plant sources are often divided into phenols, carotenoids, and vitamins (Baiano et al., 2015).

2.1. Phenolic structure

The chemical structure of the phenolic compounds includes one or many aromatic rings which have annexed one or numerous AOH (hydroxyl) groups in different, free, combined, or bound statuses (Wang et al., 2015; Telles et al., 2017). Phenolic compounds have many health benefits, they act as antioxidants, anticarcinogenic antimutagenic, antifungal, and anti-inflammatory (Alshikh et al., 2015). Considering that, legumes are a rich source of bioactive phenolic compounds, some of these phenolic compounds are concentrated in the seed coat of legumes (Gan et al., 2016;) and include phenols, flavonoids, and phenolic acids (Hossain et al., 2021). Legume seeds are essential in the human diet because they are excellent sources of protein, fiber, minerals, vitamins, and bioactive compounds (Magalhães et al., 2017). Essentially sources of legumes are categorized into mature legumes, including dry beans, dry peas, lentils, and fava beans; and immature legumes which are considered green beans and peas, respectively (FAO, 2018). Regarding Kalili et al., 2022, the fava beans concentrations ranged from 49.5 to 594.4 mg

GAE/ g for the total phenols, a range of 0.7 mg to 3.4 mg QE/g for flavonoids, and for tannins was on average from 4.9 mg to 73.91 mg TAE/g dry weight.

2.1.1. Phenolic acids

Phenolic acids (Table 2) are similar to phenol carboxylic acids, being an organic compound, which belongs to the aromatic carboxylic acids class with a C6-C1 type skeleton (Heleno et al., 2015). However, phenolic acids are the main source of bioactive chemical substances, from the class of phenolic components, which are discovered in various natural foods or even beverages intended for human consumption (Zhang et al. 2020). Furthermore, this metabolite occurs in second place, after the flavonoids, which concurs with the distinctive organoleptic characteristics of food (Zhang et al., 2016).

The phenolic acids are a different class which includes hydroxybenzoic acid derivatives (p-hydroxybenzoic, protocatechuic, gallic, vanillic, and syringic acids) and hydroxycinnamic acid derivatives (caffeic, chlorogenic acid, ferulic, p-coumaric) (Singh et al., 2017). PCA (protocatechuic acid) is a water-soluble benzoic acid derivative that reduces metabolic disorders associated with obesity (Ormazabal et al., 2021) and has anti-atherosclerotic, anti-inflammatory, antineoplastic, analgesic, antibacterial, hepatoprotective and antiviral effects (Kakkar et al., 2014). Gallic acid has antioxidant, antimicrobial, anti-inflammatory, anticancer, cardioprotective, gastroprotective, and neuroprotective effects (Choubey et al., 2015). Chlorogenic acid is a good antioxidant, but it is also involved in several biological activities, antifungal activities, and antimutagenic activities, provides protection against cardiovascular diseases (Mirali et al., 2014) and reduces the process of carcinogenesis (Shin et al., 2015; Telles et al., 2017). The chlorogenic acid present in cereals, as well as other legumes, has been shown that inhibits the activity of digestive amylase, and prevents the degradation of food and its contamination with microorganisms (Heidtmann-Bemvenuti et al., 2011; Pagnussatt et al., 2013; Telles et al., 2017). Ferulic acid, like chlorogenic acid, is known for its antioxidant activity, anticancer properties and helps in the prevention and therapy of diabetes. (Kumar & Prothi, 2014; Telles et al., 2017).

Common as well as fava beans (*Vicia faba* L.), like food in human nutrition, gathered its study in countless research projects, both for the component rich in phenolic compounds (Sigh et al., 2017), especially for their oxidative activity, as well as for the role of preventing countless diseases (Beninger et al., 2003; Menga et al., 2023). In addition, a total of 10 phenolic compounds were identified in the fava bean extracts, comprising four hydroxybenzoic acids (protocatechuic, p-hydroxybenzoic, vanillic, and syringic), three hydroxycinnamic acids (chlorogenic, p-coumaric and trans-ferulic) and three flavonoid-related compounds (Johnson et al., 2021).

For instance, lentils (*Lens culinaris* L.) are well known for their antioxidant effect (through polyphenols concentrations) within the human metabolism and protective outcome from diseases such as cardiovascular degeneration or different types of cancer. Additionally, depending on the varieties of the colors, lentils range from black, brown, red, and green, and this characteristic is given a different antioxidant report (Tienda-Vasquarez et al., 2023). In lentils extracts, p-hydroxybenzoic acid and gallic aldehyde were reported as major hydroxybenzoic acids, and trans-ferulic, trans-p-coumaric, and sinapic acids were reported as major hydroxycinnamic acids (Amarowicz et al., 2009; Mustafa et al., 2022).

Moreover, phenolic acids identified from water-based extracts from green peas (*Pisum sativum* L.) are quinic acid, 5-caffeoylquinic acid, gallic acid and p-coumaric acid, ferulic acid, protocatechuic acid were not identified (Castaldo et al., 2022). In soybean (*Glycine max*), 8 phenolic acids were identified, among them three in a larger quantity such as chlorogenic acid having values from 78.35 to 221.04 µg Gallic acid equivalents (GAE)/g DW, p-hydroxybenzoic acid 38.92 to 196, 40 µg GAE/g DW and caffeic acid 137.43 to 240.28 µg GAE/g DW for black soybean (Zhu et al., 2018).The total phenolic content (TPC) in selected legume seeds is reported in Table 2. The highest values of TPC were recorded in beans, with a range that varies from 92.85 to 151.04 mg GAE/g (Chaeib et al., 2011), and the legumes with the lowest content in phenolic acids are green peas and yellow peas with values between 0.65 to 1.14 mg GAE/g (Han and Baik, 2008; Xu et al., 2007). Moreover, TPC for soybean is lower than other relevant studies that showed total phenolic acid content for soybean ranged from 13.35 to 21.49 mg GAE/g DW (Zhu et al., 2018). Other studies showed that total phenolic content in chickpeas, lentil, and soybean was recorded as 3.12 ± 0.07, 9 ± 0.02, 10.22 ± 0.01 mg GAE g^{-1} (Naz et al., 2023).

2.1.2. Flavonoids

Flavonoids (Table 2) as a secondary metabolite (De Luna et al., 2020) which contribute to health benefits (Juca et al., 2020) especially in humans' bioavailability and biological activity (Maleki et al., 2019). Besides, flavonoids were intensely studied for their nutritional benefits with anti-oxidant activity (Shen et al., 2022). Moreover, the well-known role of flavonoids in plants is the implication in giving coloration to fruits and flowers (Williamson et al., 2018). However, the general classification of the flavonoids is structured in flavonols, flavones, isoflavones, anthocyanidins, flavanones, flavanols, and chalcones and each of them is distributed in different food sources (Shen et al., 2022).

Table 2. Total phenolic content (TPC), total flavonoid content (TFC), and condensed tannin content (CTC) were reported in various legume beans

Legumes	TPC	TFC	CTC	References
Faba beans	92,85 to 151,04 mg GAE/g	11,87 to 43,86 mg RE/g	309,28 to 958,77 mg CE/g	Chaieb et al. (2011); Baginsky et al. (2013)
Soybeans	0,81 to 5,89 mg GAE/g	1.06 to 4.04 mg CE/g	0,37 to 1,96 mg CE/g	Kumar V. et al. (2010) ; Xu et al. (2007)
Lentils	4,86 to 9,60 mg GAE/g	3,04 to 4,54 mg CE/g	3,73 to 10,20 mg CE/g	Xu et al. (2007)
Green peas	0, 65 to 0,99 mg GAE/g	0,05 to 0,15 mg CE/g	0,23 to 0,61 mg CE/g	Han and Baik (2008) Xu et al. (2007)
Yellow peas	0,85 to 1,14 mg GAE/g	0.09 to 0,17 mg CE/g	0,22 to 0,59 mg CE/g	Singh et al. (2017) ; Xu. et al. (2007)
Chickpeas	0,98 to 2,2 mg GAE/g	0,72 mg CE/g	0,52 mg/CE g	Han and Baik (2008); Xu. et al. (2007)

Furthermore, legumes such as Fava beans have been considered one of the oldest plants in the world, being nutritionally efficiently used fresh or dry (Kalili et al., 2022), for being rich in flavonoids with up to 16 in total (El Feky et al., 2018). On the other hand, studies on legumes such as common bean (*Phaseolus vulgaris* L.*)* reported flavonoid concentrations up to 252 mg CE/100 g DW (dry weight) (Yang et al., 2020). Legume seeds including lentils, soybeans, common beans, or peas are nutritionally rich in flavonoids and also contribute to reducing the risk of human affection, respectively type 2 diabetes, and obesity (Zhang et al., 2015). In addition, the potential nutraceutical properties of peas (*Vicia faba* L.) could be considered a healthy option used in human nutrition for the antioxidant compounds (Loizzo et al., 2021). Yellow or green peas are cultivated and scientifically studied worldwide for their pivotal nutrients (Kumari & Deka, 2021) and are extremely useful in curing diabetes, cardiological affections, cancers, and numerous degenerative diseases for their antioxidant properties (Oh et al., 2019; Roy et al., 2020; Kumari & Deka, 2021). Regardless of the pea's color classification, isoflavones commonly accumulate in these legumes. On the other hand, soybeans are rich in isoflavones as well (Shen et al., 2022).

2.1.3. Tannins

Tannins (Table 2) are plant compounds produced by the condensation of simple phenolic compounds and have a complex chemical structure. They are generally divided into hydrolyzable and condensed/non-hydrolyzable tannins (Delavan-3-ol polymers) (Xu et al., 2007). Condensed tannins (proanthocyanin) are phenolic compounds that are found in large amounts in legume seeds such as lentils, yellow or green peas, soybeans, and common beans (Xu et al., 2021).

The condensed tannin content (CTC) in selected legume seeds is reported in Table 2. Beans and lentils are rich in tannins. Beans have the highest content of condensed tannins among the six legumes with content of 309.28 to 958.77 mg CE/g (Chaieb et al., 2011), and the lowest content was observed in peas, 0, 23 to 0.61 mg CE/g for green pea (Xu et al., 2007) and 0.22 to 0.59 mg CE/g for yellow pea (Singh et al., 2017). Other studies have shown that the content values of condensed tannins in Fava beans can vary from 1.9 mg/g (Karatas et al., 2017) to 2586 mg/100 g catechin equivalents (Weihua et al., 2015), these variations can be explained by environmental conditions, geographical area, genetic variability, and quantification methods (Martineau-Côte et al., 2022).

Tannins have been shown to reduce the digestibility and bioavailability of bean proteins because they bind proteins and form insoluble complexes (Karatas et al., 2017; Martineau-Côté et al., 2022).

2.2. Carotenoids

Carotenoids are natural bioactive compounds found in legumes and are known to be important antioxidants with pigmentation properties, these functions establish their role as valuable nutritional additives associated with numerous health benefits (Myrtsi et al., 2023; Altuner et al., 2022). Carotenes typically are hydrocarbons (Qudah, 2009) that contain only carbon and hydrogen. Carotenes are divided into two main groups: carotenes or hydrocarbon carotenoids composed only of carbon and hydrogen atoms and xanthophylls are oxygenated hydrocarbon derivatives that contain at least one oxygen function (Grigore et al., 2023). Beta-carotene and lutein are known as carotenoids and lutein and zeaxanthin as xanthophylls (Qudah, 2009; Kumar et al., 2015). In the human diet, the most important carotenoids are β-carotene (Figure 2), α-carotene, β-cryptoxanthin, lutein, zeaxanthin, and lycopene (Rao & Rao, 2007).

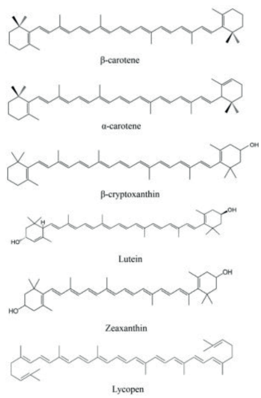

Figure 2. Chemical structures of the carotenoids found most often in the legumes

Legumes are included in the diet for their important sources of carotenoids (including provitamin A) and tocopherols (vitamin E), which play pivotal roles in the prevention of inflammatory processes, as well as coronary, and neuromuscular disorders and maintaining eye health (Turco et al., 2016). The most important carotenoids identified in legumes were beta-carotene, lutein, zeaxanthin, neoxanthin, and violaxanthin (Qudah, 2009). Another study has identified 8 lutein and zeaxanthin isomers from legumes where the highest content of carotenoids identified were in red beans (8.29-20.95 μg/g) and lentils (4.53-21.34 μg/g), followed by black soybeans (4.41-6.09 μg/g) and cowpea (6.62-9.46 μg/g) (Kan et al., 2018). Fava beans contain three important carotenoids beta-carotene, lutein and zeaxanthin. In bean seeds, the highest concentration of carotenoids is represented by lutein (Qudah, 2009). Concerning the content of the total carotenoids in Fava beans, this was 1.97 μg (Qudah, 2009), in another article, varying from 5.7 mg/g at 8.4 μg FW (fresh weight) (De Cillis et al., 2019). Soybeans are mostly considered an important source of carotenoids compared to other legumes such as corn and peas. Furthermore, lutein was detected in all varieties studied, while only 37% and 65% of the varieties contained β-carotene and zeaxanthin, respectively. Regarding the carotenoid content of lentils in the study of Zang et al. 2014, lutein and zeaxanthin were identified in almost 20 varieties of lentils. The total carotenoid content (TCC) varied between 5.32 and 28.1 lg/g DW and the total carotenoid index (TCI) varied from 4.64 to 19.6 lg/g DW (Zang et al., 2014). The total content of lutein and zeaxanthin (total of all trans and cis isomers) ranged between 4.32-17.3 lg/g DW and 0.32-2.73 lg/g DW and were significantly different ($p < 0.05$) among the 20 lentil cultivars studied. The highest content of carotenoids in lentils is lutein (all-trans-lutein 64%-78%) followed by zeaxanthin (all-trans-zeaxanthin 5%-13%) (Zang et al., 2014). In pea seed and cotyledons violaxanthin, lutein, and β -carotene were identified and in chickpeas, lutein was reported as the main carotenoid, followed by zeaxanthin and β -cryptoxanthin. Another study has identified, violaxanthin, lutein, zeaxanthin, and b-carotene were identified in the mature pea and chickpea seeds și a low concentration of b-cryptoxanthin was detected in chickpeas (Arevalo et al., 2020).

2.3. Vitamins

Vitamins represent natural substances essential in small quantities to normal metabolism. Oxidative stress occurs due to the inability of the body's antioxidant defense mechanism; therefore, it is recommended to administer exogenous antioxidant supplements (Julia et al., 2019) such as vitamin A, vitamin C (Mohamed et al., 2020) and vitamin E (Kan et al., 2018). Moreover, in vegetables can be included the well-known and numerous benefits of fava beans for human health, after the content of carbohydrates, proteins, and antioxidants, to be taken into account are the vitamins, mostly B complex, ascorbic acid (Alghamdi, 2009; Mohamed et al., 2020), retinol (Didier et al., 2023) and tocopherol (Kan et al., 2018).

2.3.1. Retinol (vitamin A)

Taking into account the general function of retinol, this is a vitamin A which is essential for human metabolism and development, being involved in the immune system, reproduction function, skin defense and numerous other functions about memory or vision. The group named Vitamin A, include retinol derivatives which are known as retinoids used on the whole medical and beauty domain (Ferreira et al., 2020). Furthermore, bioactive forms of retinol which are metabolized at the human cell level, are represented by retinal and retinoic acid, retinyl being intended for depository (Ferreira et al., 2020).

On the other hand, retinol is a nutrient component found in soybean sprouts, with a concentration of up to 34 RE/100 g (Plaza et al., 2003). Additionally, legumes with content of vitamin A are soybeans with 22UI/100g (USDA, 2019), lentils has 8 UI/100 g (USDAa, 2019), fava beans being the most rich legume in vitamin A, with 333 UI/100 g (USDAb, 2019).

2.3.2. Ascorbic acid (vitamin C)

The oxidative role of ascorbic acid, frequently known as vitamin C, makes this subject important for numerous research (Njus at al., 2020). Studies in the field show that vitamin C is a multifunctional metabolite (Bilska et al., 2019) that has many properties, however, three biological functions are proper to be mentioned. Among these is counted the function of being a reducing agent for enzymatic reactions, a scavenger of free radicals, and for the action as antioxidant which eliminates reactive oxygen species. In addition, antioxidants could be taken from natural exogenous sources such as legumes, fruits, seeds, and many others (Hossain et al., 2021). For decades, people use food processing in different ways, microwaves, boiling, heat, and autoclaving; however, cooking processes make physicochemical changes of nutrient composition and vitamins too. Moreover, vitamin C can easily degrade under usual cooking treatments (Uherova et al., 1993). Nevertheless, published research results regarding the nutritional composition of fava beans, that taking ascorbic acid with a high antioxidant potential is a good option for the production of healthy foods (Hossain et al., 2021). Additionally, legumes such as pigeon peas (*Cajanus cajan* (L.) Huth), contains 39.00 g/100 g of vitamin C for immature seeds (Hossain et al., 2021).

2.3.3. Tocopherol (vitamin E)

Vitamins represent a small amount of the total nutrients in vegetables compared to phenols or flavonoids. Tocopherol, also known as vitamin E, as an exogenous compound, has an important antioxidant effect on human metabolism (Baiano et al., 2015; Menga et al., 2023). In addition, scientists in the field discovered that vitamin E, includes several isoforms such as: α-, β-, γ-, and δ-tocopherol and α-, β-, γ-, and δ-tocotrienol. Apart fromthe antioxidant effect of α- and β-tocopherol, in human health a big contribution has γ-tocopherol, especially for its anti-inflammatory and antitumor impact; and δ-tocopherol for preventing many cancerous types (pulmonary, breast, colon etc.) and lipid accumulation (Azzi, 2018; Azzi, 2019).

Vitamin E, is more abundant in beans, peas and lentils, being found particularly as γ-tocopherol (Amarowicz et al., 2009; Martín-Cabrejas et al., 2019). Moreover, regarding to a study of Kan et al., 2018, where they studied almost 29 legumes, including soybean, pea and lentil, the total tocopherol composition was in different ranges: 120.96±2.48a; 58.40±0.7g and 41.28±0.24i, respectively. Furthermore, the antioxidant effect of fava bean, could be taken into account as legumes for its 0.08 mg of alpha tocopherol which were found in a study conducted by Jahreis et al. (2016). Gamma tocopherol being not determined (Jahreis et al., 2016). Additionally, legumes such as pigeon pea contain 0.39 g/100 g of vitamin E in immature seed, regarding Kuraz Abebe 2022.

3. Conducted assays for determining the antioxidant activity and antioxidant capacity

The antioxidant capacity of legumes differs according to biological variation (Table 3) and is reported throughout a wide range (Ketnawa et al., 2022). The highest natural endogenous antioxidant levels can be influenced by technological processing (Garrido-Galand et al., 2021) including seed germination (coatings) (Gu et al., 2022) and are important. The high number of phenolic antioxidants in seed hulls is an essential concern and it is important to evaluate the methods and instruments that can be specific and cost-effective for establishing the antioxidant potential. Analyzing the antioxidant content of legumes typically involves laboratory methods that measure the levels of specific antioxidant compounds or antioxidative capacity. The antioxidant capacity of legumes refers to their ability to neutralize or scavenge free radicals and prevent oxidative damage (Pisoschi et al., 2021; Mihai et al., 2022). Antioxidants in legumes primarily include phenolic compounds, flavonoids, vitamins (such as vitamin C and vitamin E), carotenoids, and other bioactive components. These antioxidants work synergistically to protect cells and tissues from oxidative stress and contribute to overall health and well-being (Crupi et al., 2023). Various methods have been developed to measure the antioxidant content in legumes. These methods include spectrophotometric assays (Diniyah et al., 2020), such as the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, ferric reducing antioxidant power (FRAP) assay, and oxygen radical absorbance capacity (ORAC) assay, among others. These assays provide quantitative measurements of the antioxidant capacity of legumes by assessing their ability to scavenge free radicals or donate electrons. In addition to spectrophotometric methods, chromatographic techniques (Adebo et al., 2021; More et al., 2022; Zhu et al., 2020).

4. Benefits of antioxidants for human and livestock

Antioxidants in legumes help neutralize harmful free radicals and reduce oxidative stress in the body (Zhang et al., 2016). Oxidative stress (Surai, 2020) has been linked to various chronic diseases, and a diet rich in antioxidants can help protect against their development. They can help reduce inflammation, lower blood pressure, improve blood lipid profiles, and enhance blood vessel function, thereby reducing the risk of heart disease and stroke. Some antioxidants found in legumes, including phytochemicals like flavonoids and polyphenols, have been shown to have anti-cancer properties, and provide protection against certain types of cancer, such as colon, breast, and prostate cancer (Almatroudi et al., 2023). The antioxidants in legumes can help reduce inflammation by neutralizing free radicals and modulating inflammatory pathways, contributing to a lower risk of chronic inflammatory conditions, including arthritis and inflammatory bowel diseases (Zhang et al., 2016). Legumes, with their high antioxidant content and low glycemic index, are beneficial for individuals with diabetes or those at risk of developing diabetes. Legumes are rich in dietary fiber, which promotes healthy digestion and prevents constipation. The antioxidants in legumes may also have prebiotic properties, nourishing beneficial gut bacteria and supporting a healthy gut microbiome, which is essential for digestive health (Kalili, 2022). Legumes can serve as a cost-effective feed ingredient for livestock production. Using legumes as a feed ingredient can help reduce feed costs, especially in regions where legumes are locally grown and readily available (Formato et al., 2022; Corino et al., 2021). Providing a diverse and nutritious diet is essential for maintaining optimal animal health and welfare. Legumes have a relatively lower environmental impact compared to some other feed ingredients. Their nitrogen-fixing ability reduces the need for synthetic nitrogen fertilizers, which can help minimize nitrogen runoff and its associated environmental consequences (Formato et al., 2022; Wang et al., 2022). Including legumes in livestock feed may contribute to improved meat quality, increased omega-3 fatty acid content, enhanced flavor profiles, and other desirable characteristics in animal products, thereby meeting consumer demands for high-quality and nutritious food (Corino et al., 2021).

Table 3. Conducted assays for determining the antioxidant activity and antioxidant capacity

Spectrophotometric methods			
Assays	Wavelength	Mechanisms of detection	References
2,2-diphenyl-1-picrylhydrazyl (DPPH)	510-520 nm	Measures the ability of antioxidants to scavenge the stable free radical DPPH.	(Diniyah et al., 2020)
Total antioxidant capacity (TAC)	450-490 nm	Measures the overall antioxidant capacity of a sample. These assays assess the ability of antioxidants to neutralize free radicals or inhibit oxidation in a chemical reaction	(Y. Zhang et al., 2020)
Folin-Ciocalteu (FC)	760-765 nm	Determines the total phenolic content in legumes. The reaction between phenolic compounds and the Folin-Ciocalteu reagent produces a color that can be measured.	(AU - Apea-Bah et al., 2022)
2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS)	645-650 nm	Method to assess the antioxidant activity of samples. ABTS is oxidized by the action of antioxidants, resulting in a color change that can be measured spectrophotometrically. This assay provides information about the ability of antioxidants to neutralize free radicals.	(González-Osuna et al., 2023)
Ferric Reducing Antioxidant Power (FRAP)	593 nm	Measures the reducing capacity of antioxidants in a sample. It involves the reduction of a ferric-tripyridyl triazine complex to a ferrous form by antioxidants, leading to a color change that can be quantified using a spectrophotometer. The FRAP assay provides information on the electron-donating capacity and overall antioxidant power of a sample.	(X. Zhang, Zhang, et al., 2021)
Trolox Equivalent Antioxidant Capacity (TEAC)	450-490 nm	Measures the overall antioxidant capacity of a sample. These assays assess the ability of antioxidants to neutralize free radicals or inhibit oxidation in a chemical reaction.	(Arevalo et al., 2020)
Oxygen Radical Absorbance Capacity (ORAC)	485-535 nm	Measures the antioxidant reaction with peroxil radicals.	
Nitric Oxide (NO) Scavenging Assay	540 nm	Measures the ability of antioxidants to scavenge nitric oxide radicals. The reaction between antioxidants and nitric oxide leads to the formation of a stable-colored product, which can be quantified spectrophotometrically.	(Xu et al., 2021)
Lipid Peroxidation Assay (TBARS)	535 nm	This assay involve the reaction between lipid peroxidation products and a chromogenic reagent, resulting in the formation of a colored complex that can be measured.	(Kasaiyan et al., 2023)
Hydroxyl Radical Scavenging Assay	460 nm	Evaluates the ability of antioxidants to scavenge hydroxyl radicals. Hydroxyl radicals are highly reactive and can cause oxidative damage.	(Verni et al., 2019)
Superoxide Radical Scavenging Assay	560-562 nm	Measures the ability of antioxidants to scavenge superoxide radicals. Superoxide radicals are highly reactive and can contribute to oxidative stress.	(Haile & Kang, 2019)
Cupric Reducing Antioxidant Capacity (CUPRAC)	455-490 nm	Measures the reducing capacity of a sample by its ability to reduce cupric ions (Cu2+) to cuprous ions (Cu+).	(Mitic et al., 2023)
Total Flavonoid Content (TFC) Assays		Measures of the concentration of flavonoids in legume samples, which contribute to their antioxidant activity.	(Liu et al., 2022)
Total Carotenoid Content	450-460 nm	Mseasues the concentration of carotenoids in legume samples. Spectrophotometric methods are commonly employed for this purpose.	(Vieira et al., 2016)
Prussian blue	725 nm	Detection of ferric (Fe^{3+}) ions. It involves the formation of a blue-colored complex called Prussian blue when ferric ions react with potassium ferrocyanide.	(Ahmad, 2021)

Aluminum chloride flavonoid	415-425 nm	Determine the total flavonoid content in samples	(Sharma & Giri, 2022)
Sodium borohydride reduction	500-800 nm	The method for determining the total reducing power or antioxidant capacity of a sample	(Singh et al., 2021)
		Electrochemical methods	
Cyclic Voltammetry		The electrochemical technique that measures the current as a function of applied potential-redox behavior of antioxidants in legumes and electrochemical activity.	(Sakinah & Fikri, 2023)
Potentiometric Titration		Measures changes in electrical potential as a titrant is added to a sample to determine the antioxidant content by titrating against a known oxidative reagent or measuring the potential changes associated with the oxidation or reduction of antioxidants.	(Rico et al., 2021)
Electrochemical Impedance Spectroscopy		Measures the impedance of an electrochemical system as a function of frequency. It can provide information about the charge transfer processes and surface properties of antioxidants in legumes, offering insights into their antioxidant capacity.	(Tolun & Altintas, 2023)
Square Wave Voltammetry		Measures the resulting current. It can be used to determine the concentration of specific antioxidants or assess their redox behavior.	(Diniyah et al., 2020)
Chemiluminescence Detection		It is used in conjunction with electrochemical methods to detect the antioxidant capacity of legumes - inhibiting or reducing the chemiluminescence reaction, providing an indirect measure of their antioxidant activity.	(Minguillón et al., 2022)
Electrochemical Sensor Arrays		By using different electrodes, each selectively sensitive to a specific antioxidant or antioxidant group, sensor arrays can provide simultaneous detection of multiple antioxidants in a sample.	(Kumar Mehata et al., 2022)
Conductometric Measurements		Assess changes in the electrical conductivity of a sample due to redox reactions or interactions with antioxidants. This method can be used to determine the antioxidant capacity or monitor the antioxidant content in legumes.	(Szparaga et al., 2021)
		Chromatographic Methods	
High-performance liquid chromatography (HPLC) analysis Gas chromatography (GC) Mass Spectrometry (MS)		Used to separate and quantify individual antioxidants in legumes. These methods provide high specificity and sensitivity for the identification and quantification of antioxidant compounds. MS is often coupled with chromatographic techniques (such as HPLC-MS or GC-MS) to identify and quantify specific antioxidants in legumes. MS provides highly accurate molecular weight determination, allowing for the identification of antioxidant compounds based on their mass spectra.	(Adebo et al., 2021; AU - Apea-Bah et al., 2022; More et al., 2022; Zhu et al., 2020) (Farag, Sharaf El-Din, Aboul-Fotouh Selim, et al., 2021)

CONCLUSIONS

The consumption of beans can meet the challenges of balanced diets, both individually and globally. With the aim of changing eating habits, we need to offer consumers a range of products that are less outdated and more practical to cook.

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REPRODUCTION, PHYSIOLOGY, ANATOMY

Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

EFFECT OF DIETARY SUPPLEMENTATION OF *Nile tilapia* WITH SEA BUCKTHORN AND VITAMIN E ON THE HEMATOLOGICAL AND SERUM BIOCHEMICAL INDICES

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Abstract

The purpose of this study was to investigate the effect of dietary supplementation with sea buckthorn and vitamin E on the physiological status of Oreochromis niloticus species. The experimental design consists in a four experimental diet: V1 - control, V2 - 1% sea buckthorn/kg feed, V3 - 500 mg vitamin E/kg feed and V4 - 1% sea buckthorn+500 mg vitamin E/kg feed. The results revealed that dietary supplementation with this phytobiotic and vitamin contributed to the emergence of significant changes compared to the control variant at the level of the number of erythrocytes, erythrocyte constants (MCV, MCH, MCHC), glucose concentration and total protein. In variant V4 it was observed an increase of erythrocyte constants (MCV and MCH) and a reduction of the number of erythrocytes. Regarding to the leukocyte reaction, in V2, V3 and V4 variants was observed an improvement of the fish physiological status compared to the fish from control variant. In conclusion, the dietary supplementation with sea buckthorn and vitamin E (V4) presented a synergistic effect on the welfare status of Nile tilapia reared in a recirculating aquaculture system.

***Key words**: hematological profile, Hippophae rhamnoides, leukocyte reaction, Nile tilapia, vitamin E.*

INTRODUCTION

To supply the demand for the human nutrition the aquaculture practices have become more intensively, this aspect is due to the reduction of the fish stock in the natural environment, day by day Because of the intensive growth, the risk of pathogens and disease has increased.

Other problems are represented by high production costs, diseases, stress, environmental impact, animal welfare problems and demand for organic production (Sonmez, 2017; Arslan et al., 2018; Elbesthi et al., 2020). The use of antibiotics and other chemotherapeutic agents for controlling diseases can reduce mortality and improve growth rates; however, at the same time they are quite expensive and at the same time they can cause a damage to the body (Ferguson et al., 2010).

Thus, to ensure the fish physiological status, it is sought to put more emphasis on the prevention than on the treatment of diseases.

For this reason, in order to obtain a higher production and to reduce the occurrence of diseases, nutraceuticals began to be introduced into the fish diet.

Nutraceuticals are natural bioactive or chemical compounds which have therapeutic properties on the organism welfare. So, by using nutraceuticals, an attempt is made to maintain an optimal state of health by preventing illness or reducing the symptoms/stagnation of a disease. Nutraceuticals can include phenolic substances, flavonoids, vitamins, fatty acids, carbohydrates and their derivatives, amino acids, minerals, etc. Among their mechanisms of action, we can say that they have properties as: anti-inflammatory, anti-carcinogenic, antioxidant, antimicrobial, anticholesterolemic, antihypertensive, etc. (Bem et al., 2021).

Hippophae rhamnoides L. is an ancient crop that was used as herbal medicine and as food additive for disease prevention. The increase in antioxidant activity in the case of sea buckthorn fruit administration is mainly due to the presence of large amounts of vitamin C, vitamin E, carotenoids (Ranjith et al., 2006), polyphenols (Ranjith et al., 2008), but also of some antioxidant enzymes such as the isoenzyme: superoxide dismutase (Xing et al., 2002). Withal, sea buckthorn fruits have a special effect at the physiological level, namely that of inhibiting the oxidation of low-density lipoproteins (Bao & Lou, 2006). It seems that seabuckthorn have some properties as: cholesterol reduction, role in the hemostasis process, reduction of blood pressure, glucose, anticancer, antibacterial, antiviral and radioprotective potential (Sahu et al., 2007). The introduction of sea buckthorn in the fish diet was done to observe its effect on the growth performance indicators and on the survival rate. For a good feeding efficiency, but also to obtain a quality product, it is important to obtain a fish with a fairly large body mass, in a relatively short time, in order to be able to be marketed. Antache et al., 2013, shown interesting results on lipid peroxidation, analysis which determines the freshness of fish meat, an important matter in fish quality, what corresponds to a high percentage of unsaturated fatty acids found in meat.

In this experiment, in addition to administering seabuckthorn in the Nile tilapia diet, we chose to administer a vitamin, respectively vitamin E. Fish feed is usually supplemented with a certain concentration of vitamin E because it has positive effects on the immune system in fish, improving specific and non-specific immune response, reducing mortality and optimizing growth performance (Ortuno et al., 2001; Shiau & Hsu, 2002; Puangkaew et al., 2004). Vitamin E has a strong antioxidant action that protects the organism from possible changes that can lead to oxidation process of cell membranes and lipoproteins at the level of different types of tissues (Adham et al., 2000), reduces the fragility of the erythrocyte membranes and improves leukocyte functions (Kiron et al., 2004). Other studies have also shown the beneficial effect of vitamin E, administered in higher concentrations, on reproduction, disease resistance, meat quality and nutrient digestibility (Lohakare et al., 2006; Samanta et al., 2006; Galaz et al., 2010).

In fish reproduction, vitamin E administration plays a very important role. Studies have shown that vitamin E deficiency could lead to a reduction in growth performance indicators by decreasing the protein efficiency ratio, weight gain, as well as feed coefficient (Bai et al., 1998; Lee & Dabrowski et al., 2003; Huang & Lin, 2004; Paul et al., 2004; Sau et al., 2004).

Therefore, the purpose of this research was to investigate the effect of a phytobiotic, in our case sea buckthorn, vitamin E and their combination on the hematological profile, on some blood biochemical parameters and on leukocyte reaction in case of Nile tilapia.

MATERIALS AND METHODS

The research took place, for six weeks, in a pilot aquaculture recirculating system station from "Dunarea de Jos" University of Galati, Romania. The biological material used in the experiment was represented by 684 specimens of two-month-old of *Oreochromis niloticus* species with 1.81±0.01 g/specimen. The specimens were shared in twelve growth units. The experimental design involved the testing of four experimental variants. The experiment was carried out in triplicate, therefore each experimental variant received a number of three growth units. So, the experimental variants were: V1 variant - the control variant, V2 variant - fish feed was enriched with 1% sea, V3 variant - fish feed was added with 500 mg vitamin E/kg feed and V4 variant - fish feed was added with 1% sea buckthorn and 500 mg vitamin E/kg feed.

Regarding to the feeding management, the biological material was fed during the experiment with NUTRA PRO "0" granulated feed, which has a content of 54% crude protein. It should be noted that we chose a feed that was not supplemented with vitamin E. The feeding intensity was 10% of biomass per day (BW/day) for three weeks then with 5% BW/day until the end of the experiment. During the experiment, the frequency of feeding was five times per day, the food being administered manually.

During the experiment, the water temperature was kept constant with the help of twelve heaters of the 1C HEATER Indicator type (200W; 200/240V; 50-60Hz). For the growth of Nile tilapia fry under optimal conditions during the experiment, the water quality was ensured by daily monitoring of temperature (T - °C) and oxygen (DO - mg/L) using the portable oximeter Hannah HI 98186 and the pH using the WTW inoLab series device (Terminal 740). For two times per week ammonium, nitrates and nitrites concentration were measured using Merck kits and the spectrophotometer Nova 400.

The results of the water quality parameters reflect that they remained within normal range for the *Oreochromis niloticus* species throughout the duration of the experimental research (Ross, 2000; El-Sayed, 2006; DeLong et al., 2009; Peterman, 2011). The average values for OD were 6.78±0.68 mg/L; for pH 7.49±0.22 units, for water temperature 25.99±0.99˚C, the ammonium concentration was 0.21±0.11 mg/L, the nitrites concentration was 0.61±0.38 mg/L and the nitrates concentration was 159.54±62.26 mg/L.

Blood sampling and analysis

To evaluate the physiological state of the fish, were collected blood samples only at the end of the experiment because at the beginning the fish were too small. For the blood prelevation as anticoagulant was used heparin, 2-phenoxyethanol was used for the fish anesthesia and and the collection was made from the caudal vein. Some researchers reported that this anesthetic does not interfere with the analysis of blood samples (Velisek et al., 2007).

The analysis of hematological parameters involves the determination of erythtocytes number (RBCc - x 10^6cell/μL), hemoglobine concentration (Hb - g/dL), hematocrit (Ht - %) and of some biochemical parameter such as concentration of total protein (TP - g/dL) and glucose (GLU - mg/dL). For the red blood cell counting was used Vulpian reactive (solution to achieve the dilution) and the Neubauer hemocytometer. For the hematocrit determination we used heparinized hematocrit capillary tube and a micro hematocrit centrifuge (Haematokrit 24 - Hettich). The centrifugation time was for 5 minutes at 12000 rpm. For the determination of hemoglobin concentration were used Drabkin reagent and SPECORD 210 Analytikjena spectrophotometer. These analyzes were carried out according to Blaxhall (1973). Based on these indices, the erythrocyte constants (mean corpuscular volume - μm^3, mean corpuscular hemoglobin - pg and mean corpuscular hemoglobin concentration - g/dL) were calculated (Ghergariu et al., 1985; Svobodova, 2001).

The concentration of glucose and total protein serum were made with a colorimetric methods and the readings were done at 635 nm (GLU), respectively at 546 nm (TP). For the GLU determination was used the o-toluidine reagent and the Biuret method was used for the TP concentration.

Leukocyte reaction

Relative and absolute number of the white blood cells determination were made by means of the blood smears which were microscopically examined using the Zeizz Axio Imager microscop. The smears staining was done by the MayGrünwald Giemsa panoptic method (MGG) (Mogodan et al., 2020). The identification of the type of leukocytes it was made according to Svobodova et al. (1991) description.

Statistical analysis

The results were statistically analyzed using descriptive statistics and ANOVA test. The results are showing as as mean ± standard deviation. Programs used were SPSS Statistics 17.0 and Microsoft Excel 2010.

RESULTS AND DISCUSSIONS

Assessment of physiological state through blood analysis shows us information about fist metabolism, we mean to the oxygen transport capacity and oxygen consumption, disease potential, immune status, the degree of stress, nutritional status etc. (Witeska et al., 2022) From this reason determination of hematological parameters represent a useful tool for fish welfare assessment (Grant, 2015; Fazio, 2019).

Following the analysis of hematological parameters, the following aspects were noted:

Regarding to the erythrocytes number was registered a significant increase in variant V2 ($p<0.05$) in which feed was supplemented with sea buckthorn (Table 1). Thus, compared to the count obtained in the V2 variant, it was observed a reduction with 6.30% in the V1 variant; 21.59% in the V3 variant and with 30.31% in the V4 variant. Nevertheless, obtained results falls within the range of 0.950-2.835 RBCc x10^6 cell/μL (Table 1). Although significant differences recorded between the variants, proving that the diet administered to cultured biomass significantly influenced the number of erythrocytes. However, the obtained values fell within the optimal range described for the *Oreochromis niloticus* species, between 0.7 and 2.8 RBCc x 10^6 cell/μL blood (Bittencourt et al., 2003).

Table 1 The results for the hematological parameters obtained in the experimental variants

Experimental variant	Hematological parameter (Average±SD)					
	RBCc (x10^6/μL)	Ht (%)	Hb (g/dL)	MCV (μm^3)	MCH (pg)	MCHC (g/dL)
V1	2.096±0.29	32.50±2.39	10.26±1.05	158.27±24.76	49.68±7.21	31.65±3.09
V2	2.237±0.21	30.41±3.78	10.86±0.68	137.20±20.76	49.01±5.29	36.27±5.29
V3	1.754±0.35	32.39±2.27	10.07±0.46	191.84±38.06	59.67±11.27	31.25±2.50
V4	1.559±0.28	32.06±2.76	10.80±0.87	212.70±44.73	71.65±14.60	33.77±1.82

Note: V1 - control variant; V2 - 1% Hippophae rhamnoides; V3 - 500mg vitamin E/ kg, V4 - 1% Hippophae rhamnoides + 500 mg vitamin E per kg feed; MCV - mean corpuscular volume; MCH - mean corpuscular hemoglobin; MCHC - mean corpuscular hemoglobin concentration.

The hematocrit recorded values between 21.00% and 36.00%. However they did not registered significant changes ($p>0.05$) between the experimental variants. Comparing the results with the values obtained in control variant (V1), in variant V2 was recorded a reduction with 6.43% in hematocrit, in the variant V4 with 1.35% and in the variant V3 with 0.34%. It must be specified that the obtained results regarding to the hematocrit are within optimal limits for tilapia, 15%-45% (Bittencourt et al., 2003) (Table 1).

Regarding to the hemoglobin concentration, a significant reduction was recorded ($p<0.05$) in variant in which vitamin E was included in fish feed (V3). Thus, compared to the V3 variant, was observed an increase in hemoglobine concentration with 1.89% in V1 variant (control), 7.25% in the V4 variant, respectively with 7.85% in the V2 variant. The values recorded in our research are within the reference range for the species *Oreochromis niloticus*, for hemoglobin concentration is between 6.58 g/ dL and 15.98 g/dL (Bittencourt et al., 2003) a fact that demonstrates that the results obtained (in range between 7.45 and 12.32 g/dL) are normal for tilapia, although significant differences were recorded between the experimental variants (Table 1).

Concerning to the mean erythrocyte volume significant differences were recorded between variants ($p<0.05$), minimum average value was obtained in the V2 variant (1% sea buckthorn), and the maximum value in the variant in which sea buckthorn and vitamin E were administered (V4) (Table 1). If we refer to the average value obtained in the control variant (V1), there was observed a reduction in the average erythrocyte volume with 13.31% in the V2 variant and an increase in it with 21.21% in the V3 variant, respectively with 34.39% in the V4 variant. The MCV concentration obtained in our experiment were between 87.50 μm^3 and 357.90 μm^3, these being within the reference range for Nile tilapia describde by Bittencourt et al. (2003) (12.36 μm^3 and 528.57 μm^3).

Also, significant differences ($p<0.05$) between variants were obtained in case of mean erythrocytar hemoglobin (MCH). Similar to the MCV concentration, the lowest concentration was recorded in the variant in which the feed was added with sea buckthorn (V2) and the maximum value in the V4 variant (Table 1). By comparison with V1 variant, the MCH concentration was reduced with 1.34% in the V2 variant and increased with 20.11% in the V3 variant, respectively with 44.22% in the V4 variant. The obtained results (between 49.01 pg and 71.65 pg, Table 1), fell within the range for the Nile tilapia species, described by Hamid et al. (2013), between 5 and 80.4 pg.

In case of MCHC concentration, a significant increase was observed in V2 variant ($p<0.05$) (Table 1), compared to the other experimental variants. Thus, the MCHC concentration increased with 7.70% compared to the V4 variant, with 14.91% compared to the V1 variant, respectively with 16.38% compared to the V3 variant. The mean erythrocyte hemoglobin concentration values fell within optimal range described for the *Oreochromis niloticus* species by Bittencourt et al., (2003), respectively between 19.84-87.73 g/dL, due to the fact that the minimum value was 23.29 g/dL, and the maximum value recorded was 52.97 g/dL.

Based on the results obtained in the V2 variant, we can state the fact that the reason why the highest value of the number of erythrocytes and hemoglobin concentration, respectively the lowest value of hematocrit which was recorded, is represented by the high vitamin C content of sea buckthorn fruits (Ranjith et al., 2006). Same results were recorded at the *Arapaima gigas* species fed with a feed supplemented with vitamin C (Menezes et al., 2006).

Following the analysis of hematological parameters and erythrocyte constants, it was noted that the dietary supplementation with *Hippophae rhamnoides*, vitamin E and with *Hippophae rhamnoides* in combination with vitamin E contributed to the emergence of significant changes as against to the control (V1) at the level of the red blood cells count, hemoglobin concentration, MCV concentration, MCH and MCHC. Dietary supplementation with vitamin E in combination with sea buckthorn contributed to the increase of erythrocyte constants (MCV and MCH) and to the reduction in red blood cells count.

For the determination of the relative and absolute number of leukocytes, lymphocytes (small and large), monocytes, granulocytes and the absolute number of platelets, the microscopic analysis of a number of 72 blood smears was used.

The leukogram of the fish taken in the study indicated only small lymphocytes (Lm), large lymphocytes (LM), monocytes (M) and neutrophil granulocytes (N). The leukograms obtained for each experimental variant, at the end of the experiment, are presented in figure 1. During the experiment a series of changes appeared at the level of the leukogram depending on the supplementation of Nile tilapia with *Hippophae rhamnoides*, vitamin E and combination between this phytobiotic and vitamin compared to the control variant.

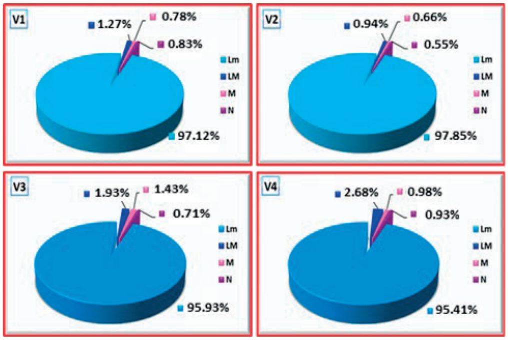

Figure 1. Relative number of leukocytes of Nile tilapia from each variant

Note: Lm - small lymphocytes, LM - large lymphocytes, M - Monocytes, N - neutrophil granulocytes.

In case of small lymphocytes (Lm), a significant decrease ($p<0.05$) of them was registered in the V4 variant (95.41±0.79%) compared to the other experimental variants. In comparison with V1, there was registered a reduction with 1.23% in V3 variant and with 1.76% in V4 variant and an increase in small lymphocytes (%) with 0.75% in V2 variant. The highest value was recorded in the V2 variant.

Unlike little lymphocytes (Lm - %), the highest value of the large lymphocytes (LM - %) registered a significant increase ($p<0.05$) in the V4 variant in which sea buckthorn with vitamin E was administered (2.68±0.37% - Figure 1). Thus, in case of large lymphocytes, we can state that in the V4 variant was registered an increase with 111.02%, and with 51.97% in the V3 variant in comparison with V1 variant. In the V2 variant was noted a reduction with 25.98% in comparison with V1 variant (Figure 1).

Regarding to the monocytes (%), a significant increase ($p<0.05$) was found in the variant in which the diet was supplemented with vitamin E (V3 variant). If we refer to the results registered in V1 variant we can say that there was a reduction of monocytes (%) with 15.38% in V2 variant and an increase of them with 25.64% in the V4 variant and with 83.33% in V3 variant.

But, in case of relative number of neutrophil granulocytes (%) insignificant differences ($p>0.05$) were recorded between the variants. The highest value was registered in V4 variant (0.93±0.36%) and the lowest in V2 variant (0.55±0.15%) (Figure 1).

In order to present in detail the changes in the leukocyte reaction, the absolute number of leukocyte and platelets were determined. In this sense, the results can be seen in the Table 2.

Table 2. Absolute leukocytes number obtained in the experimental variants

Experimental variant	Absolute leukocytes number (x10^3 cell./µL)					
	Leukocytes	Lymphocytes		Monocytes	Neutrophil granulocytes	Platelets
		small	large			
V1	418.96±68.81	406.38±64.19	5.52±2.46	3.40±2.09	3.66±2.52	59.72±28.71
V2	115.14±32.00	112.76±31.60	1.05±0.49	0.71±0.25	0.62±0.18	6.93±3.14
V3	81.45±16.58	78.36±16.46	1.58±0.43	1.16±0.55	0.54±0.14	11.76±4.77
V4	60.81±6.11	58.03±5.94	1.62±0.24	0.60±0.15	0.56±0.20	5.63±1.55

Note: V1 - control variant; V2 - 1% Hippophae rhamnoides; V3 - 500mg vitamin E/ kg, V4 - 1% Hippophae rhamnoides + 500 mg vitamin E per kg feed.

The absolute number of leukocytes it fell within the range of 45.56-526.65 x10^3 leukocytes/µL. The values obtained in the V1 variant are significantly higher ($p<0.05$) by comparison with the results registered in the V2, V3 and V4 experimental variants (Table 2). Thus, the values recorded in the control variant (V1) are higher than the optimal interval described for the Nile tilapia by Hrubec et al. (2000), respectively between 21.56 and 154.69 x10^3 leukocytes/µL.

In case of small lymphocytes a significant increase ($p<0.05$) was observed in V1 variant by comparison with the other experimental variants (Table 2). So, a reduction was observed with 72.25% in V2 variant, 80.72% in V3 variant and with 85.72% in V4 variant (Table 2). It must be specified that the results obtained in control variant - V1 are not in the interval for the *Oreochromis niloticus* species, 6.78-136.39x10^3 small lymphocytes/µL (Hrubec et al., 2000), these being much larger.

The results of absolute number of large lymphocytes registered significant differences ($p<0.05$) between V1, V2, V3 and V4 variants. The highest value was registered in V1 variant and the lowest value in V2 variant. Absolute number of large lymphocytes was recorded in the range of 0.45-10.53 x 10^3 large lymphocytes/µL. But this time the values obtained in the control variant (V1) fall within the reference range: 2.85-30.83 x 10^3 large lymphocytes/µL (Hrubec et al., 2000). However, compared to the average value recorded in the control variant, a reduction in the absolute number of large lymphocytes was recorded with 80.98% in the V2 variant, with 71.38% in V3 variant and with 70.65% in V4 variant (Table 2).

Regarding to the absolute number of monocytes, in the V4 variant was recorded the lowest value, this being statistically significant ($p<0.05$). Thus, by comparison with the control variant, the absolute number of monocytes was decreased with 79.12% in V2 variant, with 65.88% in V3 variant and with 82.35% in the V4 variant (Table 2). However, the obtained results fall within the range described by the Hrubec et al., (2000), (0.40-4.29 x 10^3 monocytes/µL).

In case of neutrophil granulocytes the absolute number recorded an increase V1 variant ($p<0.05$) compared to the other experimental variants. However, the obtained results are found in the reference interval for this species, between 0.56-9.87 x 10^3 neutrophils/µL (Hrubec et al., 2000). It should be specified that the mean value recorded in the V3 variant is below the lower limit of the reference range. By comparison with V1 variant the reduction was with 83.06% in the V2 variant, with 85.25% in the V3 variant and with 84.70% in the V4 variant (Table 2).

Regarding to the absolute platelet count the lowest number was obtained in V4 variant and the highest number in the V1 variant (Table 2). Between the variants were registered significant differences ($p<0.05$). If the results obtained in V1 variant falls within the range for the platelet

count (25.06 x10^3 cells/μL and 85.24 x10^3 cells/μL) described by Hrubec et al., 2000, the values recorded in V2, V3 and V4 variants are in the range described by Tavares-Dias and Oliveira (2009) (2.00-78.90 x10^3 cell/μL).
The research showed that under the influence of stress, due to the increase in corticosteroid hormones (cortisol, catecholamines), platelets count registered an increase and the coagulation time recorded a reduction (Docan, 2010).
The significant increase in the leukocytes, respectively lymphocytes, from the circulating blood in V1 variant (control) signifies the appearance of a strong immunomodulatory effect which may be due, among other things, to the presence of a stress factor (Martins et al., 2002). However, research has shown that the absolute number of leukocytes is much higher in juveniles than in adults (Hrubec et al., 2000).
In case of monocytes, absolute number is normal because it was obtained in the reference range described by the literature, but also because some researchers reported that monocytes represent a percentage of 10% of the leukocytes count (Table 2).
Evaluation of the leukocyte count by the absolute number of cellular elements highlighted the fact that the addition of feed from the V2, V3 and V4 variants led to the improvement of the fish compared to the variant in which the feed was not supplemented (V1).
In fish, the stress response is characterized in particular by the stimulation of the hypothalamus, which contributes to the activation of the neuroendocrine system and subsequently to the appearance of some metabolic and physiological changes (Lowe & Davison, 2005).
In terms of blood biochemistry parameters the concentration of glucose and total proteins were analyzed, in this research.
Comparing the results obtained in the case of glucose concentration a significant reduction ($p<0.05$) of it were registered in V2, V3 and V4 variants compared to V1 variant (control) (Figure 2). Thus, there was a reduction with 14.16% in V4 variant, with 18.99% in V3 variant and 22.82% in V2 variant compared to V1 variant. The obtained results fell within the interval of 59.81-142.38 mg/dL, but Bittencourt et al. (2003) reported for *Oreochromis niloticus* a reference range between 22.7-107.0 mg/dL. The values recorded in the control variant (V1) do not fall within the reference range, being more higher.

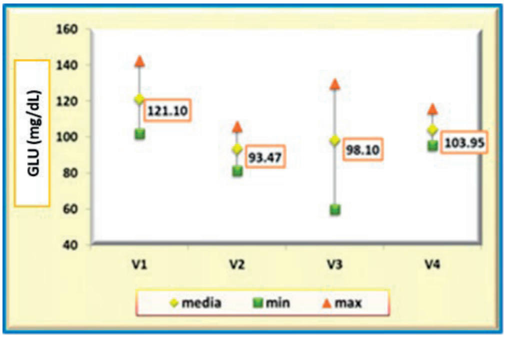

Figure 2. Glucose concentration in case of Nile tilapia from each experimental variant

Sharif (2012) reported that the administration of vitamin C in Nile tilapia feed contributed to a significant reduction in glucose concentration compared to variants in which the feed was not supplemented with vitamin C. It is proven once again by this research the therapeutic value of administration sea buckthorn in fish, precisely because of the high content of vitamin C. In the V2 variant (sea buckthorn) the lowest glucose value was recorded.
In case of total protein concentration the highest value was registered in V3 variant ($p<0.05$) (Figure 3) and by comparison with the other experimental variants were registered a significant differences.

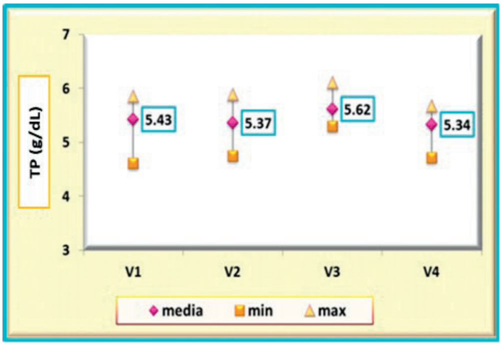

Figure 3. Total protein concentration for the Nile tilapia from each experimental variant.

By comparison with the control variant (V1) there was a decrease of total proteins with 1.11% in the V2 variant and 1.66% in the V4 variant, respectively an increase with 3.50% in V3 variant. The values of total protein concentration fell within the range of 4.62 - 6.11 g/dL and the optimal interval described by Hrubec et al. (2000) was between 2.9 and 6.6 g/dL for Nile tilapia.

Studies have shown that the administration of vitamin C in the Nile tilapia diet led to lower total serum protein concentrations compared to the variants in which vitamin C was not administered (Sharif, 2012), same values were obtained in V2 variants (1% *Hippophae rhamnoides*/kg feed), respectively in V4 variant in which the feed was supplemented with 1% *Hippophae rhamnoides* and 500 mg vitamin E/kg feed.

CONCLUSIONS

In conclusions assessment of hematological parameters and erythrocyte constants highlighted the fact that supplementing the diet with the phytobiotic *Hippophae rhamnoides* , vitamin E and with the phytobiotic *Hippophae rhamnoides* in combination with vitamin E contributed to the appearance of changes in a significant mode ($p<0.05$) in comparison with the control (V1) in case of the number of erythrocytes, hemoglobin concentration and erythrocytar constants (MCV, MCH and MCHC). Addition of food with vitamin E and sea buckthorn (V4) contributed to the increase of erythrocyte constants (VEM and HEM) and reduction of the number of erythrocytes. The biochemical analysis of the blood indicated an increase the glucose concentration ($p<0.05$) in the V1 variant (control) in which the feed was not added a phytobiotic or vitamin. The values obtained are much higher than those presented in the literature. Among variants V2, V3 and V4, the lowest value of glucose concentration was recorded in V2 variant (1% *Hippophae rhamnoides*) (93.47±7.48 mg/dL).

Regarding to the leukocyte count analysis by the absolute number of cellular elements noted that administration of 1% *Hippophae rhamnoides*, 500 mg vitamin E and 1% *Hippophae rhamnoides* in combination with 500 mg vitamin E in *Oreochromis niloticus* diet led to the improvement of the Nile tilapia welfare compared to the variant in which the feed was not added.

ACKNOWLEDGEMENTS

The present research was supported by the project An Integrated System for the Complex Environmental Research and Monitoring in the Danube River Area, REXDAN, SMIS code 127065, co-financed by the European Regional Development Fund through the Competitiveness Operational Programme 2014–2020, contract no. 309/10.07.2021.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

THE ACTION OF THE MINERAL SUPPLEMENT "PMVAS" AND THE THERMAL FACTOR ON SOME TRACE ELEMENTS IN CALVES IN THE POSTNATAL PERIOD

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Abstract

This paper reflects the research carried out regarding the status in copper, zinc and iron depending to the intake of the mineral supplement `PMVAS` and establishing the correlation between the action of the food factor and the action of the low temperature of a moderate stress intensity on the organism of the calves in the early postnatal period. Thus, the paper presents the results of the separate action of the mineral premix `PMVAS` and its conjugate with the thermal factor on some indices of saline metabolism in calves in postnatal period carried out in order to correct the deficiency of trace elements and determine the parameters that can act beneficially on homeostasis, resistance and adaptive capacities of animals to the action of the environment. At the separate and combined application of the factors studied in dynamics (7, 30, 60, 90 days) on the organism of the calves were obtained representative data regarding the functional state of the organism demonstrating a moderate intensification of the metabolism of trace elements.

Key words: *calves, mineral supplement, stress, temperature, trace elements.*

INTRODUCTION

The state of salt metabolism of calves in the early postnatal period especially of trace elements depends on the liver reserves that they obtained at birth from their mothers and from the milk consumed. These sources could be insufficient in farms where intensive growing technologies are practiced because young animals are particularly sensitive to trace element deficiencies, which manifest themselves more pronounced and in a more acute form than in adult animals (Lamand & Lamand, 2013; Usachiov & Strelitzov, 2019). Trace elements exert their effect in the composition of enzyme systems and participate in various metabolic processes, which are especially intense in young, fast-growing animals. The occurrence of micronutrient deficiency in an animal depends on the intensity of the growth process (Furdui, 1986; Furdui et al., 1992; Cheghina, 1993; Lorenz et al., 2011; Iakushkin, 2012).

Essential trace elements are present in an almost constant amount in the body, they cause structural and metabolic anomalies by their absence, they prevent or correct these anomalies by their intake. The intake of an optimal amount of trace elements is generally ensured by a balanced diet, but it may be insufficient if the composition of the diet mainly consists of foods from intensive crops or the foods are intensively processed, preserved or thermally processed (Strutinschi, 1997). There are physiology situations such as growth in which an additional supply of trace elements is required. Long-term exposure to certain risk factors such as unbalanced nutrition, stress, pollution, can also increase the need for trace elements (Kurbanalieva, 1982; Mashkina & Stepanenko, 2017; Medvedev & Sokolova, 2019).

The success of raising productive animals depends on the effectiveness of prophylaxis and correction of various deviations of homeostasis in adult cattle and particularly in calves in the early postnatal period (Jegou et al., 2006; Gonsales Martin & Elivira Partida, 2011; Bociarov, 2015). This can be easily achieved by supplying the young organisms with qualitative nutrients in optimal quantities and eliminating from the organism the useless products obtained as a result of metabolism (Dorosh, 2007; Dronov, 2000). However, at the present time, there are frequently recorded deficiencies of trace elements that affect the functions of hematopoiesis, endocrine glands, defense reactions of the body, microflora of the digestive tract, regulation of metabolism and biosynthesis of proteins (Heinrichs & Radostits, 2001; Kraskovo, 2005; Kucinskii, 2007; Zavalishina et al., 2011). The acute trace element deficit requires expenditure to correct and undertake veterinary medical prophylactic measures to combat various complications which in turn lead to additional animal losses (Kurbanalieva, 1982; Gaidukova et al., 2003; Karashaev, 2007; Marie-Vinciane, 2008). The most important trace elements for the organism of animals in the early postnatal period are "essential trace elements with demonstrated risk of deficiency" such as iron, copper and zinc. The importance of iron for the body is determined by its ability to bind and transport oxygen and participate in cellular respiration (Shedrunov et al., 1989). Iron is a fundamental constituent of hemoglobin and its absence can cause iron deficiency anemia. Iron is also part of the structure of myoglobin (it ensures the oxygen reserves of the muscles) and of numerous enzymes. The periods in which preventive supplementation of iron intake is necessary is the period of early postnatal ontogenesis (Karashaev, 2007; Zavalishina et al., 2011).

Copper is considered an anti-infectious, anti-inflammatory, and anti-oxidant agent, plays a significant role in the process of hematopoiesis as a biocatalyst that stimulates the formation of hemoglobin from inorganic iron compounds. At a lack of copper in the diet of cows and young animals, immature forms of erythrocytes appear in the blood which lead to aggravation of anemia. Copper is also essential for the normal development of the skeleton, plays an important role in the synthesis of cartilaginous tissues and intervenes in bone mineralization, in the regulation of nerve signal transmission at the brain level. With a copper deficiency in the feed, infectious states and rickets-like phenomena are observed in calves. Copper is also considered a powerful factor in reducing oxidative stress (Nazdrachova, 2004; Kurdenko et al., 2017).

Zinc is an essential trace element involved in protein synthesis, it is part of the structure of more than 300 hormones and enzymes. It has an important role in cell division, being involved in the synthesis process of DNA and RNA. It is indispensable for the action of vitamin A, influences the activity of the pituitary hormones regulating the activity of the gonads (ovaries or testicles) FSH and LH, enters in the composition of insulin, helps to transform thyroxine (T4) into triiodothyronine (T3). Zinc also intervenes in maintaining the integrity of the immune system. Although the daily requirement is small, it is indispensable for the proper functioning of the calves' organism.

Supplementing with minerals of cattle rations is important also for the farmer who could benefit from a better productivity of the owned animals and therefore a better financial gain if the status in trace elements of the animals would be adequate. From the moment the animals are deficient, the products obtained from them are also deficient. There are studies that demonstrate that supplementing animal rations with macro- and microelements has a positive effect on the human organism.

In order to correct the deficiency of trace elements, we proposed to study the action of the mineral premix "PMVAS" on the organism of calves in the early postnatal ontogenesis, applied separately and conjugated with the abiotic environmental factor (temperature) of moderate stress intensity. The additional application of the thermal factor of a moderate stress intensity on the calves was carried out with the aim of stimulating the adaptive capacities and resistance of the animals to environmental factors and normalizing the physiological parameters of the organism in the early postnatal period.

MATERIALS AND METHODS

As a research object in this study, 30 calves of the Black-and-White breed were selected, divided into the control group (LM) and two experimental groups (LEP and LETP).

In order to correct the saline metabolism and increase the adaptive capacities of the organism, the action of the mineral compound "PMVAS" was studied, applied separately or

conjugated with the thermal factor on calves in the postnatal period. The premix "PMVAS" was developed in the Institute of Physiology and Sanocreatology and contains cobalt carbonate, copper sulphate, iron sulphate, potassium iodate, manganese sulphate, zinc sulphate, sodium humate and calcium phosphate. The quantity of mineral substances was calculated based on the recommended norms, the content of mineral substances in the blood of animals and their feed value. This concentration constituted various values contained in the range from 0,1 mg to 100,0 g.
The study was conducted from the 7th day to the 90th day of postnatal ontogenesis.
The animals from LM, LEP and LETP during the research period were in similar maintenance conditions. Calves received the same ration, which was composed of hay, silage and concentrated fodder according to existing norms. In addition, each calf in all batches during the entire study period consumed 300 liters of whole milk.
The differences between the rations of the calves consist in the fact that the animals in the experimental groups LEP and LETP in addition to the basic ration received a compound of mineral substances "PMVAS" in the amount of 1.5 g per 1 liter of milk consumed.
At the same time, the calves in the LETP experimental group, in addition to the procedures carried out in the LEP, were subjected to the action of the low temperature. As a stress factor the temperature of +5ºC was applied. The application of the temperature on the calves was carried out during the postnatal period at the age of 3, 7, 15, 20, 25 and 30 days. After introduction into the climatic chamber "Zootron" and adaptation of the animals for 1 hour to the new conditions, the temperature is gradually lowered to +5ºC. The temperature drop lasts 30 minutes. Exposure of animals to "low temperatures" at the age of 3, 8 and 15 days lasts 1 hour, and at the age of 20, 25 and 30 days - 2 hours.
Blood samples were collected at the age of 7, 30, 60 and 90 days. In order to appreciate the separate action of the mineral premix "PMVAS" and its conjugate action with the thermal factor on the functional state, resistance and adaptive capacities of calves, the amount of iron and copper in the blood was studied by the photoelectrocolorimetric method in the modification of Cuznetzov, zinc in the blood by the photoelectrocolorimetric method according to Cebotariova. The processing of the obtained data was carried out using methods established for the biological field (Merkurieva, 1963; Ivanter, 2010).

RESULTS AND DISCUSSIONS

In the present study was investigated the dynamics of the concentration of iron, copper and zinc, trace elements considered important for the organism. The obtained results of the amount of iron in the blood plasma of calves are presented in Figure 1.

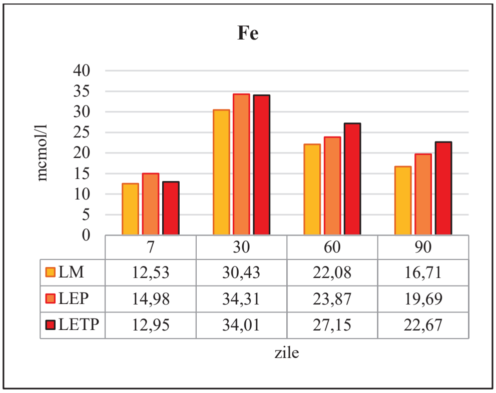

Figure 1. The amount of iron in the blood of calves subjected to the separate and conjugated action of the food factor with the thermal factor

Note: Here and hereafter: LM - control group; LEP - experimental group in which the "PMVAS" premix was administered; LETP - experimental group in which the "PMVAS" premix and the thermal factor were administered.

The data of Figure 1 demonstrates that the amount of iron in the blood plasma of calves varies depending on the period of application of the studied factors and the nature of these factors. Iron is an important and effective index in the prophylaxis of iron-deficiency anemia (Anokhin et al., 2003). The analysis of the iron content in the animals subjected to the experiment demonstrates a significant increase in its level in blood plasma in calves of all groups subjected to the experiment at the age of 30 days ($P<0,05$). In the later periods of the experiment (60 and 90 days) the amount of iron in all three groups is reduced in comparison with its value at the age of 30 days. Although the amount of iron in the blood plasma with age tends to decrease in all groups, however, its higher values were recorded in the experimental groups. For example, at the age of 30 days, the amount of iron in LEP was 34.31±1.82, in the LETP group - 34.01±4.51, while in LM - 30.43±2.74 mcmol/l. At 60 days, the iron level in LM decreased to 22.08±2.60, in LEP to 23.87±1.58 ($P<0.05$) and in LETP to at 27.15±1.96 mcmol/l. A similar dynamic was preserved at the age of 90 days, constituting values of 16.71±1.15 ($P<0.05$), 19.69±1.03 ($P<0.05$) and 22.67±3.63 mcmol/l, corresponding to LM, LEP and LETP.

At the same time, it is noted that in the age periods of 7 and 30 days the amount of iron in LEP is higher than in LETP, then its content in LEP decreases in the following age periods of 60 and 90 days compared to LETP. This state of affairs demonstrates that initially a more pronounced influence on the iron content in the blood serum is provided by the separate administration of the food factor, then in the second half of the experiment in the upper position is placed the conjugate action of the food factor with the thermal one. The higher amount of iron recorded in the experimental groups according to the properties exerts an important action in respiration and tissue nutrition thus contributing to the prophylaxis of anemia and enhancement of the immunological reactivity of animals.

Next, the amount of copper in the blood plasma of the animals subjected to the experiment was studied. The obtained results are presented in Figure 2.

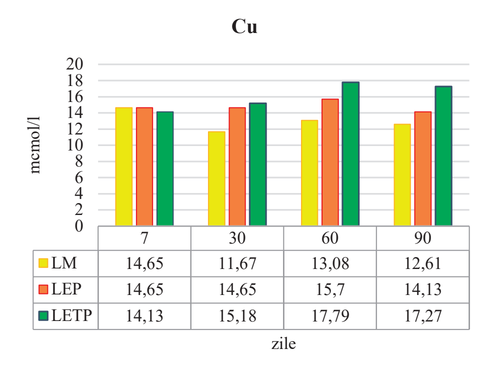

Figure 2. The amount of copper in the blood of calves subjected to the separate and conjugate action of the food factor with the thermal factor

Analyzing the amount of copper in the blood serum (Figure 2) it is marked that throughout the research period the amount of copper in the experimental groups is higher compared to that in the control group. At the same time, we mention that the conjugate action of the researched factors is more pronounced on the quantity of copper compared to the separate action of the food factor. The amount of copper in LETP is higher than its amount in LEP during the entire duration of the experiment and at the age of 60 and 90 days it constituted 17.79 and 17.27 mcmol/l.

Thus, the high level of copper in all experimental groups conditions the increase of hemopoiesis, the activity of the rumen microbiota, the functional state of the endocrine and nervous system, the development of bone system and increase of productivity of animals.

Since zinc is involved in protein synthesis, it is part of the composition of many hormones and enzymes, we considered it appropriate to study its evolution to the action of the investigated factors. The obtained results are presented in Figure 3.

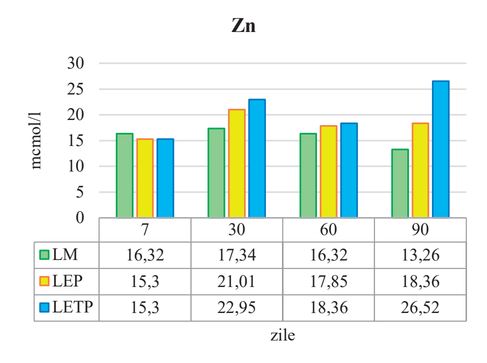

Figure 3. The amount of zinc in the blood of calves subjected to the separate and conjugated action of the food factor with the thermal factor

From the data in Figure 3, it is denoted that the concentration of zinc in the blood of the animals in all groups subjected to the experiment oscillates, registering maximum values at the age of 30 days. In LEP and LETP the zinc values at the beginning of the experiment are equal (15.3 and 15.3 mcmol/l). Then, under the conjugate action of the studied factors (LETP), its quantity essentially increases throughout the study period, exceeding the value of zinc in the blood plasma in calves from the experimental group (LEP), in which only the food factor was applied (at 90 days $P<0.05$). The large amount of zinc obtained experimentally according to its properties influences the activity of the prestomach microbiota, adjusts the reproductive function and participates in the formation of bone tissue. Zinc deficiency in calves depends to a certain extent on the content of this trace element in cow's milk, that is, on the presence of this trace element in the mother's body. Meanwhile, despite the fact that zinc is very well absorbed by the calf from milk, it happens that the calf subsequently shows symptoms of zinc deficiency, probably due to a change in the absorption of this element by the calf. In this case under such circumstances, it is desirable to increase the zinc content in the diet of calves (up to 40-50 mg/kg SM).

CONCLUSIONS

Following the evaluation of the action of the studied factors, applied either separately or conjugately, on the organism of the calves in the postnatal ontogenesis and especially on the quantity of trace elements, it is possible to conclude that they provide a more optimal oscillatory level of iron, copper and zinc in the blood. The changing nature of the level of these elements in the blood plasma of experimental animals reflects not only their quantity, which arrives in the body through the food ration, but also peculiarities of their metabolism.

At the same time, we mentioned that the amount of the elements studied in LETP is higher compared to LEP. This increase is recorded throughout the experimental period, which confirms a beneficial and synergistic action of the studied factors on the organism of the calves in the early postnatal period, which is expressed by potentiating of their effects on the studied trace elements.

The obtained results demonstrate that the elaborated mineral premix, administered to animals in the amount of 1.5 g/l of milk, and the application of the thermal factor show positive effects on the productivity of animals in postnatal period. Thus, the daily weight gain of calves in LEP and LEPT was higher, correspondingly, by 70 and 62 g than in LM.

Thus, the researches carried out open numerous perspectives both at a practical and fundamental level. It is appropriate to review the intake and evolution of other trace elements in calves in the early postnatal period. Analysis of the effectiveness of a supplement in trace elements could be performed by comparing blood status and health, production and reproductive performance, depending on the form and amount of minerals consumed by animals. The quantitative parameters of trace elements should be recalculated according to the breed and performance of the animals, but also depending to the form of minerals ingested.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Institute of Physiology and Sanocreatology and was financed from the Project 20.80009.7007.25 "Methods and procedures for maintenance and conservation of biodiversity depending on the integrity of gametogenesis and food variability".

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

THE INFLUENCE OF POLYPHENOL EXTRACT FROM DANDELION ON THE PHYSIOLOGICAL STATE OF THE ORGANISM OF BREEDING ROOSTERS

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Abstract

This paper includes the study of specialized scientific bibliographic sources and the research of the influence of polyphenols on the improvement of the state of oxidative stress on the organism of breeding rooster. It is established that dandelion polyphenols have a beneficial influence and have the ability to stop and block reactive forms of oxygen through fermentative and non-fermentative antioxidant systems. All these changes are observed in the obtained results. It is established that there is an increase of superoxide dismutase (SOD) in the experimental group compared to the corresponding control group 159.6±0.69 and 110.93±0.30 u/c, as well as catalase indicates significant changes in the experimental group versus the corresponding control group 35.0±0.53 and 26.23±0.37 µM/L, which provides reliable protection of the organism against the toxic effects of high concentrations of superoxide anion radical and hydrogen peroxide.

Key words*: breeding roosters, polyphenols, antioxidants, oxidative stress, food ration.*

INTRODUCTION

Fundamental and applied research is currently being carried out in multiple research institutions, directed in all directions of studying biologically active compounds of plant origin. In particular, research on antioxidants (AO) is of particular interest. These substances are able to significantly diminish or modify the oxidation of the substrate (Bekker et al., 2004), which can be widely used in the pharmaceutical, food, cosmetology, veterinary medicine and other industries. It is known that plants can be rich sources of antioxidants, such as phenols, flavonoids, carotenoids, tocopherol, ascorbic acid etc. (Katalinic et al., 2006).

Living organisms are protected from the influence of free radicals by the antioxidant system, which contains fermentative and non-fermentative substances, capable of completely neutralizing the harmful influence of these toxic substances (Iashin, 2008). The decrease in the activity of the antioxidant system and therefore the increase in the concentration of free radicals in the organism is linked to a multitude of unfavorable factors, such as radioactive and ultraviolet irradiation, worsening of the ecological situation, permanent stresses, unsupervised administration of veterinary drugs, use of feed contaminated with different pollutants etc.

The harmful effects of free radicals in the case of oxidative stress can be reduced by regular consumption of certain nutrients with antioxidant activity. The main natural antioxidants are flavonoids, aromatic hydroxy acids, anthocyanins, vitamins C and E, carotenoids etc.

Anthocyanins are of exceptional importance because due to the charge of the oxygen atom in the C ring, anthocyanidins and anthocyanins penetrate cell membranes more easily (Ehlenfeldt et al., 2001).

Polyphenols occupy a significant place among the most important classes of natural compounds, which determine the biologically active influence on the tissues and organs of the body with the maintenance of their functions at an optimal level of their activity. Most flavonoids give living organisms anti-inflammatory influence, antioxidant protection and many other positive effects for their proper functioning. Currently, it is considered that flavonoids are indispensable components of the food ration of animals and humans. In plants flavonoids are found in free form and in the form of glycosides, their content varies from 0.5 to 5% (Petrova, 1986; Smirnova, 1986). According to research (Kalashnikov et al., 1985) the content of flavonoids in the aerial part of the plant does not exceed 3.5%.

According to modern concepts, free radicals and other forms of reactive oxygen species (ROS) play a significant role in regulating the basic functions of the cell. It should be noted that ROS, depending on the strength of the pathogenic factor affecting the cell, can act either as inducers of adaptation processes or as inducers of apoptosis. In addition, ROS are able to directly influence the destructive effect on cellular structures, as well as the initiation of free radical oxidation of lipids, proteins, nucleic acids, which is the basis of the pathogenesis of many disorders of organs and systems of living organisms (Sazontova et al., 2005; Shabanov et al., 2010). ROS realizes its physiological and pathological effects in close interaction with other regulatory factors of the intra - and extracellular metabolic process, modifying their activity (Lukyanova et al., 2013; Novikov et al., 2013).

Of all the cellular components most subject to attack by ROS are mitochondria, as a result of damage to membrane lipids, proteins, DNA and even their death. Moreover, the death of mitochondria does not require any additional proteins other than those that are present in itself.

Mitochondria possess a protective system against ROS, which includes the enzymes superoxide dismutase (neutralization of superoxide anion into hydrogen peroxide), peroxidase and glutathione peroxidase (degradation of hydrogen peroxide), as well as glutathione, reduced form of coenzyme Q, ascorbic acid and other low molecular weight antioxidants.

When mitochondria cease to cope with the problem of ROS detoxification that they form, regardless of the listed defense mechanisms, the so-called "oxidative stress" develops in the cell. As a result of excessive formation of oxygen radicals, the latter begin to perform mainly destructive functions, rather than serve as signaling molecules. Specific changes in cellular components take place: membrane structures are damaged due to lipid peroxidation (LPO), proteins are oxidized to tyrosine, cysteine and serine residues, DNA damage, a change in the redox potential of the cell due to oxidation of glutathione and NAD(P)H. Is observed the destruction of mitochondrial structures from the membrane to mitochondrial DNA (mtDNA) (Murphy, 2004).

ROS have a damaging effect primarily on mitochondrial membranes. In particular, under the action of ROS in the internal membrane protein of mitochondria, which provides conjugate ATP/ADP transfer, oxidation of Sh-Group Cys-56, which promotes the formation of a nonspecific mitochondrial channel (mPTP), permeable to low molecular weight substances (Pojilova et al., 2014). ROS significantly affects the concentration of calcium ions in the matrix of mitochondria and cell cytoplasm, causing the pumping of Ca^{2+} into the cytoplasm from the extracellular and intracellular space, and into the matrix - from the cytoplasm, by activating calcium transporters (Ghosh et al., 1995).

Mitochondrial DNA (mtDNA) is also a vulnerable target for the pathogenic action of ROS. The high concentrations of reactive oxygen species in mitochondria and the weak repair system between these organelles increase the frequency of mtDNA mutations compared to nuclear DNA. Oxygen radicals cause specific substitutions in the DNA molecule. Thus, the hydroxyl radical has a harmful effect on DNA due to oxidation of bases, their modification and damage to chromosomes. Such mutations can lead to pathology and cell death or to its malignant transformation. MtDNA damage is particularly dangerous due to the gradual, long-term accumulation of mutations under the long-term effect of ROS. A number of important and unique mitochondrial proteins are encoded in the mitochondrial genome, and the damage to the genes responsible for them leads to the dysregulation

of their expression and their subsequent functioning (Skulachev, 2012).
Since the formation of ROS in the cells of aerobic organisms occurs continuously, then in the cells there is a system of protection against their harmful influence. Protecting cells from excess ROS, and the oxidative damage caused by them, is achieved through the functioning of the antioxidant system, which includes oxidizing antienzymes, low molecular weight compounds and those that form a redox buffer, vitamins, albumins, free fatty acids and metal ion complexes.
Antioxidant enzymes that neutralize ROS include superoxide dismutase (SOD), catalase and peroxidase. SOD catalyzes the dismutation of two superoxide molecules to form hydrogen peroxide and oxygen. Isoforms of this enzyme are present in all cellular compartments, where superoxide can form. Hydrogen peroxide formed during superoxide dismutation is neutralized by catalase or glutathione and thioredoxin-peroxidases in peroxisomes.
The intracellular redox status is assisted by the thiol system and primarily by glutathione (GSH) and thioredoxin (TRX), which create a buffer system to maintain a more reduced amount compared to the conditions of the extracellular environment. Glutathione is one of the main intracellular antioxidants, which participates in maintaining the redox status due to the neutralization of hydrogen peroxide: $H_2O_2 + 2GSH \rightarrow 2H_2O + GSSG$. Regeneration of reduced glutathione (GSH) from glutathione disulfide (GSSG) occurs by means of glutathione reductase: $GSSG + NADPH + H^+ \rightarrow 2GSH + NADP^+$. Under the conditions of oxidative stress due to the rapid oxidation of glutathione, the ratio GSH/GSSG decreases, but it can very quickly restore to the initial level. In the case of depletion of GSH in any tissue, it can be supplied due to its release into the blood from the storage (liver) (Urso et al., 2003). Thioredoxin acts as a reducer of disulfide bonds in proteins and an electron donor for TRX-eroxidase, at the same time it does not affect the production of ROS or the reduced amount of glutathione. Thioredoxin is reduced by thioredoxin reductase and NADPH (Li et al., 2002).
The free radical formation processes hypochlorite anion and hydroxyl radical are located in the cytoplasm and are monitored by cytoplasmic enzymes or natural water-soluble antioxidants. For example, taurine is able to block the hypochlorite anion in the form of a chloramine complex, carnosine (dipeptide) and its derivatives will neutralize the hydroxyl radical. A major importance for the prevention of lipid peroxidation, which is initiated in the hydrophobic space of cell membranes and the destruction of fatty acid radicals, is α-tocopherol, being located in membranes. Having a high concentration in biological membranes prevents their damage by free radicals. Tocopherol interrupts the chain reactions of lipid peroxide formation, transforming into a radical, which regenerates both with the help of water-soluble active reducing agents such as ascorbate and glutathione, and with the help of hydrophobic ubiquinol. From the point of view of antioxidant protection, ubiquinol is the most effective form of coenzyme Q_{10}.
The regulation of ROS formation can be achieved in two ways: through the direct influence on oxygen free radicals and their binding (direct antiradical action) and through the way of fortifying of antioxidant activity (first of all, the activity of antioxidant protection enzymes). However, the use of nutrients rich in anthocyanins, phenols, polyphenols, etc. it can be aimed both at eliminating the primary damage induced by ROS, which are the basis of the pathogenesis of functional disorders of the organs and systems of the animal and human organism, and at blocking the apoptosis induced by them (Kiricek et al., 2004).
The negative role of ROS and oxidative stress has been demonstrated in many dysfunctions of living organisms. For example, during hypoxia, it is confirmed that antioxidants weaken the disorders associated with hypoxia (Marcova et al., 2013; Novikov et al., 2011). Therefore, antioxidants are used in the complex correction of hypoxic states (Levcenkova et al., 2012; Cekman et al., 2014). Antioxidant therapy is widely used today for ischemic diseases in cardiology and neurology, for toxic liver damage, systemic connective tissue diseases and other ailments (Kriucova et al., 2013; Shabanov et al., 2010).

Special attention in scientific research is attracted by the so-called "physiologically compatible antioxidants" (PCAO), representing in themselves modified natural antioxidants (Novikov et al., 2007). PCAO are conjugated redox factors, maintaining homeostasis indices within normal physiological limits and returning homeostasis indices to normal values in pathological situations or extremals. A particularity of PCAO is their ability towards compatibility, which is represented as more important compared to the antioxidant activity. They are able as a component part of one or another physiological system to influence molecular targets and cause deviations in the oxidation-reduction potential of the cell, synchronized with cell cycles or other biological cycles. PCAO very effectively regulates free radical oxidation reactions and the redox status of the cell (Novikov et al., 2013).

Therefore, the research of these biologically active substances, the development of methods for their isolation, the determination of their chemical structure and the study of the dependence of biological activity on chemical structure in order to create new preparations with antioxidant effect is an important and urgent problem of biomedicine. The interaction of free radicals of oxygen with proteins leads to a variety of reticular disorders and other cellular components, such as protein fragmentation, specific damage to the protein molecule, which changes its structure and affects the functional activity of the protein.

Inhibitors of reactive oxygen species (ROS) form the basis of the body's defense system against the excessive generation of active oxygen metabolites (AOM). In fact, the body's defense against AOM is a universal complex system of chemical and biochemical reactions that occur at different biological levels and taking place through different mechanisms, involving various high and low molecular weight compounds, such as redox enzymes, polypeptides, some vitamins, amino acids, polyphenols etc. (Zencov et al., 2001; Budnikov et al., 2005).

MATERIALS AND METHODS

The study was carried out on 10 breeding roosters and they were divided into two groups of five roosters each: a control group and an experimental group, which were administered *per os* in a dose of 2 ml hydroalcoholic polyphenol extract from dandelion with a total amount of antioxidants of 0.27 g gallic acid equivalent per 100 gr. The extract was administered during two cycles of spermatogenesis. Hematological, biochemical indices and amino acids in blood serum and seminal plasma were studied. Spectrophotometric methods and the Folin-Ciocalteu method were used to determine the total amount of antioxidants. The total amount of antioxidants was determined on the SF-5400 UF spectrophotometer.

RESULTS AND DISCUSSIONS

In order to determine the influence of biologically active substances obtained by hydroalcoholic extraction from dandelion, hematological indices of roosters from the experimental group and the control group were studied. The research results are presented in Table 1.

Table 1. Hematological indices of the reproductive roosters included in the experiments for study the action of dandelion polyphenols

Groups	Leukocytes, 10^9/L	Erythrocytes, 10^12/L	Hemoglobin, g/L	Hematocrit, %	Mean corpuscular hemoglobin MCH, pg	Mean corpuscular volume MCV, fl	MCH concentration g/dL
Control	38.6± 4.3	3.45±0.25	139.3±5.5	46.3±2.25	39.4±0.57	131.5±0.87	30.5±0.16
Experimental	41.3±3. 2	3.67±0.08	143.3±2.08	47.2±1.22	39.0±0.21	128.4±1.58	30.3±0.45

Leukocytes represent those components of the blood that are involved in the defense against pathogens and in the identification of active pathologies. Although they represent only 1%

of the blood volume, being, nevertheless, key elements of the immunity of each individual organism, their role in the immune response is a huge and indispensable. From the obtained results we observe changes in their content, in the control group having a content of 38.6±4.3, and in the experimental group the value is 41.3±3.2 - 10^9/L, which testifies to us about a positive influence of dandelion extract on these blood elements.

Erythrocytes, in turn, also have different roles in living organisms, such as: transporting oxygen and carbon dioxide, amino acids, hormones (through their absorption on the surface of erythrocytes), participate in immunological processes, in maintaining blood pH etc., also changes are observed in their content for the control group having a value of 3.45±0.25, and for the experimental group indicating a value of 3.67±0.08 - 10^12/L.

The hemoglobin also shows changes in its content with a value for the control group of 139.3±5.5, and for the experimental group 143.3±2.08 g/L.

Based on the experimental results that are presented in table 1, a change and stabilization of all the investigated hematological indices is observed.

In addition to studying the hematological indices, some biochemical indices of the antioxidant system were also studied. The research results are presented in Table 2.

Table 2. The fermentative antioxidant status in the blood serum of the reproductive roosters included in the experiment to study the action of dandelion polyphenols

Groups	SOD, u/c	G-GTP, u/L	Catalase, μM/L	G-S-T, nM/sL
Control	110.93±0.30	9.33±0.40	26.23±0.37	19.21±0.38
Experimental	159.6±0.69	11.4±0.44	35.0±0.53	30.41±0.52

Superoxide dismutase (SOD) plays an important role in protecting cells from the damaging effect of the radical anion superoxide and is rightfully considered the main enzyme of the intracellular antioxidant system. SOD not only stabilizes cell membranes by preventing lipid peroxidation, but by decreasing oxygen levels, it protects catalase and glutathione peroxidase. The results of the research show an increase of the SOD value up to 159.6±0.69 compared to the control group with a value of 110.93±0.30 u/c.

SOD activity is regulated by compounds containing SH groups: glutathione, cysteine and others, as well as indirectly by glutathione metabolism enzymes. The results are presented in table 3. The latter, together with catalase and peroxidases have different substrate specificities, providing the detoxification of hydrogen peroxide. However, a noticeable disadvantage of this enzymatic process is the formation of hydrogen peroxide, which is prone to the generation of highly reactive hydroxyl radicals, but is reduced to water mainly by catalase and glutathione peroxidase.

Catalase in combination with SOD forms a reliable protection of the body against the toxic effects of high concentrations of superoxide anion radical and hydrogen peroxide. At the same time, the minimum controlled level (<50 μM) of these biomolecules, which are necessary under physiological conditions for the implementation of many cellular processes, is maintained. The results of the research show us a higher activity of catalase in the experimental group with a value of 35.0±0.53, compared to the control group with a value of 26.23±0.37 μM/L.

Glutathione peroxidases (GPO) are the most important enzymes that ensure the inactivation of reactive oxygen species by destroying both H_2O_2 molecules and lipid hydroperoxides. These enzymes catalyze the reduction of peroxides with the participation of the tripeptide - glutathione (γ-glutamylcysteinylglycine). GPOs are able to neutralize not only H_2O_2, but also various lipid peroxides synthesized in the body upon activation of POL processes. Glutathione peroxidase protects proteins, lipids, nicotinamide coenzymes from oxidative attack and restores lipid peroxides.

In addition to the ability to reduce hydrogen peroxide and fatty acid hydroperoxides, GPO provides protection to aerobic organisms against the highly toxic peroxynitrite by reducing it to the nitrite anion. In addition, glutathione-S-transferases are involved in the isomerization of steroids and prostaglandins and are involved in the metabolism of other endogenous substances. In particular, GST may be involved in the synthesis of leukotrienes,

supporting the inflammation process. Therefore, a positive influence of the polyphenol extract of dandelion is observed on the indices of the fermentative antioxidant status in the blood serum of breeding roosters.
In addition to studying the hematological indices and the fermentative antioxidant status, the comparative content of the combined functional groups of free amino acids in the blood serum, seminal plasma and reproductive cells of breeding roosters and their distribution depending on the biological material studied were also investigated. The research results are presented in Table 3.

Table 3. Comparative content of combined functional groups of free amino acids in blood serum (mcm/100 ml), seminal plasma (mcm/100 ml) and reproductive cells (mcm/100 g) of breeding roosters

Amino acids	Blood serum	Seminal plasma	Reproductive cells
Σ Nonessential amino acids	301.59±34.10	1642.21±106.50	70.66±0.27
Σ Essential amino acids	104.15±15.90	154.57±11.90	33.31±2.80
Σ Immunoactive amino acids	136.53±23.95	846.57±56.29	46.01±0.18
Σ Glycogenic amino acids	164.79±22.06	330.33±10.49	35.66±0.91
Σ Ketogenic amino acids	40.54±8.49	56.87±6.96	10.84±0.20
Σ Proteinogenic amino acids	405.74±48.74	1796.78±118.40	103.96±3.07
Σ Sulfur-containing amino acids	23.54±7.43	79.16±12.07	6.74±0.23

From the results of the research, it can be seen that the proportion of immunoactive amino acids in the blood serum is 31.23%, while in seminal plasma and reproductive cells it is much higher - respectively 44.90% and 41.57% of the total amino acid content. Likewise, from the obtained data, we observe that the proportion of sulfur-containing amino acids is almost the same in the blood serum (5.38%), seminal plasma (4.20%) and reproductive cells (6.09%).
The ratio of proteinogenic amino acids to the total volume of free amino acids is 92.82% in blood serum, 95.30% in seminal plasma and 93.94% in reproductive cells, that is, it is practically identical. Interestingly, at the same time, the share of the fund of essential amino acids in the blood serum is 23.83%, seminal plasma 8.20%, and in reproductive cells they are much higher - 30.09%.

CONCLUSIONS

The inclusion in the food ration of antioxidant substances of plant origin beneficially influences the protein, lipid and carbohydrate metabolism at any level of organization of the tissues and systems of living organisms.
The relevance of studying this problem is constantly increasing due to the convincing scientific results regarding the role of ROS not only in maintaining homeostasis, but also in the development of cellular pathology and dysfunctions of the animal organism in general.
It is important to note that, despite the great interest in the use of antioxidants to prevent and reduce the consequences of biological disorders associated with oxidative stress, further research is needed to study the effect of these compounds on the antioxidant status and on the level of oxidative stress in the body of bioobjects.
The results of this study allow us to conclude that the spectra of free amino acids of both seminal plasma and spermatozoa indicate changes in which they can cause pathologies of metabolic processes in spermatozoa.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Institute of Physiology and Sanocreatology and was financed from the Project 20.80009.7007.25 “Methods and procedures for maintenance and conservation of biodiversity depending on the integrity of gametogenesis and food variability”.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

STUDY OF THE IMPACT OF SOME FACTORS ON THE GESTATION LENGTH OF ANGLO-NUBIAN GOATS REARED IN FOOTHILLS

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Abstract

Data is presented from 140 records of pregnancies and births of goats of the Anglo-Nubian breed, bred in the farm of the Research Institute of Mountain Stockbreeding and Agriculture - Troyan, Bulgaria. The data was used to determine the influence of sex, type of delivery and parity on the gestation length (GL) in goats. The delivery season is not taken into account, as it is always in the winter. There was a significant difference in the GL of goats that gave birth to singles and twins (p<0.001). The GL for all kids born as singles was 151.1±0.4 and twins 149.2±0.3 days. No significant difference was observed between the GL of goat that gave birth to male (151.2±0.7) and female (151±0.6) singles. A difference of more than one day was found between the GL of goats that gave birth to male and female twins (148.2±0.6 and 149.9±1, respectively). The GL in goats that gave birth to twins of different sexes was 149.4±0.4 days, and in goats from the first to the fifth parity (149.2±0.6; 149.9±0.5; 149.0±0.6; 150.5±0.6; 150.9±0.7 days) respectively.

Key words: *gestation length, goats, parity, sex, type of birth.*

INTRODUCTION

Anglo-Nubian is a British breed of goat that is the product of a composite crossbreeding between native British breeds, East Asian goats with pendulous ears and Nubian bucks (Lazarov et al., 1995).
The Anglo-Nubian breed is spread on all continents. In some places, it is raised as purebred or more commonly is used in crossbreeding programs in different regions of the world (Stemmer et. al., 2009).
The first import of the breed to Bulgaria took place in 1985 in RIMSA in the town of Troyan (Zunev, 1991), and over the years the breed was used for improvement and reproduction in crossbreeding with Bulgarian White Dairy goat breed (Todorova et al., 2021) (Figure1).
In recent years, the breed has become more and more relevant for farmers in Bulgaria, and for this reason it is of interest for research work.
Gestation length is a physiological variable that is of economic importance in most animal species (Satue et al., 2011). This value is strictly defined for each animal species, but varies within the breed and is affected by various factors such as season of delivery and

Figure 1. Cross F1 Anglo-Nubian x Bulgarian White Dairy Goat

age of the mother (Mellado et al., 2000; Haldar & Ghosh, 2015).
Bushara (2010) summarized several studies on the gestation length in Nubian goats in Sudan, such as El-naim (1979), who reported a gestation of 146.5±1 days, Jubartalla (1998) with 143-149 days and Elabid (2002) with 148.57±3.6 days.
Yagoub et al. (2013) reported 147.1±0.8 days of gestation length for Nubian goats reared in Sudan. Praharani et al. (2016) reported

150.25±1.39 days of gestation for Anglo-Nubian goats in Indonesia, and Mia et al., (1996) - 148.41±0.84 days for Anglo-Nubian goats reared intensively in Bangladesh.
Peaker (1978) reported that the type of delivery reliably affected the gestation length in Saanian goats. In contrast, Zunev (1991) reported that in goats of the Bulgarian White Dairy breed bred under the same climate conditions and technology as those in the present study, the type, sex, birth weight of the kids and the age of the mother did not have a significant effect on gestation length of the goat mothers.
According to Dickson-Urdaneta et al. (2000), gestation length was also greatly affected by farm management. The feeding of the goats and the season of delivery are also factors to be taken into account.
According to Bushara (2010), provision of amount of energy level in pregnant doe feed would accelerate in growth rate of fetus that ultimately result in shortening gestation period.
Last but not least, Joshi et al. (2018) draws attention to the fact that the farm location affects the productive and reproductive performance of goats and in particular the length of pregnancy. The author summarizes the results of studies by Sharma et al. (2017) and Parajuli et al. (2015) who reported that goats reared at higher altitudes had a longer gestation length compared to goats reared at lower altitudes.
The aim of the present study was to determine the impact of sex, type of delivery and parturition order on the gestation length in Anglo-Nubian goats reared in foot-hill regions.

MATERIALS AND METHODS

Geographical location

The study was conducted on the farm of the Experimental Base of the Research Institute of Mountain Stockbreeding and Agriculture of Troyan, Bulgaria. The site is located 380 m above sea level, (42° 53' 39" N /24° 42' 57" E). Climate is temperate-continental with a pronounced mountain influence, with four seasons, without fogs and strong winds. The average temperature for February and March in the last ten years has varied between 3.5 - 6.01°C, and the average precipitation amount has been 49.7 - 59.5 mm.

Animals

All animals were reared under the same conditions. During the period from May to October, the animals were pasture-raised and supplemented with concentrated feed, whereas during the winter period the animals were barn-raised and fed with a ration containing 2 kg of hay and 0.8 kg of concentrated feed per head. Free access to water and salt was provided. The goats were vaccinated against enterotoxemia, dewormed and injected with vitamins A, D and E (Vialiton, Biovet). Goats were inseminated by hand as each “jump” recorded in an insemination dairy. The gestation length was calculated from the date of insemination to the date of delivery of the goats.
A few days before the expected delivery, the goats are separated into individual boxes and are under the supervision of breeders. Immediately after delivery, the weight of the kids was measured with electronic scales and recorded in a diary. The newborn kids together with their mothers stayed in the individual pens for five days. After that, they were separated into pens of ten kids, where they remained until weaning.

Experimental setup

Data from a total of 140 gestation and delivery records of Anglo-Nubian goats are presented. The data were used to determine the influence of sex, type of delivery and parturition order on the gestation length in goats. The season of delivery is not taken into account, as it is always in February and March.
According to the type of delivery, the gestation length of goats that gave birth to single (48), twin (77) and triplet kids (15) was examined According to parity, the data is distributed as follows - goats of first, second, third, fourth and fifth parity on the first parity - 33 heads, on the second - 29 heads, on the third - 28 heads, on the fourth - 22 heads and on the fifth - 28 heads. The impact of birth weight on the gestation length of goats that gave birth to singles was examined, as the weight of the kids was divided into <3.3 and ≥3.3 kg.

Statistical analysis

One-way ANOVA was used for statistical comparison. The differences were tested by Student t-test.

RESULTS AND DISCUSSIONS

Table 1 shows the impact of the type of delivery on the gestation length of studied goats.

The present results show that the pregnancy of goats thah had single kids was significantly longer by 1.9 days than that of goats with twins ($p<0.001$). No significant difference was observed in the gestation length of goats that gave birth to twins and those with triplets. As can be seen from Table 1, the maximum period of gestation length in goats that gave birth to twins and those that gave birth to triplets was the same - 157 days These results are consistent data by Zunev (1991) for Bulgarian White Dairy goat breed raised under identical climate conditions and technology as those of the present study with 151.5 days for goats that gave birth to single kids and 150.05 days of gestation for goats that gave birth to twins.

Table 1. Impact of the type of delivery on the gestation length

Goats giving birth to	n	x ± Sx	min.	max.
singles total	48	^{a***}151.1±0.4	144	160
twins total	77	^{a***}149.2±0.3	141	157
triplets total	15	^{NS}149.2±0.9	140	157

Note: a - singles in total/twins in total; ***p<0.001, NS- not significant.

The author points out that animals that had triplets had 2.23 days shorter gestation than those that had singles.

For their two-year study of gestation length in Bulgarian White Dairy goats reared under identical conditions to the present study, Stoycheva et al. (2018) reported 151.8 days of gestation for goats that had single kids and 150.8 days for goats with twins.

In their 15-year study of Anglo-Nubian goats in Peru raised at 20 m above sea level and in a temperate climate, Naver & Custodi (1984) reported 150.8 days of gestation for goats with singles, 150.8 days for goats with twins, and 147.1 days for goats with triplets.

Contrary to the present finding, Ageeb (1992) found for Baggara pasture-raised goats in Sudan that the gestation length was significantly affected by the type of delivery ($p < 0.05$), as goats that were pregnant with twins had a longer gestation (149.9 days) compared to those who gave birth to one kid (145.1 days).

The present results are also in conflict with what was found by Moaeen-ud-Din et al. (2008) who reported a gestation length of 149.9±1.9 days for goats bearing singles, 150.0±2.1 days in goats with twins and 151.0±2.5 days in goats of Matou breed in China bearing triplets reared in subtropical climate. These differences are likely determined by factors such as the age of the goats, the season of delivery, and others. According to Asdell (1929), Mellado et al. (2000), Kudouda (1985), and Bushara (2010), in Sudanese Nubian goats, gestation length was not reliably affected by the number of kids born. Table 2 shows the impact of the gender of the kid on the gestation length

Table 2. Impact of the sex of the kid on the gestation length

Goats giving birth to	n	x ± Sx	min.	max.
male singles	22	151.2±0.7^{NS}	144	160
female singles	26	151±0.6^{NS}	146	158
male twins	18	148.2±0.6^{NS}	145	155
female twins	14	149.9±1^{NS}	141	157
same-sex twins	45	149±0.4^{NS}	141	157

Note: NS- not significant.

To examine the effect of kid gender on gestation length, we also divided goats according to delivery type. There is almost no difference in the gestation length between goats that gave birth to single males and single females. Goat mothers that gave birth to female twins had a 1.7 day longer gestation than those who gave birth to male twins. The gestation of goats that gave birth to twins of different sexes was shorter than that of goats that gave birth to twins of the same sex (Table 2). The present findings are in agreement with those reported by Stoycheva et al. (2018) in Bulgarian White Dairy goats. The authors reported 151.9 days of gestation for male singles and 151.7 days in female, 150.3 days for male twins, 151.3 days for female and 150.9 days in heterosexual, respectively.

According to Hafez (1993), ewes that gave birth to male lambs had longer gestation than those that gave birth to females. According to Ageeb (1992), the gender of the kid has a significant effect on the gestation length in Baggara goats. In contrast to Hafez (1993), the aforementioned author found that goats that gave birth to female kids had a longer gestation period than goats that gave birth to male kids. This conclusion in the present study can only be confirmed in goats that gave birth to same-sex twins.

Bushara (2010) summarized the studies of El-naim (1979) and Kudouda (1985) in Sudanese Nubian goats and Mishra et al. (1979) in Indian Sirohi goats - according to them, the sex of the newborn kid has no significant effect on the gestation length in goats.

Table 3 shows the influence of parity on the gestation length.

Table 3. Effect of parity on the gestation length

Goats giving birth to	n	x ± Sx	min.	max.
first	33	149.2±0.6^{NS}	141	160
second	29	149.9±0.5^{NS}	146	157
third	28	149.0±0.6^{NS}	140	155
fourth	22	150.5±0.6^{NS}	145	157
fifth	28	150.9±0.7^{NS}	141	159

Note: NS - not significant.

The gestation length, according to parities in the present study, varied between 140 and 160 days. The widest range of variation in first-parity goats and fifth-parity goats was observed. The longest gestation was registered in fifth-parity goats and the shortest in third-parity goats. The difference between them is 1.9 days. The present findings show that as maternal age/parity increases, so does the gestation length.

Present data are in agreement with those found by Zunev (1991) for Bulgarian White Dairy goat breed, reared under conditions identical to those of present study. The author reported a 149.4-day gestation period for second-parity goats, 150.3-day for third-parity goats, and 150.5 days for fourth-parity goats.

Silva et al. (2021) present data on Anglo-Nubian goats in semi-arid regions of Brazil at an altitude of 534 m. with semi-intensive farming. Deliveries were spread over December, April and August. The first-parity goats in this study had the longest gestation (152 days), and the third-parity goats had the shortest (144 days). The gestation of goats giving birth for the second, fourth and fifth time was 148, 148 and 149 days respectively.

In Hair goats raised at 1600 m above sea level in the Eastern Anatolia region of Turkey, deliveries were similar to those in the present study from February to April. Bolacali (2019) reported gestation length for first, second, third and fourth parities with 149.1; 148.5; 149 and 148.3 days respectively.

Hoque et al. (2002) reported that gestation length was strongly influenced by parity in goats from three breed groups in Bangladesh. Second-parity goats had the longest 148-day gestation period, and fifth-parity goats the shortest - 144.9 days.

According to Asdell (1929), there is a very clear difference in the gestation length between young and older mothers. The author relates this not to the number of deliveries (parity), but rather to the age of the mothers. That is, gestation is shorter in young animals than in older ones.

Table 4. Impact of the birth weight of single kids on the gestation length

Singles	x	Sx	min.	max.
≥3.3	150.9 ^{NS}	0.53	146	158
<3.3	151.3^{NS}	0.87	144	160

Note: NS - not significant.

From the point of view of the birth weight of the kids, we can see that the difference is small and insignificant. These data are in agreement with those presented by Stoycheva et al. (2018) in Bulgarian White Dairy goats.

According to Zunev (1991), in goats of the Bulgarian White Dairy goat breed, the kids born with the lowest live weight had the shortest embryonic period.

CONCLUSIONS

It was established that the gestation length of goats of the Anglo-Nubian breed raised in the foothills of the Republic of Bulgaria is a relatively constant value, with slight variations depending on some factors.

A significant difference was found in the gestation length of goats that gave birth to single kids and those that gave birth to twins. The average gestation length for all goats that gave birth to single kids was 151.1±0.4, and for twins 149.2±0.3 days.

As the age of the mother/parities increased, so did the gestation length, as it was the longest in the fifth parity with 150.9±0.7.

The present research could be useful in organizing work processes and managing goat farms.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

THE COMPOSITION OF HEAVY METALS AND THE CONTENT OF ESTERIFIED FATTY ACIDS IN BEE TISSUES DEPENDING ON THE ENVIRONMENTAL CONDITION

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Abstract

The work aimed to record the content of heavy metals, including toxic and esterified forms of fatty acids in tissues, and the honey productivity of bees in different natural zones of the Carpathian region. The level of dangerous elements of the first class of toxicity - Lead and Cadmium - increases significantly in the tissues of honey bees of the foothills and forest-steppe zones, compared to the conditionally clean mountain environment. In the Carpathian region, all heavy metals accumulate in more significant quantities in the abdominal tissues of honey bees than in the tissues of the chest and head. In the direction from the mountain to the foothills and further to the forest-steppe zone of the Carpathian region, a decrease in the content of esterified forms of fatty acids is observed in the tissues of honey bees. The intensity of conversion of the esterified form of linolenic acid into its longer-chain and unsaturated fatty acids of the omega-3 family in the tissues of the head of honeybees in the foothills and especially in the forest-steppe zones is sharply reduced.

Key words*: bioindicator, fatty acids, heavy metals, tissues.*

INTRODUCTION

The sources of heavy metal emissions and the ways of their entry into the environment are diverse. Still, they are generally of artificial origin due to urbanization and industrialization (Slivinska et al., 2018; 2019; 2020; 2021). Urbanization and industrialization, in particular, the activities of industry, agriculture, energy, and transport, as well as intensive extraction of minerals – all led to the release of heavy, including highly toxic, lead, cadmium, and arsenic into the air, water, soil and plants (Gutyj et al., 2017; 2019; Kovka & Nedashkivskyi, 2019; Tikhonova et al., 2020; Briffa et al., 2020).

The migration of heavy metals in objects of the external environment caused their accumulation in soils and plants (Krainiukov et al., 2020; Tikhonova et al., 2020; Briffa et al., 2020; Bashchenko et al., 2020; Prychepa et al., 2021). As a result, some types of plants gave way to others, and the terms of their flowering changed, thus the conditions of honey collection by bees (Kryvyi et al., 2018; Kovka & Nedashkivskyi, 2019; Krainiukov et al., 2020; Kobysh et al., 2021; Al-Kahtani et al., 2021). The above also led to the accumulation of heavy metals in the tissues of honey bees and beekeeping products (Didaras et al., 2020; Mărgăoan et al., 2021; Ricigliano et al., 2021).

As is known, heavy metals, including toxic ones, are involved in synthesizing, desaturation, and peroxide oxidation of long-chain fatty acids in plant and animal tissues and fluids. As a result, the supply of plant tissues, in particular pollen and bees, with energetic, structural, biologically active, and antimicrobial material changes (Desbois & Smith, 2010; Giri et al., 2018; Corby-Harris et al., 2021; Butsiak et al., 2021). All this affects bee colonies' productivity and their products' quality indicators (Matin et al., 2016; Kovalskyi et al., 2018; Vishchur et al., 2019; Gizaw et al., 2020; Monchanin et al., 2021).
Therefore, the issue of producing ecologically safe beekeeping products is urgent. Moreover, the production of honey bees occupies a prominent place in human life (Matin et al., 2016; Didaras et al., 2020; Costa et al., 2021; Ricigliano et al., 2021; El-Seedi et al., 2022). Very high requirements are placed on the quality indicators of honey bee products because Ukraine has become the leading exporter of honey to Europe.
In the literature, there needs to be more data on the content of heavy metals and different forms of fatty acids in bee pollen and tissues of honey bees kept in different natural zones of the Carpathian region (Klym & Stadnytska, 2019; Saranchuk et al., 2021).
Given the above, studies of the content of heavy metals, including toxic and esterified forms of fatty acids in tissues, and honey productivity of worker bees in different natural zones of the Carpathian region are of significant scientific and practical interest.
The goal of the work. To record the content of heavy metals, including toxic and esterified forms of fatty acids in tissues and honey productivity of bees in different natural zones of the Carpathian region.

MATERIALS AND METHODS

Experimental apiaries of clinically healthy honey bees of the Carpathian breed (*Apis mellifera* (L.) *carpatica*) were selected based on the private mountain (Slavsko village, Stryi district), foothills (Nizhnya Stynava village, Stryi district) and forest-steppe (village Myklashiv, Lviv district) zones of Lviv region, where natural and climatic conditions and ecological situation are different.
To assess the intensity of technogenic load on the environment where experimental honey bee apiaries are located, the content of heavy metals (Ferrum, Zinc, Copper, Chromium, Cobalt, Nickel, Lead, and Cadmium) in the topsoil, bee pollen, and tissues of honey bees was determined.
In each of the above-described natural zones of the Carpathian region, the honey productivity of worker bees was studied in 3 apiaries and each of 5 hives (Kovalskyi & Kyryliv, 2011). In particular, the honey productivity of bees was reviewed in early June, mid-July, and mid-August by pumping out honey frames. These studies were conducted based on one bee colony of average strength and the same queen age. In addition, samples of bee pollen and honey bees were taken for laboratory research at each apiary from 3 hives in the spring-summer period. At the same time, examples of the arable layer of the soil were born in the radius of the available flight of honey bees.
The content of heavy metals, including toxic ones, was determined in selected samples of the topsoil, bee pollen, and tissues of the abdomen, chest, and head of honeybees and the pieces of the tissues above of honeybees – esterified forms of fatty acids. At the same time, the value of the level of heavy metals, including toxic and esterified forms of fatty acids, in tissues for the body of honey bees in different natural zones of the Carpathian region was analyzed.
The content of heavy metals (Ferrum, Zinc, Copper, Cobalt, Chromium, Nicol, Lead, and Cadmium) in the selected samples of the arable layer of the soil, bee comb, and tissues of the abdomen, chest, and head of honey bees was determined according to the currently valid state standard (DSTU 4405: 2005) on an atomic absorption spectrophotometer - Selma-115 (Vlizlo, 2012). The content of esterified forms of fatty acids in the tissues mentioned above of honey bees was determined by gas-liquid chromatography (Rivis et al., 2022).
The obtained digital material was processed by the method of variational statistics using the Student's criterion (Ibatullin & Zhukorskyi, 2017).

Arithmetic mean values (M) and errors (±m) were calculated. Differences were considered probable at $p < 0.05$. The computer program Origin 6.0 and Microsoft Excel were used for calculations.

RESULTS AND DISCUSSIONS

It was established that in the arable soil layer of the foothills and forest-steppe zones of the Carpathian region, compared to the conditionally clean mountain zone, there is probably a higher content of the studied heavy metals (Table 1). At the same time, the arable layer of the soil of the forest-steppe zone of the Carpathian region contains the highest level of the studied heavy metals. The content of hazardous elements of the first toxicity class - Lead and Cadmium - in the arable layer of the soil in the above zone is 1.1 times higher than the maximum permissible concentration (Yatsuk & Balyuk, 2019).

Table 1. Gross content of heavy metals, including toxic ones, in the topsoil in different natural zones of the Carpathian region, g·10-3/kg of air-dry mass (M ± m, n = 3)

Metal and its symbol	Natural zones of the Carpathian region		
	Mountain	Foothills	Forest-steppe
Ferrum, Fe	14325.00 ± 294.214	15184.29 ± 454.862*	16573.04 ± 294.429**
Zinc, Zn	47.58 ± 4.488	78.52 ± 3.722**	96.13 ± 4.890***
Cuprum, Cu	21.60 ± 1.391	34.56 ± 1.828**	45.64 ± 2.264***
Cobalt, Co	11.76 ± 0.375	13.63 ± 0.560**	17.20 ± 1.830***
Chrome, Cr	41.69 ± 2.283	63.65 ± 3.584**	87.53 ± 4.163***
Nicole, Ni	21.24 ± 1.625	41.33 ± 2.512***	59.42 ± 3.214***
Lead, Pb	19.37 ± 0.784	25.83 ± 1.442*	33.30 ± 2.870***
Cadmium, Cd	2.03 ± 0.088	2.60 ± 0.115*	3.20 ± 0.271***

Note. Here and further, differences are likely compared to the mountainous zone: * - $p < 0.05$–0.02; ** - $p < 0.01$; *** - $p < 0.001$

It is believed that the increase in the content of lead in the arable layer of the soil is associated with the intensive movement of motor vehicles (Loretta et al., 2015) and cadmium - with the introduction of meliorants and mineral fertilizers, primarily phosphogypsum and superphosphate, respectively (Razanov et al., 2015; Vozhehova et al., 2021). It can be seen that phosphoric acid residues in phosphogypsum and superphosphate bind solid elements and carriers for cadmium.

The obtained data characterize artificial environmental pollution in the experimental territories. The high level of heavy metals, including toxic ones, in the soil, is the reason for the increase in their concentration in bee pollen (plant pollen) obtained in the foothills and forest-steppe zones of the Carpathian region (Table 2). All this is a consequence of greater urbanization and industrialization of the above territories.

Table 2. The content of heavy metals, including toxic ones, in bee pollen in different natural zones of the Carpathian region, g·10-3/kg air-dry mass (M ± m, n = 3)

Metal and its symbol	Natural zones of the Carpathian region		
	Mountain	Foothills	Forest-steppe
Ferrum, Fe	33.52 ± 0.830	37.11 ± 0.781*	43.39 ± 2.253**
Zinc, Zn	34.39 ± 1.91	39.20 ± 0.900*	42.72 ± 0.872**
Cuprum, Cu	2.01 ± 0.089	3.02 ± 0.169*	4.20 ± 0.170***
Cobalt, Co	1.01 ± 0.029	1.14 ± 0.050*	1.44 ± 0.112***
Chrome, Cr	4.10 ± 0.177	5.02 ± 0.180*	6.68 ± 0.149***
Nicole, Ni	0.58 ± 0.015	0.65 ± 0.015*	0.74 ± 0.023**
Lead, Pb	0.12 ± 0.007	0.18 ± 0.009*	0.26 ± 0.012**
Cadmium, Cd	0.04 ± 0.003	0.07 ± 0.007*	0.10 ± 0.009**

It should be noted that in the forest-steppe zone of the Carpathian region, compared to the mountain zone, the arable layer of the soil and bee pollen has a relatively high content of probiotic heavy metals - Zinc, Copper, Cobalt, Chromium, and Nickel. The above-mentioned heavy metals in acceptable quantities are essential for plant tissues and bees (Hsu et al., 2021). However, the increased level of toxic lead and cadmium in the arable layer of the soil and bee hives can neutralize the positive effect of probiotic heavy metals on the mentioned tissues (Purać et al., 2019).

The high level of Ferrum, Zinc, Copper, Cobalt, Chromium, Nicol, Plumbum, and Cadmium in bee honey, in turn, is the reason for the increase in their content in the tissues of honey bees. In particular, it was established that in the tissues of the abdomen (respectively 163.76 g·10-3/kg of raw mass and 191.91 against 127.61), breast (80.10 and 97.24 against 65.27), and head (respectively 100.32 and 119.32 against 81.24 g·10-3/kg of raw weight) of honey bees of the foothill and forest-steppe zones, compared to the mountain zone, there is a higher total content of the studied heavy metals (Tables 3, 4 and 5). The level of dangerous elements of the first class of toxicity - Lead (by 1.33-4.00 times) and Cadmium (by 1.78-4.00 times) - is especially significant in the tissues of honey bees of the foothills and forest-steppe zones, compared to the conditionally clean mountain environment). The concentration of the element of the second toxicity class - Chromium - also increases noticeably (by 1.18-1.60 times).

Table 3. The content of heavy metals, including toxic ones, in the abdominal tissues of honey bees in different natural zones of the Carpathian region, g·10-3/kg raw weight (M ± m, n = 3)

Metal and its symbol	Natural zones of the Carpathian region		
	Mountain	Foothills	Forest-steppe
Ferrum, Fe	46.48 ± 1.046	63.72 ± 1.220***	77.03 ±1.630***
Zinc, Zn	77.08 ± 1.190	91.32 ± 1.536**	104.24 ± 2.060***
Cuprum, Cu	0.34 ± 0.012	0.47 ± 0.014**	0.59 ± 0.014***
Cobalt, Co	0.31 ± 0.009	0.36 ± 0.014*	0.43 ± 0.017**
Chrome, Cr	2.43 ± 0.070	3.12 ± 0.082*	3.78 ± 0.112**
Nicole, Ni	2.43 ± 0.035	3.40 ± 0.067***	4.13 ± 0.059***
Lead, Pb	0.88 ± 0.035	1.21 ± 0.038**	1.50 ± 0.046***
Cadmium, Cd	0.09 ± 0.009	0.16 ± 0.006**	0.21 ± 0.012**

Table 4. The content of heavy metals, including toxic ones, in the breast tissues of honey bees in different natural zones of the Carpathian region, g·10-3/kg of raw mass (M ± m, n = 3)

Metal and its symbol	Natural zones of the Carpathian region		
	Mountain	Foothills	Forest-steppe
Ferrum, Fe	36.10 ± 0.931	42.18 ± 0.812**	49.06 ± 0.555***
Zinc, Zn	17.51 ± 0.587	23.50 ± 0.625**	31.46 ± 0.507***
Cuprum, Cu	1.93 ± 0.041	2.82 ± 0.061***	3.17 ± 0.070***
Cobalt, Co	1.84 ± 0.035	2.19 ± 0.077*	2.40 ± 0.049***
Chrome, Cr	3.08 ± 0.046	3.51 ± 0.058**	4.34 ± 0.186**
Nicole, Ni	4.00 ± 0.049	4.80 ± 0.085**	5.47 ± 0.128***
Lead, Pb	0.78 ± 0.023	1.04 ± 0.068*	1.25 ± 0.038***
Cadmium, Cd	0.03 ± 0.003	0.06 ± 0.003*	0.09 ± 0.003**

Table 5. The content of heavy metals, including toxic ones, in the tissues of the head of honey bees in different natural zones of the Carpathian region, g·10-3/kg of raw mass (M ± m, n = 3)

Metal and its symbol	Natural zones of the Carpathian region		
	Mountain	Foothills	Forest-steppe
Ferrum, Fe	26.79 ± 0.607	31.16 ± 0.979*	36.59 ± 1.324**
Zinc, Zn	30.13 ± 0.630	36.30 ± 0.564**	44.00 ± 0.280***
Cuprum, Cu	8.39 ± 0.319	14.35 ± 0.417***	17.66 ± 0.400***
Cobalt, Co	7.22 ± 0.055	7.56 ± 0.078*	7.97 ± 0.084**
Chrome, Cr	6.87 ± 0.098	8.15 ± 0.117**	9.47 ± 0.254***
Nicole, Ni	1.00 ± 0.055	1.47 ± 0.041**	1.78 ± 0.058**
Lead, Pb	0.81 ± 0.030	1.25 ± 0,044**	1.73 ± 0.050***
Cadmium, Cd	0.03 ± 0.003	0.08 ± 0.003***	0.12 ± 0.006***

It should also be noted that the tissues of the abdomen of honeybees are much more active accumulators of heavy metals than the tissues of the chest and head. Practically all heavy metals are accumulated in 1.61-2.04 times greater quantities in the tissues of the abdomen of honey bees than in the tissues of the chest and head.

Heavy metals at a physiologically determined level are involved in metabolic processes and the content of fatty acids in the body tissues of bees. In particular, cobalt-initiated protein synthesis in bee tissues, due to the activation of transport and information nucleic acids (Osman et al., 2021), is usually accompanied by the accumulation of polyunsaturated fatty acids necessary for the construction of cytoplasmic and cell membranes.

Since it is part of 9-desaturase, cuprum in the tissues of the body of bees at a physiologically determined level contributes to the formation of palmitic and stearic monounsaturated fatty acids of the omega-7 (palmitooleic) and omega-9 (oleic) families, respectively (Di et al., 2020; Takic et al., 2021).

Linoleic and linolenic acids, sequentially synthesized in plant tissues from oleic acid, are considered indispensable for bees and, therefore, must enter their bodies with food (Hajiahmadi et al., 2020; Hsu et al., 2021; Takic et al., 2021). In the tissues of bees from linoleic and linolenic acids because zinc is part of 2-, 3-, 4-, 5- and 6-desaturases, even longer-chain and more unsaturated fatty acids, respectively, of the omega-6 families (eicosatriene, eicosatetraenoic-arachidonic, docosadiene, and docosatetraenoic) and omega-3 (eicosapentaenoic, docosatrienoic, docosapentaenoic, and docosahexaenoic) (Takic et al., 2021; Hsu et al., 2021).

Longer chains and more unsaturated fatty acids of the omega-3 and omega-6 families are precious for the bee body. As mentioned above, polyunsaturated fatty acids in the body tissues of bees are a source for constructing cytoplasmic and cell membranes and synthesizing biologically active derivatives - prostaglandins, thromboxanes, and leukotrienes (Trinkl et al., 2020; Mărgăoan et al., 2021; Matuszewska et al., 2021). Thus, polyunsaturated fatty acids of the omega-3 and omega-6 families affect the health and vitality of bees.

Precious polyunsaturated fatty acids for bees are fatty acids of the omega-3 family (eicosapentaenoic, docosatrienoic, docosapentaenoic, and docosahexaenoic) (Trinkl et al., 2020; Matuszewska et al., 2021; Takic et al., 2021). These acids in the body tissues of bees are the initiators of synthesizing very strong and fast-acting anti-inflammatory peptide substances - cytokines (Ranneh et al., 2021).

Based on the above, our next task was to study the concentration of esterified fatty acids in the tissues of the abdomen, breast, and head of honey bees obtained from hives located in the mountain, foothill, and forest-steppe zones of the Carpathian region.

The content of esterified forms of saturated fatty acids with an even (caprylic, capric, lauric, myristic, palmitic, and stearic) and odd (pendecanic) amount of carboxylic acids was studied in the tissues of the abdomen, breast, and head of honey bees of the foothills and forest-steppe zones of the Carpathian region, compared to the mountain zone. Atoms in the chain, monounsaturated fatty acids of the omega-7 (palmitoleic) and omega-9 (oleic and eicosaenoic) families, and polyunsaturated fatty acids of the omega-3 (linolenic, eicosapentaenoic, docosatrienoic, docosapentaenoic and docosahexaenoic) and omega-6 (linoleic, eicosadienoic) families, eicosatrienoic, eicosatetraenoic-arachidonic, docosadiene and docosatetraenoic). Data on their content are presented in Tables 6, 7, and 8.

It should be noted that the most significant amount of esterified forms of fatty acids is found in the tissues of the abdomen, breast, and head of honey bees (Desbois & Smith, 2010; Giri et al., 2018; Burdge, 2018; Saranchuk, 2020; Corby-Harris et al., 2021). Esterified forms of fatty acids in the mentioned tissues of honey bees are included in the composition of phospholipids, esterified cholesterol, mono-, di- and triacylglycerols (Desbois & Smith, 2010; Corby-Harris et al., 2021).

It was established (Tables 6, 7 and 8) that the total concentration of esterified forms of fatty acids in the tissues of the abdomen, chest and head of honey bees obtained from hives located in the foothills (respectively 17.67, 18.57 and 18.33 g /kg of raw mass) and forest-steppe (16.98, 17.69 and 17.31) zones of the Carpathian region, compared with the tissues of the abdomen, breast and head of honey bees selected from hives located in the mountain zone (21.72, 22, 69 and 23.17), due to the lower level of saturated fatty acids in their composition (2.24, 2.32 and 1.99 and 2.09, 2.12 and 1.96 versus 2.93, 2.97 and 2.97) and an odd (0.07, 0.07 and 0.09 and 0.06, 0.05 and 0.08 versus 0.09, 0.09 and 0.13) number of carbon atoms in the chain, of monounsaturated fatty acids of the omega-7 (0.05, 0.05 and 0.05 and 0.04, 0.04 and 0.04 versus 0.08, 0.07 and 0.07) and omega-9 (3.53, 3.31 and 3.12 and 3.36, 3.20 and 3.03 vs. 4.12, 4.00 and 3.78) and polyunsaturated fatty acids of omega-3 families (6.18, 6, 62 and 7.29 and 5.97, 6.42 and 6.98 against 7.61, 8.01 and 9.10) and about mega-6 (respectively 5.60, 6.10 and 5.79 and 5.46, 5.86 and 5.22 against 6.89, 7.55 and 7.12 g/kg raw weight).

In terms of energy, lipids are much more valuable than proteins and carbohydrates (Ruedenauer et al., 2021; Stabler et al., 2021). Literature sources indicate that the greater the number of fatty acids contained in tissues, the greater their energy value for the body of honey bees (Ruedenauer et al., 2021).

It was established (Tables 6, 7, and 8) that the most significant amount of esterified forms of saturated, monounsaturated, and polyunsaturated fatty acids are contained in the tissues of the abdomen, breast, and head of honey bees in the mountainous zone of the Carpathian region (respectively 21.72, 22.69 and 23, 17 g/kg of raw weight), a smaller amount of them is in the tissues of the abdomen, chest, and head of honey bees in the foothills (17.67, 18.57 and 18.33), and even less in the forest-steppe (respectively 16.98, 17, 69 and 17.31 g/kg raw weight).

Table 6. The content of esterified fatty acids in the abdominal tissues of honey bees in different natural zones of the Carpathian region at the beginning of the summer period, g/kg of raw mass (M ± m, n = 3)

Acid and its code	Natural zones of the Carpathian region		
	Mountain	Foothills	Forest-steppe
Caprylic acid, 8:0	0.08 ± 0.003	0.04 ± 0.003**	0.03 ± 0.003**
Capric acid, 10:0	0.05 ± 0.003	0.03 ± 0.003*	0.02 ± 0.003**
Lauric acid, 12:0	0.06 ± 0.003	0.04 ± 0.003*	0.03 ± 0.003**
Myristic acid, 14:0	0.07 ± 0.003	0.05 ± 0.003*	0.04 ± 0.003**
Pentadecanoic acid, 15:0	0.09 ± 0.003	0.07 ± 0.003*	0.06 ± 0.003**
Palmitic acid, 16:0	1.20 ± 0.028	0.93 ± 0.024**	0.92 ± 0.012***
Palmitoleic acid, 16:1	0.08 ± 0.003	0.05 ± 0.003*	0.04 ± 0.003**
Stearic acid, 18:0	1.30 ± 0.032	1.04 ± 0.045**	0.95 ± 0.024***
Oleic acid, 18:1	3.91 ± 0.085	3.36 ± 0.031**	3.21 ± 0.059**
Linoleic acid, 18:2	2.79 ± 0.074	2.28 ± 0.080**	2.23 ± 0.038**
Linolenic acid, 18:3	4.05 ± 0.113	3.31 ± 0.052**	3.20 ± 0.067**
Arachinic acid, 20:0	0.17 ± 0.007	0.11 ± 0.005**	0.10 ± 0.006**
Eicosaenoic acid, 20:1	0.21 ± 0.006	0.17 ± 0.003*	0.15 ± 0.003**
Eicosadienoic acid, 20:2	0.21 ± 0.009	0.17 ± 0.003*	0.15 ± 0.003**
Eicosatrienoic acid, 20:3	0.31 ± 0.009	0.23 ± 0.009**	0.21 ± 0.006**
Arachidonic acid, 20:4	3.18 ± 0.076	2.67 ± 0.035**	2.60 ± 0.032**
Eicosapentaenoic acid, 20:5	2.17 ± 0.071	1.74 ± 0.023**	1.71 ± 0.017**
Docosadienoic acid, 22:2	0.28 ± 0.012	0.21 ± 0.006**	0.19 ± 0.007**
Docosatrienoic acid, 22:3	0.32 ± 0.010	0.23 ± 0.006**	0.21 ± 0.006**
Docosatetraenoic acid, 22:4	0.32 ± 0.009	0.25 ± 0.006**	0.23 ± 0.007**
Docosapentaenoic acid, 22:5	0.53 ± 0.015	0.43 ± 0.006**	0.40 ± 0.007**
Docosahexaenoic acid, 22:6	0.54 ± 0.009	0.47 ± 0.015*	0.45 ± 0.007**

Table 7. The content of esterified fatty acids in the breast tissues of honey bees in different natural zones of the Carpathian region at the beginning of the summer period, g/kg of raw weight (M ± m, n = 3)

Acid and its code	Natural zones of the Carpathian region		
	Mountain	Foothills	Forest-steppe
Caprylic acid, 8:0	0.03 ± 0.003	0.02 ± 0.003*	0.01 ± 0.000**
Capric acid, 10:0	0.04 ± 0.003	0.02 ± 0.003*	0.01 ± 0.003**
Lauric acid, 12:0	0.05 ± 0.003	0.03 ± 0.003*	0.02 ± 0.006*
Myristic acid, 14:0	0.09 ± 0.003	0.06 ± 0.003**	0.04 ± 0.003***
Pentadecanoic acid, 15:0	0.09 ± 0.006	0.07 ± 0.003*	0.05 ± 0.003**
Palmitic acid, 16:0	1.29 ± 0.026	1.08 ± 0.022**	0.97 ± 0.098**
Palmitoleic acid, 16:1	0.07 ± 0.003	0.05 ± 0.003*	0.04 ± 0.003**
Stearic acid, 18:0	1.30 ± 0.032	1.02 ± 0.050**	0.97 ± 0.047**
Oleic acid, 18:1	3.74 ± 0.066	3.13 ± 0.073**	3.04 ± 0.084**
Linoleic acid, 18:2	2.95 ± 0.064	2.37 ± 0.049**	2.28 ± 0.052**
Linolenic acid, 18:3	3.58 ± 0.118	3.02 ± 0.058*	2.92 ± 0.04**
Arachinic acid, 20:0	0.17 ± 0.006	0.11 ± 0.006**	0.10 ± 0.006**
Eicosaenoic acid, 20:1	0.26 ± 0.014	0.18 ± 0.009**	0.16 ± 0.012**
Eicosadienoic acid, 20:2	0.28 ± 0.009	0.20 ± 0.015*	0.19 ± 0.015*
Eicosatrienoic acid, 20:3	0.21 ± 0.009	0.16 ± 0.006**	0.15 ± 0.006**
Arachidonic acid, 20:4	3.51 ± 0.082	3.00 ± 0.050**	2.80 ± 0.128**
Eicosapentaenoic acid, 20:5	2.43 ± 0.050	1.98 ± 0.041**	1.97 ± 0.044**
Docosadienoic acid, 22:2	0.23 ± 0.009	0.18 ± 0.007*	0.17 ± 0.007**

Docosatrienoic acid, 22:3	0.27 ± 0.012	0.19 ± 0.007**	0.17 ± 0.009**
Docosatetraenoic acid, 22:4	0.37 ± 0.014	0.29 ± 0.009**	0.27 ± 0.009**
Docosapentaenoic acid, 22:5	0.81 ± 0.034	0.67 ± 0.009*	0.63 ± 0.019*
Docosahexaenoic acid, 22:6	0.92 ± 0.029	0.76 ± 0.019**	0.73 ± 0.017**

Table 8. The content of esterified fatty acids in the tissues of the head of honey bees in different natural zones of the Carpathian region at the beginning of the summer period, g/kg of raw weight (M ± m, n = 3)

Acid and its code	Natural zones of the Carpathian region		
	Mountain	Foothills	Forest-steppe
Caprylic acid, 8:0	0.03 ± 0.003	0.02 ± 0.003*	0.01 ± 0.003*
Capric acid, 10:0	0.04 ± 0.003	0.02 ± 0.003*	0.01 ± 0.003**
Lauric acid, 12:0	0.05 ± 0.003	0.03 ± 0.003*	0.02 ± 0.003**
Myristic acid, 14:0	0.09 ± 0.003	0.06 ± 0.003**	0.05 ± 0.003**
Pentadecanoic acid, 15:0	0.13 ± 0.003	0.09 ± 0.003**	0.08 ± 0.007**
Palmitic acid, 16:0	1.52 ± 0.084	0.98 ± 0.030*	0.94 ± 0.029**
Palmitoleic acid, 16:1	0.07 ± 0.003	0.05 ± 0.003*	0.04 ± 0.003**
Stearic acid, 18:0	1.05 ± 0.038	0.86 ± 0.014*	0.83 ± 0.015**
Oleic acid, 18:1	3.54 ± 0.089	2.95 ± 0.045**	2.87 ± 0.026**
Linoleic acid, 18:2	2.77 ± 0.068	2.15 ± 0.036**	2.03 ± 0.037***
Linolenic acid, 18:3	3.54 ± 0.075	3.00 ± 0.054**	2.92 ± 0.046**
Arachinic acid, 20:0	0.18 ± 0.012	0.11 ± 0.007**	0.10 ± 0.007**
Eicosaenoic acid, 20:1	0.24 ± 0.012	0.17 ± 0.006**	0.16 ± 0.003**
Eicosadienoic acid, 20:2	0.19 ± 0.006	0.14 ± 0.006**	0.13 ± 0.006**
Eicosatrienoic acid, 20:3	0.13 ± 0.003	0.10 ± 0.003**	0.09 ± 0.003**
Arachidonic acid, 20:4	3.35 ± 0.072	2.85 ± 0.087*	2.44 ± 0.042***
Eicosapentaenoic acid, 20:5	2.47 ± 0.081	1.94 ± 0.058**	1.87 ± 0.037**
Docosadienoic acid, 22:2	0.22 ± 0.009	0.17 ± 0.006**	0.16 ± 0.006**
Docosatrienoic acid, 22:3	0.23 ± 0.006	0.18 ± 0.006**	0.16 ± 0.006**
Docosatetraenoic acid, 22:4	0.46 ± 0.014	0.38 ± 0.006**	0.37 ± 0.006**
Docosapentaenoic acid, 22:5	1.24 ± 0.038	0.91 ± 0.029**	0.86 ± 0.032**
Docosahexaenoic acid, 22:6	1.62 ± 0.058	1.26 ± 0.029**	1.17 ± 0.038**

Esterified forms of polyunsaturated fatty acids of the omega-3 and omega-6 families in the composition of phospholipids are included in the structure of cellular and cytoplasmic membranes of the body and ensure their functional activity and, ultimately, the vital activity of bees (Trinkl et al., 2020). At the same time, the esterified form of linoleic acid and its longer-chain and unsaturated fatty acids in the body of bees is the initiator of pro-inflammatory processes (Mărgăoan et al., 2021), and linoleic acid and its longer-chain and unsaturated fatty acids are anti-inflammatory (Ranneh et al., 2021). Esterified forms of linoleic and linolenic acids and their longer-chain and unsaturated fatty acids act on the bees' bodies through the corresponding pro-inflammatory and anti-inflammatory peptide cytokines (Ranneh et al., 2021).

It was recorded (Tables 6, 7, and 8) that the most significant amount of esterified forms of polyunsaturated fatty acids of the omega-3 and omega-6 families are contained in the tissues of the abdomen, breast, and head of honey bees of the mountain zone of the Carpathian region (respectively 7.61, 8.01 and 9.10 and 6.89, 7.55 and 7.55 g/kg of raw weight), a smaller number of them are in the tissues of the abdomen, chest, and head of honey bees in the foothill zone (6.18, 6.62 and 7, 29 and 5.60, 6.10 and 5.79), even less in the forest-steppe (respectively 5.97, 6.42 and 6.98 and 5.46, 5.86 and 5.22 g/kg raw weight). The mentioned esterified forms of polyunsaturated fatty acids of the omega-6 and omega-3 families in the exact quantities in bee tissues are initiators of pro-inflammatory and anti-inflammatory processes, respectively.

Esterified forms of polyunsaturated fatty acids linolenic and linoleic in the tissues of the bee body are primarily the precursors of a whole series of longer-chain and more unsaturated fatty acids, respectively, of the families of omega-3 (eicosapentaenoic, docosatrienoic, docosapentaenoic and docosahexaenoic) and omega-6 (eicosatrienoic, eicosatetraenoic-arachidonic, docosadiene and docosaterpene). Zinc takes an active part in the reactions of conversion of linoleic and linoleic acids into their longer-chain and more unsaturated derivatives in bee tissues, as it is part of 2-, 3-, 4-, 5-, and 6-desaturases (Di et al., 2020; Hsu et al., 2021). At the same time, the ratio of the content of esterified forms of linolenic and linoleic acids to the content of their longer-chain and unsaturated fatty acids of the omega-3 and omega-6 families, respectively, indicates the intensity of transformation of the less functionally and biologically active former into more active latter (Hsu et al., 2021).

It was established (Tables 6, 7, and 8) that the esterified form of linolenic and linoleic acids in the abdominal tissues of honey bees kept in the foothills and forest-steppe zones of the Carpathian region, compared to the mountain ones, are less wholly transformed into their longer-chain and unsaturated fatty acids of the families omega-3 (1.15 and 1.16 vs. 1.14, respectively) and omega-6 (0.69 and 0.69 vs. 0.68, respectively). The same direction of transformations of the esterified form of linolenic acid is observed in the breast tissues of honey bees (0.82 and 0.83 versus 0.81). At the same time, in the breast tissues of honey bees of the forest-steppe zone of the Carpathian region, compared to breast tissues of honey bees of the mountain zone, the intensity of conversion of the esterified form of linoleic acid into its longer-chain and unsaturated fatty acids of the omega-6 family is sharply reduced (0.64 vs. 0.60). A similar direction of transformations of the esterified form of linoleic acid is observed in the tissues of the head of honey bees of the foothill zone of the Carpathian region (0.64 versus 0.59). At the same time, in the tissues of the head of honey bees, which are kept in the foothills and especially in the forest-steppe zones of the Carpathian region, compared to the mountains, the intensity of the conversion of the esterified form of linolenic acid into its longer-chain and unsaturated fatty acids of the omega-3 family (respectively 0.70 and 0.72 vs. 0.64).

The above-mentioned indicates the negative influence of several heavy metals present, primarily toxic Lead and Cadmium, on the synthesis in the body tissues of honeybees of very active biologically and functionally longer chain and unsaturated fatty acids of the omega-3 and omega-6 families.

The low total content of esterified polyunsaturated and monounsaturated fatty acids of the omega-3, omega-6, omega-7, and omega-9 families can contribute to the increase in the fragility of bee comb walls through the bee body, in particular the wax glands (Didaras et al., 2020; Castaños et al., 2022). At the same time, the shallow content of the above fatty acids in the tissues of the abdomen, chest, and head of honeybees can cause a decrease in the permeability of its structural components to water and water-soluble substances and thereby inhibit the intensity of metabolic processes (Bakour et al., 2022; Castaños et al., 2022). It can also contribute to a decrease in the functional activity of cellular and cytoplasmic membranes of the body of honey bees and thereby inhibit their vital activity (Corby-Harris et al., 2021; Ricigliano et al., 2021; Wang et al., 2021).

It was established that the total content of esterified polyunsaturated and monounsaturated fatty acids of the omega-3, omega-6, omega-7, and omega-9 families in the tissues of the abdomen, breast, and head of honey bees obtained from hives located in the foothills (respectively 15.36, 16.08 and 16.25 g/kg of raw weight) and forest-steppe (14.83, 15.52 and 15.27) zones of the Carpathian region, compared to the tissues of the abdomen, breast, and head of honey bees selected from hives located in the mountain zone (18.70, 19.63 and 20.07 g/kg raw weight, respectively), is smaller (Tables 6, 7 and 8).

Saturated, monounsaturated, and polyunsaturated fatty acids caprylic, capric, lauric, myristic, palmitooleic, oleic, linoleic, linolenic, eicosaenic, eicosadiene, eicosatriene, eicosatetraenoic-arachidonic, eicosapentaenoic, docosadiene, docosatrienoic, docosatetraenoic, docosapentaenoic, and docosahexaenoic provide antibacterial protection of the body honey bees (Didaras et al., 2020; Rothman et al., 2020; Mărgăoan et al., 2021; Bakour et al., 2022). In particular, the high antibacterial activity of the above acids against bee rot was found (Rothman et al., 2020).

Caprylic and, to a lesser extent, capric and, to a lesser extent, lauric and, to a lesser extent, myristic acids have an antimicrobial effect due to their high ability to reduce the concentration of hydrogen ions (Didaras et al., 2020;

Rothman et al., 2020; Bakour et al., 2022), and palmitooleic, oleic, linoleic, linolenic, eicosaic, eicosadiene, eicosatriene, eicosatetraeno-arachidone, eicosapentaeno, docosadiene, docosatrieno, docosatetraenoic, docosapentaenoic, and docosahexaenoic - with the growth of this series, increase the surface activity of the tissues of microorganisms and thereby strongly suppress their vital activity under the normal osmotic pressure of the surrounding environment (Wang et al., 2021; Mărgăoan et al., 2021).

It was established that the total content of esterified caprylic, capric, lauric, myristic, palmitoleic, oleic, linoleic, linolenic, eicosenoic, eicosadienoic, eicosatrienoic, eicosatetraenoic-arachidonic, eicosapentaenoic, docosadienoic, docosatrienoic, docosatetraenoic, docosapentaenoic, and docosahexaenoic acids, which provide antibacterial protection of the bee organism, in the tissues of the abdomen, chest, and head of honey bees obtained from hives located in the foothills (respectively 15.36, 16.31 and 16.38 g/kg of raw mass) and forest-steppe (15.31, 15.60 and 15.36) zones of the Carpathian region, compared to the tissues of the abdomen, chest, and head of honey bees selected from hives located in the mountain zone (respectively 19.16, 19.84 and 20.28 g/kg of raw mass), there is less (Tables 5, 6 and 7). This significantly affects, as evidenced by the scientific literature (Didaras et al., 2020; Mărgăoan et al., 2021; Wang et al., 2021), the antibacterial and antifungal activity of the body tissues of honey bees.

Thus, as a result of an increase in the artificial load on the environment and the accumulation of heavy metals, primarily toxic, in the components of the ecosystem, the energy, structural, biological, and antimicrobial value of the central mass of long-chain fatty acids for the body of bees decreases.

The decrease in the content of esterified forms of fatty acids in the tissues of honey bees kept in hives located in the foothills and especially the forest-steppe zones of the Carpathian region, compared to the tissues of honey bees selected from hives located in the mountainous zone, is associated with their transition to anionic form. This is due primarily to the binding of fatty acids by heavy metal cations (Giri et al., 2018).

It was established that the honey productivity of worker bees in the mountainous zone of the Carpathian region is at the level of 46.6 ± 0.95 kg, in the foothills - 36.6 ± 1.04, $p<0.05$, and in the forest-steppe - 31.2 ± 0.56 kg, $p<0.01$ per bee colony per season. The honey productivity of worker bees decreases due to the high level of heavy metals, including toxic but low - esterified fatty acids, in the tissues. Other scientists also point to the negative influence of territories polluted with heavy metals on the productive characteristics of honey bees (Gizaw et al., 2020; Monchanin et al., 2021).

All over the world, there is a search for means of bioindication of the ecological state of the environment (Komarova, 2018; Costa et al., 2021). This is because heavy metals, like other environmental pollutants, have different transfer rates from the soil to the root system, from the root system to the stem, from the stem to the inflorescence, and from the inflorescence to pollen and nectar.

It was previously indicated (Saranchuk & Rivis, 2008) that *Taraxacum officinale* Wigg pollen can serve as a bioindicator of the ecological state of the environment in the conditions of the Carpathian region due to the optimal content of heavy metals and fatty acids. The positive thing about this bioindicator is that it allows to determine different levels of accumulation of heavy metals and fatty acids and thus gives more information. The body tissues of honey bees can also serve as a bioindicator of the ecological state of the environment in terms of the content of heavy metals and esterified forms of fatty acids.

CONCLUSIONS

The total content of the studied heavy metals in the tissues of honey bees in the foothills and forest-steppe zones of the Carpathian region, compared to the mountain zone, is 1.23-1.50 times higher. The level of dangerous elements of the first class of toxicity - Lead (by 1.33-4.00 times) and Cadmium (by 1.78-4.00 times) - is especially significant in the tissues of honey bees of the foothills and forest-steppe zones, compared to the conditionally clean mountain environment).

In the Carpathian region, all heavy metals accumulate 1.61-2.04 times more in the abdominal tissues of honey bees than in the breast and head tissues.

In the direction from the mountain to the foothills and further to the forest-steppe zone of the Carpathian region, a decrease in the content of esterified forms of fatty acids is observed in the tissues of honey bees (21.72-23.17, 17.67-18.57 and 16.98-17.69 g/kg raw mass).

Esterified forms of linolenic and linoleic acids in the abdominal tissues of honey bees kept in the foothills and forest-steppe zones of the Carpathian region, compared to the mountain ones, are less completely converted into their longer-chain and unsaturated fatty acids of the omega-3 families (respectively 1, 15 and 1.16 vs. 1.14) and omega-6 (0.69 and 0.69 vs. 0.68, respectively). At the same time, the intensity of conversion of the esterified form of linoleic acid into its longer chain and unsaturated derivatives of the omega-6. Also, the intensity of the conversion of the esterified form of linolenic acid into its longer-chain and unsaturated fatty acids of the omega-3 family in the tissues of the head of honey bees in the foothills and especially in the forest-steppe zones (0.70 and 0.72 vs. 0.64, respectively) is sharply reduced.

As a result of the accumulation of heavy metals in tissues, energy (by 21.0-44.6%), structural (21.2-45.7), biological (8.7-24.7), and antimicrobial (by 21.6-32.1%) values of esterified forms of fatty acids for the body of honey bees kept in hives located in the foothills and especially forest-steppe zones of the Carpathian region, compared to the tissues of bees kept in apiaries located in the mountainous zone.

The honey productivity of worker bees per beehive per season is lower in the foothills and forest-steppe zones of the Carpathian region, compared to the mountain, by 27.3% and 49.4%, respectively.

Body tissues of honey bees can serve as a bioindicator of the ecological state of the environment in terms of the content of heavy metals and esterified forms of fatty acids

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

THE INFLUENCE OF POLYPHENOLS OF GREEN WALNUT EXTRACT ON ZINC HOMEOSTASIS AND ITS ROLE IN THE ORGANISM OF BREEDING ROOSTERS

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Abstract

In this paper were analyzed the scientific bibliographic sources regarding the physiological and biochemical role of zinc on the tissues and organs of the organism of breeding roosters. In addition, in laboratory conditions, our researchers studied hematological, biochemical indices as well as glycine, glutamate and cysteine content in blood serum, seminal plasma and reproductive cells of breeding roosters. It is established that this element has a beneficial action on the organism of breeding roosters, maintaining and stabilizing the indices listed above at the level of physiological norms, compared to the roosters from the control group, and at the same time participating in the antioxidant protection of cells and maintaining cell homeostasis by balancing transmembrane metabolism.

Key words: *blood serum, cells, cell membranes, metabolism, zinc.*

INTRODUCTION

Being a necessary element for living organisms, zinc participates in all metabolic processes of the body, being a component of more than 7200 enzymes (Kimura & Kambe, 2016). One of the main roles of this element is the synthesis of protein and nucleic acids, at the same time stabilizing the structure of DNA, RNA and ribosomes, playing an important role in the translation process (Juravliova et al., 2007). It also has an important role in cell growth and division, participates in the stabilization and permeability of cellular and intracellular membranes, in membrane transport processes (Williams, 2012), the formation of antioxidant status as a protector of free radical reactions (Panasenko et al., 2018), possesses significant influence on the immune system (Daaboul et al., 2012) and apoptotic processes (Pang et al., 2013), osteogenesis, hemopoiesis, tissue respiration, growth, brain formation and its neurotransmitter function advocating as a neuromodulator and neuromediator (Li et al., 2001), reproduction and development of the fetus (Sheibak, 2015). In the cell zinc cations have a catalytic, structural and regulatory function. They catalyze the hydrolysis of peptides, proteins of some etheric substances and aldehydes (Salinikova, 2012). Zinc enhances the activity of osteoblasts and inhibits the activity of osteoclasts. In osteoblasts zinc can enhance cell proliferation, alkaline phosphatase activity and osteogenic effect (Hie & Tsukamoto, 2011).

Zinc plays an important role in the normal development of the testicles, as well as in spermatogenesis and possibly in the hormonal regulation of male reproductive function (Favier, 1992).

Zinc is a cofactor for more than 80 enzymes and is of great importance for the stability of such macromolecules as ribonucleic acid and DNA, as well as for protein synthesis, cell division and the stability of cell membranes (Favier, 1992). In addition, zinc is a component of superoxide dismutase, one of the key antioxidants.

The concentration of zinc in the male reproductive system significantly exceeds the same in other organs and tissues. Zinc is mainly eliminated by the prostate, but is also found in significant amounts in maturing spermatozoa, where its concentration is correlated with the level of oxygen consumption and the stability of nuclear chromatin (Kruczynski et al., 1985).

A series of scientific works prove to us that the use of antioxidants in the correction of male infertility is completely justified (Favier, 1992; Kruczynski et al., 1985). The fact that the effectiveness of many antioxidants has not been confirmed in all studies may indicate that their effects were not strong enough to be identified in these studies. There are regimes for monitoring the stable functioning of the reproductive system and the spermatogenesis process, which use various combinations of antioxidant substances and which, of course, in turn will allow to obtain a maximum effect of blocking reactive oxygen species.

With the discovery of the zinc-finger structural motif in proteins, the presence of the structural function of zinc was proven. Zn^{2+} cations regulate both the fermentative activity and the stability of proteins, acting at the same time as an activator ion or an inhibitory ion. The regulation of zinc accessibility in eukaryotes is primarily achieved by the compartmentalization of zinc and the functioning of the metallothionein/thionein system, which allows to control the zinc content in cells. The biological necessity of zinc is confirmed by the existence of homeostatic mechanisms which regulate its absorption, distribution, cellular needs and excretion (Gammoh & Rink, 2017).

In order to maintain an adequate homeostasis of zinc, a sufficient daily consumption is necessary, because compared to iron, the body lacks a specialized system for its deposition. The highest concentrations of zinc are found in muscles, bones, skin and liver (Mills, 2013). The required amount of zinc depends on age, sex, weight, direction of animal exploitation and food ration (Jeong & Eide, 2013).

Relatively, the richest nutrients in the zinc content of the food ration are bran, grists and food yeasts. Therefore, most food rations need to be evaluated in the content of the amount of polyphenols in the food ration of animals in order to obtain a more effective metabolism of micro- and macroelements.

Zinc deficiency can be caused by the following causes: unfermented plant ligands and some dietary fibers, which inhibit zinc absorption. Other factors, which influence the assimilation of zinc, are the ions of different metals. In particular, the absorption of zinc is inhibited by bivalent ions, such as Co^{+2}, Ni^{+2}, Cu^{+2}, Fe^{+2} and Cd^{+2}, while at the same time the ions of Mg^{+2} do not influence this process. A positive influence on the absorption of zinc are casein, histidine and methionine. Biological additives with zinc possess different bioavailability. Zinc bound with amino acids such as aspartate, cysteine and histidine have the highest absorption concentration, followed by chloride, sulfate and zinc acetate, while zinc oxide has the lowest bioavailability (Reiber et al., 2017).

Metallothioneins (MT) are cysteine-rich proteins that bind metal ions, particularly zinc and copper (Maret, 2000). There are four different classes of MT: MT-1 and MT-2 are spread throughout the organism; their basic function is to maintain cellular homeostasis of zinc and chelation of heavy metals. The expression of MT-3 and MT-4 is limited by the paternal cell type, where MT-3 the priority is detected in the brain, but MT-4 in the stratified epithelium (Kimura & Kambe, 2016; King, 2011).

The basic functions of metallothioneins in the body are related to the transport of metal ions, the maintenance of redox reactions and protective functions. Binding of heavy metals in various bioenergetic complexes takes place momentarily at the introduction of metals into the body by any method and regardless of concentration (Shafran et al., 2003). This bond has a dynamic character, that is, the metal "migrates" from complexes with a weaker resistance to complexes with a stronger resistance (Pihteeva, 2009). MT are reasonably considered to be proteins involved in the detoxification of essential and non-essential metals (Ruttkay-Nedecky et al., 2013).

A study by Formigari A. et al. pointed out the protective effects of zinc and the zinc-MT complex in oxidative stress induced by copper and iron and the apoptosis observed during it (Formigari et al., 2007).

In addition to metallothioneins there are other proteins that bind zinc and act as a system, ensuring the storage and control of zinc release. Albumin binds around 80% of the zinc in the plasma and is considered the basic transporter of zinc. The interaction between albumin and Zn^{+2} have a special importance for the supply of tissues and organs with this microelement. Albumin carries out the transporter of zinc absorbed in the liver, facilitates the consumption of Zn^{+2} by endothelial cells and erythrocytes. Complexes of zinc with albumin have increased kinetics of metabolism and facilitate the modulation of free zinc in plasma.

In any case, the increase in the content of fatty acids, observed in various metabolic disorders or pathological states of the organism, which can cause a hypoalbuminemia, causes a significant decrease in the binding and the stoichiometric ratio Zn^{+2}/albumin, but at the same time the zinc cations not influencing the stoichiometric binding of fatty acids (Sheibak, 2015; Lu et al., 2008). Also, among the zinc-binding proteins are alpha-2-macroglobulin, histidine-rich glycoprotein and protein family S100 (Gilston et al., 2016). Besides these proteins in the metabolism of zinc also participate some chemical elements and some vitamins, such as: copper, iron and folic acid.

It is scientifically proven that the ingestion of large amounts of zinc over a period of several weeks can interfere with the metabolic processes of copper bioavailability. Massive ingestions of Zn cause the intestinal synthesis of metallothionein, which in turn captures copper in the intestinal cells and prevents its systematic absorption. Balancing the food ration in the relative content of zinc does not affect the absorption of copper, as well as any deviations to increase the concentration of copper do not influence the absorption of zinc.

Excess iron can decrease the absorption of zinc, this can happen in pregnant sows or newborn piglets during the treatment with iron preparations of iron deficiency anemia, which is very common in these animals during the given period.

Large amounts of dietary calcium in the ration of animals decrease zinc absorption not only in mature animals, but also in youth.

The bioavailability of folic acid from food is increased by the action of a zinc-dependent enzyme, suggesting a possible interaction between zinc and folic acid. Older studies claimed that small doses of zinc decrease the absorption of folic acid and conversely large amounts of folic acid affect the amount of zinc taken up by the body.

Apart from this, numerous scientific researches have shown that zinc also has antioxidant properties (Rostan et al., 2002). So, it has been demonstrated that zinc diminishes the damage of cells and their genetic apparatus, as a result of the influence of ultraviolet irradiation and increases the resistance of skin fibroblasts to oxidative stress damages (Richard et al., 1993). To a certain extent, this effect is conditioned by the influence of zinc-containing enzymes and proteins that participate in the elimination of ROS (active oxygen radicals), in particular these are superoxide dismutases (SOD) and MT (Abel & de Reuiter, 1989), but the antioxidant potential of zinc is not limited to them. It is assumed that zinc can replace the metals themselves, which actively participate in the reactions of free radical formation (iron, copper), but alone does not participate in the redox reactions (Rostan et al., 2002). The given hypothesis is confirmed by observations, which prove to us, that the activity of SOD in those systems, in respect of which it is proved, that the protective influence of the action of zinc is insufficient to achieve such a level of protection against free radicals.

MATERIALS AND METHODS

To carry out the research in the study were included 10 breeding roosters, which were divided into two groups of five birds each (control group and experimental group). Roosters in the control group were administered *per os* hydro-alcoholic polyphenol extract from green walnuts in a dose of 1 ml with a total antioxidant activity of 1.7 g gallic acid equivalent per 100 gr. The extract was administered during two cycles of spermatogenesis. Hematological, biochemical indices and amino acid content in blood, seminal plasma and reproductive cells were studied.

RESULTS AND DISCUSSIONS

In Table 1 are presented the hematological indices of reproductive roosters included in the experiments to study the influence of zinc on the functional state of the immunoreactive blood components.

Table 1. Hematological indices of reproductive roosters included in experiments to study the influence of zinc on the functional status of immunoreactive blood components

Groups	Leukocyte, 10^9/L	Erythrocytes, 10^12/L	Hemoglobin, g/L	Hematocrit, %	Lymphocytes, 10^3/L	Monocytes, 10^3/L	Eosinophils, 10^3/L
Control	37.6±4.1	3.22±0.33	138.6±3.51	44.9±1.6	92±15.4	6.6±4.46	5.0±1.0
Experimental	41.9±3.62	3.64±0.32	151±2.0	49.5±1.73	86±14.0	5.0±1.73	3.0±1.0
Reference values	20-40	3.4	138	36.8	70.06	6.7	6.0

The supply of the organism with the necessary amounts of zinc is of great importance for the blood components of the immune system. Zinc influences the response of lymphocytes, mitogens and cytokines, serves as a cofactor of the thymus hormone - thymulin, participates in the transduction of leukocyte signals. In cell culture high concentrations of zinc in serum-free medium stimulate monocytes to secrete anti-inflammatory cytokines (Wellinghausen et al., 1996).

In the result of the research, it is observed that the leukocytes, also called white blood cells, under the influence of zinc concentration from the hydroalcoholic polyphenol extract from green walnuts have a higher mathematical and biological value compared to the control group, indicating the figures of 41.9±3.62, while in the control group indicating a value of 37.6±4.1.

Erythrocytes also play a very important and decisive role in the health of birds and animals, in that they carry fresh oxygen throughout the organism. These cells show a value of 3.22±0.33 (10^12/L) compared to 3.64±0.32 (10^12/L) in the experimental group, which testifies about a beneficial influence on blood components of zinc in combination with copper and by activating some fermentation systems.

Hemoglobin is the protein inside erythrocytes, and it is also the one that ensures the transport of oxygen throughout the body. In addition to transporting oxygen, erythrocytes also have the role of removing carbon dioxide from the organism, transporting it to the lungs, to be eliminated with the exhaled air.

The lymphocytes present in most vertebrates, being also a component of the immune system, responsible for the body's defense reactions and the production of antibodies against substances they consider foreign and are presented with the following values in the experimental group 86±14.0, compared to the control group with 92±15.4 10^3/L. An insignificant effect of zinc on the proliferation of lymphocytes is demonstrated in the presence of mitogens - phytohemagglutinin or concanavalin A. It has been shown that zinc has an immunoregulatory effect, that is, it reduced the response of lymphocytes at high doses and increased it at low doses (Faber et al., 2004).

Monocytes being involved in the defense against bacterial infections, maintain their values within the limit of 5.0±1.73 in the experimental group, compared to the control group with a value of 6.6±4.46 10^3/L.

Eosinophils are involved in several pathological processes, such as allergic, parasitic and neoplastic ones (cancer), they are considered destructive effector cells in the final stage that have a role in parasitic infections and allergic reactions by releasing acid hydrolases of the lysosomal type and histones. However, eosinophils are also multifunctional leukocytes involved in inflammatory and physiological immunity. Under homeostatic conditions, eosinophils are particularly abundant in their own lamina of the gastrointestinal tract, where they are involved in various biological processes within the gastrointestinal tract.

At the same time, the influence of zinc on the antioxidant and protein status was evaluated and researched. The results are presented in Table 2.

So as is known from research results and bibliographic data, superoxide dismutase (SOD) is the key enzyme of the antioxidant system, which manifests important protective roles against cellular and histological damage produced by ROS (reactive oxygen species).

Table 2. Influence of zinc on antioxidant and protein status

Groups	SOD, u/c (min/L)	G-GTP, u/L	Catalase, μM/L	G-S-T, nM/sL	Prot. tot., g/L
Experimental	127.5±0.4	10.8±0.66	29.2±0.82	22.0±0.51	68.0±0.31
Control	110.93±0.30	9.33±0.40	26.23±0.37	19.21±0.38	61.21±0.74

Superoxide dismutase is the antioxidant factor, which catalyzes the dismutation of superoxide anion into hydrogen peroxide and molecular oxygen, and prevents the formation of peroxynitrite and hydroxyl radical, which can damage tissue components. Taking into account the fact that, depending on the metal ion in the active center of the ferment, several SOD isoenzymes are highlighted, of which the greatest activity is possessed by Cu -, Zn-SOD. From the data of the table, we observe changes between the SOD content in the experimental group indicating a value of 127.5±0.4 u/c (min/L)compared to the control group with a value of 110.93±0.30 u/c (min/L), which testifies to the changes, which take place under the influence of zinc from extract of green walnuts.

Catalase is one of the key enzymes of the antioxidant system, which performs the function of antiperoxide protection in conditions of oxidative stress and the intensified formation of active forms of oxygen. This enzyme of major importance has the property of being produced when the organism needs it. From the obtained results we observe a difference between the values of the control group 26.23±0.37, compared to the experimental one which has a value of 29.2±0.82 μM/L.

Glutathione-S-transferases (GSTs) are a group of enzymes that are important in blocking many xenobiotics in living organisms. Enzymes protect cells against toxic substances by conjugating the thiol group of glutathione with electrophilic xenobiotics and therefore, defend cells against the mutagenic, carcinogenic and toxic effects of the compounds. GST activity has been shown to be present in plants, insects, yeast, bacteria and in most animal and human tissues, especially in the liver, which plays a key role in detoxification. From the obtained results we notice that this enzyme changes in the experimental group and has a value of 22.0±0.51 nM/sL compared to the control group with a value of 19.21±0.38 nM/sL. Total protein, represents the total sum of all serum proteins, which circulate in the vascular bed and part of the basic component of the blood, the main proteins being free albumin bound with zinc and globulin, which has firm bonds with zinc, in turn being an active element in more than 300 metallo-fermentative reactions, it has a decisive role in the synthesis and decomposition of nucleic acids and protein metabolism, nitrogen metabolism, as well as participates in antioxidant protection (Fredricks et al., 1960). From the research results, it can be observed that experimental data were obtained, which indicate a value for the control group of 61.21±0.74 g/L, and for the experimental group – 68.0±0.31 g/L, which demonstrates an influence of zinc on the amount of protein in the blood serum.

Glutathione is a tripeptide consisting of three amino acids: glycine, cysteine and glutamine, which participates in the synthesis of leukotrienes and is a cofactor of the enzyme glutathione peroxidase.

In these studies, amino acids, which are closely related to glutathione, were determined in the blood serum, seminal plasma and reproductive cells. The research results are presented in Table 3.

As follows, from the obtained results, it is observed, that glutathione is a valuable antioxidant and is produced by almost all cells of the organism. However, a higher concentration of amino acids is observed in the seminal plasma, which are dependent on glutathione, which in turn depends on the level of zinc in the body.

Table 3. The content of glycine, glutamate and cysteine in blood serum, seminal plasma and reproductive cells of roosters

Amino acids	Blood serum, (mcm/ 100 ml)	Seminal plasma, (mcm/ 100 ml)	Reproductive cells, (mcm/ 100 ml)
Cysteic acid	1.41±0.79	2.04±0.34	0.66±0.04
Glutamic acid	8.10±2.05	207.54±17.36	7.57±0.22
Glutamine	32.23±9.18	757.26±42.11	21.75±0.18
Glycine	46.47±2.27	37.56±2.01	8.88±0.08
Cysteine	1.84±0.34	9.76±0.97	1.56±0.07

CONCLUSIONS

Zinc is an indispensable microelement for the functioning of every cell in the human body. Zinc deficiency affects the processes of growth and development. The best way to prevent zinc deficiency is a well-balanced ration and supplemented with vegetable polyphenolic components to prevent zinc deficiency in cells and tissues of the body, also once obtaining a more efficient metabolism and detoxification of the body by stimulating fermentative reactions and other zinc-dependent processes in living organisms.

Studying and analyzing the research results, we notice that zinc is an element that participates in the antioxidant protection of cells and the maintenance of cell homeostasis by balancing transmembrane metabolism.

From the research carried out by researchers, it appears that it is necessary to monitor the level of zinc content in living organisms by balancing the food ration and maintaining an adequate metabolism, thus ensuring the organism a good functioning of all organs and systems.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Institute of Physiology and Sanocreatology and was financed from the Project 20.80009.7007.25 “Methods and procedures for maintenance and conservation of biodiversity depending on the integrity of gametogenesis and food variability”.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

IMMUNOGLOBULINE-G LEVEL AND BODY WEIGHT OF BALB/C RECEIVING COMBINATION TREATMENT OF LYOPHILIZED *Curcuma longa* AND *Curcuma xanthorrhiza* DURING AN ANGIOGENESIS EXPERIMENTAL

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Abstract

*Immunoglobuline synthesis is largely determined by the presence of the type of antigen that enters the body. However, various biological bioactive substances have a potential to boost individual immunity processes. In this study, 5 weeks old BALB/c was used. This study aims to evaluate the role of the combination of Curcuma xanthorrhiza and Curcuma longa lyophilisate on serum immunoglobuline-G level, angiogenesis process and BALB/c body weight. The bio-active substances were distrubuted through drink water. This study used a randomized complete design arranged with factorial 2*4. The results showed that treatment had no significant effect on IgG levels and the process of angiogenesis (P>0.05), but had a significant effect on body weight (P<0.05). We concluded that combination of Curcuma xanthorrhiza and Curcuma longa lyophilisate could be used in wound healing during an angiogenesis process.*

***Key words**: BALB/c, Curcuma, immunity, liopylisate.*

INTRODUCTION

Excellent health in animals is one of the key determinants of being able to produce and reproduce optimally. Health problems could disrupted a biochemical processes in tissues which negatively affected various body organs to carry out systemic physiological functions.

If an organ or part of the body is damaged, for example in the form of injury, especially to a muscle tissue, repairs needed to the cells of that tissue (Wilkinson et al., 2020; Marin et al., 2017) through the process of angiogenesis (Sorg et al., 2017; DiPietro et al. , 2016).

According to Li et al. (2003), angiogenesis at normal levels will play a role in cell regeneration to heal tissue damage in the wound area, especially open wounds on body surface which having a potential to become an 'entrance' for pathogenic microbes or infections (Leapar et al., 2015) so strategies are needed through appropriate treatment (Veith et al., 2019).

An individual's immune condition is very needed to protect body from a threat of microbes infectious (Yu et al., 2017; Cunningham, 2019; Goenka & Kollmann, 2015).

The individual immunity of young mammalian animals is highly dependent on the success of obtaining maternal antibodies contained in colostrum (Toar et al., 2019; Lewis et al., 2017; Hanson et al., 2007). This mechanism is made possible by the natural passive transfer process of antibodies (Rumokoy et al., 2017), becoming an important problem if this process fails (Staněk et al., 2019).

Mother colostrum antibodies contain many IgG antibodies which are widely used for human and animal health (Arslan et al., 2021; Stockler et al., 2021).

As time increases, day by day, the mammalian body will easily synthesized antibodies (Goenka & Kollmann, 2015). The level of ability to synthesize some types of antibodies depends on various factors such as environmental conditions (Amasawa et al., 2021) and nutritional intake, various bio-molecules that enter the body either through drinking water or through food as well as the type of antigens exposed to the body (Toar et al., 2021; Cuop et al., 2004).

Various scientific reports showe that various bio-molecules from plants have a significant role in helping body to synthesize antibodies (Weström et al., 2020; Rumokoy et al., 2016; Breijo et al., 2018; Toar and Rumokoy, 2021).

The *Curcuma longa* plant is a natural resource which is mainly known as a kitchen spice. Apart from that, the rhizomes of this plant are widely used in traditional medicine in various regions to help maintain body stamina in relation to endurance (Rosidi et al., 2014). Another type of plant that is similar to *C. longa* is *Curcuma xanthorrhiza* which was used for various health benefits. This substance has a great potential in controlling various threats of pathogenic microorganisms and parasites in traditional livestock farming which has recently received a lot of attention through research activities, including being able to control the synthesis of VEGF in overcoming angiogenesis abnormalities (Melincovici et al., 2018; Rumokoy et al., 2023).

MATERIALS AND METHODS

This study used 24 neonates of five weeks old experimental BALB/c mice; lyophilized extract of *Curcuma longa* and *Curcuma xanthorrizha*; rearing box with dimensions 30 cm in length, 20 cm in width and 20 cm in high, each cage was placed four mice.

The cages were equipped with a feeder and nippled drinking bottle. The others main materials used in this research were: measuring cup, petri-dish, micro-tube, micro-pippet, alcohol (70%), MDP digital scale max. 500 g; wide rolls of tissue; gel plate, radial immuno-diffusion plate, 0.1 M buffer solution pH 7, 0.1% nitric acid, 1 µg/ml amphotericin B, 0.002 M ethylene-diamide-etheric tetraacid and serum immunoglobulin for antibody analysis. AD1 of commercial feed (70%) and grounded yellow corn were mixed and used as mice feed to this experiment.

The variables observed were: total of serum immunoglobulin-G (IgG); tissue healing and body weight. The quantity of serum antibody total was detected through a single radial immuno-diffusion (SRID) test. The IgG blood sample serum was obtained by using a technic of tail blood collection.

This step of wound healing was evaluated during the angiogenesis which measured the percentage level of epidermal tissue recovered after a wound done purpose in tail base (TB) of mice.

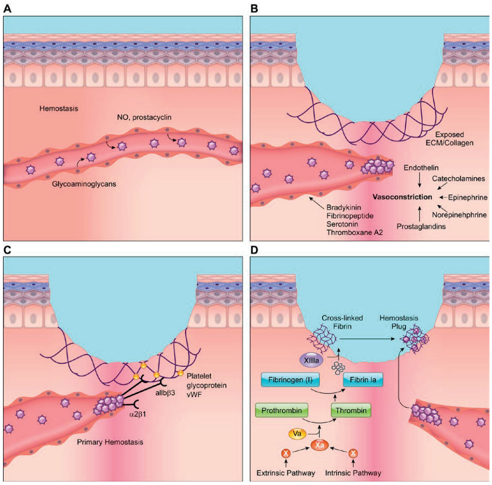
The final body weight of the experimental animals was measured after two weeks of experiment at seven weeks old.

The material of fresh *C. xanthorrhiza* (Cx) and *C. longa* (Cl) were purchased from local agriculture production then its rhizomes were selected and cleaned to get the best rhizomed.

The next steps were *broyage*, mixing, and then precipitation. Dry lyophilization were proccesed for about two weeks before using.

This study used a completely randomized design arranged by factorial A*B (2x4), factor A was the level of *C. longa* (A1 0 mg*L^{-1} and A2 5 mg*L^{-1}) and factor B was the level of *C. xanthorriza* as immuno-enhancer per litter of drinking water: B1 0 mg*L^{-1}; B2 5 mg*L^{-1}; B3 10 mg*L^{-1}; B4 15mg*L^{-1}

The data were statistical analysis to evaluate the significance effect of treatment by using SPSS software.



Source: Rodrigues et al. (2019)

Figure 1. The process in wound healing

RESULTS AND DISCUSSIONS

The results of this study were presented by the following figures.

Figure 2 presented the effect of combination combination of *C. longa* and *C. xanthorrhiza* on IgG serum level of BALB/c. We found an significant interaction in this experiment ($P<0.05$).

The treatment Cx15-Cl15 caused an highest effect on IgG serum level (172.75 mg IgG in 1 dl of blood serum) followed by effect of treatment of Cx-Cl15 that reached 181.25 IgG in blood serum while the lowest response shown in Cx0-Cl15 treatment obtained in 165 mg of IgG in a litter of serum.

These responses indicate the important role of these combination substances in supporting the body's defense against infection in its environment.

This effect could be caused by the role of immunoregulators and neuromodulators of the susbtances of curcumin in animals when exposed to pathogenic agents that lead to the immune system, especially in IgG synthesis as related to the scientific report of Szelényi (2001). Widjaja et al. (2022) has shown a modulator effect of curcumin on boosting the immunity of people received a COVID-19-vaccination.

A non significance effect of a combination treatment of lyophilized *C xanthorriza* and *C. longa* ($P>0.05$) on tissues healing of BALB/c shown in Figure 3. Tissue healing depends more on balanced nutritional intake (Timms, 2011). An epidermal growth factors, stem cell therapies could be an aliternative solution in tissues healing (Vyas and Vasconez, 2014). The steps of wound healing presented by Rodrigues et al. (2019) as show in Figure 1.

The highest response tended to dominate in the treatment of combination of Cx15-Cl10 (15 mg *C. xanthorrhiza* per 1 litter of drink water combined with 10 mg *C. longa* per drink water).

These results indicate that the application treatment level of bioactive substances in this study from the two types of curcuma does not have a significant role in the angiogenesis process for tissue repair.

Another possibility is that the size of the damaged tissue area in this experiment did not involved the function of the curcuma used.

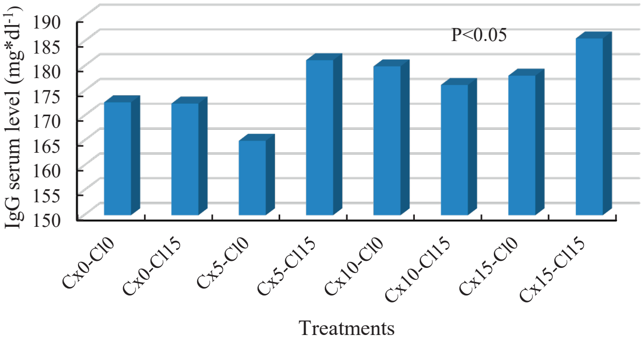


Figure 2. IgG Level of BALB/c

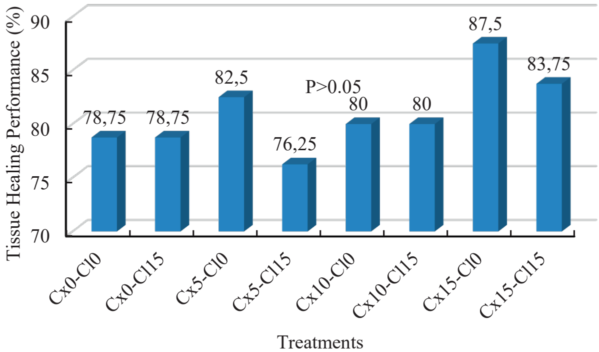


Figure 3. Treatment Effect on Tissues Healing

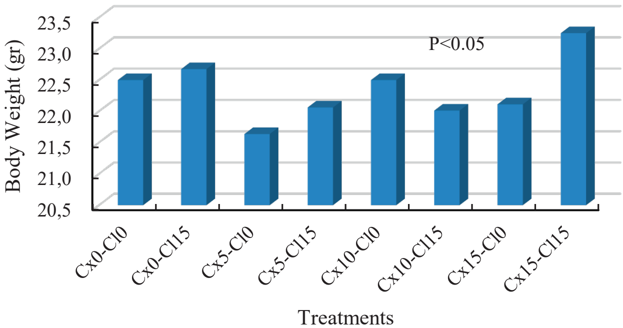


Figure 4. Treatment Effect on Body Weight of BALB/c

The Figure 4 represented significance effect of combination treatment of lyophilized *C. xanthorriza* and *C. longa* ($P<0.05$) on body weight of BALB/c after two weeks of treatment. The highest response was found in the treatment of combination of Cx15-Cl15 (15 mg *C. xanthorrhiza* per 1 L of drink water combined with 15 mg *C. longa*). This results related to the report Al-Sultan (2003) that an utilization 5% of *C. longa* in diets could increased a body weight. The curcuma contained antioxidant that has an important role for body protection to ensure biochemical processes in the body.

CONCLUSIONS

The treatment of combination substances of *Curcuma longa* and *Curcuma xanthorrhiza* has a positive effect on IgG antibody level and body eight gain in drink water of BALB/c.

ACKNOWLEDGEMENTS

The authors fully expressed they gratitude to Sam Ratulangi University: This *R_MAPALUS* realization was financed by BLU-PNBP 2023 - DIPA of Sam Ratulangi University *(No: 1398/UN12.13/LT/2023)* through LPPM Unsrat in Academic Year of 2023.

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TECHNOLOGIES OF ANIMAL HUSBANDRY

Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

RESEARCH ON EFFECT OF MILKINGS FREQUENCY ON COWS' MILK PRODUCTION

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Abstract

In the present socio-economic context and environmental sustainability trend it is a high accent on milk production related with increased milk yield per cow. This study aims to explore the influence of milking technology for Holstein dairy cows on "milk" productivity and quality, at the farm level. Over three consecutive years, the dynamics of milk quantity and its quality parameters (% fat, % protein, somatic cells count, % dry matter) were followed. The values of these parameters changed as a result of the increase of milkings frequency per day, from two in the first year of study, to three milkings per day in the following years. The results were statistically analyzed, from the point of view of significance, using the Fisher and Student tests. The comparative analysis of the 3 years of production shows that milk production had a positive evolution, both in terms of quantity and quality.

Key words: *dairy cow, Holstein, milking technology, qualitative milk parameters.*

INTRODUCTION

Animals belonging to the taurine species hold the first place among the species bred in Romania, with particular importance due to their growth and exploitation technology and productions performances and the social and economic impact, in the context of Romania's integration into the European Union.

Dairy cows use in the most profitable way the consumed feed, which gives an advantage for their growth. It is 5-10 times more profitable to produce milk than to produce meat.

It is essential that the production capacity of the animals, the financial aspect and the elements of organizational management should be taken into account in the breeding technology of dairy cows at the current level (Vidu, 2006).

These aspects must be transferred into breeding techniques applied in farms in order to achieve large milk productions at a low cost. Everything depends on the good understanding of this economic sector and the mastery of the knowledge intended in breeding animals for milk (Maciuc, 2006; Maciuc et al., 2015; Huțu et al., 2020).

The importance of knowing the level of milk production is due to the fact that it is the main indicator for assessing cows from dairy breeds and those with mixed production abilities.

Setting out the productive capacity of the animals allows their ranking in order to create the selection groups, by identifying and nominating cow candidates for the status of bull mothers (Alexoiu & Roșu, 1988; Dinescu & Ștefănescu, 1997; Vintilă & Dronca, 2000; Velea & Mărginean, 2004).

Holstein cows have very good skills for both milk production and meat production.

The Holstein-Friesian breed has a good precocity, the age of morphological maturity being around 4 years old. The young females are admitted to reproduction at the age of 13-14 months, once the optimal weight for insemination is reached (380-400 kg live weight). The maximum milk production is recorded at the fourth lactation, and the average period of exploitation is approximately 3-4 lactations, with the production level being high throughout the duration of exploitation. Animals have docile behaviour.

The specific feed consumption is 0.9 - 1 UN/kg of milk, which makes the economy of the breed highly appreciated, and the milk index (somato-productive index) to be over 1/8.

Improper growing and exploitation conditions and lacks in the feeding and breeding of Holstein cows lead to a decrease of the productive performance, increasing the specific consumption of food and the risk of disease manifestation (Diaconescu & Nicolae, 2012).

In the present socio-economic context and environmental sustainability trend it is a high accent on milk production related with increased milk yield per cow.

That is why it is important to measure milk productions and carry out qualitative analyzes of these productions, so that the improvement of these parameters can be achieved,

This requirement can be obtained by reducing the number of animals in the farms, by improving the breeding and exploitation conditions of animals and by applying a suitable milking technology, in order to maintain the health of the animals and the udder.

MATERIALS AND METHODS

Over three consecutive years (2018-2020), the dynamics of milk quantity and its quality parameters (% fat, % protein, somatic cells count, % dry matter) were followed, depending on the milking technology applied, changing from two milkings per day in 2018, to three milkings per day in the following years (2019, 2020).

The biological material studied was represented by lactating cows, whose milk production was subject to the Official Performance Control. The size of the statistically analysed samples was given by the number of controls performed annually (1283 controls in 2018, 2011 in 2019 and 1696 in 2020). The collected milk samples were analysed, from a qualitative point of view, with the help of the Milkoscan analyser. The results of the quantitative and qualitative parameters of the farm's milk production, obtained during the research period, were statistically analysed. The programs used were Microsoft Excell (Office 2010) and SPSS Statistics 20.0 for Windows.

In order to obtain valid results from a statistical point of view and to be able to say accurately whether or not there are significant differences regarding the amount of milk and qualitative parameters during the analysed period, the Student and Fisher statistical tests were used.

The Student test was calculated according to the following formula (Sandu, 1995):

$$\hat{t} = \frac{\bar{X}_1 \cdot \bar{X}_2}{\sqrt{\dfrac{(\sum X_1^2 + \sum X_1^2) \cdot (n_1 + n_2)}{(n_1 + n_2 - 2) \cdot (n_1 \cdot n_2)}}}$$

Also, for the Fisher test, the analysis of variance (ANOVA) was carried out with two sources of variation, in order to establish whether or not there were significant differences in the performances achieved between the groups of animals (Table 1) (Grosu, 2022).

Table 1. ANOVA (Analysis of Variance)

Source of variation (SV)	Between groups (I)	Within groups (i)	Total
Degrees of freedom (DF)	$DF_I = p - 1$	$DF_i = N - p$	$DF_T = N - 1$
Sum of squares (SS)	$SS_I = \sum C - CT$	$SS_i = \sum\sum X^2 - \sum C$	$SS_T = \sum\sum X^2 - CT$
Mean of squares (MS)	$MS_I = SS_I/DF_I$	$MS_i = SS_i/DF_i$	
Fisher	$\hat{F} = MS_I/MS_i$		

where:

p - number of groups;
N – total number of animals;
DF – degrees of freedom;
SS – sum of squares;
MS – mean of squares;
∑C – sum of corrections;
TC – total correction;
∑∑X2 – the sum of the squares of the values.

RESULTS AND DISCUSSIONS

1. Dynamics of milk production and quality parameters in the period 2018-2020

The data obtained through the Official Performance Control, for some of the milk quality parameters (fat percentage, protein percentage, number of somatic cells and dry matter), were subjected to primary analysis. The data taken and the units of measurement are according to the milk analyzer, Milkoscan (Table 2 and Figures 1-5).

Table 2. Descriptive statistics for the qualitative and quantitative parameters of milk production in 2018-2020 period

Specification	2018	2019	2020
Number of samples	1283	2011	1696
Milk (l/day/head)	24.65±0.16	26.98±0.157	29.10±0.13
Fat (%)	3.95±0.02	4.01±0. 018	4.32±0.01
Protein (%)	3.30±0.01	3.48±0. 010	3.41±0.01
Somatic Cells Count (x 1000/ml)	57.99±1.21	61.21±1.003	68.32±1.24
Dry matter % (m/m)	12.81±0.03	13.10±0.024	13.23±0.02

Figure 1 shows the ascending evolution of the amount of milk from one year to another. The amount of milk is higher in 2019 (26.98 l/day/head) compared to 2018 (24.65 l/day/head). In 2020 the average amount of milk/day/head of cow (29.10 l/day/head) is higher both compared to 2018 and compared to 2019. This is due to the change from 2 milkings/day in 2018 to 3 milkings/day in 2019, respectively 2020.

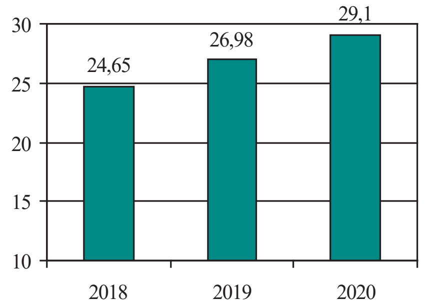

Figure 1. The dynamics of milk quantity in 2018-2020 period (l/day/head)

In the farm, the animals were selected with the aim of obtaining a new generation with a higher productive potential, and the feeding of the animals was constantly evolving and updating, which also led to an increase in milk production

It is very important to mention that the percentage of fat has also increased. The evolution of the fat percentage registers a continuous progress, thus in 2020 is observed the highest increase, due to the good quality of the volume feed, which indicates that the milk is not only in a high quantity, but also with a better quality (Figure 2).

The evolution of the protein percentage is different, the highest value being registered in 2019, then a decrease occurs in 2020 (Figure 3). This can be attributed to the fact that the two parameters, milk fat and protein, are inversely proportional, so that with an increase in the percentage of fat, there is a decrease in that of protein.

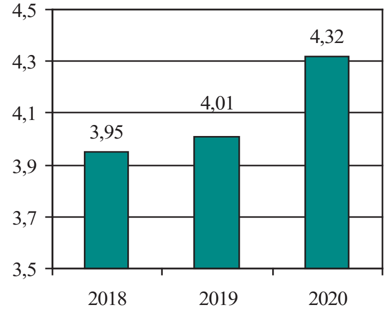

Figure 2. The dynamics of fat percentage per years

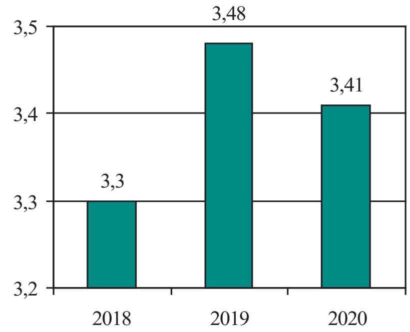

Figure 3. The dynamics of the protein percentage per years

The percentage of protein and the percentage of fat are parameters with high importance. It can be seen that the fat/protein ratio is normal, both for the 2018/2019 and 2019/2020 comparisons, and as a percentage, both characters are within the limits of the breed.

Another analysed parameter was the somatic cell count, which has major implications in udder health. Its average values show that the udder health of the studied animals is good (Figure 4).

An ascending evolution of the somatic cells count can be observed from year to year, but this fact can be attributed to the reaching of the cow's productive maturity, and the values remained within normal limits.

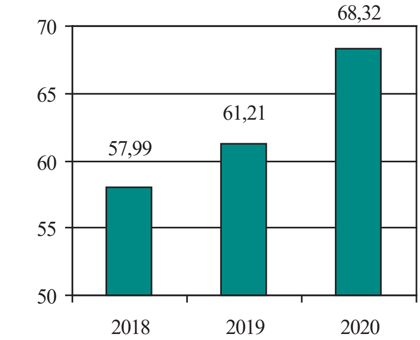

Figure 4. The dynamics of SCC per years

The increase of the dry matter percentage, as can be seen from the graphic representation, is higher from one year to another, due to the increase in the amount of milk (Figure 5).

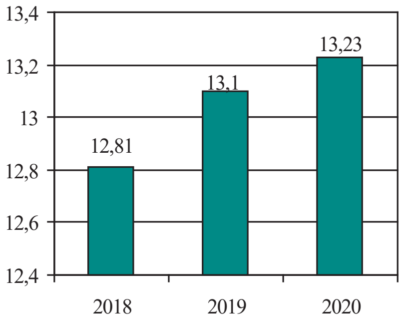

Figure 5. The dynamics of the percentage of dry matter per years

The comparative analysis of the 3 years of production shows that from one year to another, the Holstein cows' herd had a positive evolution, which was seen both in the quantity of milk and in its quality.

2. Testing the results for significance of differences

In the case of experimental research, there were compared the recorded performances throughout the analysed period, 2018-2020, using the Fisher and Student test.

In order to determine whether or not there were significant differences in milk quantity from year to year for the period 2018-2020, there was calculated Fisher's test with analysis of variance.

In Table 3 it can be observed that the calculated Fisher value is much higher than the tabulated Fisher value for the corresponding degrees of freedom. Thus, analysing the value of P, it can be concluded that for the amount of milk there are very significant differences between the 3 years of production, for Holstein cows.

Table 3. Fisher's test by Analysis of Variance (ANOVA) for homogeneity of variances in milk quantity

Source of variation	DF	SS	MS	$\hat{F}$ calculated	P value	$\hat{F}$ tabulated
Between groups	2	14566.92	7283.46	191.70	0.001	2.99
Within groups	4987	189474.10	37.99	-	-	-
Total	4989	204041	-	-	-	-

DF – degrees of freedom;
SS – sum of squares;
MS – mean of squares.

Because the Fisher test provides information only at general level, that is there are significant differences between the 3 years of production, further the Student test was applied to check if the differences are only for certain years of production (Table 4).

Table 4. Student test for milk quantity

Specification	2018/2019	2018/2020	2019/2020
t-calculated	10.45	21.68	10.43
t-table	1.96	1.96	1.96
p value	0.001	0.001	0.001

Thus, all possible combinations between the 3 years were taken into account in order to establish if there are statistical differences between them.

As expected, and supported by the Student's test, it can be observed that P values are all adequated to the highest degree of significance, namely that there are very significant differences for all combinations of the 3 years of production.

Following, all 4 qualitative milk parameters were analysed and, as it is shown in Table 5, for fat percentage the differences are very significant (p - 0.001).

Table 5. Fisher's test by Analysis of Variance (ANOVA) for homogeneity of variances for % of milk fat

Source of variation	DF	SS	MS	$\hat{F}$ calculated	P value	$\hat{F}$ tabulated
Between groups	2	136.30	68.15	113.70	0.001	2.99
Within groups	4987	2988.90	0.59	-	-	-
Total	4989	3125.25	-	-	-	-

DF – degrees of freedom;
SS – sum of squares;
MS – mean of squares.

When one year was compared with another, using the Student's test, it was found that for the combination between 2018 and 2019 the differences were distinctly significant (p - 0.02), and for the other two combinations, 2018 with 2020 and 2019 with 2020, the differences were very significant (p - 0.001) (Table 6).

Table 6. Student's test for milk fat values

Specification	2018/2019	2018/2020	2019/2020
t-calculated	2.20	13.77	12.29
t-table	1.96	1.96	1.96
p value	0.02	0.001	0.001

Analysing the protein percentage from the variances homogeneity point of view, between the years 2018, 2019 and 2020, it can be found that, as in the case of the fat percentage, the differences also show that they are very significant (p - 0.001) (Table 7).

Table 7. Fisher's test by Analysis of Variance (ANOVA) for homogeneity of variances for % of milk protein

Source of variation	DF	SS	MS	$\hat{F}$ calculated	P value	$\hat{F}$ tabulated
Between groups	2	25.32	12.66	67.91	0.001	2.99
Within groups	4987	929.60	0.18	-	-	-
Total	4989	954.92	-	-	-	-

DF – degrees of freedom;
SS – sum of squares;
MS – mean of squares;

In Table 8 it can be observed that when the years are analysed in groups of two, there is a slight change in the relevance of milk protein.

Table 8. Student's t test for milk protein values

Specification	2018/2019	2018/2020	2019/2020
t-calculated	11.97	7.86	3.92
t-table	1.96	1.96	1.96
p value	0.001	0.01	0.01

Thus, for the percentage of protein, the Student test shows that there are very significant differences when we compare the years 2018 with 2019. When comparing 2018 with 2020 and 2019 with 2020, the differences are only distinctly significant.

As a general conclusion, there are statistically significant differences between the 3 years of production, regardless of how they are evaluated.

Table 9 shows that for the parameter with major importance in udder health (somatic cells count - SCC) there are very significant differences between the 3 years in which Holstein cows were analysed and evaluated.

Table 9. Fisher's test by Analysis of Variance (ANOVA) for checking homogeneity of variances for SCC

Source of variation	DF	SS	MS	$\hat{F}$ calculated	P value	$\hat{F}$ tabulated
Between groups	2	86527.59	43263.79	19.67	0.001	2.99
Within groups	4987	10963623	2198.44	-	-	-
Total	4989	11050151	-	-	-	-

DF – degrees of freedom;
SS – sum of squares;
MS – mean of squares.

Although the Fisher test, through the analysis of variance, showed that there are very significant statistical differences between the values of the somatic cells count from one year to another, when the evaluation is done by groups (Table 10), the differences are distinctly significant (with a p value between 0.01 and 0.04).

Table 10. Student's t-test for SCC

Specification	2018/2019	2018/2020	2019/2020
t-calculated	2.04	5.94	4.43
t-table	1.96	1.96	1.96
p value	0.04	0.01	0.01

The last character analysed (dry matter, %), but not the last one in terms of importance, shows that when the 3 years are analysed at the same time, the statistical differences are very significant (Table 11).

The data obtained after the statistical analysis by comparing the years two by two, show that for the combination of 2018-2019 and that of 2019-2020, the differences were distinctly significant, and for the years 2018-2020 very significant statistical differences resulted (Table 12).

Table 11. Fisher's test by Analysis of Variance (ANOVA) for checking homogeneity of variants for % of milk dry matter

Source of variation	DF	SS	MS	$\hat{F}$ calculated	P value	$\hat{F}$ tabulated
Between groups	2	133.86	66.93	61.06	0.001	2.99
Within groups	4987	5465.98	1.09	-	-	-
Total	4989	5599.85	-	-	-	-

DF – degrees of freedom;
SS – sum of squares;
MS – mean of squares.

Table 12. Student's t-test for % milk dry matter

Specification	2018/2019	2018/2020	2019/2020
t-calculated	7.79	11.13	3.85
t-table	1.96	1.96	1.96
p value	0.01	0.001	0.01

From the analysed data, related to the quantity and quality parameters of milk, it can be observed that the measures and decisions that were taken every year at the farm level, in terms of selection, nutrition and management, led to significant changes in milk quality.

CONCLUSIONS

Milk production increased significantly by performing three milking per day, compared to the milking technology based on two milking per day.

All quality parameters taken into account (fat percentage, protein percentage, dry matter percentage and somatic cells count) of the milk have improved. Even if there are increases in the values of somatic cells count, this fact has nothing to do with the health of the udder, but is correlated with reaching the productivity maturity of the cows.

Milk production, both quantitative and qualitative, is critically influenced by the genetic value of the animals and the feeding technology, but also by the milking technology, which can be improved (udder preparation, daily number of milkings, interval between milkings etc.).

The transition to three milking per day had a positive impact both on milk production and on the animals in general. In order to increase milk production and a good state of health of the animals, it is recommended to continue milking the dairy cows three times a day, thus ensuring appropriate conditions for breeding and exploitation.

ACKNOWLEDGEMENTS

This research work was carried out with the support of the Faculty of Animal Productions Engineering and Management, University of Agronomic Sciences and Veterinary Medicine of Bucharest, and is part of the elaboration of the doctoral thesis

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

INVESTIGATIONS CONCERNING THE EXCRETION OF ANTIBIOTIC RESIDUES IN THE MILK OF COWS TREATED WITH ANTIBIOTICS

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Abstract

The somatic cell count in the mixed milk of the 4 quarters has dropped from 1.155 million to 200 thousand within 120 hours of the last treatment. In cow 5390 somatic cell count tends to decrease after 48 hours of treatment and this can be interpreted as a success of treatment. However, 72 hours after treatment, the somatic cell count increases again, in the affected quarter causing an increase in the somatic cell count in the mixed milk of the 4 breast quarters from 565 thousand to 812 thousand somatic cells. The amount of residue excreted via milk as a percentage of the total amount applied was from 5.2% to 45.3%. In the group of cows milked 1.5 times a day, the percentage of residue excreted via milk was 17.75% compared to 27.51% in the group of cows with two milkings per day. The average milk yield of the group of cows milked twice a day was 23.55 ± 4.8 kg standard deviation and in the group of cows milked 1.5 times a day was 26.4 ± 2.59 kg.

Key words*: antibiotic, milked, somatic cells, udder.*

INTRODUCTION

The presence of antimicrobial substances in raw milk may have toxicological consequences (Dewdney et al., 1991; Currie et al., 1998; Cola et al., 2022), but also technological consequences (Molina et al., 2003).

Ideal tests are those that give positive responses as close as possible to the “Maximum Residue Limit” (MRL), defined as levels of interest. Tests giving positive results at much higher MRL are questionable. Tests giving positive results below the MRL require an excessive number of samples necessary for confirmation.

There are several screening tests. These tests were evaluated under various experimental conditions (Seymour et al., 1988; Bachmann et al., 2019; Andrew, 2000; Anika et al., 2019).

Andrew (2001) reported false-positive results of some screening tests, tests in which milk from individual cows was used. False-positive results represent losses for producers (milk may be rejected from consumption). In contrast, Bachmann et al. (2019) reported a lower incidence of false-positive results in three out of four screening tests evaluated.

The screening tests were accepted, because they met the standards for the low incidence of false-positive results but also of false-negative results (FDA, 1996). Along with these standards, there are also a few principles that must be taken into account. Those are:

- a positive result of a screening test is a presumption that the analyte (antibiotic residue) is present in the milk sample;
- the screening test does not identify a specific analyte or measure it quantitatively;
- the accepted screening tests must give positive results when the antibiotic concentration is below the safety/tolerance level; this is a false tolerance result and not a false-positive result;
- screening tests are the fastest tests for detecting residues of antibiotics in milk.

The microbial growth inhibition test uses standard cultures to test the growth of a micro-organism (e.g. *Bacillus stearotermophilus*) in solid or liquid medium. The milk sample is added to the surface of the agar and left in the medium, and if the sample contains inhibiting agents, the growth of the micro-organism is completely reduced or inhibited (Navratilova, 2008).

Microbial growth inhibition tests differ according to the type of micro-organism used, the duration of incubation and the temperature and detection levels of the residues analysed (Currie et al., 1998).

The most used commercial tests with spores of *Bacillus stearotermophilus var calidolactis* are:

Delvotest SP (DSM, Netherlands), Copan test (Copan, Italia), Charm Farm 960 Test (Charm Sciences, Inc. USA).

Tests with *Streptococcus thermophiulus* are: Valio T 101-test, Valio T 102 - test (Valio, Finland).

The most used enzyme tests are: Penzym and Penzym S (UCB Bioproducts, Belgium), and among the most used immunological tests are: Delvo- X Press β-lactum (DSM Netherlands), β star (UCB Bioproducts, Belgium), Rosa test (Charm Science, Inc USA).

Sykorova et al. (2012) compare the sensitivity of the detection of five assays for the assessment of milk aminoglycosides (gentamicin, neomycin, streptomycin, kanamycin, and spectinomycin). The sensitivity of these assays was evaluated based on the experimental determination of the detection limits (LOD). The detection limits for the STAR assay were MRL for neomycin (1.5 μg/g), gentamicin (0.10 μg/g), streptomycin (0.20 μg/g) and kanamycin (0.15 μg/g). Spectinomycin (0.20) was not detected at MRL level. Modern biotechnologies and genetic engineering represent promising solutions in the near future (Bonciu, 2020) and the discovery and application of these solutions will remain valid for the situations created by the emerging pathologies in the animal cell determined by bacteria with significant potential for the development of antibiotic resistance. On the other hand, livestock production forms an integral part of the units that practice organic farming and must contribute to the balance of agricultural production systems (Bonciu, 2022a). The management of ecological animal growth forbids the use of antibiotics, with some exceptions (Bonciu, 2022b).

MATERIALS AND METHODS

Investigations regarding the excretion of antibiotic residues in cows with clinical mastitis were made at S.C. Fenov S.R.L.

Parameters used for the characterisation of cows: registration number of the cows; lactation number; days after calving; daily average production during experiments; body weight; somatic cell count in cow's milk at the onset of the mastitis; severity of clinical symptoms (1 = slightly modified milk with small and large clots; 2 = modified milk with large clots; 3 = abnormal milk; 4 = abnormal milk plus body temperature above 39.5ºC); number of quarters with subclinical mastitis; success of treatment: somatic cells below 200,000/ml at the end of the experimental period.

Milking frequency: twice a day: at 06:00 and 17:00, interval between milkings of 11 hours and 13 hours, 8 cases;

1.5 times a day: at 05:00 and 21:00 every other day and at 13:00 the next day, interval between milkings of 16 hours.

Milk sample collection

The milk samples were collected daily for the experimental period as follows:

- milk samples for each quarter of the udder;
- milk samples per cow (mixed milk from the 4 quarters).

The milk samples were kept in the refrigerator (6ºC) for a maximum of 30 hours.

Medicines used

Intramammary treatments with Cobactan LC, Cefquinome 75 mg. One syringe has 8 grams and the treatment scheme was of 3 successive treatments after each milking in the affected quarter.

In order to apply the same amount of antibiotic substance over the period of time, the following scheme was applied (Table 1).

Table 1. Treatment scheme

Mastitis detection	Two milkings per day		1.5 milkings per day	
	Morning	Evening	Morning	Evening
Anamnesis	Day 1 06:00	Day 1 17:00	Day 1 06:00	Day 1 17:30
Treatments three times in 24 hours	Day 1 06:00 Day 1 17:00 Day 2 06:00	Day 1 17:00 Day 2 06:00 Day 2 17:00	Day 1 06:00 Day 1 14:30 Day 2 06:00	Day 1 17:30 Day 2 03:30 Day 2 15:00
Further milkings	06:00 17:00	17:00 06:00	21:00 13:00 05:00	05:00 21:00 13:00

Methods of analysis

1. Udder health: the somatic cell count was determined by the fluoro-opto-electronic method with the help of the SOMASCOPE MK II counter.

2. Antibiotic residue detection and quantification: identification was carried out using the EKOTEST method (EON

TRADING-USA); quantification of antibiotic residues was done by disc method.
3. Setting the waiting time: time when safety concentration is reached. For this purpose, a tolerance limit is calculated for a number of milkings per animal. This limit is the time taken for the residue concentration in milk, in the vast majority of animals, to reach safety levels (MRL).
Variance analysis: variance was used to determine which factors had a systematic influence on the waiting time.

RESULTS AND DISCUSSIONS

The status of the animals included in the experiment is presented in Table 2.
Figures 1, 2 and 3 show the evolution of somatic cell count on each quarter of the breast and on the entire mammary gland.
In Figure 1, the treatment had an effect, and the somatic cell count decreased in the milk of the affected (left anterior) breast quarter from 4.5 million to 710 thousand at the end of the 120-hour waiting period after the last treatment. The somatic cell count in the mixed milk of the 4 quarters has dropped from 1.155 million to 200 thousand within 120 hours of the last treatment.
In cow 5390 (Figure 2) the somatic cell count tends to decrease after 48 hours after treatment and this can be interpreted as a success of treatment.
However, 72 hours after treatment, the somatic cell count increases again, in the affected quarter causing an increase in the somatic cell count in the mixed milk of the 4 breast quarters from 565 thousand to 812 thousand somatic cells (Figure 3), unsuccessful treatment.

Table 2. Status of the cows included in the experiment

No.	Registration number (No. of cases)	Lactation no.	Days after calving	Kg milk/day (kg)	Body weight (kg)	Quarter affected	Somatic cell count/cow x1000	Severity	Extra infected quarters	Treatment success (somatic cells below 100,000)
Cows with two milkings per day										
1	4091	3	200	22.8	652	Left anterior	800	1	1	Yes
2	5353	3	240	14,6	664	Left anterior	2100	3	1	No
3	5354	2	180	23,2	598	Right anterior	1022	2	0	Yes
4	5362	2	244	18,8	609	Right posterior	3780	3	2	Yes
5	5378	2	68	27,8	710	Left anterior	4520	2	1	Yes
6	5390	1	109	28,4	596	Left anterior	5100	4	1	No
7	5400	1	6	26,6	620	Right posterior	982	1	0	Yes
8	5830	1	66	26,2	634	Left posterior	2660	2	0	Yes
Cows with 1.5 milkings per day										
1	2231	4	1	28.8	712	Left anterior	1612	2	1	Yes
2	5851	1	82	27.6	688	Right anterior	3812	3	0	Yes
3	2239	2	61	26.4	708	Left posterior	6012	4	1	No
4	5861	1	128	22.8	640	Right anterior	1654	2	0	Yes

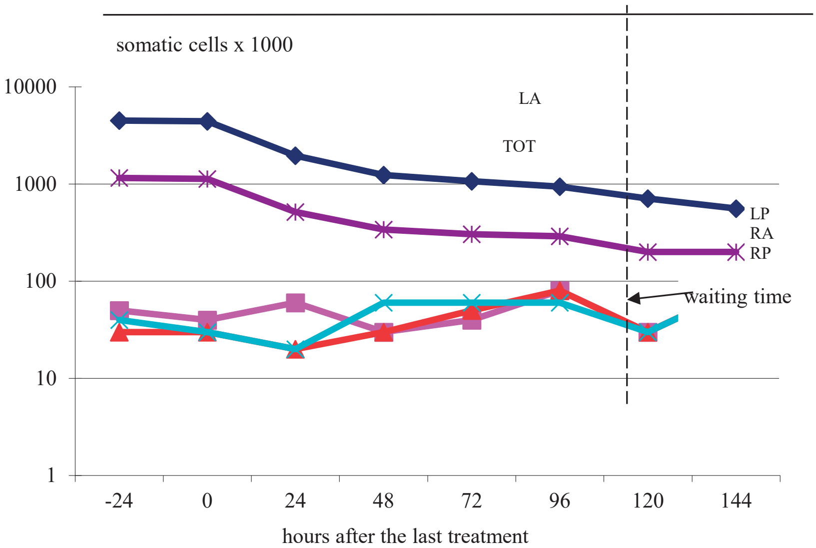

Figure 1. Evolution of the number of somatic cells in the milk of mammary quarters and from mixed milk, after treatment (cow 5378)

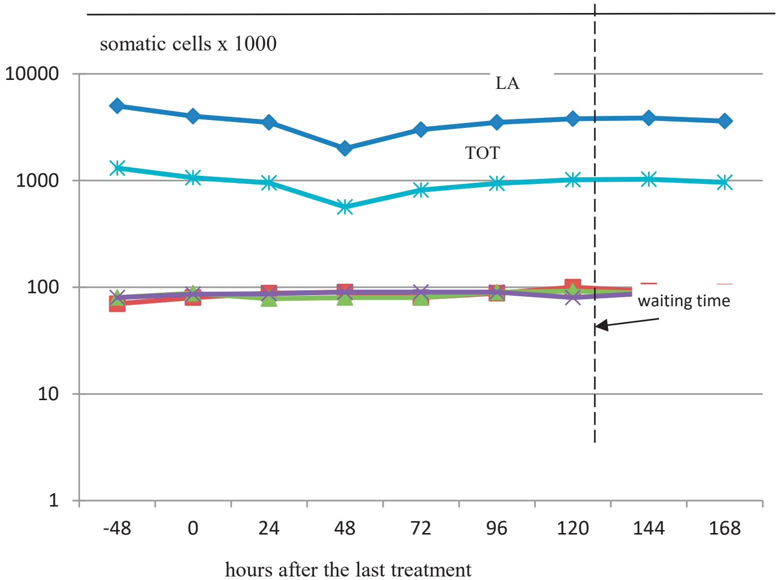

Figure 2. Evolution of the number of somatic cells in the milk of mammary quarters and from mixed milk, after treatment (cow 5390)

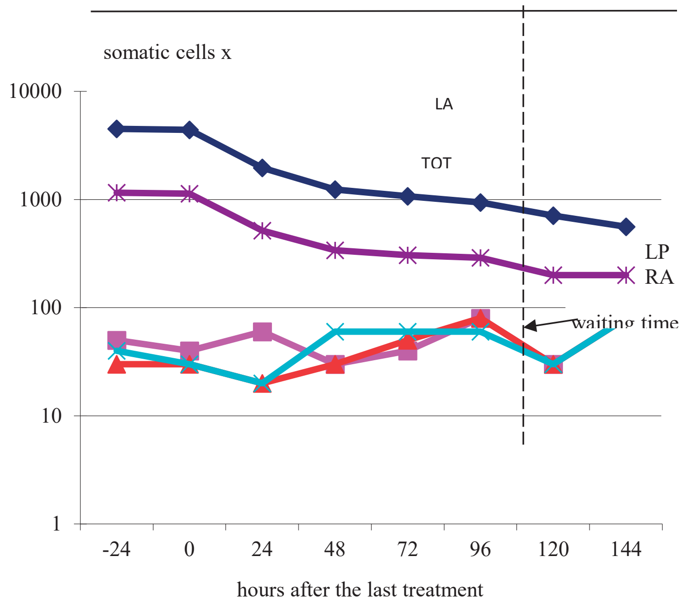

Figure 3. Evolution of the number of somatic cells in the milk of mammary quarters and from mixed milk, after treatment (cow 5378)

Table 3. Residue excretion of Cefquinome via milk

Cow registration number	Milk production (kg)	Cefquinome excreted	
		mg	% of dose applied
4091	22.8	70	30.2
5353	14.6	12	5.2
5354	23.2	32	38.2
5362	18.8	102	45.3
5378	27.8	40	20.8
5390	28.4	60	32.4
5400	26.6	55	27.8
5830	26.2	48	20.2
Average with two milkings/day ± Standard deviation	23.55 ± 4.8	52.38 ± 26.88	27.51 ± 12.3
Coefficient of variation	20.3	51.3	44.7
2231	28.8	42	17.3
5851	27.6	25	14.7
2239	26.4	65	28.0
5861	22.8	32	11.0
Average of 1.5 milkings per day ± Standard deviation	26.4 ± 2.59	41 ± 17.45	17.75 ± 41.1
Coefficient of variation %	9.8	42	41.1

In cow 5353 the treatment was not successful. Somatic cell count tends to decrease. However, the number of somatic cells in the mixed milk increases from 727 thousand to 1,650 million at 72 hours after treatment due to an increase in the number of somatic cells from another breast quarter. Table 3 shows the amount of Cefquinome excreted per each cow and milking group (with different periods between milkings). The amount of residue excreted via milk as a percentage of the total amount applied was from 5.2% to 45.3%.

In the group of cows milked 1.5 times a day, the percentage of residue excreted via milk was 17.75% compared to 27.51% in the group of cows with two milkings per day.

The average milk yield of the group of cows milked twice a day was 23.55 ± 4.8 kg standard deviation and in the group of cows milked 1.5 times a day was 26.4 ± 2.59 kg standard deviation.

Quantitatively, Cefquinome excreted via milk was 52.38 mg in the group of cows milked twice daily and 41 grams in the other group of cows. In cows with two milkings, the excretion of Cefquinome residue was 7.8% higher than in cows with 1.5 milkings per day.

CONCLUSIONS

The sensitivity of the ECOTEST method guarantees the rapid detection of antibiotic residues in cow's milk.

Milk from S.C. Fenov S.R.L. poses no health risks to consumers.

Some components of milk, after the treatment of severe mastitis, influence the test for the detection of antibiotic residues. These include: somatic cells, lactoferrin, lysozyme, free fatty acids or sodium.

The use of antibiotic overdoses for the treatment of sick animals must be associated with the detection of antibiotic residues after the waiting period.

It is recommended to test milk from treated animals on the first day after the waiting period to detect milk with antibiotic residues from animals with severe diseases or overdoses.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

OPTIMIZATION OF INDOOR MICROCLIMATE PARAMETERS IS AN IMPORTANT FACTOR IN STIMULATING METABOLISM IN THE BODY AND INCREASING PIG PRODUCTIVITY

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Abstract

Numerous scientific studies conducted in recent years have proven that, along with genetic and feeding factors, the provision and control of optimal microclimate parameters is closely related to the physiological state of animals, the course of the main metabolic processes in the body of pigs of different ages and productive groups. It is known that the potential productivity of animals under unsatisfactory housing conditions is realized only by 70-80%. Among these indicators, an important role in the process of growing pigs belongs to the microclimate of the premises - temperature, humidity, speed of air movement, its gas composition, concentration of harmful gases, microorganisms and dust in it. When pigs deviate from the optimal parameters, thermoregulation and metabolism are disturbed, the digestibility and assimilation of feed nutrients deteriorates, and as a result, productivity decreases, which ultimately negatively affects the quality of pork and the efficiency of production. Taking into account the constant intensification of pork production processes and climatic changes on the territory of Ukraine, the study of the influence of indoor microclimate parameters on metabolic processes and productive qualities of pigs of different age groups is relevant and of both scientific and practical interest.

Key words: *indoor microclimate, metabolism, pig productivity, stress.*

INTRODUCTION

Countries with a developed pig-breeding industry are constantly improving the technologies of keeping, feeding, selection and breeding of animals. It should be noted that in recent years, significant progress has been made in our country in improving the technologies of this branch of animal husbandry and increasing the efficiency of pork production.

Along with this, it should be emphasized that climate changes create a number of problems in ensuring proper microclimate standards in premises for keeping pigs, especially under modern, industrial technologies of their cultivation.

The microclimate of livestock premises is a set of various parameters, primarily such as: temperature, humidity, speed of air movement, chemical composition of air, content of dust, microbes, and harmful gases in it. The microclimate of the premises largely depends on the seasonality of maintenance, technology, livestock, supply of feed and water, removal of manure.

Modern pig farms today look more like intensively working industrial facilities than the traditional farms of the past. Rooms for keeping pigs are equipped with complex systems for creating and controlling optimal microclimate parameters, which take into account the growing requirements for new genotypes of pigs, comfort, economy and environmental friendliness of their keeping and breeding technologies.

Taking into account global climate changes, which cause a significant and long-term increase in temperature, causes the development of heat stress in pigs, it is an urgent problem that requires the creation of optimal microclimate maintenance systems in rooms for keeping all technological groups of pigs.

MATERIALS AND METHODS

The article has a review and analytical nature, in which the results of numerous scientific studies of domestic and foreign scientists in recent years are analyzed and summarized regarding the violation of metabolic processes in the body of pigs when the optimal parameters of the microclimate are not observed, as well as the relationship with the state of health and productivity of animals.

RESULTS AND DISCUSSIONS

In the conditions of intensification of the pig breeding industry, it is necessary to evaluate the effect of all factors affecting the welfare of animals. First of all, this concerns the microclimate of the premises and its most important parameters, such as temperature, humidity and air movement speed, as well as the presence of harmful gases. In this connection, the requirements for the creation and operation of automated microclimate systems are increasing.

The formation of microclimate in the premises is influenced by various factors, in particular the climate and topography of the area, the ecological condition of the soil, heat-insulating and other properties of building materials, the type and age of animals, the technology of their maintenance and other factors. In order to maintain proper health and productivity, full manifestation of physiological functions in the body of pigs, it is necessary to maintain optimal parameters of the microclimate in the premises and take into account the effect of seasonal factors, both positive and negative effects of which have been confirmed by many scientists.

In particular, Gerasimchuk (2018) believes that in order to increase the profitability of pig farming in Ukraine, as in the whole world, more and more attention is being paid to the development of new technological approaches to the issues of livestock conservation, growth intensity, health animals and veterinary well-being, and increasing the level of these factors is possible only under the condition of a comprehensive solution to the issues of the influence of microclimate conditions, the action of stressogenic factors, maintenance, feeding for the growth and development of pigs of various technological groups.

In turn, Mylostyviy (2019) asserts that high air temperatures in the hot period of the year are an acute problem in the conditions of keeping a large number of animals in a limited space, and taking into account the seasonal dynamics of the content of pests gases in premises for keeping pigs, it is the transitional periods of the year that are problematic for maintaining the optimal content of ammonia, hydrogen sulfide and especially carbon dioxide in it, even in conditions of significant air movement.

In the works of Mikhalko & Povod (2019) it was established that at high outdoor air temperatures, the geothermal type ventilation system makes it possible to create more comfortable temperature conditions in the room for keeping sows and realizing their productive qualities. However, the increase in temperature and low air humidity in the premises for sows require the implementation of additional measures to normalize their regime.

According to Bugaevskii et al. (2010), temperature is closely related to relative air humidity and has a significant impact on pig development indicators. Increased or decreased humidity negatively affects the health of piglets. When the air temperature in the pigsty deviates from the norm and decreases, the moisture concentration automatically increases. Condensate settles on the walls and ceilings in the room, which leads to the freezing of the room, the development of fungi and pathogenic microorganisms. At elevated temperatures, air humidity drops catastrophically and the air dries out. In such conditions, pigs overheat, which also negatively affects their general condition. The optimal moisture content in the room where the pigs live should be in the range of 60-70%. Such an indicator contributes to the active development of healthy animals and the increase of their population.

The long-term harmful effect of an unfavorable temperature and humidity regime often remains imperceptible, so the damage caused by such an effect is usually not taken into account, and the overspending of feed to support heat exchange processes at low temperatures and high air humidity leads to a decrease in efficiency. the difficulties of running the pig industry, especially at enterprises with small herds (Mylostyviy et al., 2019). Thus, in the climatic conditions of Ukraine, the study of the influence of the above parameters of the indoor microclimate on the reproductive qualities of sows, the live weight of newborn piglets and the course of oxidation-reduction processes in the body of animals are relevant and require a more detailed study.

Foreign researchers, in particular, Baumgard (2015) and others note that non-compliance with the optimal parameters of the microclimate can contribute to the spread of diseases, a decrease in the natural resistance and productivity of pigs. In turn, compliance with regulatory parameters in premises for keeping pigs contributes to the full course of physiological processes in the body, supports its homeostasis, strengthens adaptive capabilities, prevents the occurrence of stressful conditions, ensures high productivity of animals and profitability of the industry.

The research of Muns (2016) proved that under the influence of seasonal factors, phenotypic fluctuation is observed for some productive and reproductive characteristics of pigs. In particular, high ambient temperature negatively affects feed consumption in sows and the weight of weaned piglets. High temperatures during farrowing worsen the welfare of the brood stock, which negatively affects the growth and health of the offspring.

The scientific works of Claus & Weiler (2020) show that under the influence of seasonal temperature fluctuations, boars of different genotypes often show a reduced synthesis of steroids, the number of sperm and libido in the summer, compared to the optimal indicators that are characteristic of the winter period. In sows, ovarian anestrus can be affected mainly in summer, occasionally in February - March. In addition, the frequency of ovulation in sows is lower in summer, and its frequency increases in late autumn and winter. As a result, the interval from weaning piglets to estrus in sows increases in the summer, during this period mating causes a decrease in the level of fertilization and, as a result, a smaller number of piglets at birth.

It was also established by De Rensis (2017) that, in firstborns and sows, the summer-autumn season is characterized by reduced fertility, and heat stress in the warm season can cause a decrease in feed consumption, while the imbalance of activity disrupts the physiological development of follicular and luteal bodies, reduces the quality oocytes and increases the mortality of embryos.

Bloemhof (2013) notes that heat stress has a more pronounced effect on reproductive performance in gilts than in mature sows. In particular, heat stress during the third week (from 14 to 21 days) before the first insemination significantly affects the farrowing speed. Heat stress in the period between the 7th day and before successful fertilization, i.e. up to the 12th day, significantly affects the total number of piglets born.

Studies by Kerr (2003), Renaudeau (2012) and Johnson (2015) showed that heat stress negatively affects live weight gains in pigs and suggested that this may be related to the course of physiological processes in the body caused by an increase in body temperature.

According to Pearce (2012), an increase in body temperature causes morphological changes in the intestines of pigs, since animals under heat stress have a higher body temperature and may have more intestinal damage. than in animals that are in comfortable conditions. In addition, under the influence of high temperatures, the secretion of gastric juice is suppressed, its bactericidal function is insufficient, the activity of pepsin decreases and the concentration of total protein in the blood increases. The proteolytic group of enzymes – pepsin, trypsin, enterokinase – is most inhibited, as a result of which digestion processes worsen and the rate of absorption of amino acids decreases. In addition, pancreatic enzyme secretion is inhibited, intestinal motility is suppressed. Accordingly, the appetite decreases, and therefore the productivity of animals. Kluzáková (2013) also found a significant decrease in sulfur gain in piglets raised in machines located away from fans and air intakes of the microclimate system.

Renaudeau et al. (2008) found that mitigating the negative impact of heat stress on pigs by creating an optimal microclimatic environment in a piggery can be achieved by designing premises, ventilation systems and using methods of evaporative cooling taking into account the peculiarities of local climatic conditions. However, Morello (2018) notes that despite any technical improvements of modern indoor microclimate systems, the air composition of the environment in pig farms is significantly affected by unpredictable external climatic changes, which can negatively affect the productivity of pigs.

In the scientific research of Costa (2014), it is reported that there is a difference in microclimate indicators for typical ventilation systems in the same rooms for growing pigs, which can have different effects on the thermoregulation of animals. According to Close et al. (2014), air velocity is important in convective heat loss, and its decrease can reduce the power of heat removal in piglets, which causes an increase in body temperature and, as a result, temperature stress.

Krommweh et al. (2014) established that increasing air mobility at high ambient temperature has a positive effect on the body of pigs, increasing heat output and preventing overheating. With an uneven distribution of air flows in the room, there are dead zones, that is, zones with a reduced air movement speed (less than 0.05 m/s) and a high concentration of harmful gases, dust and microorganisms, which has a negative effect on the health of animals. In the cold and transitional periods of the year, the optimal speed of air movement in pig houses is 0.15-0.3 (m/s). In summer, the speed of air movement can be up to 1 m/s or more, depending on the season and climatic zone.

The results of numerous scientific studies indicate that as a result of the vital activity of animals, the decomposition of nitrogen and sulfur-containing substances in manure and bedding, as well as insufficient air circulation in the premises, significant concentrations of ammonia and carbon dioxide can accumulate, hydrogen sulfide, mercaptans, methane and other gases. As research by Patel et al. (2018) shows, long-term keeping of pigs in closed rooms with an increased concentration of these gases causes deviations and disorders in the course of metabolism in the body, the development of deep morpho-functional disorders in organs and tissues, and a decrease in natural resistance and immunological reactivity of the body.

It has also been studied that in livestock premises where the excessive level of ammonia, hydrogen sulfide, and carbon dioxide in the air reduces the productivity of pigs and increases the percentage of culling, as well as increases the level of overspending of feed per unit of production.

Research by Parker (2010) indicates that elevated concentrations of ammonia have a pronounced effect on the ethological interactions of pigs, while animals in such conditions demonstrate greater aggression. In addition, pigs exposed to a high level of mechanical noise, which is a feature of artificial ventilation, are less prone to aggressive actions than pigs kept under elevated ammonia levels. It is noted that increased concentrations of ammonium can worsen social stability in technological groups, although the mechanisms of its influence are currently unknown.

According to Philippe (2011), high density of animal housing or inadequate design of machines can increase floor pollution and cause an increase in NH3 concentration, which is statistically correlated with the ambient temperature and ventilation rate in the room for keeping pigs.

The research results of Saha (2014) proved that the concentration of NH_3 in the pig complex changes seasonally and depends on the values of the external temperature. During the year, significant correlations ($P<0.001$) of NH_3 concentration with external seasonal climatic fluctuations were established, including external temperature, humidity, wind speed and direction, hour of the day and day of the year.

The above literary data generally indicate that the creation of comfortable conditions for keeping pigs supports the normal course of metabolism in the body, reduces morbidity, reduces feed costs and improves the economic efficiency of pork production.

CONCLUSIONS

Experimental studies conducted in recent years have established that the creation of comfortable housing conditions and appropriate microclimate standards at pork production enterprises is an important component of modern intensive pork production. The given literary data also show that the regulatory provision of such microclimate parameters as temperature, humidity, air in the room with the optimal concentration of harmful gases in them are extremely important factors for ensuring the welfare of pigs, optimizing the course of metabolism in their organism, the proper productivity of animals and obtaining high-quality products of the pig breeding industry.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

STUDY REGARDING THE IMPROVEMENT OF MILK PRODUCTION ACCORDING TO THE SIRES VALUES

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Abstract

The research proposed to evaluate the influence of the seminal material on milk production. Also was studied seminal material from different countries in order to determinate if the origin influence as well, genotype - environment correlation. The study was carried out on Holstein cows from Romania and data provided by Holstein Ro association. For sires values were consulted international website, recognized worldwide, like dairybulls.com, where the data are published by CDCB (Council on Dairy Cattle Breeding) or INTERBULL. The cows that took part at the research were all at their first lactation, from farms located throughout Romania. After establishing the groups of contemporaries, the real production achieved on standard lactation was compared with the surplus amount of milk due to the sire's values, in order to see if the expected result was achieved. For avoiding error conclusions were compared cows from the same farm, same sire, in that working hypothesis the feeding influence is removed.

Key words*: dairy cows, Holstein, milk production, Romania.*

INTRODUCTION

Nowadays, at national level, milk, together with dairy products, constitutes a range of products highly appreciated by consumers. This deed arises as a result of the fact that the milk has exceptional nutritional values and contains: all the essential amino acids, 5 types of protein, fat acids, 4 types of lactose, mineral elements and enzymes.

The national milk production is ensured by the animals exploited in individual households, but only in a very small extent and most part of the milk requirement being provided by intensive industrial farms. However, ensuring the national needs varies from one season to another or from one year to another. This happens as a result of the lack of a long-term strategy regarding dairy cows in Romania, of the political conflicts in the neighborhood and because of imports, which in certain conditions for processors represent more advantageous solutions than purchasing milk from Romanian farmers. In our country the farmers have only one option left, to improve their milk production, to improve their herd in order to produce a larger amount of milk with the same herd under the same technological conditions. There are of course many methods by which the breeder can improve milk production, however to use imported semen from the most valuable contemporary bulls represents the best and fastest solution. Normally, dairy farmers select bulls that will increase the profitability of the exploitation (Schneeberger et al., 1982; Allaire & Thraen, 1985; Rogers, 1990; Tozer & Stokes, 2001).

If in the past there were certain impediments regarding the artificial insemination (the possibility to import top quality semen, the reduced number of operators, the possibilities of storing the material, reticence of farmers regarding this procedure and of course the costs). In the present, things have changed farmers choose artificial insemination, globalization allows the import of semen from anywhere in the world, at any time, and problems related to operators and stored can no longer be discussed. The only debatable aspect therefore remains the price of the doses of semen. The price of a dose can start from a few euros and increases substantially for doses from top bulls, which show superior characters, and if we are also talking about sexed material, the costs are very high. Following these presented aspects, of course, the question arises as to why

farmers should still choose for something like this. The answer is simple, it facilitates the improvement of the livestock in the fastest way, with the help of a dose of sexed material, coming from such a bull, you can get a cow with a higher milk production than the farm average, in a time interval of only 3 years. In other conditions this fact would require a much longer time (native bulls do not have as high a breeding value, 50% chance of getting a female). In the same time inbreeding is avoided, in dairy cattle this has direct and indirect economic consequences for a dairy producer, such as reduced milk and milk component yields, lower reproduction rates, and reduced survival of offspring, (Wiggans et al., 1995; Thompson et al., 2000).
The aim of this paper is to analyze if, in fact, the milk production of the daughters takes place according to the values of the sires.

MATERIALS AND METHODS

The data that are the basis for the realization of this study come from Holstein Breeding Association (ACV Holstein Ro), official control production (COP) and herdbook.
In sight of the present study three farms from Romania were selected, all raising Holstein cows, only cows at the first lactation were selected in the research.
Furthermore, their sire was identified, and of course it's value of improvement on milk production. Regarding the recorded milk production of the cows, the standard lactation (305 days) was used.
Further, by comparing the production of the cows taked in study with the average milk production of contemporaries and with the average of the dams first lactation the objective is analyzed.
In the same time correlating the milk values of the sires with their daughters milk productions we can observe a trend.
The correlation formula is:

$$r\,(x,y) = \frac{\sum(x-\bar{X})(y-\overline{y})}{\sqrt{\sum(x-\bar{X})^2\sum(y-\bar{y})^2}}$$

$\overline{X}$ - the mean of the first sample;
$\overline{Y}$ - the mean of the second sample.

Before that, since the values of the sires are expressed in pounds (lbs), they have been converted into kilograms (kg), to be expressed in the same unit of measure as the cows milk production.
In order to establish if are significative differences between daughters – mothers production T-Test (Student's T-Test) was used.
T-Test formula:

$$t = \frac{\overline{X_1} - \overline{X_2}}{\sqrt{\frac{s_1^2}{n_1} \quad \frac{s_2^2}{n_2}}}$$

RESULTS AND DISCUSSIONS

In order to prepare the study, 3 farms from the territory of Romania were selected (east, south and west). Afterwards, 3 sires from each farm were analyzed, sires from different countries of origin were chosen (from U.S.A., Netherlands, Canada and Italy), in order to see if there are significant differences through the lens of the genotype-environment relationship (Granaci et al., 2021).
Initially, the results of the analysis of the first farm, located in the east of the country, will be presented (Table 1)

Table 1. Analysis of daughters production in farm A

Specification	Milk quantity (kg)
Contemporaries average L305	12225
Milktime daughters - average L305	13025
Milktime - Milk value	1244
Dam 1st lactation average	10728
Difference Milktime daugh. - contemp.	800*
Difference Milktime daugh. - dam 1st lactation	2297
Frazzled daughters - average L305	13010
Frazzled - Milk value	652
Dam 1st lactation average	11939
Difference Frazzled daugh. - contemp.	785
Difference Frazzled daugh. - Dam 1st lactation	1071***
Jedi daughters - average L305	14005
Jedi - Milk value	933
Dam 1st lactation average	11922
Difference Jedi daugh. - contemp.	1780
Difference Jedi daugh. - dam 1st lactation	2083***

Farm A, like all the farms that are part of this work, use the intensive growing system. They place great emphasis on genetics, continuously importing genetic material of the highest quality.

First sire analyzed is *Milktime*, originally from Canada, with indentification number - CA 12609045.

STE ODILE MILKTIME

Interbull ID: CANM000012609045
NAAB: 200HO10904

Production Traits [CDCB 12/2022]: G

% Rel.	Milk Lbs.	% Fat	Fat Lbs.	% Prot.	Prot. Lbs.
99	2765	.14	64	-0.03	76

Figure 1. Milktime - milk value
(source: http://www.dairybulls.com)

As can be observed in Figure 1, the bull Milktime has a milk value of 2765 lbs, approximate 1244 kg. Milktime's daughters, from farm A, in the first lactation recorded a mean of 13035 kg of milk on standard lactation. Compared to the average of contemporaries which is 12225 kg, we notice a difference of 800 kg of milk, a difference that cannot be overlooked. We also followed the production of mothers during their first lactation, to highlight whether there have been significant changes on this line as well. We note the fact that the mothers had an average of 10728 kg during the first lactation, in this case, the difference between the production of milktime's daughters and their mothers is 2297 kg of milk. After applying the T-test, the result obtained is p = 3.8204*, there are significant differences between daughters - mothers production.

MELARRY JOSUPER FRAZZLED

Interbull ID: USAM000074261651
NAAB: 007HO12788

Production Traits [CDCB 12/2022]: G

% Rel.	Milk Lbs.	% Fat	Fat Lbs.	% Prot.	Prot. Lbs.	NM$	FM$	CM$	GM$
99	1448	.03	64	-0.03	36	729	748	736	574.0

Figure 2. Milktime - milk value
(source: http://www.dairybulls.com)

The second bull studied in farm A is called *Frazzled*, it's coming from U.S.A. with identification number – US74261651 and has a milk value of 1448 lbs/652 kg (Figure 2). Frazzled's daughters recorded an average of 13010 kg of milk on their first standard lactation, with 785 kg more compared with the contemporaries average. Their dams, at their first lactation had an average of 11939 kg of milk (a very good one), however Frazzled daughters obtained with 1071 kg of milk more. The differences are very significant, p = 4.2081***.

S-S-I MONTROSS JEDI-ET

Interbull ID: 840M003123886035
NAAB: 007HO13250

Production Traits [CDCB 12/2022]: G

% Rel.	Milk Lbs.	% Fat	Fat Lbs.	% Prot.	Prot. Lbs.	NM$	FM$
99	2073	0.20	18	0.00	65	605	586

Figure 3. Jedi - milk value
(source: http://www.dairybulls.com)

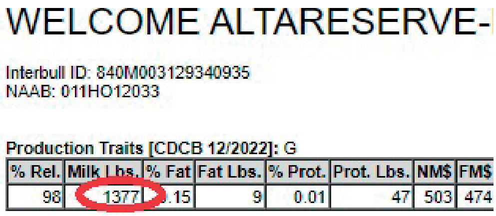
The last bull studied in Farm A it's *Jedi*, it has a milk value of 2073 lbs/933 kg (Figure 3). Originally from U.S.A., with identification number US 3123886035, it currently registers a number of 36386 daughters, 64% of them in U.S.A.

In farm A, Jedi's daughters, at their first lactation recorded an average of 14005 kg of milk on standard lactation. Their mothers, during the first lactation as well had an average of 11922 kg. Thus, we find a milk production higher by 2083 kg of milk at the level of Jedi's daughters.

It is also interesting that the mothers of the daughters of the previous bull, Frazzled, registered a similar value 11939, but the Frazzled's daughters are not at same level. We thus deduce the fact that the extra milk recorded at Jedi's daughters is mainly due to the value of the bull, which is higher than Frazzled's (the daughters benefiting from the same conditions in the rest, being in the same farm). In the same time the result of the T-student is similar with the previously one, p = 3.9064***, the differences are very significant.

All cows that are daughters of the bulls studied recorded very good results, exceeding the average of their contemporaries, their productions being considered good even worldwide.

In the second table are presented the results from the farm located in the south of the country (farm B). If in the first one the bulls were from U.S.A. and Canada, in the second one the bulls were analyzed from U.S.A. and Netherlands.
The first bull studied in this farm is *Altareserve*, originally from U.S.A. has a milk value of 1377 lbs/798 kg with the identification number US 3129340935 (Figure 4).


WELCOME ALTARESERVE-

Interbull ID: 840M003129340935
NAAB: 011HO12033

Production Traits [CDCB 12/2022]: G

% Rel.	Milk Lbs.	% Fat	Fat Lbs.	% Prot.	Prot. Lbs.	NM$	FM$
98	1377	.15	9	0.01	47	503	474

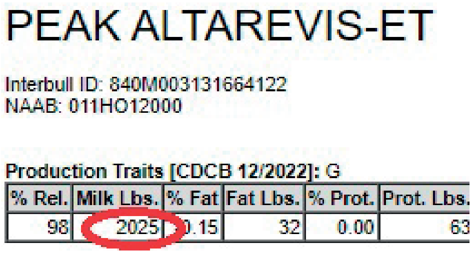
Figure 4. Altareserve - milk value
(source: http://www.dairybulls.com)

It's daughters, recorded at their first lactation in the farm B an average of 12131 kg of milk in 305 days of lactation (Table 2).

Table 2. Analysis of daughters production in farm B

Specification	Milk quantity (kg)
Contemporaries average (L305)	11333
Altareserve daughters - average L305	12131
Altareserve - milk value	620
Dam 1st lactation average	10458
Difference Altareserve daugh. - contemp.	798
Difference Altareserve daugh. - dam 1st lactation	1673**
Altarevis daughters - average L305	12224
Altarevis - milk value	911
Dam 1st lactation average	10061
Difference Altarevis daugh. - contemp.	891
Difference Altarevis daugh. - Dam 1st lactation	2163***
Altashockwave daughters – average L305	11988
Altashockwave - milk value	447
Dam 1st lactation average	10923
Difference Altashockwave daugh. - contemp.	655
Difference Altashockwave daugh. - dam 1st lactation	1065^{NS}

The contemporaries of Altareserve's daughters recorded an average of 11333 kg, 798 kg less compared to the production of its daughters. A good production was also obtained by mothers, 10458 kg of milk on standard lactation, however the daughters surpassing them with 1673 kg. In this case, p = 2.9882**, distinctly significant differences being noted.
Further, the second bull studied in farm B it's *Altarevis*. Coming for U.S.A. with the identification number US 3131664122 and having a milk value of 2025 lbs/911 kg (Figure 5).


PEAK ALTAREVIS-ET

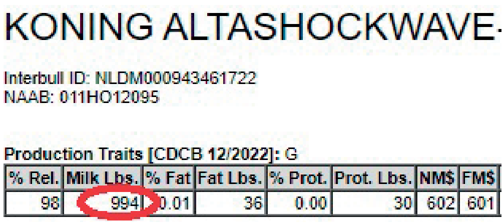
Interbull ID: 840M003131664122
NAAB: 011HO12000

Production Traits [CDCB 12/2022]: G

% Rel.	Milk Lbs.	% Fat	Fat Lbs.	% Prot.	Prot. Lbs.
98	2025	.15	32	0.00	63

Figure 5. Altarevis - milk value
(source: http://www.dairybulls.com)

The daughters of this bull realized in average a production of 12224 kg of milk in ther first standard lactation. Compared with the productions realized by their contemporaries, we can notice an addition of 891 kg of milk. Even a bigger difference is notice between the daughters of Altarevis production and their mothers production, a difference of 2163 kg, the mothers recorded an average of 10061 kg on their first lactation. This difference is proved also by the result of T-test, p = t = 3.9660***, very significant differences.


KONING ALTASHOCKWAVE-

Interbull ID: NLDM000943461722
NAAB: 011HO12095

Production Traits [CDCB 12/2022]: G

% Rel.	Milk Lbs.	% Fat	Fat Lbs.	% Prot.	Prot. Lbs.	NM$	FM$
98	994	.01	36	0.00	30	602	601

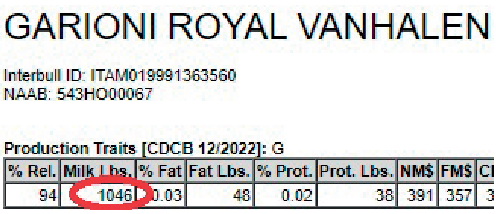
Figure 6. Altashockwave - milk value
(source: http://www.dairybulls.com)

Altashockwave is the last bull studied from the farm B. It's originally from Netherlands, having the identification number NL 943461722 and milk value equal with 994 lbs/ 447 kg (Figure 6). Having the lowest value of milk among the bulls from farm B, this is also reflected in the production of daughters, 11988 kg of milk, lowest compared with the production of Altareserve or Altarevis daughters. This fact does not mean that its daughters did not perform, on the contrary, the daughters produced 655 kg of milk more compared with their contemporaries and with 1065 kg ok milk more compared with their mothers. However, p = 1.8236^{NS}, with others words, the differences are not significant in this case. In other words, they performed, but in correlation with the value of the sire, more above the farm average, but under the daughters of more valuable bulls.

The last farm that took part to the study, farm C, located in the west of the country propose for the study two bulls coming from Italy and one from Netherlands (Table 3).

Table 3. Analysis of daughters production in farm C

Specification	Milk quantity (kg)
Contemporaries average (L305)	11831
Van Halen daughters	12623
Van Halen - milk value	471
Dam 1st lactation average	6310
Difference Van Halen daugh. - contemp	792
Difference Van Halen daugh. - dam's 1st lactation	6313***
Fusion daughters	13115
Fusion - milk value	693
Dam 1st lactation average	8528
Fusion's daugh. - contemp.	1284
Diff Fusion daugh– dam 1st lactation	4587***
Sound system daughters	12565
Sound system - milk value	581
Dam's 1st lactation average	8065
Difference Sound system's daugh. - contemp.	734
Difference Sound system's daug. - dam's 1st lactation	4500***


GARIONI ROYAL VANHALEN

Interbull ID: ITAM019991363560
NAAB: 543HO00067

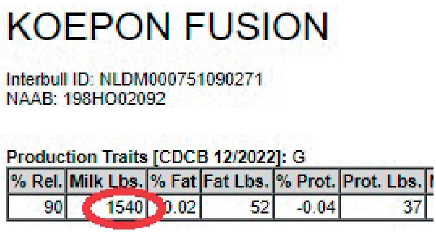
Production Traits [CDCB 12/2022]: G

% Rel.	Milk Lbs.	% Fat	Fat Lbs.	% Prot.	Prot. Lbs.	NM$	FM$
94	1046	0.03	48	0.02	38	391	357

Figure 7. Van Halen - milk value
(source: http://www.dairybulls.com)

In the beginning, we will present the information regarding the bull named *Van Halen*. Originally from Italy, with identification number IT 19991363560 and a milk value of 1046 lbs/471 kg (Figure 7).

We note the fact that in farm C, the daughters of Van Halen recorded a mean of milk productions of 12623 kg, 792 kg more than the mean of their contemporaries which was 11831 kg. Remarkable is the big difference between the production of Van Halen's daughters and their mothers. The differences are very significant, p = 12.3586***, Van Halen’s daughters recorded a double production, 6310 kg of milk being the mean of the mothers production.


KOEPON FUSION

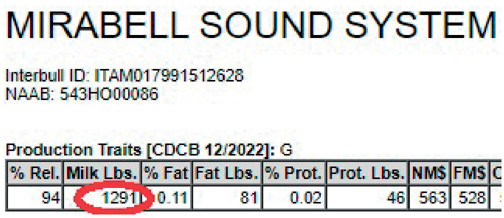
Interbull ID: NLDM000751090271
NAAB: 198HO02092

Production Traits [CDCB 12/2022]: G

% Rel.	Milk Lbs.	% Fat	Fat Lbs.	% Prot.	Prot. Lbs.
90	1540	0.02	52	-0.04	37

Figure 8. Fusion - milk value
(source: http://www.dairybulls.com)

The second bull is a Dutch one, named *Fusion* having the identification number NL 751090271 and a milk value of 1540 lbs / 693 kg. It’s milk value reflects at its daughters production, 13115 kg of milk in average, compared with contemporaries production we notice a plus of 1284 kg of milk. A big difference is noticed also between Fusion’s daughters and their mothers production (8528 kg), 4587 kg of milk in the favor of the descendants, p = 5.4165***, so very significant differences.


MIRABELL SOUND SYSTEM

Interbull ID: ITAM017991512628
NAAB: 543HO00086

Production Traits [CDCB 12/2022]: G

% Rel.	Milk Lbs.	% Fat	Fat Lbs.	% Prot.	Prot. Lbs.	NM$	FM$
94	1291	0.11	81	0.02	46	563	528

Figure 9. Sound System - milk value
(source: http://www.dairybulls.com)

The last bull that took part to the study is an Italian one, named Sound System, having a milk value of 1291 lbs/581 kg, being identify by IT 17991512628 (Figure 9). Sound system daughters recorded at their first lactation an average of 12565 kg of milk, with 734 kg above the contemporaries average. Compared with their mothers production, these recorded 4500 kg of milk more (the mothers average production at first lactation being 8065 kg of milk), p = 5.3390***, again same result obtain, very significant differences being noticed.

We conclude as in the case of the third farm the value of improving milk production, due to the sires, turns in real production in the milking room.

As previously mentioned, correlations between the bulls milk value and their daughters milk production were also made for each farm. For the first farm, the result of correlation, r is equal with – 0.02, in other words, non-existent correlation (Figure 10).

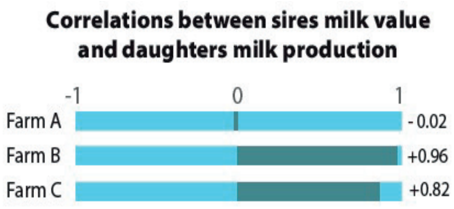


Figure 10. Correlation results

At the second farm, r is equal with +0.96, almost 1 (maximum value). This result is telling us that the correlation is very high, being a very close relationship between the variables.

Similar result is recorded at farm C, r=0.82, again showing a very high correlation between the sire milk value and their daughters milk production.

CONCLUSIONS

The use of semen from world-class bulls represents the best method of improving milk production at the national level at the moment.

In this study it was found that the daughters of such bulls will produce a higher amount of milk compared to their contemporaries. Also compared to the mothers productions, impressive results are noted, there are cases where the production effectively doubled. The results from T-test proved (except one single case) that the differences are significant and very significant regarding the milk production of daughters – mothers.

Regardless of the origin of the sires (U.S.A., Netherlands, Italy or Canada) the results were positive.

Based on the results obtained from the correlations, except for farm A where the result is 0 (the production of cows in this farm being balanced) we deduce the fact that between sire milk value and daughter milk production exist a positive and highly relationship.

ACKNOWLEDGEMENTS

This present study was supported by Holstein RO Breeding Association (ACV Holstein Ro).

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

AI BASED DEVELOPMENT OF A LOW COMPUTATIONAL INTENSITY ALGORITHM FOR CATTLE HEART RATE (HR) ESTIMATION

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Abstract

The main goal is to estimate the HR value from the activity sensor 3D acceleration measurements of the cattle rumen bolus. During the development of the algorithm it was intended to execute the primary calculations on the device's microcontroller and the additional calculations could be performed on the server. The proposed HR estimation algorithm is based on simple data cleaning and peak detection, but the validation and postprocessing of the detection uses AI methods, namely MLP artificial neural network with different cell numbers. The accuracy of the period estimation (IBI) was ±50ms, which means an 8% error. This allows basic alerts to be implemented.

Key words*: 3D accelerometer data, Artificial Intelligence methods, cattle rumen bolus, HR estimation.*

INTRODUCTION

PLF is one of the leading technologies in agriculture (Knight, 2020b). One of the main directions of this is to collect relevant information from the animals (Szabo & Alexy, 2022) and their environment with the appropriate sensors, process it with an information system (Cabrera & Fadul-Pacheco, 2021; Caja et al., 2016; Dado & Allen, 1994; Daum et al., 2022; El Bilali et al., 2020; Khanal et al., 2010) and use the obtained results for the purpose of automation and/or decision support. These processes are of great economic importance, as they make animal husbandry more profitable, and at the same time solve the partial replacement of the missing human workforce and help to ensure the well-being of the animals (Alsaaod et al., 2012; Caja et al., 2016; Knight, 2020a; Michie et al., n.d.). A rumen bolus sensor can be used as a sensor in such a system, and it is already used in dairy cattle. Rumen bolus sensors typically measure temperature, pH and/or activity, as this is usable technical solution for operating such sensors at the current technological level (Borchers et al., 2017; Cabrera & Fadul-Pacheco, 2021; Caja et al., 2021; Campos et al., 2018; Dado & Allen, 1994; Hajnal et al., 2022; Hamilton et al., 2019; Hanušovský et al., 2017; Ipema et al., 2008; Knauer et al., 2016; Knight, 2020b; Mottram, 2010; Vakulya et al., 2022; Zhang et al., 2018). The current systems can also be used for alarms, but I thought it was possible to expand the range of measured characteristics with new ones. In this article, it was examined whether there is a realistic possibility to determine the heart function parameters (Heart Rate –HR, Interbeat Interval –IBI) with the help of the rumen bolus. This intention is meaningful, because the heart function in cows (Caja et al., 2021; Kovács et al., 2014), just like in humans (Piros et al., 2023), is an important characteristic that gives information about the state of health, indicates the level of stress and certain events, such as the start of calving. As a result, this information can be extremely valuable in PLF systems. In the case of cows, no such tests have yet been carried out, but in the case of humans, there is a lot of literature with results available, as a common target function of modern wearable technology is the examination of heart function (Bruser et al., 2013; Curone et al., 2010; Galli et al., 2018; Hernandez et al., 2015; Kwon et al., 2011; Lahdenoja et al., 2016; Nakano et al., 2012; Zhao et al., 2021). The publications show that it is very difficult to accomplish the task precisely and efficiently. For human use, the heart rate is typically determined from optical data from pulse oximeters, and in case of activity measurement, the devices work with a high sampling frequency and often with an additional sensor. Many publications deal with

the methodology of data processing, which often uses signal processing methods with high computational demands due to the significant noise in the measurement(Alzahal et al., 2009; Galli et al., 2018; Nakano et al., 2012).
The aim of this research is to develop an algorithm with low computational requirements that can be run on the rumen bolus microcontroller and is able to estimate cardiac IBI values. Since a real device to be developed does not necessarily have a way to continuously measure and process data, it was necessary to determine how large a series of measured data could be processed with the highest accuracy. The aim is therefore to establish the optimal period durations on the basis of shorter measured data series of a few seconds. According to previous experience, the algorithms can be made sufficiently accurate with the help of some post-processing step. In this paper, an artificial neural network was used for post-processing. The research question is, with which parameters a simple algorithm gives the best estimation, as well as whether it is possible to post-process the data with the help of the neural network and whether it is possible to achieve a usable result with the relatively low sampling frequency and processing steps with little computational demand.

MATERIALS AND METHODS

In this paper, 3D acceleration data measured by the rumen bolus sensor were used. The experimental set-up and the method of data processing are described in details in the previous publications (Vakulya et al., 2022). The accelerometer was used to measure 3D acceleration data with a sampling frequency of 25Hz. The data was sent via radio communication to the receiver, which recorded the measurements supplemented them with a time stamp. Parallel ECG monitoring measurements were carried out (Hajnal et al., 2022; Kovács et al., 2014). We had no way of synchronizing the timer of the two devices, but at the same time we confirmed with another experiment that the difference was within 2s, so the ECG values can be considered as actual control values.

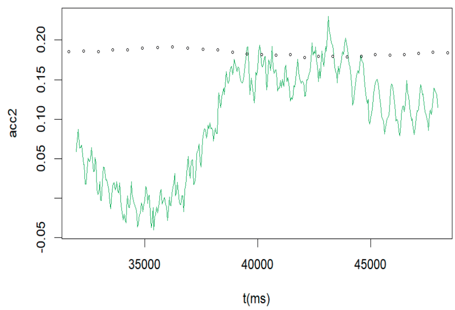


Figure 1. Raw accelerometer data of 20s long data acquisition period (green) and the control Interbeat Interval (IBI) values (dots)

The algorithm was developed using R scripts within the RStudio 2022.07.1+554 environment and Orange Datamining 3.35 software (*Orange DataMining*, n.d.).
The Figure 1. shows the raw data. It can be seen that the periods belonging to the heart can be clearly discerned in some parts of the curve, for example in the last 3s, but they are almost barely perceptible in certain parts of the curve (at 35s). It can be seen that the movement activity of the animal is strongly superimposed on the curve, and elsewhere the curve becomes detached, often resulting in completely false period length detection. The basic idea behind detection is a method based on standard pre-processing, peak detection and post-processing steps (Zhao et al., 2021). The details of the algorithm and the method of data processing can be seen in Figure 2. During the data processing, in the first step, it was a trial to reduce motion artefacts (MAs) originates from the animal's body movement using a thresholding procedure of the acceleration data. Values that differed from the average by more than the threshold were cut to the threshold level. The thresholds were established on the basis of the distribution statistics of the acceleration derivative. This was followed by a low-pass filter to remove the high-frequency signal components, it was resolved by a moving mean computation with a window of a given width. For the peak detection the two highest acceleration axes were used, because technically the cow's heart is located almost vertically upwards to the rumen, so the

accelerations resulting from the heart's action are mostly indicated on the vertical axis. The principle of peak detection was to locate the zero points of the derivative. The peak detection was performed on the basis of the minimums and maximums of the function for each data series of a few seconds in length, so final two data series were resulting in a series of IBI values detected based on the minima and maxima.

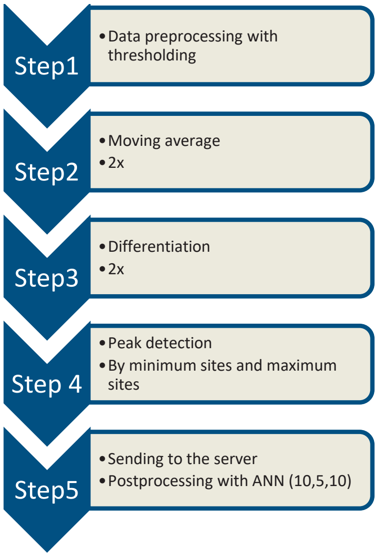


Figure 2. Data processing workload

Unfortunately, higher frequency harmonics and period lengths multiplied due to detection errors can also be included in this series. It has been described in the literature that MAs can also generate false period lengths. In this way, the minimum, maximum, average and median values from each data series were determined, so total of 16 data for the two channels were calculated which in an ideal data series are all the equal and according to the 25Hz sampling frequency the 1/40 part of the IBI values. This data set was used for post-processing. This system was investigated for the ideal data series length in which the detection based on 3-4-5 s long data series. The accuracy after detection and the accuracy after postprocessing of trained neural network were determined. An important pre-requisite for neural networking is that the dataset must be balanced. Figure 3 shows the histogram of the control ECG data set, and it is concluded that the prerequisite is not met.

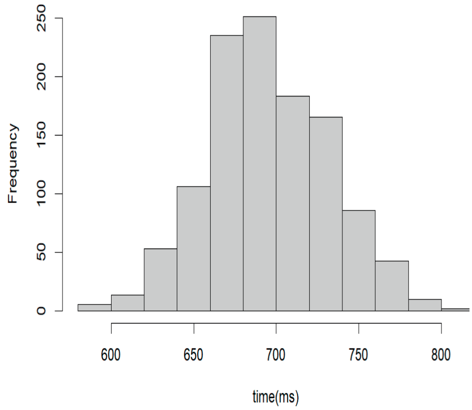


Figure 3. Histogram of control ECG Interbeat Intervals (IBI)

The training dataset of the neural network was balanced using the random walk oversampling (RWO) method (H. Zhang & Li, 2014). The neural network used is an MLP with three hidden layers with 10,5,10 neurons, RELU activation function and ADAM solver. The data was share to teaching (70%) and testing (30%) datasets. The neural network training was performed as a classification and evaluated in the usual way (ACC, MAE, ROC, F1, Confusion matrix). The presented metrics were get in all cases on testing data.

RESULTS AND DISCUSSIONS

Figure 4 shows a sample dataset after preprocessing and peak detection. We can see that in certain cases it was possible to find the real peak very accurately. Since the curves have small subpeaks (shoulders), we set the peak detection so that it does not detect two peaks close to each other, but because of this, the algorithm often does not find the most prominent peak, but instead produces the shoulder as a result. It can be concluded that the peaks are detected, but the accuracy with the specified parameters should be further improved in the future, or perhaps supplemented with an algorithm that helps to separate the main and secondary peaks. If the algorithm detects several secondary peaks, then the correct value can be obtained from the average of two or three sections, so the average of the peak distances can provide an approximately good solution in this case. If several peaks are included in the analysis, the false peaks cause the shortening of the period length on one side and the increase of the period length on the other side. If there is a correctly identified period in the data set, it will be located near to the median of the series. As more ideal the data series, as more homogeneous the resulting IBI series, ideally all members are equal, and contrary, if there are large differences in the detection, it can definitely indicate a detection error. In such cases, it is possible to discard the data series as a bad detection, or to try to decide what the real value is in the post-processing (with the MLP artificial neural network in this paper). The distribution of errors after the first detection is shown in Figure 7 B. It can be seen that the error function has a roughly normal distribution, because many parameters cause the error, but the function has shifted a little to the left, so typically the IBI values are underestimated by the algorithm. The average error is unacceptably large, and in this form it cannot be used even for a rough estimation.

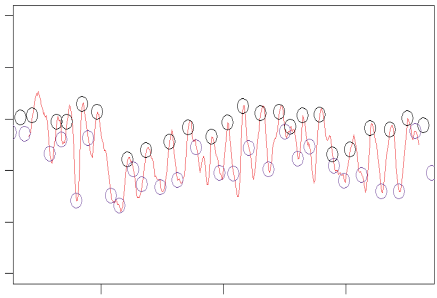


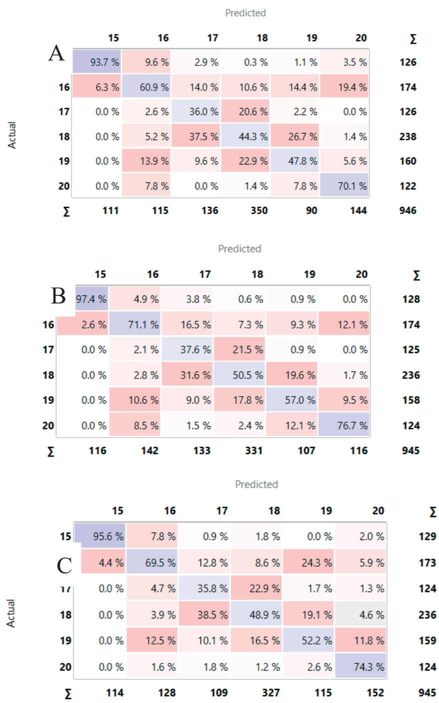
Figure 4. Result of the data processing algorithm. The red curve is the preprocessed acceleration data supplemented with the detected maximum and minimum peaks

The primarily obtained values were processed with a neural network. The primary detection can be implemented on the microcontroller of the bolus sensor and the result of the measurement is a data set of a size that can already be sent using a radio data transmission standard protocol. Post-processing using an intelligent method can already be implemented on the server side. During the implementation, we consciously tried to use a relatively small neural network in order to avoid overtraining as much as possible, taking the risk that the obtained results would be weaker. I solved the neural network data processing as a classification task, because in this way the processing of individual IBI period categories can be evaluated in more detail. Table 1 contains the evaluation of the post-processing of data sets of size 3s, 4s, 5s. It can be seen that the Classification Accuracy (CA) value is relatively small. The best case is the processing of a 4s long data series, but even here the detection rate accuracy is only 61.5%. The same is true for other metrics like F1, Precision, Recall, MCC. At the same time, the AUC (Area Under Curve) value, which assesses the validity of the detection, the size of the area under the ROC curve, produced significantly better results. The best case here also occurred in case of processing the 4s long data series, with a value of 0.874, which is quite close to the ideal value of 1. The explanation for the two types of results is that, although in many cases it is not possible to accurately categorize the data, at the same time, in most cases, the result is not fundamentally bad at the end of the processing, only one category mistake, which is approximately corresponds to an error of 40ms. The actual IBI values fall between 600ms and 1200ms, that is, the difference of the detection means an error of 5-10%.

Table 1. Quality metrics of the ANN processing of 3s, 4s, 5s long data series primary results

Model	AUC	CA	F1	Prec	Recall	MCC
NN3s	0.845	0.552	0.545	0.567	0.552	0.456
NN4s	0.874	0.615	0.616	0.635	0.615	0.532
NN5s	0.859	0.603	0.598	0.612	0.603	0.518

A detailed evaluation of the ANN post-processing can be done with the help of Figures 5 and 6. Figure 5 shows the confusion matrix for the processing of 3s, 4s, 5s long data series. Naturally, here too, the processing of the 4s long data series is the most effective in terms of almost all values. In the main diagonal, the proportion of correctly categorized data is better in all values compared to the other two data sets. The ratio of the worst categorized data can be seen in the bottom left and upper right corners, in this respect the processed data series of 5s is the best, it contains the fewest gross errors. It can be seen that the largest numerical values are found in the main diagonal and in the band immediately next to it, which means that the majority of the data can be classified with no more than 1 category error. Detection of extreme categories is better than the average. The detection of category 15 (600ms IBI) is 93.7-97.4% accurate.



A

Actual \ Predicted	15	16	17	18	19	20	Σ
	93.7 %	9.6 %	2.9 %	0.3 %	1.1 %	3.5 %	126
16	6.3 %	60.9 %	14.0 %	10.6 %	14.4 %	19.4 %	174
17	0.0 %	2.6 %	36.0 %	20.6 %	2.2 %	0.0 %	126
18	0.0 %	5.2 %	37.5 %	44.3 %	26.7 %	1.4 %	238
19	0.0 %	13.9 %	9.6 %	22.9 %	47.8 %	5.6 %	160
20	0.0 %	7.8 %	0.0 %	1.4 %	7.8 %	70.1 %	122
Σ	111	115	136	350	90	144	946

B

Predicted	15	16	17	18	19	20	Σ
	97.4 %	4.9 %	3.8 %	0.6 %	0.9 %	0.0 %	128
16	2.6 %	71.1 %	16.5 %	7.3 %	9.3 %	12.1 %	174
17	0.0 %	2.1 %	37.6 %	21.5 %	0.9 %	0.0 %	125
18	0.0 %	2.8 %	31.6 %	50.5 %	19.6 %	1.7 %	236
19	0.0 %	10.6 %	9.0 %	17.8 %	57.0 %	9.5 %	158
20	0.0 %	8.5 %	1.5 %	2.4 %	12.1 %	76.7 %	124
Σ	116	142	133	331	107	116	945

C

Actual \ Predicted	15	16	17	18	19	20	Σ
15	95.6 %	7.8 %	0.9 %	1.8 %	0.0 %	2.0 %	129
	4.4 %	69.5 %	12.8 %	8.6 %	24.3 %	5.9 %	173
17	0.0 %	4.7 %	35.8 %	22.9 %	1.7 %	1.3 %	124
18	0.0 %	3.9 %	38.5 %	48.9 %	19.1 %	4.6 %	236
19	0.0 %	12.5 %	10.1 %	16.5 %	52.2 %	11.8 %	159
20	0.0 %	1.6 %	1.8 %	1.2 %	2.6 %	74.3 %	124
Σ	114	128	109	327	115	152	945

Figure 5. Confusion matrix of (A) 3s, (B) 4s, (C), 5s long data series. Each category is 40ms wide

This category is particularly important for implementing alarms related to elevated heart rate. It should be mentioned that this category can only be mistaken in one direction in the system. Figure 6 shows the ROC curve belonging to class 15 (600ms IBI). It can be seen that the shape of the curve is almost ideal and enables a good categorization of the class. Unfortunately, we cannot ignore the fact that this category was rather underrepresented in the learning dataset, and the required amount of learning data is the result of oversampling. In such cases, relying on statistical considerations, we can hope that the processing gave a real result, but this can only be confirmed with further measurements.

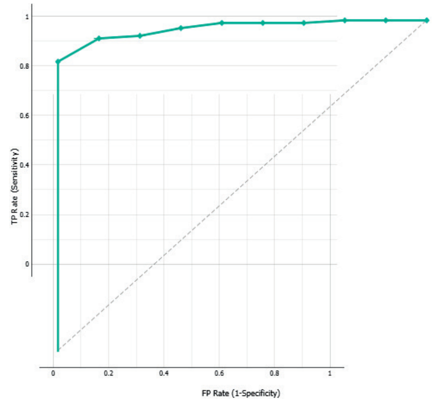


Figure 6 ROC curve for class 15 which corresponds the 600ms IBI

Figure 7 shows the histogram of error values after primary data processing (A) and after neural network post-processing. It can be seen that the detection accuracy has improved by almost an order of magnitude.

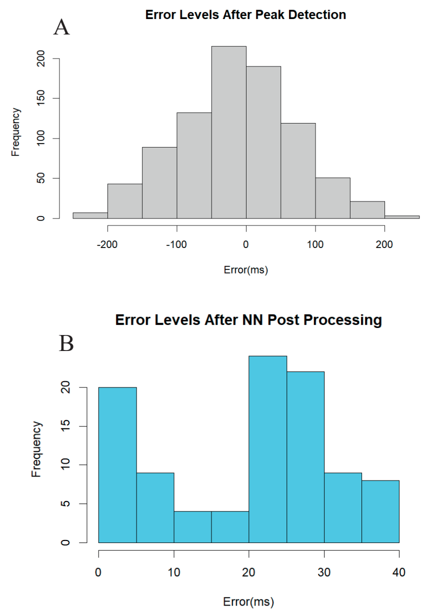


Figure 7. Histogram of IBI detection errors: (A) errors of the 4s long detection period by the median of detected maxima, (B) errors of the ANN result

Table 2. Detected IBI categories and the corresponding HR values

Cat	IBI (ms)	HR (BPM)
1	800	75
2	760	79
3	720	83
4	680	88
5	640	93
6	600	100
7	560	107
8	520	115
9	480	125

Table 2 shows the detectable IBI and HR values. Among them, it was possible to detect categories 1-6 in the current experiment.
The average error value is 20-40 ms, which is not suitable for very accurate animal health measurements, but it is already suitable for the implementation of important health warnings or alarms related to significant HR changes (disease, increased stress, initiation of calving) in PLF-related animal husbandry support applications.

CONCLUSIONS

In this paper, an algorithm development process was presented, of which the IBI and the HR values can be calculated from the measured 3D accelerometer data in a cow rumen bolus sensor. The algorithm to be implemented must be of low computational intensity that it can also be implemented on the sensor's microcontroller. In such a case, the first question is the quantity of data to be collected, for the optimum calculation can be carried out. Experiments have revealed that the collection of 4-5 seconds of data is optimal for calculations. It could be seen from the results that 4s are ideal according to most metrics, and 5s is the best in terms of gross error rate, but processing a longer sequence with such a simple algorithm no longer improves the result. The 4s-5s data series can mean 100 or 125 measured data, considering the 25 Hz sampling frequency. The measurement error is 40ms in this case, which theoretically can be improved to a 10 ms detection accuracy assuming 3-4 cycles of detection, but unfortunately, due to the significant noise of the measurement, such good results cannot be obtained on real data. Measurements were evaluated with a simple O (N) data cleaning and peak detection algorithm. The error of the results obtained is of a magnitude greater than the theoretical minimum. Based on the results, it can be concluded that it would be worthwhile to use a slightly more skillful data cleaning and peak detection algorithm in the future, but it also appears that due to the high noise ratio of the measurement, this alone is not sufficient. It is absolutely necessary to subject the measurement to some kind of post-processing. In this work, a neural network was examined as a post-processing method and it was found that the neural network is a suitable solution for post-processing. A relatively small sized neural network was consciously chosen to avoid the risk of over teaching. Postprocessing with a neural network significantly improved detection accuracy and enabled detection with a ±40ms error. The resulting method is thus appropriate for classifying cardiac function into six categories.
The accuracy of the obtained results can be improved in the future and the current accuracy is not yet suitable for veterinary purposes. On the other hand, the system is suitable for use in information systems related to PLF, it is probably suitable auxiliary data for detection of stress on animals, for alarms related to calving and diseases.
The HR values above 100 could not be measured by the control ECG measurement, so there is currently no information on the detectability of these values.
The next step of the research is to conduct additional measurements and gather the corresponding control data. After that, the refinement of the data cleaning and peak detection algorithm and the examination of the neural network solution in the detection of values that are underrepresented or not measured at all in the current experiment.

ACKNOWLEDGEMENTS

Thanks for Albacomp Zrt for creation of the bolus and for Levente Kovács (MATE) for help in control measurements. Lot of thanks for Etyek Farm to support us as an experimental place.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

LIMOUSIN BREED – CREATION, APPROVAL, SPECIFICATIONS AND CHALLENGES. REVIEW

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Abstract

Limousin is a cattle breed created and selected in France, more than 150 years ago in the region of the cities of Limoges, Albussac, La Courtine in the regions of Limousin and Marche that are part of the central massif of southwestern France, through artificial and natural selection, by the way of selection of the local Blonde d'Aquitaine, distinguished by a rough constitution and used for work. Local natural and climatic conditions affected on its creation. The aim of the present study was to analyze the creation, consolidation, exterior and constitution and related selection, available gene pool and the trend of distribution of Limousin breed worldwide, in Europe, and particularly Bulgaria. This is a breed with excellent meat production qualities. The cattle of this breed are unpretentious in feeding and care, having good utilization of pastures, and have normal fertility. They are resistant to diseases, and show intensive growth. They are used for the production of lean, high-quality beef and for industrial cross-breeding. The study is based on analysis of scientific developments and concepts dedicated to beef cattle breeding. General scientific research methods, information-logical analysis of scientific and scientific-practical information were used as methodological basis for its implementation.

Key words: *breeds, consolidation, constitution, exterior, trends.*

INTRODUCTION

Historical data on the creation of the breed.
This breed is not called 'the butcher's breed' by accident. Chefs also appreciate meat for its low surface fat and marbling. The history of the breed is believed to be as old as the history of Europe. Rock paintings found in the cave of Lascaux, near Montignac, depict tours likened to the contemporary Limousin breed. Recent DNA studies of European and Eurasian cattle, as well as fossils found at various archaeological sites, show that Limousin, Gascon, Aubrac, Salers and Charollais breeds have a common ancestry linked to cattle that lived in past geological periods in Eurasia.

Limousin is a cattle breed created and selected in France, more than 150 years ago in the region of the cities of Limoges, Albussac, La Courtine in the regions of Limousin and Marche, a part of the central massif of the Southwestern France, using artificial and natural selection, by the way of selection of the local Blonde d'Aquitaine, distinguished by rough constitution and used for work. That is a specific administrative region characterized with harsh winters, poor stony soils, sloping and rough terrains and scarce pastures.

Astigariaga and England (2005) indicated that the region is located at 45.69˚ latitude and 1.62˚ longitude. The altitude is 350 m. The population is about 137,000 people. The city of Limoges, which is the administrative center of the district, was founded by Emperor Octavian Augustus in the 1st century. This area is known for its well-developed animal husbandry.

Lafarge (1698) reported that Limousin oxen were universally known and respected as pack animals. At the end of their lives, they were usually fattened and used for slaughter and food.

The first written evidence of the existence of Limousin breed dates back to the end of the 18th century. In the first half of the 19th century, the selection was carried out systematically to increase the meat-producing qualities and turned the breed into a combined one for work, milk and meat. In 1770, Lieutenant general

Antoine de Sartini, police commandant of Paris sent a letter asking – Can he count Limousin oxen after Easter?
In 1791, Jacques-José Saint-Martin, an agronomist by profession, realized the great significance of Limousin breed for the markets of the following cities: Paris, Lyon and Toulouse. Subsequently, it gradually began to improve and develop as a meat-producing breed. Charles de Luobari and his shepherd Roer, through systematic selection, developed the selected Limousin herd in the period 1854-1896, which won 265 ribbons in the prestigious breeding competition of the city of Bordeaux. In 1856, the first herd book of the Limousin breed was registered by Louis Michel. Annual national competitions for the best specimens began. The selection was carried out in a planned way, with an evaluation of fathers for the quality of the offspring. In 1914, the total number of animals registered in the book was 5,416. The book was reorganized twice in 1923 and 1937. In 1926, a closed herd book began to be kept. From July 2007 to June 2008, in connection with the Legislation of the European Union, under increased pressure from French farmers, according to the requirements of the European Association Limousin-EUROLIM, having 11 countries as members, a planned restructuring of the herd book was carried out. In France, 4.1 million beef cattle are raised, 25% of which are of Limousin breed. The number of Limousin cows in the country is about 1 022,000. The controlled Limousin cows are 131,000. There are 51,000 registered in the herd book. Due to its qualities, Limousin cattle breed has received awards at prestigious competitions, such as the title 'Good European Cow', the main prize 'Cup for Good Meat' at the general agricultural competition in Paris in 2003.
There are many associations of Limousin breed, such as: Limousin Cattle Group France, Swedish Limousin Association, British Limousin Cattle Society, North American Limousin Foundation, Canadian Limousin Association, Columbia Limousin Association, Australian Society of Limousin Breeders, Texas Limousin Association, Bundesverbdndes Deutscher Limousinzucher, Associacao Brasiieira doc Criadores de Limousin, Irish Limousin Association, Hungarian Limousin Association, etc.
In Bulgaria, about 5,500 purebred Limousin cattle are bred. There are 3,300 controlled cows. The control is carried out by two breeding associations: National Association for Beef Cattle and Association for Breeding Beef Cattle Breeds in Bulgaria (Nikolov & Karamfilov, 2021).
The purpose of the present study was to investigate the main elements of the breeding of Limousin beef cattle breed raised under different technologies, around the world, in Europe and in Bulgaria.

MATERIALS AND METHODS

The study is based on an analysis of scientific developments and concepts dedicated to Limoisin beef cattle. As a methodological basis for conducting the study, general scientific research methods, information-logical analysis of scientific and scientific-practical information, as well as materials for marketing research on the market of different cattle breeds and crossings in beef cattle breeding were used. Descriptive, monographic and retrospective analyses were also provided. To achieve this goal were used summarized data concerning Bulgarian, European and worldwide cattle breeding in the period 2010-2021. The information used is mainly referred to publications of the FAO, Ministry of Agriculture, Food and Forestry (MAFF), Department of Agrostatistics, agricultural reports. The paper cites pieces of scientific works by Bulgarian and foreign authors. Summaries and conclusions were made.

RESULTS AND DISCUSSIONS

Growth and development

Growth is increasing the body proportions, through the deposition of structurally and functionally, full-fledged live weight. Weight development is a major, decisive factor for cattle meat productivity. The study of weight and linear development is of great significance for the breeding process (Karamfilov, 2020).
Koots (1994) analyzed published genetic evaluations as a parameter for beef production traits. According to him, low to moderate

hereditability was observed in weaning calves at a given live weight.

Gordyyanets (2010) found that calves from local genotypes showed an advantage compared to calves from milk productivity, significantly more efficiently using one kilogram of fodder to obtain one kilogram of growth.

Limousin breed is characterized by a harmonious physique, strong constitution and good growth capacity. It is a leader in the efficiency of muscle growth. It has a fine head with a broad forehead. The breed has horns, but hornless individuals are also found. The chest is wide, but not deep enough. The back is broad and flat. The body has well-defined carnivorous forms. The abdomen is voluminous but tight. The skin is colourless, fine and elastic. The hair coat varies from golden beige, fawn to brownish red, with light, white areas around the eyes, and muzzle and belly and groin. It is not pretentious in terms of feeding and rearing (Panayotova, 2011; Kosilov et al., 2013; Gorinov & Lidzhi, 2016).

Karlikov (2010) reported that the linear evaluation of exterior is moderately inherited (H = 0.32). According to Sinivirski (1988), the most common exterior defects for Limousin breed are saddle back, weak hind limbs and delicate bone system.

The live weight standard of Limousin breed shows that female animals reach 650-700 kg. The maximum weight achieved in a cow is 950 kg. Bulls weigh 900-1100 kg. The maximum weight achieved by a bull is 1560 kg. The age of first calving is 24-30 months. The weight of calves at birth varies between 36-40 kg. The weight of a 120-day-old male calf is 165 kg. The weight of female calves at 210 days is 242 kg. The weight of male calves at 210 days is 268 kg (Panayotova, 2011, Nikolov & Karamfilov, 2021).

Marces et al. (2001) analyzed the genetic parameters of live weight at weaning in Limousin calves. Data from this study were obtained from 41 calves born to 50 Limousin heifers and cows raised in the Samaiayuca desert in Mexico. The animals were pasture-raised. The results of the analysis are as follows: the average live weight at weaning of calves from Limousin heifers was 204.31 kg. The weight of calves obtained from cows varied from 207.79 to 215.06 kg. Female calves were lighter than males by 5.7-5.9%. The hereditability value of the weaning trait at certain live weight is H = 2.23 ± 0.006.

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Sudarev (2010) analyzed the birth weight, at 18 months and in adults of Hereford, Limousin and Charollais breeds in relation to the acclimatization abilities of the animals in the Tver region and the role of the breeds in industrial cross-breeding.

Panin (2010a) studied the growth dynamics of experimental animals in the harsh continental climate of the South Urals, Russia, and found that industrial cross-breeding created new opportunities to increase productive qualities. Three groups of 10 bulls were formed. In the 1^{st} group there were Limousin bulls, in the 2^{nd} one some crossings of first generation of Simmental x Limousin, in the 3^{rd} group there were Simmental bulls. Until the age of 8 months, the calves were reared on the pasture according to the classic ‘cow-calf’ technology. The growth indices of the experimental calves were studied monthly. Feeding was according to certain rates.

Table 1. Live weight at different ages of beef breeds in the Tver region according to Sudarev (2010)

Breed	At birth		At 18 months	Adult animal		Yield
	Female	Male		Cows	Bulls	%
Hereford	32	28	475	575	900	58-62
Limousin	42	35	525	550	1,050	60-70
Charollais	43	37	575	750	1,100	60-70

The data given in the table above (Table 1), shows the author's conclusion that calves live weight at birth was almost the same 26.7-32.6 kg. There were some differences in live weight of 2-month-old calves.

The difference between purebred animals and bulls of 1st and 3rd group is 6.2 kg, or 22.1% ($P<0.99$) in favour of Limousin.

Crossings took an intermediate position between them with 2.0 kg, or 2.9% compared to the Simmental breed ($P<0.95$) and 4.2 kg, or 5.5% compared to the Limousin. During the entire experiment, the highest increase was found in the Limousin breed. 4-month-old calves surpassed their Simmental peers by 7.7 kg, or 6.5% ($P<0.99$) and the crossings by 4.4 kg, or 3.7% ($P>0.95$). 6-month-old Limousin bulls reached a live weight of 181.4 kg, and surpassed the crossings, their peers by 5.1 kg or 2.9%, the Simmental bulls by 11.3 kg or 6.6% ($P>0.95$). 8-month-old Limousin bulls surpassed the crossings and Simmental calves – by 9.9 kg, or by 4.2%, respectively ($P>0.99$).

10-month-old animals in 1stgroup (Limousin breed) surpassed the bulls in 2ndgroup (crossings) and 3rdgroup III (Simmental breed), respectively by 12.9 kg or 4.4% ($P>0.95$) and 25.5 kg or 9.2% ($P>0.99$).

The same difference among groups was preserved for 12-month-old animals and the live weight was as follows: 1st group – 372.7 kg, 2nd – 344.3 kg and 3rd – 329.4 kg. The experimental 14-month-old bulls in the 1st group surpassed their peers from the 2nd group by 43.4 kg, or 10.7% ($P>0.99$), from the 3rd group by 65.0 kg, or 16.9% ($P>0.999$). In 16-month-old bulls, the superiority increased and represented 51.0 kg., or 11.0% ($P>0.999$) and 80.1 kg, or 18.5%, respectively.

They surpassed their peers from the other two groups, as and in the previous age periods with 87.0 kg, or 17.9% ($P>0.999$) and 55.0 kg, or 10.6% ($P>0.999$), respectively. The author's main conclusion is that Limousin bulls in the conditions of a sharply continental climate show higher absolute growth and high growth intensity compared to their crossings and peers from the Simmental breed.

Laninoy (1973) reported that Limousin breed has excellent meat-producing qualities. Cattle of this breed are considered as unpretentious in feeding and care, having good utilization of pastures and having high fertility. They are resistant to diseases and show intensive growth. They are used for the production of lean high-quality beef, as well as in hybridization schemes as a sire breed, providing easy calving and excellent quality indicators.

Akhmetov (2005) claimed that purebred Limousin animals in Russia were 2,114 or 1.4% of the total population of the Russian Federation. 597 cows, 958 bulls, 393 heifers and 306 calves were bred in the country. They are mainly used for industrial cross-breeding with Black-and-White and Simmental cattle, as the resulting F1 crossings form a meat-producing herd and are crossed with the Charollais and Blonde d-Aquitaine, and the males are fattened. The purebred assessed cows showed in the first year live weight of 492 kg, as it was in the 537 kg in the second, and in the third 597 kg. The weight of the bulls was 958 kg. Pedigree male calves from the state farm 'Kumskoi', in the city of Stavropol, showed average daily gain of 1,301 g and weight of 453 kg at 12 months age.

Umnov (2005) found that from crossings of Black-and-White cows with bulls of Limousin breed, young animals with intensive growth and good meat productivity were obtained. Male crossings at 15 months reached a weight of 490.4 kg and by this indicator they exceeded the control group of their peers by 22.5 kg.

Gizatullin & Khaziakhmetov (2010) observed good heterosis effect, intensive growth, high meat productivity and fodder utilization in crossings of Black-and-White, Simmental in Bestuzhev cattle with Limousin bulls in Bashkortostan. Crossings surpassed the maternal forms of their peers by up to 20% in terms of live weight, 8-12% in terms of warm carcass, and feeding costs are reduced by 6-10%.

Smirnova (2010) studied fattening results of Hereford, Aberdeen Angus and Limousin beef male calves in the Leningrad Region. The average live weight of Limousin calves was 520 kg at 18 months, which is 66 kg more than Hereford calves and 144 kg more than Aberdeen Angus.

Gudymenko et al. (2010) conducted an experiment with bulls from Limousin, Aubrac and Simmental breeds. Three groups of 15 animals were formed. The animals were purebred and reared on deep permanent litter, according to the technology. The calves were selected from cows that gave birth in the autumn-winter season. Until the age of 7 months, the calves were raised with their mothers on the pasture, and from 7 to 18 months they were fattened on specialized feeding grounds. The feeding was full, and the daily ration was according to Kalashnikov standards (2003). The ration structure was the same for all animals. The calves, depending on the genotype, reacted differently to the environmental conditions, which had an impact on their live weight. In the analysis made by the authors, it can be seen that 15-month-old bulls from Limousin and Aubrac breeds exceeded Simmental breed by 25.8 kg or by 6.2%. Limousin and Aubrac animals are characterized by compactness and well-developed musculature and meat forms.

Videv et al. (2001) reported that while crossing Bulgarian Brown cattle x Limousin x Charollais, animals are obtained for fattening with high average daily gain of 1200 g, and the best individuals in the experimental setup gave 1400-1500 g and were realized as bulls for industrial cross-breeding.

Gordynec et al. (2010) reported that the economic efficiency in breeding Limousin calves and its crossings with Black-and-White cattle and Maine-Anjou is higher compared to calves from dairy breeds, and it shows that calves with meat genotype have higher growth and fodder utilization as a result. 5.5-6-month-old Limousin calves and their crossings showed high growth energy and high average daily gain. They surpassed Black-and-White calves by 23%, Limousin x Black-and-White cattle by 8%, and Limousin x Maine-Anjou crossing by 12%.

Fertility

A significant indicator predetermining the production of meat from the population as a whole is the number of calves obtained and weaned.

Petrushko (2010a) reported that the fertility of the Limousin breed was 98%, and in individual herds it reached up to 100%. Limousin cows had normal fertility, and in three-year-old cows, fertility sometimes reached 100%. Calves were extremely light compared to other breeds.

Dop (2009) indicated the following fertility of cows from different breeds: Limousin – 98%, Hereford – 94%, Charollais – 91%. Limousin cows generally had longer gestation period of 287-297 days. Perinatal mortality in France for Limousin cattle was only 3%. The interval between calving and first mating period greatly affects fertility. He considered that the average interval should be about 60 days for mature cows and about 80 days for two-year-old cows. With good feeding, this interval is usually short, and with bad nutrition, it is significantly extended. Spring-calving herds had higher overall fertility level than autumn-calving herds except when hay was limited to 40% (Todorov, 2001).

Panin (2010a) concluded that Limousin cows in the Southern Urals are distinguished by good fertility, which shows regularity in calving. Calving interval was 380 days, which was 77.6% in pedigree herds. 94.8 calves were obtained from 100 cows. Difficult births were 1.7%

Meat productivity

The quantitative and qualitative indicators of meat productivity in cattle are the following: breed and individual characteristics of animals, breeding technology and other non-hereditary factors.

Sinivirski (1988) considered that breed affiliation has great significance for the quantity and quality of beef. Specialized beef breeds mature earlier, fatten better and have higher meat quality than dairy breeds. The same author expressed the opinion that in cattle with the advancing age there are significant changes in metabolism, in the establishment of nutrients and in the intensity of growth. The consumption of nutrients per 1 kg of gain gradually increases. With advancing age, the slaughter yield increases, the amount of fat and energy content in the meat decreases, the number of bones in the carcass decreases, the tenderness and juiciness of the meat deteriorates, and the taste improves.

During the creation and improvement of Limousin breed, meat productivity was the main, essential feature.

Levantin (1996) found that high growth intensity, low fat content and high muscle tissue content in the carcass characterize the French-Italian breeds, as one of them is Limousin.

Pitchford (2008) commented on the values of the F94L gene. The author made the following conclusions:

- This gene produces 20% more meat on the same amount of food;
- The gene increases the tenderness of the meat by 15%;
- The gene is of very high purity in animals of Limousin breed, but of very low purity in the others.

In studies in Adelaide, Australia, they found changes in myostatin CRC3 under the influence of the F94L gene. This gene occurs at very high frequency in Limousin cattle and does not affect light calving and fertility. A highly resistant form of the gene is with prevalence of 83% in Limousin and only 3% in Belgian Blue, 0.6% in Aberdeen Angus and 0% in Hereford. The 83% prevalence gives reason to believe that 69% of all individuals are homozygous for this trait, 28% are heterozygous and only 3% do not carry this gene. Animals carrying the gene showed 13% higher muscle eye size and 4.4% higher meat quality.

The yield was 64-67%, muscle/fat ratio was 7, muscle/bone ratio was 4.7 according to Dop (2009).

Petrushko (2010b) reported that meat of Limousin cattle was with thin fibres, tender, juicy, well-marbling, tender and tasty. Meat productivity of calves was 75%, Category E and U according to the European classifications.

For 1 kg of bones, there were 6.5 kg of meat. The meat content of the carcass varied between 82-83%. Filippova (2009) stated that fat content in the meat was 7-10%, and protein content was 19-20%.

According to the American Herd Association, one-three-ounce serving of lean Limousin beef contains an average of 73 mg of cholesterol. The same measure of chicken contains 76 mg of cholesterol, pork has 77 mg of cholesterol, fried shrimp has 178 mg of cholesterol, and one egg has 274 mg of cholesterol.

According to a study by the University of Texas at Iowa, Limousin meat contains significantly less fat, 2.43%, instead of 6.37%, which is found in other beef cattle breeds. This represents an impressive 61% reduction in fat. It is said that in a study among five beef breeds, Limousin meat is first in tenderness and juiciness, and equal in taste among others.

Mokhov and Sharokin (2007) reported that beef contains: muscle tissue 64.5-72.3%, fat tissue 10.4-12.9%, connective tissue 9.3-10.1%. 100 g of beef contain from 100 to 196 Kcal. or 410-804 KJ.

Ivanov et al. (2001) studied the fattening of female calves of Black-and-White breed and crossings between Limousin and Brown breed. Upon reaching 400 kg of live weight, calves were slaughtered and a full slaughter analysis was performed. Calves from the 2nd group gave 10.7% higher average daily gain. They also had better slaughter performance.

Bikbulatova (1998) conducted a series of experiments on fattening male calves from Limousin, Simmental, Bestuzhev and Black-and-White cattle breeds. 18-month-old calves from Limousin breed reached weight of 638 kg, which was by 48.8 higher than their peers from the Simmental breed, by 87.7 kg than their Black-and-White cattle peers and by 59 kg compared to Bestuzhev breed.

Vinogradov (2007) reported that in November 2006 to March 2007 he studied 170 Aberdeen Angus and Limousin calves obtained by embryo transfer. The objective of the study was to observe the live weight and growth dynamics in transplant calves. The experiment was conducted in Samara Region, Nefrostorsky District. During the conducted research, different live weight was found between female and male calves. For Aberdeen Angus it was 4.1 kg or 14% in favour of male individuals, and for Limousin – 2.8 kg or 12.7% also in favour of male calves. The live weight of male calves at birth was 32.3 kg, and that of females – 28.2 kg. The calves felt well and did not suffer from dyspepsia or pneumonia. The average daily gain of 6-month-old calves of

Aberdeen-Angus breed was 1138 g and 1087 g for Limousin breed.
According to Panin (2010b), the slaughter indicators of 15-month-old Limousin bulls are as follows:

Live weight, kg	572.6
Pre-slaughter, kg	561.0
Carcass weight, kg	344.2
Pure meat, %	61.3
Weight of internal fat, kg	22.1
Percentage content of internal fat	3.9
Meat in the carcass, kg	366.3
Meat in the carcass, kg	65.2

According to Hakimov (2011), 15-month-old transplant Limousin bulls had the following slaughter parameters:

Pre-slaughter, kg	531.7
Carcass weight, kg	311.2
Meat in the carcass, kg	58.6
Weight of the internal weights, kg	14.5
Percentage content of internal fat	2.74
Meat in the carcass, kg	325.8
Meat in the carcass, %	61.3
Meat in the half carcass, kg	150.8
Meat in the half carcass, kg	122.7
Meat in the half carcass, kg	81.4
Bones, %	15.8
Tendons and ligaments, kg	3.90
Tendons and ligaments, kg	2.60
Ratio of edible and inedible parts	4.35

The above-mentioned data testify to obtained meat with good nutritional qualities and technological indicators. The good culinary properties, the energy value, the high content of protein and amino acids distinguish Limousin breed as one of the leaders among modern beef breeds.

Milk productivity
Milk productivity is a function of the mammary gland, the development and action of which is controlled by the Central Nervous System. Milk yield of cows is a significant trait of economic importance, determining to a large extent the live weight of calves until weaning. There is a high value of positive correlation between milk productivity of beef cows and their 3-month-old calves (H = 0.69-0.75) and calf growth and fodder consumption (H = 0.51-0.69).
Mokhov and Sharohin (2007) reported that cow's milk has the following composition: 88.5% water, 3.1% proteins, 3.5% fat, 4.7% milk sugar, 0.7% mineral substances, ferments, etc. 100 g of milk contains 58 Kcal or 238 kJ.
The main method of determining milk yield of cows in beef cattle breeding is the control weighing of calves before and after lactation. Using this method, they found that the average daily milk production of Limousin cows was 5.99 kg, and it changed in June and September, as in individual cows it fluctuated from 1.8 kg to 9.1 kg daily milk yield. The most common method of milk yield research in beef cattle is manual milking, after intravenous administration of 10 IU oxytocin, one minute before milking.
Lanina (1973), Levantin and Smirnov (1970), Cherkashchenko (1975) and Prakhov (1975) expressed the milk productivity of beef cows in terms of absolute or equivalent live weights of calves at weaning. They recommend to assess the mothers according to calf live weight at weaning, citing that the correlation coefficient between calf live weight at weaning and maternal milk yield is relatively high.
Lowson (1981) noted that calf live weight at weaning was closely related to the availability of pasture and concentrate feeding. He reported that the correlation between calf live weight at the age of 100 days and maternal milk yield (r = 0.85) is much greater. At this age, the assessment is less affected by nutrition.
Lowson (1982) believed that when evaluating cows, milk yield should be adjusted for milk composition. He used a formula to calculate the milk weight, where 1 lb of adjusted milk contains 340 Kcal of energy. The author found that milk productivity, and hence calf weight at weaning depends the most on the conditions of the year (the availability of pasture). This affects both the total productivity of milk and its ingredients. The corresponding year also influences with its climatic conditions.

CONCLUSIONS

Limousin breed is with excellent meat production qualities. The cattle of this breed are unpretentious in feeding and care, having good utilization of pastures and have normal fertility. They are resistant to diseases and parasites.

They are characterized by a harmonious body structure, strong constitution and good growth ability.
The breed is a leader in the efficiency of muscle growth. It is used for the production of lean, high-quality beef and veal, as well as for industrial cross-breeding.
It is introduced well on all continents and shows good economic results.

ACKNOWLEDGEMENTS

I would like to express my gratitude to the management body of the Research Institute of Mountain Stockbreeding and Agriculture in Troyan, for the logistic and material support in the study.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

INFLUENCE OF THE TEMPERATURE-HUMIDITY INDEX ON SOME PHYSIOLOGICAL PARAMETERS IN DAIRY GOATS

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Abstract

The aim of this paper is to study the microclimate in a dairy goat farm during the warmest (July and August) and coldest months of the year (January and February) and evaluate its impact on animal welfare and health. Temperature, relative humidity and illuminance were monitored. The severity of temperature stress was determined by calculating the temperature-humidity index (THI). The average value of THI for the month of August exceeded that for the month of July by 1.5 and it also remained above the threshold accepted for extremely high heat stress (28.6). During the cold days of the year (January and February), when the goats were mainly in the barn, THI did not exceed 17, varying between 5 and 16.8. The average relative humidity and illuminance remained within their permissible values. As a physiological adaptive response during the hot months, animals responded with an increase in rectal and skin temperature, pulse rate and respiratory movements while rumen contractions decreased.

***Key words**: goats, temperature stress, temperature-humidity index, physiological parameters*

INTRODUCTION

The welfare and good health condition of goats are of utmost significance for unleashing their genetic potential in terms of milk production. The temperature, humidity, air movement, illuminance, etc. are extremely important environmental factors. (Aleena et al., 2018; Archana et al., 2018; Pragna et al., 2018).

Aleena et al. (2018) and others specify goats as animals with a large adaptation potential with reference to the changing environmental conditions. Nevertheless, upon non-observance of fundamental requirements towards the rearing conditions, problems related to the growth (Pragna et al., 2018), productivity (Archana et al., 2018), as well as the immunity (Dangi et al., 2015) are observed.

Regardless of the growing climate changes, goats are considered to be drought-tolerant animals (Serradilla et al., 2018; Stone et al., 2020), which are capable of surviving (Chebli et al., 2020) and fighting multiple disease agents (Pal & Chakravarty, 2020). In this respect, their breeding is considered to be more cost-effective than that of cattle. Goats use relatively low-quality feeds to create valuable high-quality food products (Peacock & Sherman, 2010). According to Darcan & Silanikove (2018), they also do not require expensive buildings.

The high-producing dairy goats, however, require optimal conditions on the pastures and in the barns so as to be safeguarded against stressful situations (Amamou et al., 2019). To this regard, Ribeiro et al. (2018) are of the opinion that it is necessary the zoo-hygienic parameters to be analysed and evaluated due to the fact that each breed has different adaptability characteristics and welfare problems (Gelasakis et al., 2017). Upon heat stress, there is redistribution of the available resources of the body, which affects the growth, reproduction, productivity and health. Consequently, the ambient temperature dictates the intensity of the metabolic processes, the heart and respiratory rate et al. (Fonseca et al., 2016).

In Bulgaria, the high summer temperatures of not only the external environment but also in the barns prove critical as they considerably exceed 28ºC, which is the optimum limit for goats. On that account, we set ourselves the task to examine the microclimate in a dairy goat farm during the warmest (July and August) and coldest months of the year (January and February) and evaluate its impact on the animals' welfare and health.

MATERIALS AND METHODS

The farm we studied keeps 170 goats of the Bulgarian White Dairy breed and is situated in South Bulgaria. The construction and technological characteristics of the building are displayed in Table 1:

Table 1. Construction and technological characteristics of the building

Element of the building	Characteristics
Length, m	38
Width, m	8
Height, m	3.2
Roof Structure	Wooden
Roof	Tiles
Walls	Bricks + adobe, without external or internal mortar
Floor	Rammed clay
Doors:	Wooden
Windows	Wooden
Building orientation	East-west
Method of breeding	free range
Cleaning	Manual
Sewerage	No
Feeding	Manual
Watering	Metal troughs in the building
Milking	Mechanically in a churn
Ventilation	Natural
Area per 1 animal: From the building (304 m²) From the yard (760 m²)	 1.74 m 4.34 m

The building is a typical brick-adobe masonry with walls width of 0.24 m and has no internal and external insulation. There is a tile roof mounted on the wooden roof construction. The goats are bred freely on a floor of rammed earth and straw bedding. Each animal is ensured 1.74 m² in the building and 4.34 m² in the yard. The building has southern exposure and 'east-west' orientation. The ventilation is natural and the cleaning of the manure- manual.

During most of the year (190-240 days), the goats are grazing with the exception of the days when the weather conditions are inappropriate. In the winter, the feeding and watering are performed in the building, and in the summer- in the yard. During the lactation period the goats are given 600 g concentrated feed extra.

The control over the temperature (ºC) and relative humidity (%) in and outside the building was exercised by measuring them with the Assman psychrometer and the Six's maximum and minimum thermometer at 9:00, 13:00, 17:00 and 21:00 in January, February, July and August. The illuminance level was determined with a lux meter PU 150 Praha. The temperature humidity index was determined using the equation reported by Marai et al. (2007):

$$THI = T - [(0.31 - 0.0031\ RH)\ (T - 14.4)],$$

where T is the dry bulb thermometer temperature (ºC), and RH is the relative humidity (RH %/100). Upon THI values of < 22.2, the animals are outside the heat stress zone; from 22.2 to < 23.3 there is a moderate heat stress; from 23.3 to < 25.6 there is a severe heat stress, and upon THI of 25.6 and more, the animals are subjected to extremely severe heat stress.

The physiological parameters of one and the same animals were reported during all months. The following recordings were made three times at 11:00, 13:00, 15:00 and 17:00 each month during the period of the study:

Rectal temperature (ºC) - with an electronic thermometer "Kebl", model 2130.

Arterial pulse rate (beats/min)- with a chronometer on the femoral artery, on the inside of the rear leg.

Respiratory movements (n/per minute)- with a chronometer by tracking the chest and the flanks movements of the animal.

Skin temperature (ºC) - with a Compact infrared thermometer 105518 with a range from -50 to + 550ºC and a resolution of 0.1ºC. The values are the mean of the temperature measured at the forehead, back and the stomach of the animals.

Rumen contractions (n/5 min)- by means of a moderate pressure on the inside of the left flank for 5 minutes. The data obtained was processed in compliance with the statistical methods and the margin of fluctuation, the arithmetic mean and the maximum limits are displayed.

The THI effect on the physiological parameters studied was reported by means of a one-way analysis:

$$Y_{ij} = \mu + THI_i + e_{ij},$$

where: Y_{ij} – is the dependent variable (each of the physiological parameters studied), μ is a mean effect, THI_i – the effect of the average THI for the month and e_{ij} is the random residual effect.

RESULTS AND DISCUSSIONS

The goats are reared in a standard brick-adobe building which provides both sufficient barn and yard space for each animal (Table 1).

Table 2 displays data about the microclimatic parameters inside and outside the building.

The microclimate in the dairy goat farms is of key importance for the health of the animals and the production of milk. The stocking density, the feeding and watering front, the cleaning and ventilation systems are all of significance for the microclimate's keeping within the permissible limits. Marciniak (2014) further adds that the design and the structure of the buildings are also important. According to the set norms in our country (Ordinance No. 44, 2006), the optimal temperature range related to goats is between 10 and 17ºC, and temperatures above 35ºC are considered critical. Upon ventilation malfunction, it is possible that the goats are overcooled or overheated.

We consider that the transient minimum temperatures of –2ºC which we registered in the winter months were not perilous for the goats. Bøe & Ehrlenbruch (2013) also take a similar stand by stating that this is due to the goats ability to adapt quickly (for approximately 2 days) to the low ambient temperature. However, the summer temperatures which exceed the optimal limits are critical. Having analysed the average temperatures inside and outside the barn (Table 2), we can assume that there are conditions for heat stress. The highest outdoor temperature was recorded in August (37.6ºC). High temperatures were also registered inside the barn in both summer months (36ºC). The average relative humidity inside the barn was within the maximum limits only during the summer months and exceeded them significantly during the others. The luminance intensity, with a few exceptions, was within the set norms.

Table 2. Temperature, relative humidity and luminous intensity mean values inside and outside the building during the different months

Parameters	Margin of fluctuation		Mean		Maximum limit
	inside	outside	Inside	outside	
Temperature, ºC					
January	-6 - 18.4	0 – 20.2	5.7 ± 1.8	10.5 ± 2.1	
February	-10 - 17.4	-2 - 21	12.3 ± 2.3	9.8 ± 1.7	
July	16 - 36.4	25 - 36	26.5 ± 1.4	30.5 ± 1.9	6-20-28
August	20.7 - 37.6	27 - 36	29.2 ± 1.1	31.2 ± 2.2	
Relative humidity, %					
January	38 - 90	58 - 100	64.5 ± 2.1	93 ± 4.1	
February	35 - 92	43 - 100	63.7 ± 2.7	95 ± 4.5	
July	42.8 - 47.4	58 - 77	45 ± 0.8	67.5 ± 2.2	50-80
August	36 - 43	45 - 72	39.5 ± 1.8	58.5 ± 1.8	
Luminous intensity, lux					
January	21 - 2120	3 - 160	1720	104	
February	30 - 1960	11 - 175	1605	111	
July	25 - 235000	12 - 688	107500	189	50-150
August	32 - 241000	13 - 705	120500	204	
Temperature-humidity index,THI					
January	0 - 16.8	3.5 - 19.2	5.1	12.5	
February	0 - 15.6	2.1 - 18.1	3.8	11.2	
July	16.1 - 32.8	15.7 - 35	24.5	32.8	22.2
August	17 - 33.3	15.8 - 36.9	25.2	33.3	

Summarizing the results of the examinations carried out, we may consider that the building studied has low to average temperature resistance both during the coldest winter (-15ºC) and the warmest summer days (above +35ºC).

We used the temperature-humidity index to assess the barn environment factors and the extent to which they form its comfort or discomfort (Marai et al., 2007) (Figure 1). Upon index values of < 22.2 - there is no heat stress, from 22.2 to < 23.3 there is a moderate heat stress, from 23.3 to < 25.6 there is a severe heat stress, and upon values of 25.6 and more, the stress is extremely severe. The data analysis indicates that both the temperatures in the barn (25º - 36ºC) and in the region (35º - 42.1ºC) form THI values which exceed the threshold for not only moderate but also extremely severe heat stress (28.6).

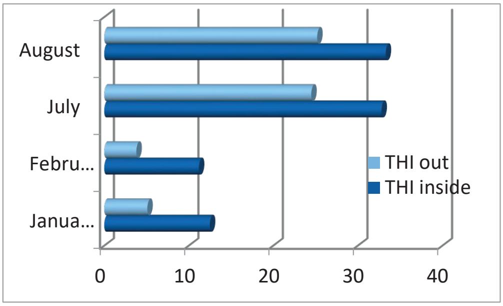

Figure 1. THI mean values inside and outside of the goat building

In the literature it is indicated that the small ruminants deal with the heat stress better than the cattle. The comparisons are made on the basis of THI values between dairy goats and dairy cows and prove the more flexible adaptability capacity of the goats (Silanikove, 2000). However, in this case the THI effect during grazing is not considered as it is in our country for example, where the small ruminants are mostly pasture-raised.

According to Kapgate et al. (2016), the type of building where the goats are kept during the summer does not have a significant effect on their physiological parameters.

Another study proves that goats in fact react to the changes in the microclimate by displaying changes in their physiological parameters, and the building characteristics also have an effect on them (Wadhwani et al., 2016). Bhatta et al. (2005) also ascertain a positive correlation between the THI and the physiological values of the goats.

Figure 2 displays the mean values of the physiological parameters examined by months. In our study, the internal body temperature varies within the reference parameters for the type, with the values being around the upper limits in July and August. Marai et al. (2007) also report an increase in the rectal temperature, pulse rate and respiration. According to (Al-Haidary, 2000), the high THI values are the reason for the lack of appetite, thermoregulation processes activation (da Silva & Rocha, 2020; Alyamani & Koluman, 2020), changes in the hormonal background, which affect the productivity and the fertility of the goats, and decrease in the production effectiveness (Souza et al., 2012). Therefore, controlling the physiological status of the animals is of important diagnostic significance for the assessment of the degree of the heat stress. Lucena et al., 2013; Ribeiro et al., 2016, 2018; Ribeiro et al., 2018a also consider that they are suitable for studying the adaptability of the small ruminants.

The maintenance of constant body temperature depends on the synchronicity between a range of complex physiological processes. Due to the fact that the sweat glands secretion in goats is poor, the acceleration of the shallow breathing and the increased ventilation of the upper respiratory passages are of great importance for the heat loss upon high ambient temperature. When the physiological cooling mechanisms of the body fail to dissipate the excess heat, the organism unlocks supplementary morpho-logical, blood and biochemical processes.

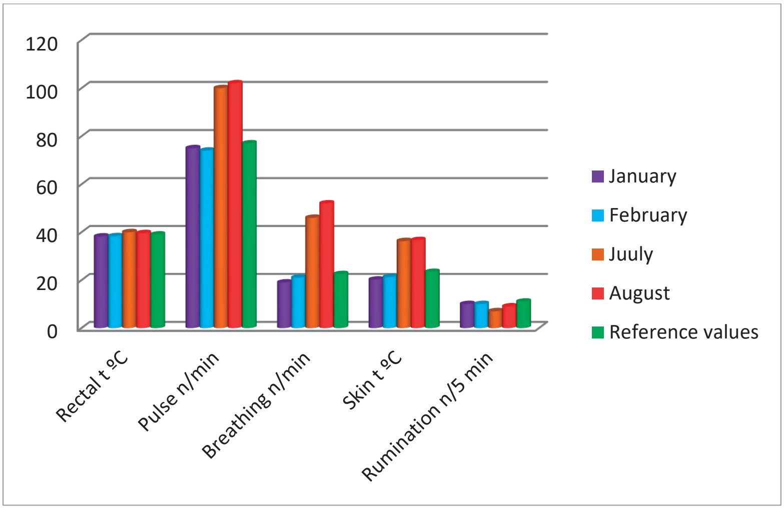

Figure 2. Mean values of the physiological parameters examined by months

We ascertained that upon increase in the THI values, the skin temperature also increases ($p<0.001$) (Table 3).

Table 3. THI influence on the physiological parameters examined and degree of reliability.

Physiological indicator	X[1]	SE[2]	F[3] p[4]
Rectal t ºC	39.02	0.15	33.9***
Pulse n/min	87.88	1.19	163.3***
Breathing n/min	34.5	1.28	178.4***
Skin t ºC	28.6	0.53	297.4***
Rumination n/5 min	9.7	0.54	6.01**

[1]X - Average
[2]SE - standard error,
[3]F - Fischer's criteria,
[4]p - Degree of reliability
***- Significance upon $p<0.001$,
** - Significance upon $p<0.01$

When the animals are bred under an optimal temperature-humidity regime, this temperature remains lower than the rectal by 5-6ºC. Upon high THI values, however, it is capable of reaching the rectal temperature (Campbell, 2011). The reason for this, according to Ligeiro et al. (2006), is the small size of their body which means that goats are forced to expose to the sun radiation surface which is larger when compared with their weight. During the day, there is a normal variation in rectal temperature from 0.3°C to 1.9ºC (Piccione & Refinetti, 2003).

The seasons change and the microclimate parameters related to it affect the body and skin temperature of the goats. In order to maintain these parameters within the norm, the animals try to dissipate the heat by accelerating their respiration (Yamani & Koluman, 2020).

The increased respiration rate upon high THI values is a temporary mechanism, which the animal uses to maintain its temperature homeostasis (Ribeiro et al., 2018a). During our study, we ascertained a change in not only the rate but also the depth of the respiratory movements. We consider that the reason for the abovementioned is the high activity of the animals in grazing conditions.

The pulse rate elevation is a reason for an increased blood flow towards the peripheral vessels. In this way a loss of heat is performed through release, transmission, convection or evaporation (Marai et al., 2007). The temperature increase over 31.6ºC according to Lucena et al. (2013) leads to heart rate elevation to 121 beats per minute which is also an indicator for thermal discomfort in goats.

The conditions in which the goats are reared, the feed content and the state of the digestive system determine the rumen movements. The higher summer values of the THI lead to slowing down of the rumen contractions ($p<0.01$). Similar results are reported by Alam et al. (2011), who also record slowing down of the ruminations when the THI values are high. In the course of time, the animals adapt and the number of the rumen contractions comes close to the reference values for the breed.

CONCLUSIONS

The accurate analysis of the factors which have effect on the health, behaviour and productivity of the goats is of importance for the production effectiveness. In conclusion, we can summarise that the high THI values reported during the summer months in South Bulgaria (24.5-25.2ºC outside, and 32.8-33.3ºC in the building) significantly affect the rectal and skin temperature ($p<0.001$), the pulse rate and the respiration ($p<0.001$) and have less effect on the rumen movements ($p<0.01$).

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

DISTRIBUTION AND PRODUCTIVE CHARACTERISTICS OF NORMANDE CATTLE BREED WORLDWIDE, IN EUROPE AND BULGARIA- REVIEW

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Abstract

The Normande breed originated from cattle brought to Normandy by Viking conquerors in the 9th and 10th centuries. For more than a thousand years, these cattle have become a dual-purpose breed to meet the milk and meat needs of the people of northwestern France. Normande cows have been exported around the world. The average height of bulls is about 152 cm and cows about 140 cm. The average body weight of male animals is about 1100 kg, and females about 700 kg. Normande cattle are an enduring French breed selected for the production of high-fat, high-protein milk, sought after for high-quality production of butter, cream and cheese and for their attractive meat properties. The Normande carry the Kappa Casein gene. The Normande breed is the only dairy breed sold as `first class` meat. The carcass weight for young bulls is 355 kg with a meat yield of 55%, for castrates - 391 kg with a 55% yield, and for slaughtered cows the carcass weight is 340 kg with a meat yield of 53%.

Key words: *fats, meat, milk, protein, Normande breed.*

INTRODUCTION

The Normande breed originated from cattle brought to Normandy by Viking conquerors in the 9th and 10th centuries. For more than a thousand years, these cattle have become a dual-purpose breed to meet the milk and meat needs of the people of northwestern France. The current book about the herd in France begins in 1883. Although the breed was destroyed by the Allied invasion of Normandy during World War II, there are currently 3 million Normande cows in France. Their current role in France is to provide rich milk for the production of various types of cheese - Camembert, Pon-Leveque and Livaro while maintaining excellent carcasses.

Normande cows are exported around the world, but received their greatest recognition in South America, where they were imported in the 1890 s. Cattle thrive there as one of the best dual-purpose breeds in the world. The total number there already exceeds 4 million purebred animals plus countless Normande crosses. There are 1.6 million purebreds in Colombia alone, and the rest mainly in Brazil (animals are crossed with zebu), Ecuador, Paraguay and Uruguay, Peru, Chile, Argentina. It is also a successful breed in Africa, Asia and Oceania. The resistance to sunlight associated with the pigmentation of the mucous membranes and the color of the coat and the health of the eyes thanks to the "glasses" makes it a major breed in the tropics and equatorial zones. Distributed in Madagascar, USA, Mexico, Belgium, Switzerland, Great Britain, Spain, the Netherlands, Bosnia and Herzegovina, Croatia, Germany and Ireland. They are also found in some countries of Eastern Europe - the Czech Republic, Lithuania, Poland, Romania, and now in Bulgaria (Figure 1). It is a highly adaptable and hardy breed. The Normande cow with its strong legs and hooves can travel long distances on uneven terrain to economically convert local roughage (North American Normande Association).

In Ireland, the breed makes the most of the grass, which is the basis of the diet and provides fertility insurance.

In Italy, it can increase cheese production by 15% for Parmesan production with a good level of milk production.

In Mexico, the breed has adapted to extreme conditions of drought and sunshine. Durability and performance have been proven to be two compatible qualities (Lanormande.com).

MATERIALS AND METHODS

The study is based on the analysis of current bibliographic sources with the subject in relation to factors that influence the productive (quantitative and qualitative composition of milk) and reproductive (calving interval, age at first calving, number of inseminations) traits of the Normande breed.

RESULTS AND DISCUSSIONS

Normande cattle are medium to large animals. The combination of three ancient dairy breeds imported from England (Ageronne, Sauchoise, Cotentine already extinct) with animals of the Durham breed (later Shorthorn) leads to a unique tricolor coat. They are usually red and white with occasional brown areas of brown fur. Brown chestnut usually has the appearance of tiger stripes or is dotted with red spots. Their eyes have dark "glasses" and dark pigmented hair around the eyes. Sun damage to the eyes is avoided by their darkly pigmented area around the eyes. They have a white head, and sometimes there are several spotted brown spots on the face. The average height of the bulls is about 152 cm, and of the cows about 140 cm. The average body weight of male animals is about 1100 kg, and females about 700 kg (https://www.roysfarm.com normande).
The Normande breed is developed on the pastures of northwestern France in the province of Normandy. More than 90% of Normans carry the Kappa Casein gene. It is known that the levels of casein beta and kappa in milk improve the quality of curdling of milk for cheese production (http://www.evolution-int.com/en/catalog/56/isu). They also show an excellent ratio of phosphorus to calcium in their milk, which is also an important aspect for the production of quality cheese (Peychevski & Chomakov, 1988).

Normandy beef is also characterized by excellent marbling, which contributes to the tenderness and taste of the meat. They have a high ratio of meat to bones, which provides a good percentage of meat at slaughter.

According to Le Guillou et al., (2019) Holstein and Normandy cows have significantly different milk production. The average Holstein milk production varies from 22.3 kg/day for first lactating cows to 24.1 kg/day for second lactating cows.

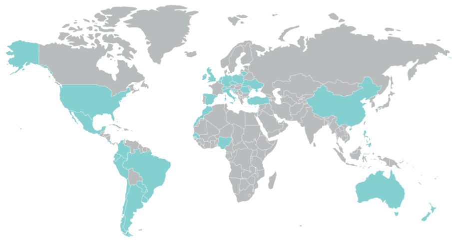

Figure 1. Distribution of Normande cattle in the world (https://www.lanormande.com/race_normande_internationale.html)

The average milk production of Normandy varies from 14.3 kg/day for cows in the first to 21.2 kg/day for cows in the second lactation. In addition, the milk protein content is higher in Normande cows than in Holstein cows - for example 35.4 g/kg compared to 30.6 g/kg, respectively, but does not differ between breeds in Normandy cows second lactation. Normande milk is rich in fat. The fat content did not differ significantly between breeds, ranging from 33 g/kg in the milk of second lactating cows in the Holstein breed to 43 g/kg in the milk of first lactating cows in Normandy. No difference in lactose, urea and acetone content was observed between breeds regardless of the age of the animal. The number of somatic cells ranged from 45.200 cells/ml to 150.600 cells/ml, with no significant differences between breeds ($p < 0.05$).
Dillon et al. (2003) conducted a study comparing Dutch Holstein-Frisian (HF), improved Irish Holstein-Frisian (CL), French Montbeliarde (MB) and French Normande (NR) dairy cows in a system based on spring calving fed with grass. HF cows give the highest milk yield; NR produce the lowest, while CL and MB are intermediate. NR produces the highest content of milk fats, proteins and lactose. The milk protein content in MB is higher than HF and CL, while the fat content in HF is higher than MB and CL.

In a study by Heins et al. (2006) milk productivity, fat content and cow protein in pure Holstein were compared with Normande/ Holstein, Montbeliarde/Holstein crosses and Scandinavian red/Holstein crosses for 305-day first lactation, calved from June 2002 to January 2005. Pure Holstein cows have significantly higher milk (9757 kg) and protein (305 kg) production than all crossbred groups, but they produce 346 kg of butter and do not differ significantly from Scandinavian red/Holstein (340 kg) crosses for fat production. Fat plus protein production was used to measure the overall productivity of pure Holsteins against crosses. Scandinavian red/Holstein (637 kg) crosses do not differ significantly from pure Holstein (651 kg) for fat plus protein production; however, the Normande/Holstein (596 kg) and Montbeliarde/Holstein (627 kg) crosses had significantly lower fat and protein production than pure Holsteins.

The interaction between the breed and the feeding system is not significant for total milk production according to Walsh et al. (2007; 2008). The breed of dairy cows has affected all variables in milk production, except for the percentage of protein. Compared to the Montbeliarde (5604 kg) and Normande (5464 kg) breeds, the Holstein-Friesian breed has the highest milk productivity (5925 kg). Holstein-Friesian cows produce more fat (226 kg), protein (202 kg) and lactose (279 kg) during lactation than Montbeliarde cows (207, 193, 266 kg, respectively) and Normandy cows breed (204, 188, 260 kg, respectively). Compared to Holstein (3.83%), the percentage of fat is similar to Normandy (3.80%), higher than that of Montbeliarde (3.71%). The percentage of lactose in Montbeliarde (4.76%) and Normandy (4.78%) is higher than in the Holstein breed.

Animals raised with a high concentrate diet achieved higher milk yield (5.840 kg), fat (220 kg), protein (200 kg), lactose (276 kg) and a percentage of lactose (4.74%) compared to those grown with a low concentrate food system (5,614; 211, 193, 265 kg; and 4.72%, respectively). The percentages of fat and protein do not differ between feeding systems. The feeding system and breed affect both average lactation and live weight (Backley et al., 1995).

Milk production for HF, MB, NM and NRF in the present study is lower than previously reported by their country of origin (Sigwald & Dervishi, 2002; Heins et al., 2006, Østerås et al., 2007). This is primarily the result of pasture-based feeding systems used in the study. However, Horan et al. (2005) demonstrate that cows with high genetic qualities have a greater milk reaction when concentrate is added within a grazing system, leading to a genotype × environment interaction for milk production. The present study did not identify genotype × environment interactions for milk production. The difference in the addition of concentrate between the feeding systems imposed by Horan et al. (2005) is greater than for the present study (approximately 1100 vs. 500 kg, respectively). Therefore, the differences between the feeding systems tested in the present study may not have been sufficient to induce genotype × environment interactions.

According to Le Guillou et al. (2019) Holstein and Normande cattle are two dairy breeds with different characteristics for milk production. Holstein is the first dairy breed in the world with higher production in terms of quantity. Normande cattle are a hardy French breed, selected for the production of high-fat and protein milk, sought after for high-quality production of butter, cream and cheese and because of their attractive meat properties. The Normande breed has lower milk production than Holstein, but higher fat and protein content and higher adaptability to durable conditions according to Ducroco (1994).

In France, most dairy cows are purebreds. Therefore, the non-additive genetic effects expressed by heterosis, loss of recombination, and depression in inbreeding have not received much attention to date (Dezetter et al., 2015). French genetic assessments are currently looking at data from different breeds separately, excluding information from crossbreds. Although crossbreeding has remained marginal until recently, it has developed over the last 10 years. Indeed, crossbred cows may be more profitable than cows of parent purebred breeds (McAllister, 2002; Heins & Hansen, 2012).

Dairy farming systems in Western Europe are based on a simple feeding system consisting of grazing and canned grass, corn silage and concentrates in varying proportions. However, there is a wide variety of feeding strategies between dairy farms (Delaby et al., 2009). Five year study by Delaby et al. (2009) examined the direct and delayed effects of four feeding strategies on lactation and the reproductive characteristics of Holstein and Normandy dairy cows. The four dietary strategies labeled Hh (high protein, high fat), Hl (high protein, low fat), Lh (low protein, high fat) and Ll (low protein, low fat) correspond to two common mixed rations in winter (corn silage with 30% concentrate or grass silage with 15% concentrate), which were subsequently crossed with two levels of concentrate supplement when grazing for up to 210 days. Each year, 72 dairy cows managed in a group winter calving were allocated to the four strategies. The four strategies led to significant variations in nutrient intake and in particular in differences in concentrate consumption, with values. Total milk production (7567, 7015, 6720 and 6238 kg per cow for treatment Hh, Lh, Hl and Ll, respectively), milk fat content (39.0, 37.1, 40.3 and 38.5 g/kg), milk protein content (33.0, 31.8, 33.1 and 31.6 g/kg, respectively), and the nature of the lactation curves and body condition are highly sensitive to the applied strategies. Holstein cows respond more dramatically to any change in diet at any time than Normande cows. Winter feeding does not affect the production of milk on the pasture, while on the pasture the milk from highly fed cows in winter has a higher content of milk fat and protein. Reproductive efficiency is not affected by the feeding strategy. Holstein cows, well fed and producing the most milk (Hh and Hl), have the lowest success rate in the first artificial inseminations (21.5%). Normande dual-purpose cows had a pregnancy rate 10 points higher than that of Holstein cows. This comparison of highly contrasting feeding strategies confirms the immediate reactivity of dairy cows (in terms of milk yield and body condition) to dietary variations during lactation, with little transfer effect from feeding levels at the beginning of lactation.

Reproductive efficiency has declined in recent decades in many dairy systems. This study by Cutullic et al. (2011) aims to compare the effects of high and low feeding on the reproductive stages (cyclicity, estrus and fertility) of Holstein and Normande cows bred on pasture. Highly fed cows received a total mixed ration consisting of 55% corn silage, 15% dehydrated alfalfa pellets, 30% concentrate in winter and 4 kg/day concentrate for grazing. Low-fed cows received only 50% grass silage and 50% haylage in winter and no grazing concentrate. Low-fat cows produce less milk but lose more body condition than high-fat cows. Normande cows produce less milk and lose less fitness than Holstein. Postpartum ovarian activity is weakly affected by the level of nutrition. In both breeds, the detection rate of ovulation is higher in low-fed cows. In both breeds, the rate of re-calving after the first and second inseminations was not significantly affected by the feeding level, although less cases of infertility or early embryonic mortality were observed in low-fed cows. For the Holstein breed, this was clearly explained by later embryonic mortality in highly fed cows. Dual-purpose cows (Normande breed) had a higher pregnancy rate by the end of the breeding period than Holstein dairy cows due to better ovarian activity and a higher rate of re-calving after insemination.

Poor udder health can be a source of economic loss for both dairy farmers and processors. This can lead to reduced milk yield and milk quality (Bartlett et al., 1991), changes in milk composition (Auldist et al., 1995), increased involuntary destruction (Berry et al., 2005), and the cost of veterinary treatment (Berry & Amer, 2005). In addition, in Ireland, the pricing of milk is differentiated on the basis of sanctions imposed on milk with a high somatic cell count. In France, an average of € 35 less costs are made for veterinary medicine per cow.

The results of this study highlight the differences between dairy breeds cattle, somatic cell count and milking characteristics. They are probably related to the differences in the breeding purposes from which these breeds are made, namely the intensity of milk selection and the inclusion of traits aimed at maintaining or improving udder health.

For beef producers, the Normandy became famous for its high-quality carcasses and delicious meat. The average 570 kg Normande castrated animal has a quality class of 90%, 347 kg weight of hot carcass, 34.3 cm fat in the ribs, 0.64 cm fat on the back, 2% total fat found in the body cavity of the carcass, in particular in the kidney, pelvic, and heart regions and 2.2 yieldgrade (https://www.normandeassociation.com/what-is-a-normande). The carcases of Normande cattle are heavy and have an excellent yield; the meat is well marbled and extremely fragrant; best value of beef both quantity and quality among dairy breeds.

The average age for slaughtering cows is 6.4 years, young bulls 21 months and castrates 36 months. The carcass weight for young bulls is 355 kg with a meat yield of 55%, for castrates it is 391 kg with a 55% yield, and for slaughtered cows the carcass weight is 340 kg with a meat yield of 53%. The meat yield of Holstein cows is 48%, and of crosses between Normande and Holstein breeds is 50%. In the young castrated animals of the Holstein breed the meat yield is 51%, in the Normande breed it is 55%, and in the crossbreeds it is 53%. The Normande breed is the only dairy breed sold as "first class" meat (supermarket Carrefour - Flunch restaurants) (Internacional Evalution).

The milk produced by the Normande cow breed is naturally rich in calcium. It has the highest content, followed by the milk produced by the Montbeyard breed and the Holstein cow's milk has the lowest calcium content (Gaignon et al., 2018).

The minimum amount of calcium in milk needed to make cheese is 1.2 g/l. If the amount is <1.1 g/l, then between 15 and 22 ml should be added. The calcium content in milk is according to the diet of the cows (grass, mineral, fodder, legumes). The calcium in Normandy milk is> 1.1g/l, so no calcium addition is required.

The data in Table 1 show that the production of one kilogram of cheese and butter requires less milk from the Normandy breed than from the Holstein cows.

Data from Table 2a obtained from Minnesota farms in 2012 on 245 F1 crosses between Normande x Holstein for 305 days of lactation show that the amount of milk increased until the third lactation and then begins to decrease.

Table 1. The amount of milk as a raw material required for the production of 1 kg of butter and 1 kg of cheese (https://infortambo.cl/es/contenidos/normando-en-chile-2)

Breed	Liters of milk per 1kg butter	Liters of milk per 1kg cheese
Holstein	23	7.25
Normande	18	5.10

Table 2a. Milk productivity in F1 stock crossbreeds Normande x Holstein cattle (Hansen & Brad, 2010)

Stock crosses	milk yield (kg)
First lactation in F_1 Normande x Holstein	8602
Second lactation in F_1 Normande x Holstein	10080
Third lactation in F_1 Normande x Holstein	10634
Fourth lactation in F_1 Normande x Holstein	10611
3rd and 4th lactation in Holstein	12332

In pure Holstein cows, the milk yield for the third and fourth lactations does not differ significantly from the milk obtained from the crosses. There is a significant difference in the percentage of fat and protein for purebred Holstein cows and F1 crosses between Normande x Holstein (Table 2b).

Table 2b. Protein and milk fat content of F1 stock crosses of Normande x Holstein cattle (Hansen & Brad, 2010)

Breed	Protein (%)	Fat (%)
Holstein	3.60	3.09
F_1 Normande x Holstein	3.72	3.22

The data in Table 3 for 305 days of lactation for 416 Holstein heifers and cows and 254 F1 Normande x Holstein heifers and cows bred in the state of Minnesota show that crossbred and purebred milk yields increased as lactations progressed. The percentage of protein in purebred animals increases, and in crossbreds it remains at the same level. Fat percentage decreased as lactation progressed in both groups of animals. There is a large increase in the amount of protein and fat per kg as lactation progresses.

The studies carried out (Table 4a), with 16 purebred Holstein cows and 13 Normandy cattle cows, in the first lactation show that the differences in chemical composition and somatic cells are observed in favor of the Normandy cattle representatives (Guajardo et al., 2020).

Table 3. Milk yield and physicochemical composition of milk from Holstein heifers and cows and F1 Normande x Holstein crosses (Hansen & Brad, 2010)

Breed	Lactations	Days	Milk (kg)	Protein, %	Protein, kg	Fats, %	Fats, kg	Protein + Fats, kg
Holstein	1	305	9,899	3.10	307	3.56	352	659
F_1 Normande x Holstein	1	305	8,603	3.23	278	3.79	326	604
Holstein	2	305	11.976	3.11	373	3.57	428	801
F_1 Normande x Holstein	2	305	10.081	3.24	327	3.73	376	749

Table 4a. Milk yield and physicochemical composition of milk from heifers and cows Holstein and Normande (Guajardo et al., 2020)

Breed	Number of cows	Number of lactations	Fats (%)	Protein (%)	Lactose (%)	Density (g mL-1)	pH	Somatic cells (cell mL-1)
Holstein	16	1	3.35	2.87	4.58	1026	6.59	163 000
Normande	13	1	3.35	3.16	4.74	1028	6.65	115 000

Table 4b. Time between first and second calving of Holstein cows and crosses F1 Normandy cattle x Holstein (Heins & Hansen, 2012)

Breed	Number of cows	Time between first and second calving, days
Holstein	3845	420.9
F_1 Normande x Holstein	276	396.5

Table 4c. Age at 1st calving, months (Heins & Hansen, 2012)

Breed	Number of cows	First calving (months)
Holstein	5574	28.3
F_1 Normande x Holstein	518	27.56

A study in Chile with purebred Holstein cows and crosses of Normande with Holstein cows found a time between first and second calving in purebred animals 420.9 days and in crosses 396.5 days, and the age of first calving was 28.3 and 27.56 months, respectively (Heins & Hansen, 2012), (Tables 4b and 4c).
The data in Figure 2 show that the fertility rate after 1st insemination in Normande cows was 8% higher than in Holstein cows. Fertility after the first insemination of the Jersey breed is the same as the cows of the Normande breed. There were no significant differences in heifers.
The Figure 3 shows that the highest number of inseminations is in cows of the Holstein breed (2.08), the lowest number is in cows of the Montbeliarde breed (1.74). 1.85 inseminations are required for Normandy cows. In heifers of the presented breeds on the chart there is no significant difference. The number of inseminations is the lowest in Normande heifers, the highest in Jersey heifers (1.69), and in Montbeliarde and Holstein heifers this number is the same (1.67).

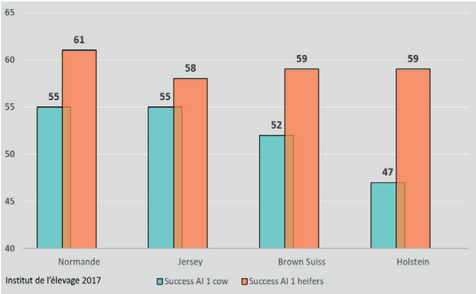

Figure 2. Level of fertility after 1st insemination in Normande, Jersey and Holstein (Evolution International, 2021)

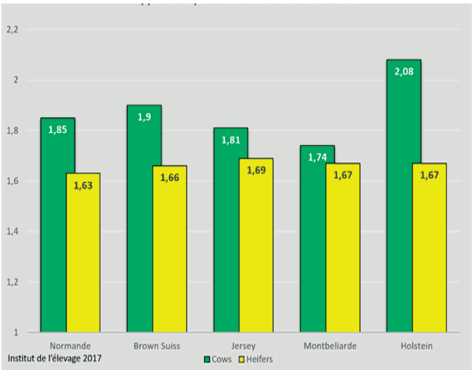

Figure 3. Number of inseminations (Evolution International, 2021)

Table 5 shows that in Normande cows there is a 96.5% ease of calving, in Holstein it is 94.6%, and in cows of the Montbeliarde breed the percentage is below 90% (84.5%). The percentage of calving without assistance is the highest among the Normande breed of cows (73.3%).

Table 5. Light calvings %, unassisted calvings % (Evolution International, 2021)

Breed	Ease of calving, %	Calving without assistance, %
Normande	96.5	73.3
Holstein	94.6	69.2
Monbeliarde	84.5	58.7

CONCLUSIONS

In conclusion, we can say that the cattle of the Normandy breed have been selected for the production of milk, rich in fat and protein for the production of butter, cream and cheese. It is naturally rich in calcium.

Meat from Normandy cattle has the best taste qualities among the bear breeds.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

RESEARCH ON THE ECONOMIC ADVANTAGES OF BREEDING AUBRAC BEEF CATTLE: A REVIEW

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Abstract

Increased interest in breeding beef cows is a result of societal trends, particularly the consumption of high-quality raw protein of animal origin. The sustainability of the beef industry requires high on-farm efficiency and productivity, as well as efficient value chains that reward achievement of target market specifications. This work reviews the most important aspects of the characterization of the Aubrac beef cattle breed, namely: productive qualities (dynamics of body weight of young animals 0-18 months, average daily gain, economic efficiency of growing breeding calves for a period of 18 months of growing, results of bulls control slaughtering), morphological parameters of the carcass (muscle tissue, fat tissue, total meat, connective tissue, bones, flashing index) and chemical composition of the meat and energy value. In the near future, this breed will be one of the most appealing options for obtaining high-quality meat without incurring prohibitively high prices, with the cattle making excellent use of our country`s meadows while also easily adapting to relief and climate.

***Key words**: beef cattle, economic efficiency, morpho-productive qualities.*

INTRODUCTION

Breeding cattle for meat production is a vital aspect of the agricultural industry. Currently, one of the most important and challenging issues facing the country's agro-industrial complex is the need to increase beef production. To achieve this, the creation of a specialized beef cattle industry is necessary. However, the existing pool of breeding animals of meat breeds, in terms of quantity and breed structure, is inadequate to meet the demands of the beef cattle industry.Therefore, it is essential to develop beef cattle breeding by utilizing domestic breeds and accessing new, promising resources from the global gene pool, such as the French-rooted breeds. The French meat breeds are preferred in many countries worldwide due to their long growth period, high growth intensity, and favorable nutrient ratios in the carcass (Mădescu et al., 2021).

The Aubrac breed is a type of cattle that originated in the Aubrac region of central France. This breed is one of the oldest breeds of cattle in France and is known for its adaptability to harsh environmental conditions. Aubrac cattle are medium to large in size and have a robust and powerful appearance, with curved horns that extend outwards. These animals are raised for both meat and milk and are considered to have particularly high-quality meat. Aubrac beef is especially prized for its flavor and low fat content. Additionally, the Aubrac breed is known for its ability to adapt to grazing conditions and produce meat efficiently in a grazing system.

Aubrac cattle are typically red or black in color, with a white or cream-colored stripe down their back. They are known for their sturdy and robust appearance, with strong legs and a deep chest. Aubrac cattle have a docile temperament and are well-suited to grazing on open pastures.

In terms of milk production, the Aubrac breed is not as high-yielding as some other dairy breeds. However, the milk that is produced is of excellent quality and is used to make high-quality cheeses.

One of the key characteristics of the Aubrac breed is its adaptability to a range of different environments. Aubrac cattle are well-suited to grazing in harsh mountainous terrain, and they are able to tolerate cold temperatures and high altitudes. They are also resistant to many common cattle diseases, making them a hardy

and low-maintenance breed. Overall, the Aubrac breed is a valuable addition to any cattle farming operation, particularly for those looking to produce high-quality beef in a grazing system.
With their robust appearance, docile temperament, and adaptability to a range of different environments, Aubrac cattle are a popular choice for farmers in many regions of the world (Madescu et al., 2022).

MATERIALS AND METHODS

In order to reach the objectives of this study, 23 bibliographic sources from the specialized literature were consulted. The main issues addressed refer to the productive qualities, morphological parameters of the carcass and chemical composition of the meat of the Aubrac cattle breed. The research methods used in this study were the observation, analysis and interpretation of data from the specialized literature.

RESULTS AND DISCUSSIONS

1. Productive qualities

The dynamics of body weight and average daily gain are important factors to consider when raising young animals, such as cattle, pigs, or sheep, for meat production.
Body weight dynamics refer to how an animal's weight changes over time. For young animals, weight gain is typically rapid as they grow and develop. The rate of weight gain can vary depending on a number of factors, including breed, genetics, nutrition, and overall health.
Average daily gain (ADG) is a measure of the average weight gain per day of an animal. It is calculated by dividing the total weight gain of the animal by the number of days it has been gaining weight. ADG can be used to track the growth and development of young animals, and it is an important factor in determining the appropriate time to market them for meat production (Paula et al., 2013).
Farmers and ranchers often monitor the dynamics of body weight and ADG of their young animals closely, as it can provide important information about their health and well-being. Regular weighing of the animals can help to track their growth and ensure that they are on track to meet production goals.appropriate time to market them for meat production (Paula et al., 2013).
To promote healthy weight gain and ADG in young animals, farmers and ranchers must provide them with a balanced diet that meets their nutritional needs. This may involve a combination of feed, forage, and supplements. It is also important to ensure that young animals have access to clean water and a comfortable living environment (Mădescu et al., 2021).
Aubrac cattle are a breed of cattle that are primarily raised for beef production. Like other breeds of cattle, the dynamics of body weight and average daily gain are important factors to consider when raising young Aubrac animals.
According to available data, Aubrac cattle are known for their moderate growth rate and their ability to convert forage into meat efficiently. As with other cattle breeds, the growth rate of Aubrac calves is influenced by various factors, including genetics, nutrition, and environmental conditions.
Research has shown that Aubrac calves typically have an average daily gain (ADG) of around 0.9 to 1.2 kg per day during the first few months of life. However, this rate can vary depending on factors such as the quality and quantity of feed, access to clean water, and environmental conditions (Madescu et al., 2022)..
To ensure healthy growth and development, it is important to provide Aubrac calves with a balanced diet that meets their nutritional needs. This may involve a combination of forage, concentrates, and supplements. Farmers and ranchers may also use growth-promoting technologies, such as implants or feed additives, to promote ADG and improve feed efficiency.
Regular weighing of the animals can help farmers and ranchers monitor their growth and make adjustments to their diet or management practices as needed. In general, young Aubrac animals are raised for about 18 to 24 months before being marketed for beef production.

Table 1. Dynamics of body weight and average daily gain of Aubrac cattle

Dynamics of body weight of young animals (x ± sx), kg			Average daily gain (x ± sx), g		
Age (months)	**Heifers**	**Bulls**	**Age (months)**	**Heifers**	**Bulls**
At birth	27.6 ± 0.79	28.3 ± 1.04	0 - 6	716.1 ± 39.47	743.3 ± 26.63
6	156.5 ± 3.50	162.1 ± 3.58	0 - 8	653.7 ± 23.08	700.4 ± 15.95
8	184.5 ± 6.20	196,4 ± 5.60	8 - 12	690.0 ± 31.84	842.5 ± 45.20
12	267.3 ± 7.68	297.5 ± 9.10	12 -15	534.4 ±29.41	783.3 ± 37.14
15	315.4 ± 9.27	368.0 ±7.47	15 - 18	622.2 ± 44.26	994.4 ± 28.30
18	371.4 ± 20.75	457.5 ± 18.34	0 - 18	716.1 ± 39.47	743.3 ± 26.63

In the Table 1, we can see that bulls register slightly higher average daily gains than females (for example, in the period 0-6 months, the heifers recorded an average of the average daily gain of approximately 716.1 ± 39.47, while the bulls recorded an average of the average daily gain of 743.3 ± 26.63), but the differences are not very significant. There can be several reasons why male Aubrac cattle have a slightly higher average daily weight gain than females:

Genetics: Male and female animals often have different genetic traits that can influence their growth rates. It is possible that the male Aubrac cattle have certain genetic traits that promote faster growth and weight gain (Soulat et al., 2016).

Hormonal differences: Male and female animals also have different hormone profiles that can affect their growth rates. Male cattle produce more testosterone, which can promote muscle growth and weight gain.

Feeding practices: It is possible that the male and female Aubrac cattle are being fed different diets or receiving different amounts of feed, which can influence their growth rates.

Social dynamics: Male and female cattle may also have different social behaviors and hierarchies that can affect their access to feed and water, and ultimately their growth rates.

However, it is important to note that the difference in weight gain between male and female Aubrac cattle during the 0-6 month period is relatively small. Overall, both males and females of this breed are known for their efficient conversion of forage into meat and their moderate growth rates.

Table 2. Economic efficiency of growing breeding calves for a period of 18 months of growing and results of bulls control slaughtering

Economic efficiency of growing breeding calves for a period of 18 months of growing		Results of bulls control slaughtering (x ± sx), g	
Received gain (kg)	429.2	Preslaughter live weight, kg	382.3 ± 13.72
Spent EFU per 1 kg of growth	6.53	Hot carcass weight, kg	209.3 ± 5.08
The cost of obtaining and growing of 1 head (€)	501.75	Weight of visceral crude fat,kg	2.23 ± 0.11
The cost of 1 centner of growth (€)	116.90	Slaughter weight, kg	211.5 ± 5.08
Proceeds from the sale of 1 head, (€)	724.65	Carcass yield %	54.8 ± 1.35
Profit on 1 head, (€)	222.89	Crude fat yield %	0.58 ± 0.22
Profitability level, %	44.4	Slaughter yield %	55.4 ± 1.36

Growing breeding calves for beef for a period of 18 months can be economically efficient, as it can result in high-quality beef and potential profits from selling breeding stock (Gagaoua M. et al., 2018).

Breeding calves raised for beef are typically selected for their genetics and growth potential, with the aim of producing high-quality beef. By raising these calves for a longer period of time, farmers can improve the quality of the beef, as the animals have more time to develop and put on weight. This can result in higher prices for the beef, which can increase profitability.

In addition, breeding calves can be sold as breeding stock, providing an additional source of income for farmers. If the calves are well-managed and have desirable genetics, they can be sold to other farmers for breeding purposes, allowing the original farmer to earn a profit on the sale of the calves as well as on the sale of their offspring (Esteve & Bouchy, 2002).

However, there are also potential drawbacks to growing breeding calves for beef for 18 months. As with any livestock production, there are risks associated with disease, weather, and other factors that can impact the health and productivity of the animals. The longer growth period can also increase production costs, particularly if the animals require more feed, water, and other resources.

The economic efficiency of growing breeding calves for beef for a period of 18 months will depend on a variety of factors, including market conditions, production costs, and the quality of the animals being raised. If managed well, breeding calves can potentially yield higher profits than calves raised for meat, as they can produce high-quality beef and also be sold as breeding stock.

The results of bulls control slaughtering include several measurements that can provide important information about the quality of the meat and the efficiency of the production process. These measurements include preslaughter live weight, hot carcass weight, weight of visceral crude fat, slaughter weight, percentage, and slaughter yield percentage.

Preslaughter live weight refers to the weight of the animal before it is slaughtered, while hot carcass weight refers to the weight of the animal's body after it has been slaughtered and dressed but before it has been cooled. The weight of visceral crude fat refers to the amount of fat that is found in the animal's internal organs (Dransfield et al., 2003).

Slaughter weight is the weight of the animal's body after it has been slaughtered and dressed, while carcass yield percentage is the proportion of the animal's preslaughter live weight that is represented by the hot carcass weight. Crude fat yield percentage is the proportion of the hot carcass weight that is represented by the weight of visceral crude fat.

Finally, slaughter yield percentage is the proportion of the animal's preslaughter live weight that is represented by the slaughter weight. These measurements can be used to assess the efficiency of the production process, as well as the quality of the meat that is produced. By monitoring these measurements over time, producers can make adjustments to their management practices to improve efficiency and quality.

A mean of preslaughter live weight of 382.3 ± 13.72 kg for the Aubrac breed is a relatively good weight. The Aubrac breed is known for producing high-quality beef, and a preslaughter weight in this range suggests that the animals are being well-managed and are receiving appropriate nutrition and care.

However, it is important to note that preslaughter weight alone is not necessarily a good indicator of the quality of the meat that is produced. Other factors, such as the animal's genetics, age, and diet, can also play a significant role in determining meat quality (Gagaoua et al., 2018; Maysonnave et al., 2020).

A preslaughter live weight of 382.3 ± 13.72 kg for the Aubrac breed is a positive sign, but it is important to consider other factors as well when evaluating the quality of the meat that is produced (Table 2).

Slaughter yield % is a measure of how much usable meat is obtained from a slaughtered animal, expressed as a percentage of its live body weight. It can be influenced by various factors such as breed, weight, age, sex, feeding, and rearing conditions.

Slaughter yield % is a measure of how much usable meat is obtained from a slaughtered animal, expressed as a percentage of its live body weight (Esteve & Bouchy, 2002). It can be influenced by various factors such as breed, weight, age, sex, feeding, and rearing conditions. Aubrac cattle are known for their high-quality beef production. The reported Slaughter yield % of 55.4 ± 1.36 for this breed is considered to be within the range of other beef breeds. However, it is important to note that there can be natural variation in the slaughter yield between individual animals of the same breed. The Slaughter yield of 55.4 ± 1.36% for Aubrac cattle appears to be a good value and can be considered a positive characteristic of this breed in terms of meat production (Maysonnave et al., 2020).

2. Morphological parameters of the carcass

Morphological parameters of the carcass in beef cattle refer to the external physical characteristics of the animal's body after it has been slaughtered and processed for meat production (Mădescu et al., 2021). These parameters are important in determining the quality and value of the meat, as well as the yield of usable meat from the carcass. Some of the commonly measured morphological parameters of the carcass in beef cattle include:

Muscle tissue: The amount of muscle tissue in the carcass is an important indicator of meat yield and quality. It is typically measured by determining the weight of the lean meat in the carcass or by calculating the rib-eye area (Astruc et al., 2014).

Fat tissue: The amount of fat tissue in the carcass is also important, as it affects meat flavor, juiciness, and tenderness. It is usually measured by determining the thickness of subcutaneous fat or by estimating the marbling score.

Total meat: This parameter refers to the total amount of meat obtained from the carcass, including both lean meat and fat.

Connective tissue: Connective tissue is an important component of meat quality, as it affects tenderness and juiciness. It is usually measured by determining the collagen content in the meat (Astruc et al., 2014; Purslow, 2005).

Bones: The amount of bones in the carcass is also important, as it affects meat yield and value. It is typically measured by determining the weight of the bone-in carcass.

Fleshing index: This parameter is a measure of the amount of muscle tissue relative to the weight of the carcass. It is usually calculated as the ratio of the rib-eye area to the weight of the carcass.

Overall, these morphological parameters of the carcass are important in determining the value and quality of meat obtained from beef cattle.

Table 3. Morphological parameters of the carcass (x ± sx)

Indicator	Morphological parameters of the carcass (x ± sx) kg	%
Muscle tissue, kg	146.3 ± 7.24	71.7 ± 1.87
Fat tissue, kg	4.27 ± 0.57	2.1 ± 0.31
Total meat, kg	150.6 ± 6.73	73.8 ± 1.55
Connective tissue, kg	9.0 ± 0.53	4.4 ± 0.29
Bones, kg	21.7 ± 1.28	21.7 ± 1.28
Flashing index	3.62 ± 0.28	-

The values you provided for the morphological parameters of the carcass in Aubrac beef cattle are indicative of a high-quality meat product. The relatively high percentage of muscle tissue (71.7 ± 1.87%) suggests that these animals have a good meat yield, while the low percentage of fat tissue (2.1 ± 0.31%) suggests that the meat is likely to be lean and healthy.

The total meat percentage (73.8 ± 1.55%) indicates that the majority of the carcass is composed of usable meat, and the connective tissue percentage (4.4 ± 0.29%) is within the normal range for beef cattle (Table 3). Connective tissue is important in determining meat tenderness, and the relatively low percentage in Aubrac beef cattle suggests that the meat may be tender and of good quality (Astruc et al., 2014). Overall, these morphological parameters suggest that Aubrac beef cattle may produce high-quality meat with a good yield of usable meat. However, it is important to note that these values may vary depending on the specific conditions of the study or experiment, and that other factors such as animal age, weight, and feeding regime can also influence the characteristics of the carcass (Begoña Panea et al., 2018).

Also, following the research carried out by a number of authors, the following morpho-productive qualities of the Aubrac cattle breed were reported in Table 4.

The values provided for the weight of Aubrac beef cattle at 18 months are indicative of the growth potential of this breed. The range of weights reported, from 414 kg to 646.5 kg, suggest that there may be some variability in growth rates within the breed, likely influenced by factors such as feeding and management practices.

The weights reported by Bakharev in 2018 are within a relatively narrow range, with bulls weighing between 457.5 ± 18.34 kg and 471.6 ± 16.34 kg. The weights reported by Sheveleva (564.8 ± 7.1 kg) and Stimbirys (646.5 kg) are notably higher than those reported by Bakharev. This may be due to differences in management practices or genetic variation within the breed (Bakharev et al., 2018a; Sheveleva et al., 2021; Stimbirys et al., 2016).

Table 4. Morpho-productive qualities of Aubrac cattle breed

Parameters	Value ($\bar{x} \pm S\bar{x}$)	References
Weight at 18 months (bulls), kg	457.5 ± 18.34	Bakharev (2018a)
	564.8 ± 7.1	Sheveleva (2021)
	414	Mordenti (2018)
	471.6 ± 16.34	Bakharev (2018b)
	646.5	Stimbirys (2016)
Average daily gain at 18 month (bulls), g	820.0 ± 20.93	Bakharev (2018a)
	794.8 ± 15.24	Bakharev (2018b)
	942.2	Sheveleva (2021)
Preslaughter live weight, kg	382.3 ± 13.72	Bakharev (2018a)
	545.4 ± 12.1	Sheveleva (2021)
	753.3 ± 23.4	Piedrafita (2003)
	686	Mordenti (2018)
Carcass weight, kg	203.9 ± 5.59	Bakharev (2018a)
	314.1 ± 3.4	Sheveleva (2021)
	451.0 ± 16.3	Piedrafita (2003)
	399	Mordenti (2018)
Muscle tissue %	71.7 ± 1.87	Bakharev (2018a)
	77.5	Sheveleva (2021)
	76.1 ± 2.3	Piedrafita (2003)
Bone %	16.2	Sheveleva (2021)
	15.4 ± 1.4	Piedrafita (2003)
	21.7 ± 1.28	Bakharev (2018a)
Total fat %	2.1 ± 0.31	Bakharev (2018b)
	3.0	Sheveleva (2021)
	7.6 ± 1.7	Piedrafita (2003)

The reported weights suggest that Aubrac beef cattle can achieve significant weight gain by 18 months of age, which may contribute to the breed's reputation for producing high-quality meat with good yields. However, it is important to note that individual animal weights can vary widely based on factors such as genetics, nutrition, and management practices.

The average daily gain values reported for Aubrac beef bulls at 18 months of age indicate the rate at which they are gaining weight during the period of growth.

The reported values range from 794.8 ± 15.24 g to 942.2 g. The higher value reported by Sheveleva (942.2 g) suggests a faster growth rate compared to the values reported by Bakharev (820.0 ± 20.93 g and 794.8 ± 15.24 g). (Bakharev et al., 2018a; Sheveleva et al., 2021). A higher average daily gain may indicate more efficient conversion of feed into body weight gain, which can lead to earlier maturity and higher carcass weights. However, it is important to note that individual animal performance can vary widely based on factors such as genetics, nutrition, and management practices. Aubrac beef bulls have the potential for good growth rates and efficient feed conversion, which may contribute to their reputation for producing high-quality meat with good yields.

The preslaughter live weight values reported for Aubrac beef cattle suggest a wide range of weights at the time of slaughter, which can have implications for carcass weight and meat quality.

The reported values range from 382.3 ± 13.72 kg to 753.3 ± 23.4 kg. The higher values reported by Sheveleva (545.4 ± 12.1 kg), Piedrafita (753.3 ± 23.4 kg), and Mordenti (686 kg) suggest a larger animal size at slaughter compared to the value reported by Bakharev (382.3 ± 13.72 kg).

Larger preslaughter live weights may result in larger carcass weights, but it is important to note that larger animals may not necessarily result in higher quality meat. The age at which an animal is slaughtered, as well as factors such as genetics, nutrition, and management practices, can also impact meat quality (Sheveleva et al., 2021; Mordenti et al., 2019; Bakharev et al., 2018; Stimbirys et al., 2016).

The reported preslaughter live weight values suggest that Aubrac beef cattle can reach a range of weights at the time of slaughter, which may provide flexibility for producers in terms of market options. However, it is important to consider factors beyond live weight, such as age and meat quality, when making management decisions for these animals.

The reported carcass weight values for Aubrac beef suggest a wide range of weights, which can have implications for meat production and market options.

The reported values range from 203.9 ± 5.59 kg to 451.0 ± 16.3 kg. The higher values reported by Piedrafita (451.0 ± 16.3 kg) and Sheveleva et al. (2021) (314.1 ± 3.4 kg) suggest that Aubrac beef can potentially produce larger carcasses compared to the values reported by Bakharev et al. (2018) (203.9 ± 5.59 kg) and Mordenti et al (2019) (399 kg).

Larger carcass weights may result in higher meat yields, but it is important to note that the quality of the meat can also be influenced by factors such as marbling, meat color, and texture. Additionally, market demands may vary based on the weight and size of the carcass, with some markets preferring smaller carcasses while others prefer larger ones.

Overall, the reported carcass weight values suggest that Aubrac beef can produce a range of carcass weights, which may provide flexibility for producers in terms of market options. However, it is important to consider factors beyond carcass weight, such as meat quality and market demands, when making management decisions for these animals.

The reported values for muscle tissue percentage in Aubrac beef suggest that this breed has a relatively high proportion of muscle tissue in the carcass, which can be a desirable trait for meat production.

The reported values range from 71.7 ± 1.87% to 77.5%, with the highest value reported by Sheveleva. The values reported by Bakharev (71.7 ± 1.87%) and Piedrafita et al. (2003) (76.1 ± 2.3%) are also relatively high and suggest that Aubrac beef can produce a carcass with a high proportion of muscle tissue.

Muscle tissue is the main source of meat, and a high proportion of muscle tissue in the carcass can result in a higher meat yield, which can be economically beneficial for meat producers. However, it is important to consider other factors such as meat quality, which can also influence the value of the meat produced. Aubrac beef can produce a carcass with a relatively high proportion of muscle tissue, which can be a desirable trait for meat production.

The reported values for bone percentage in Aubrac beef suggest some variation between studies, with values ranging from 15.4 ± 1.4% to 21.7 ± 1.28%. The highest value is reported by Bakharev et al. (2018), while the lowest value is reported by Piedrafita et al. (2003), and Sheveleva et al. (2021) reported an intermediate value.

The percentage of bone in the carcass is an important morphological parameter that can affect meat yield and quality. High bone percentages can lead to a lower meat yield and a higher proportion of bone-in cuts, which may not be as desirable for consumers. However, some bone is necessary to provide structure and support to the carcass.

It is important to note that the percentage of bone can vary depending on several factors such as age, sex, and diet. Therefore, it is difficult to compare values reported by different studies without considering these factors. Aubrac beef suggest some variation between studies, but the values are generally within the range reported for other beef breeds. It is important to consider the balance between bone and muscle tissue percentages in the carcass to optimize meat yield and quality.

The reported values for total fat percentage in Aubrac beef suggest some variation between studies, with values ranging from 2.1 ± 0.31% to 7.6 ± 1.7%. The lowest value is reported by Bakharev et al. (2018), while the highest value is reported by Piedrafita et al. (2003), and Sheveleva et al. (2021) reported an intermediate value.

The percentage of fat in the carcass is an important morphological parameter that can affect meat quality, flavor, and tenderness. Low fat percentages may result in tougher and less flavorful meat, while high fat percentages may lead to greasy and less healthy meat.

It is important to note that the percentage of fat can vary depending on several factors such as age, sex, and diet. Therefore, it is difficult to compare values reported by different studies without considering these factors.
Overall, the reported values for total fat percentage in Aubrac beef suggest some variation between studies, but the values are generally within the range reported for other beef breeds. It is important to consider the balance between fat and muscle tissue percentages in the carcass to optimize meat quality and consumer satisfaction (Chambaz et al, 2003; Dubost et al., 2013).

3. Chemical composition of the Aubrac meat

Beef is a highly nutritious food that is rich in protein, vitamins, and minerals (Oury et al., 2009). The chemical composition of beef can vary depending on factors such as the breed of cattle, age, diet, and management practices. Generally, beef contains approximately 75% water, 20% protein, and 5% fat, along with a variety of vitamins and minerals such as iron, zinc, and vitamin B12 (Web & O'Neill, 2008). The protein in beef is considered to be of high quality, containing all of the essential amino acids that humans need for growth and maintenance of bodily tissues (Begoña Panea et al., 2018).
The fat content in beef can also vary, with some cuts being leaner than others. Beef fat is a source of both saturated and unsaturated fatty acids, including the healthy omega-3 fatty acids (Chambaz et al, 2003; Dubost et al., 2013).
The chemical composition of Aubrac meat has been studied in various research articles. Generally, beef meat consists of water, protein, fat, and ash, as well as some micronutrients such as vitamins and minerals.

Table 5. Physico-chemical composition of Aubrac beef

Parameters	Value ($\bar{x} \pm S\bar{x}$)	References
Protein %	22.20	Attilio L. Mordenti
	19.41 ± 0.36	Bakharev A. A.
	23.23 ± 0.34	Vigilijus JUKNA
Dry matter %	20.8 ± 0.37	Bakharev A. A.
	25.43 ± 0.23	Vigilijus JUKNA
	30.63	Attilio L. Mordenti
Fat %	1.03 ± 0.05	Vigilijus JUKNA
	0.33± 0.07	Bakharev A. A.
Ashes %	1.17 ± 0.11	Vigilijus JUKNA
	1.05 ± 0.07	Bakharev A. A.
Cooking loss %	23.6	Attilio L. Mordenti
	26.53 ± 1.85	Vigilijus JUKNA
Drip loss %	0.39	Attilio L. Mordenti
	4.04 ± 0.18	Vigilijus JUKNA

The protein content of beef is an important nutritional parameter that contributes to the quality and value of the meat. The values reported for Aubrac beef are within the expected range for high-quality beef, with protein content ranging from 19.41% to 23.23% (Table 5). The protein content is influenced by various factors, including genetics, age, sex, and nutrition
Based on these values, Aubrac beef seems to have a relatively high protein content compared to other beef breeds. The protein content is an important indicator of the quality of meat, as it is essential for building and repairing muscle tissues. However, it is important to note that the protein content may vary depending on various factors such as age, gender, diet, and management practices. Therefore, it is necessary to consider these factors when interpreting the protein content of beef.
Based on the information provided, it seems that the authors have measured the dry matter percentage in beef Aubrac and reported their findings.
The dry matter percentage is the amount of solid material remaining after all the water has been removed from a sample. It is an important

parameter in determining the nutritional value and quality of animal feed and food products.

The reported values for the dry matter percentage in beef Aubrac are as follows: Bakharev A.A. et al., 2018: 20.8 ± 0.37%; Vigilijus Jukna et al., 2017: 25.43 ± 0.23%; Attilio L. Mordenti et al., 2019: 30.63%.

It is difficult to comment on these values without more information on the methodology used to measure the dry matter percentage, the sample size, and the variability of the samples. However, it is clear that the values reported by the authors are different from each other, suggesting that there may be variability in the dry matter content of beef Aubrac depending on various factors such as the age, breed, diet, and management of the animals.

Dry matter percentages provide useful information for researchers, producers, and consumers interested in the nutritional value and quality of beef Aubrac. However, further studies and analyses may be needed to fully understand the variability and determinants of dry matter content in this type of beef.

The reported values indicate that there is a significant difference in the fat content of beef Aubrac between the two authors. Vigilijus JUKNA reported a much higher fat percentage than Bakharev A. A. In addition, the standard deviations of the measurements also suggest that there is variability in the fat content of beef Aubrac.

The difference in the reported values could be due to various factors such as differences in the age, breed, diet, and management of the animals used in the studies, as well as differences in the methodology used to measure the fat content.

It is important to note that the fat content of beef Aubrac is an important nutritional parameter, as it affects the flavor, tenderness, and overall quality of the meat. Consumers and producers may be interested in the fat content of beef Aubrac for various reasons, such as for health and dietary considerations, as well as for marketability and quality control.

The reported values for cooking loss percentage in beef Aubrac are as follows: Mordenti: 23.6%; Vigilijus: 26.53 ± 1.85%

The cooking loss percentage is a measure of the amount of weight lost during cooking due to the evaporation of moisture. It is an important parameter in determining the yield and quality of meat products, as well as the sensory properties such as texture and juiciness (Listrat et al., 2016).

The reported values indicate that there is a difference in the cooking loss percentage between the two authors, with Vigilijus reporting a slightly higher cooking loss percentage than Mordenti. The higher cooking loss percentage reported by Vigilijus may be due to various factors such as the age, breed, diet, and management of the animals used in the study, as well as the cooking method and conditions.

The reported values provide useful information for researchers, producers, and consumers interested in the yield and quality of beef Aubrac. However, it is important to note that further studies and analyses may be needed to fully understand the determinants of cooking loss in this type of beef and to provide more accurate and consistent measurements.

The reported values for drip loss percentage in beef Aubrac are as follows: Mordenti: 0.39%, Vigilijus: 4.04 ± 0.18%.

Drip loss percentage is a measure of the amount of moisture that is lost from the meat during storage or refrigeration, and it is an important parameter in determining the quality and shelf-life of the meat.

The reported values indicate that there is a significant difference in the drip loss percentage between the two authors, with Vigilijus reporting a much higher drip loss percentage than Mordenti. The higher drip loss percentage reported by Vigilijus may be due to various factors such as the age, breed, diet, and management of the animals used in the study, as well as the processing and storage conditions.

It is important to note that high drip loss percentage can negatively affect the quality and shelf-life of the meat by reducing its juiciness and tenderness. Therefore, the reported values provide useful information for producers and consumers interested in the quality and shelf-life of beef Aubrac.

CONCLUSIONS

We can conclude that Aubrac cattle are a breed primarily raised for beef production. They are known for their moderate growth rate and efficient conversion of forage into meat. During the period of 0-6 months, female Aubrac cattle have an average daily weight gain of 716 grams, while males of the same age have an average daily weight gain of 743 grams. However, the difference in weight gain between males and females is relatively small and can be influenced by genetics, hormonal differences, feeding practices, and social dynamics.

To promote healthy growth and development of Aubrac cattle, farmers and ranchers must provide them with a balanced diet that meets their nutritional needs. Regular weighing of the animals can help to monitor their growth and ensure that they are on track to meet production goals. Overall, Aubrac cattle are a hardy and adaptable breed that can thrive in a variety of environments and management systems, making them a popular choice among beef producers.

Aubrac is a breed of cattle that is highly valued for its meat quality. The breed is known for producing meat with a high percentage of protein and a low percentage of fat, which makes it a healthy and nutritious food option. Additionally, Aubrac beef has a high slaughter yield and a good amount of muscle tissue, which is desirable for meat production.

Furthermore, the data presented suggest that Aubrac cattle have a good growth rate and can reach significant live weights at relatively young ages, making them a promising breed for meat production. However, it is important to note that some of the values presented vary across studies and may be influenced by factors such as feeding practices and breeding programs.

Overall, the information indicates that Aubrac is a breed with good meat quality and production potential, but further research is needed to fully understand the characteristics and potential of this breed.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

THE ANALYSIS OF DAIRY COWS FARM USING INTEGRATIVE NUMERICAL SYSTEM ANI 35L/2000

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Abstract

In order for dairy cows to manifest their genetic potential and for the farmer to achieve good milk production, it is necessary to respect animal welfare. The welfare of the animal means giving it the chance to manifest its behavior, but at the same time the relationship of the animal caretaker matters a lot. The integrative system ANI 35L/2000 provides a point award to each of the five system-specific criteria to determine where the weaknesses are on the farm and where the strengths are. This study shows with the help of the ANI 35 number system where there are vulnerabilities and where there are not. Also, grazing is a strong point for establishing the welfare of dairy cows.

Key words: *ANI 35, animal welfare, dairy cows, pasture, welfare.*

INTRODUCTION

The concept of animal welfare means not only animal health that is linked to a good production but also include the emotional state of the animal and expressing behavior.
For the animal to express his specific behavior and to be in a positive emotional state, must be respected welfare conditions that also brings a good health and imply a very good production.
Animal welfare is a concept that helps farmers to understand the needs of the animal but from a whole perspective.
Are two systems that manage the concept of animal welfare in farms:
1) ANI 35L/2000;
2) Welfare Quality® Assessment Protocol.

So, animal welfare can be valued from the known recommendations to be the five freedoms, according to (Szücs & Cziszter, 2010):
1) Freedom from hunger and thirst;
2) Freedom from discomfort;
3) Freedom from pain, injury and illness;
4) Freedom to express normal behavior;
5) Freedom from fear and danger.

From those two systems, ANI 35L/2000 is in English language and ANI means ´Animal Needs Index´, was invented by the researcher (Bartuseek, 1985) but was called Tiergerechtheitsindex (TGI) (https://www.tandfonline.com/doi/abs/10.1080/090647001316923036/).
Coincidentally, ANI 35L includes five criteria like the five freedoms mentioned above but this are husbandry criteria.
Also, the welfare and protection of farm animals are judged differently for each species, with significant differences between EU Member States (Martelli, 2009; Cziszter et al., 2010). This means specific legislation for dairy cows.
According to Mitchell (2001) and Cziszter et al. (2010), are two tipes of economical motivations for animal welfare legislation:
1) When consumers feel that they individually benefit from improved animal welfare;
2) When society as a whole can benefit from improved animal welfare.

From what is known so far, it can be stated that animal welfare in dairy cows is necessary.

MATERIALS AND METHODS

The research has been done on july 2023, to observe in general the level of welfare on dairy cows farm situated in South-East region of Romania, almost in the center of the Romanian plain, on Ilfov county. To establish the level of welfare, was used the integrative numerical system ANI 35L/2000. ANI 35 system used determines if a shelter meets the needs of dairy cows in more than one respect. The principles are that dairy cows needs more space for resting, movement inside and outside, more air, clean and non-slip areas, to manifest a productive life. On farm, ANI 35 must consider five criteria with parameters and points are allocated for each parameter and at end are calculated to make a sum and to obtain the ANI score. The score can have value between -9 + 45.5. Points given to each parameter show us where are vulnerabilities or poor conditions and where is invulnerability or better conditions. ANI score gives the farmer the occasion to improve the welfare of dairy cows to receive a higher production of milk.

The five criteria of ANI 35L/2000 are:

1) Locomotion or movement;
2) Social interaction;
3) Flooring (type and degree of integrity);
4) Microclimate (light, air and noise);
5) Human-animal relationship.

94 dairy cows from Romanian Black and white breed were analized. Romanian Black and white breed is known as Holstein in Romania and that because to form this breed was used cows from Holstein and seminal material.

The shelter haves 100 m with paddock. The floor is from concrete, manure accumulates on both sides of the stable in a channel, evacuated manually by caregivers. Lighting is naturally and artificially. Water is at discretion. Access to the pasture is made in spring and autumn, in the morning, 5 hours. On the criteria „social contact" at parameter ´calves´ the score will be zero because only the dairy cows were noticed. Was observed and noticed 21 parameters and 11 sections. The following are known from the farm: all 94 dairy cows are in lactation, the percentage of fecundity is 69, service period is 91 days, percentage of no returns after mating is 75, calving interval is 392 days, calving sex ratio 52 females/48 males and fertility is 82%. It is also known that on the whole year 2022 the milk production was 553224 litres and an average milk production per month of 47470 litres.

In Figure 1 is showed the Romanian Black and white breed in the shelter.

Figure 1. Dairy cows from Romanian Black and white breed

RESULTS AND DISCUSSIONS

As will be shown in the Tables 1-6, using the ANI 35L/2000 numeric system the welfare of dairy cows from Romanian Black and white breed is good.

Table 1. Movement criteria (Bartuseek et al., 2000)

Criteria	Parameters	Section	Number of points min.-max.	Points	Total
1. **Movement**	Free stabulation housing systems	Space allocation	0.0-3.0	2.5	10.5
		Possibility of lying down, lying and getting up normally	0.0-3.0	3	
	Hitch systems	Shed size and boundaries	0.0-1.0	1	
		Movement of hitch	0.0-1.0	1	
	Outdoor areas	Paddock access	0.0-3.0	3	
		Pasture access	0.0-1.5	0	

At criteria number 1 (movement) on the parameter (outdoor areas) the zero score comes from the section ´pasture access´ because pasture is done only on spring and autumn seasons.

Table 2. Social contact criteria (Bartuseek et al., 2000)

Criteria	Parameters	Section	Number of points min.-max.	Points	Total
2. **Social contact**	Free stabulation housing systems, space allocation		0.0-3.0	3	7.5
	Herd structure in free stabulation housing and hitch systems		-0.5-2.0	2	
	Management of Calves		-0.5-1.0	0	
	Outdoor areas	Access to paddock	0.0-2.5	2.5	
		Access to pasture	0.0-1.5	0	

At criteria number 2 (social contact) on the parameter (calves) the zero score comes because was not taken into account. On the parameter (outdoor areas) is also zero at the section ´access to pasture´ from the same reason mentioned above.

Table 3. Floor criteria (Bartuseek et al., 2000)

Criteria	Parameters	Section	Number of points min.-max.	Points	Total
3. **Floor**	Lying area	Soft	-0.5-2.5	0.5	3
		Cleanliness	-0.5-1.0	0.5	
		Non-slip surface	-0.5-1.0	0.5	
	Activity areas, hall ways		-0.5-1.0	0.5	
	Outdoor yards		-0.5-1.5	1	
	Access to pasture		0.0-1.0	0	

At criteria number 3 (floor) on the parameter ´access to pasture´ the score is zero showed at the two criteria above.

Table 4. Microclimate criteria (Bartuseek et al., 2000)

Criteria	Parameters	Section	Number of points min.-max.	Points	Total
4. **Microclimate (light, air, noise)**	Natural light in the shelter		-0.5-2.0	2	8.5
	Air quality and air circulation		-0.5-1.5	1	
	Air currents in the lying area		-0.5-1.0	1	
	Noise level		-0.5-1.0	1	
	Outdoor areas	Days/year of access to the paddock	0.0-2.0	2	
		Hours/day of access to the paddock	0.0-2.0	1.5	

Table 5. Contact with humans criteria (Bartuseek et al., 2000)

Criteria	Parameters	Number of points min.-max.	Points	Total
5. **Contact with humans**	Cleanliness of playpens, feed and drink areas	-0.5-1.0	1	4.5
	Technical condition of shelter facilities	-0.5-1.0	1	
	Condition of animal skin	-0.5-1.0	0.5	
	Animal hygiene	-0.5-0.5	0	
	Condition of onglons	-0.5-1.5	0.5	
	Technopathies	-0.5-1.5	0.5	
	Health of the animals	-0.5-1.5	1	

At criteria number 5 (contact with humans) on the parameters ´animal hygiene´ the score is 0 and is interpreted as medium.

Table 6. Total points of each criteria and ANI score

Criteria	Points	Total
1. **Movement**	10.5	34
2. **Social contact**	7.5	
3. **Floor**	3	
4. **Microclimate (light, air, noise)**	8.5	
5. **Contact with humans**	4.5	

As we observe, the final ANI score from all five criteria is 34 and means a good result.
The only major issues appear at pasture access because is conditioned.

Interpretation of each score from each parameter and section which was not mentioned under each table, is on the authority of Bartuseek et al. (2000):
In the first criteria (movement) on the parameter: (free stabulation housing systems) at section ´possibility of laying down, lying and getting up normally the score 3 mean comfortable, (hitch systems) at section shed size and boundaries the score 1 mean comfortable, (outdoor areas) at section ´paddock access the score 3 mean more than 270 days/year on paddock.
In the second criteria (social contact) on the parameter: (herd structure in free stabulation housing and hitch systems) the score 2 it's family herd, (outdoor areas) at section ´paddock access´ the score 3 it's the same result mentioned above.
The the third criteria (floor) on the parameter: (lying area) at section soft the score 0.5 meaning concrete, metal or plastic grids, cleanliness and non-slip surface the same score meaning medium, (activity areas, hall ways) also the score meaning medium, (outdoor yards) the score 1 meaning natural floor, dry, firm.
On the four criteria (microclimate) on the parameter: (natural light in the shelter) the score 2 stands open fronted housing, (air quality and air circulation) 1 stands good air quality, (air currents in the lying area) 1 stands none, (noise level) 1 stands no noise, (outdoor areas) at section: days/year of access to the paddock 2 stands more than 230 days and ´hours/day of access to the paddock 1.5 stands more than 6 hours.
On the five criteria and the last one (contact with humans) the parameter: (cleanliness of playpens, feed and drink areas) the point 1 equal clean, (technical condition of shelter facilities) 1 equal good, (condition of animal skin), (condition of onglons) and (technopathies) the score 0.5 equal medium, (health of the animals) the 1 equal good.
According to Sakar et al. (2022), in a study evaluating Anatolian Black cattle, after using ANI 35L, they made the statement that possibility for cows to go on the courtyard and pasture has positive benefactions to animal welfare.

CONCLUSIONS

As was presented a short introduction on the animal welfare concept and the importance of the ´five freedoms´ and five husbandry criteria from the structure of ANI 35L/2000 indicator, can be stated that welfare on dairy cows farms is required.
The score zero from criteria 1, 2, 3 and 5 which involves pasture and animal hygiene is understandable because of the farm management but ANI 35L system on practice indicate the contrary.
The lowest score 3 is at criteria (floor) because of concrete and leads to affects the onglons of dairy cows and also lead to technopathies.
To be mentioned that ANI 35 is not utilize to impose new welfare regulations but seek to be respected the standards at minimum. In this study case, grazing is allowed on two seasons and satisfies the criterion of animal welfare.

The total points of 34 which means ANI score attributed to farm show us a heightened attention for raising dairy cows from Romanian Black and white breed, which is reflected in animal welfare.

ACKNOWLEDGEMENTS

The work of Nica George was supported by the project ''PROINVENT'', postdoctoral research (POCU), Romania, financing contract nr. 62487/03.06.2022 – code SMIS: 153299.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

STIMULATIVE FEEDING INFLUENCE OVER MILK PRODUCTION AT KARAKUL OF BOTOSANI BREED

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Abstract

The main objective of the research carried out, was to determine the impact due to additional feeding on the specific performance of milk production in Botoșani Karakul sheep breed. In order to achieve the main objective of the research, two groups of adult females between three and six years of age were formed. Both lots were maintained under similar conditions, applying a traditional technology based on feeding them from the stock during the cold season and on pasture during the warm season. The experimental treatment was represented by the fact that L2 benefited from additional feeding applied 25 days before the mating date. This batch received a 170 g amount each morning consisting of a mixture of cultivated cereals (ground corn, sunflower meal, barley and oat grains). Live weight and body condition evaluation at mating time indicates a 2.41 kg higher live weight at L2 but also an improvement in body condition by 0.31 points. For live weight, a significant difference between groups was obtained for $P \leq 0.05$. Regarding the milk production obtained from the females in relation to the BCS assigned to L1 the biggest difference was between BCS = 3.0 and BCS = 1.5 and in L2 between BCS = 2.5 and BCS = 1.5, both situations being significant for $P<0.01$.

Key words: *body condition, Botoșani Karakul Sheep Breed, ewes milk, flushing, milk production.*

INTRODUCTION

The area where the research was carried out is located in the North Eastern of Romania. As in other areas of Europe, climate change is evident in this area and exerts a negative influence not only on the environment but also on the behavior and level of production obtained from small ruminants. Since the main climatological factors, represented by temperature and precipitation, contribute to the increase of annual periods in which drought and high temperatures exceed critical levels, it is found that in that area there are changes in the composition of the green mass on the pasture, affecting the body condition of the ewes exactly in the period preceding the breeding season (Nechifor et al., 2022).

In order to maintain herds in favorable conditions, especially in areas that tend to become arid after the climatic conditions have changed, and the periods of high temperatures and drought are prolonged and affect the vegetation of the plants, it is necessary to apply some activities to support the body condition in the females that form the livestock at the farm level. Therefore, alternative feeding strategies are needed in these arid areas based on supplementary feeding in critical periods with feed that varies greatly from season to season and from year to year due to the evolution of precipitation (Rekik et al., 2020). In these conditions, sheep preparation for a new production cycle will start already in the period preceding the breeding period and most of the time it is based on additional feeding by administering cultivated cereals in quantities that vary according to their nutritional value. Typically for small ruminants the source of additional energy varies greatly and includes in addition to grains and easily digestible fiber sources and other very good quality forages (Simeanu et al., 2018).

By means of additional feeding, the aim is to restore the body condition of the sheep to an optimal level. After a period of flushing, the additional food factors will have a positive effect and will have a major nutritional intake, contributing to the support of some metabolic functions and the secured reserves will be

stored in the body tissues, especially in the fat tissue and in the intramuscular fat, being able to be mobilized when necessary (Martin et al., 2009).
Sheep body condition is extremely important because metabolic factors manifest themselves intensely and can exert highly variable effects, from increasing the level of basic productions (milk, wool, meat) to increasing reproductive indices, when the ensured minimum requirements become favorable, or it can reach a complete block of reproduction when circumstances become critical (Rekik et al., 2020; Martin et al., 2009; Caton & Dhuyvetter, 1997).
The purpose of the research was to perform a detailed analysis to highlight both the impact of additional feeding and the relationship between body condition score (BCS) and some productive traits in Botosani Karakul Sheep Breed.

MATERIALS AND METHODS

The biological material belonged to the Botosani Karakul sheep breed growing at the Research and Development Station for Sheep and Goat Breeding, Popăuți - Botoșani. In order to evaluate the impact due to additional feeding on body condition and milk production, two batches (L1 and L2) were formed. Each batch consisted of 100 females from the base herd aged between three and six years. The established batches had the same experimental treatment, being maintained during the entire period of research in the traditional breeding system. This system relay on keeping ewes in shelter from December to May and on pasture during the warm season, i.e. from May to the end of November.
The experimental factor was represented by the fact that group L2 benefited for 25 days additional feed before the beginning of the breeding season. Lot L2 benefited from a supplemental feed based on administrating in each days morning of a 170 g quantity of a mixt cultivated cereals (ground corn, sunflower meal, barley and oat grains). The supplementary cereals feed mixture was intended to improve the body condition of the females before the start of the breeding season. Through this additional feeding, the aim was to ensure an additional energy intake of 15% compared to the feeding level benefited by the females that constituted L1.
Water and salt were provided at discretion. The breeding season was carried out between September and October, with managed natural breeding being used, with 25 females assigned to one ram. The calving season took place between March and April and the lambs were weaned 70 days post-partum.
The assessment of the body condition of the two groups was carried out by palpating the muscle mass and the fat deposits located on the upper line of their body (back, saddle and rump) being given marks from 1 (for the thin ones) to 5 (for the very fat ones) with subunits of 0.5. using a method developed by Russel (1991). Body condition was assessed at the time of ewes mating and the date of ewes weaning. The assessment was carried out by two experienced persons. If different opinions or certain controversies were recorded, the evaluation was extended until a total consensus and the same point of view was obtained. Live weight was determined using an electronic scale that had an accuracy of ± 100 g.
The performance evaluation for milk production resulted from lactation analyze was based on the application of successive periodic inspections, and using for the lactation period the Nica method and for the milking period exclusively the method AT4 in compliance with the technical specifications suggested by International Committee for Animal Recording.
Estimation of the average total production of milk was carried out using the Fleischmann method.

$$\mathrm{TMY} = \mathrm{L}_1.\mathrm{int}_1 + \sum_{i=2an} \left(\frac{\mathrm{L}_i + \mathrm{L}_{i-1}}{2}.\mathrm{int}_i\right) + \mathrm{L}_n.14$$

where:
TMY= Milk yield (kg)
L1 = milk yield of the 1st monthly test;
Li = milk yield of the 2th monthly test (i = 1,..., n);
Ln = milk yield of the last test;
int1 = number of days from kidding to 1st monthly test;
inti= number of days between monthly tests (i-1) and i (i = 1,...,n);
n = total number of monthly tests for a specific animal.
Data were statistically evaluated with the algorithm REML (REstricted Maximum Likelihood), which provides the achievements of the statistical parametric estimators within the normal range.

RESULTS AND DISCUSSIONS

Supplemental feeding sheep with grain, hay or silage is necessary when pasture or stubble is deficient in energy and protein. The passage of longer periods of time in which nutritional requirements are not ensured at an optimal level affects body condition, with negative effects on the main indicators specific to production or reproduction.

According to the research protocol, the main objectives referred to evaluation of supplementary feeding influence on the changes that occur during a season on sheep live weight, on body condition and on specific performances of milk production.

Evaluation of Body Condition Score and Average Body Weight as an effect of additional feeding

Achieving higher milk yields requires higher energy consumption because the amount of energy required to maintain body tissue functions and milk production exceeds what lactating sheep can consume (Pascal, 2015).

Metabolic processes increase if milk productivity increases and in conditions where nutritional requirements are not ensured, they can trigger an increase in metabolic stress that negatively affects not only sheep production but also their health (Farman et al., 2018). Milk productivity and reproductive traits then decrease. The body energy reserves mobilization during early lactation allows ewes to bridge the gap between dietary energy intake and its loss through milk production (Schroder & Staufenbiel, 2006). As changes in energy reserves have a considerable influence on productivity, health and reproduction in ruminants reared for milk (Ucar et al., 2011; Whay et al., 2003), an optimal management of energy reserves is required in all ewes in lactation. The indicators, which characterize the metabolic processes of dairy cows, are body condition score (BCS) and live weight (ELW).

So, by ensuring this energy nutritional surplus of only 15%, an improvement in body condition was obtained, which from a biological point of view was based both on the restoration of body reserves and on the storage of some energy resources that the animals metabolized in later periods.

Table 1. Descriptive statistics for Body Condition Score (BCS) and Ewe Live Weight (ELW)

Time of assessment								
	Mounting				Weaning lambs			
	L_1		L_2		L_1		L_2	
Character	$\bar{x}$	$\pm s_{\bar{x}}$	$\bar{x}$	$\pm s_{\bar{x}}$	$\bar{x}$	$\pm s_{\bar{x}}$	$\bar{x}$	$\pm s_{\bar{x}}$
BCS	2.48	0.059	2.79	0.055	2.21	0.061	2.57	0.063
ELW	46.61	0.548	49.02	0.505	45.09	0.566	47.44	0.551

Trait 1	Trait 2	Dif. med.	Q1	Q2	W1	W2	*P* value
The difference and its significance for BCS (points)							
BCSL2W	BCSL1M	0.09	0	0	0	0	ns
BCSL2W	BCSL1W	0.36	0	0	0	0	0.01
BCSL2W	BCSL2M	0.22	0	0	0	0	0.05
BCSL2M	BCSL1M	0.31	0	0	0	0	0.01
BCSL2M	BCSL1W	0.58	0	0	0	0	0.01
BCSL1W	BCSL1M	0.27	3.63	4.4	0.22	0.26	0.01
The difference and its significance for ELW (kg)							
ELWL2W	ELWL1M	0.83	0	0	0	0	ns
ELWL2W	ELWL1W	2.36	0	0	0	0	0.05
ELWL2W	ELWL2M	1.57	0	0	0	0	ns
ELWL2M	ELWL1M	2.41	0	0	0	0	0.01
ELWL2M	ELWL1W	3.93	0	0	0	0	0.01
ELWL1W	ELWL1M	1.52	3.63	4.4	1.97	2.39	ns

Notes: BCSL1M and BCSL2M: body condition score at mounting;
BCSL1W and BCSL2W: body condition score at weaning lambs.
$ELWL_1M$ and $ELWL_2M$; average body weight at mounting;
$ELWL_1W$ and $ELWL_2W$ average body weight at weaning lambs.

The research carried out clearly highlights the positive impact due to additional feeding and its effect on the body condition both at the time of lambing and at the time of lambs weaning

(Table 1). Except for the difference between BCS determined for L2 at lambing time (BCSL2W) and BCS determined for L1 at lambing time (BCSL1M) all other differences were significant for $P \leq 0.01$ and $P \leq 0.05$, respectively. Regarding live weight, only the difference between ELWL2W and ELWL1M and respectively ELWL1W and ELWL1M was insignificant for the statistical thresholds considered.

Following the evaluation of body condition, carried out in the two periods, some statistical differences were recorded between the batches for the considered thresholds. The statistical processing of the data obtained as a result of body condition assessment at the time of lambing indicates that the ewes forming L2 had a better body condition because the average score assigned was 11.11% higher than L1. This aspect was due to the fact that the additional feed provided in the period preceding the mount allowed a better restoration of body reserves.

However, by applying supplemental feed for 25 days prior to mating, an improvement in body condition was achieved. In a similar research in which the effect of flushing treatment applied for three weeks was followed, the BCS increased from 0.75 to 0.87. It is also found that following the application of a flushing based on energy intake there were significant effects on the primary follicles, the diameters of the large follicle and the corpus luteum. Plasma concentrations of glucose and cholesterol during the flushing period were significantly different ($P<0.05$) between all applied treatments (Nurlatifah et al., 2019). The role, importance and effect of additional feeding applied in the pre-mount period are special and it should be an indispensable measure used in the application of management that supports the achievement of positive results. This is possible because with increased nutrient intake, especially energy, the sheep can reach the ideal BCS so that they are ready for breeding. Animals with high BCS have better ovulation rates than animals with low BCS. Animals that have good BCS are not inhibited by the production of reproductive hormones such as GnRH, estrogen, FSH and LH (Scaramuzii et al., 2006).

Regarding the body condition evaluation at the time of lambs weaning, the score difference is higher by approximately 14% in the group that benefited from additional feed. This aspect is due to the fact that the sheep of this batch used in a more efficient way the body reserves accumulated and stored during the period in which the experimental treatment was applied.

The live weight registered different average values, being higher in the lot that benefited from flushing. Thus, at the time of mounting to L2, the average live weight was 49.09±0.505 kg. Practically, this batch recorded a 5% increase compared to L1, and this accumulated increase was based on the restoration of tissues and the establishment of some stores that will be used during the period when physiological and metabolic processes require high energy consumption.

On the day of lambs weaning, although the live weight decreased in both groups, the difference remains at approximately the same level, being superior by approximately 5% to L2, being significant for $P \leq 0.5$.

The results obtained represent a basic support for obtaining milk production superior performance. The argumentation of this statement is supported by several researches which show that the ewe body weight at mating, influences not only the number and weight of the lambs at lambing (Gordon, 1997) but also the productivity of the sheep (Vatankhah & Salehi, 2010).

In other research conducted on Kivircik sheep, significant effects of BCS on pregnancy rate, lambing rate ($P < 0.05$) and fecundity ($P < 0.05$) were found. The BCS for the highest pregnancy, lambing rate, and fecundity was determined between 2.01 and 3.00, while the lowest rates for these traits were ≤1.50. The highest rates of the pregnancy rate, lambing rate, and fecundity and gestation productivity were 75.9%, 70.9%, 1.11 and 3.34 kg, respectively (Yilmaz et al., 2011).

Evaluation the Milk Yield in relation with BCS and as an effect of additional feeding

In order to obtain conclusive data, data obtained from the BCS assessment at weaning lambs were used. Milk production resulting during the period when the sheep were in the exclusive milking situation was determined for

each sheep according to the score obtained when determining the BCS (Table 2).
In the case of the batch that did not benefit from additional feed (L1), the highest milk production obtained during controlled lactation was recorded in ewes that had BCS = 3.0 points. In this case, the total amount of milk was higher by 23.18 kg compared to the level of milk obtained from sheep that obtained BCS = 1 and by 8.27% compared to the amount of milk milked from ewes with BCS = 3.5. The obtained data also highlight the fact that the amount of milk milked was obtained from the groups of ewes that had BCS between 2.0 and 3.0.

Table 2. Statistical indicators for Milk yeld in relation with BCS (kg)

BCS Points	n	$\overline{X}$	$\pm s_{\bar{x}}$	S	V%
L1					
1.0	6	53.68	0.255	0.624	1.163
1.5	18	55.45	0.865	3.672	6.622
2.0	28	56.22	0.238	1.257	2.236
2.5	26	66.72	0.535	2.728	4.089
3.0	20	69.88	0.911	4.074	5.83
3.5	2	64.1	2.100	2.97	4.633
L2					
1.5	11	56.65	0.376	1.247	2.201
2.0	15	63.73	0.496	1.923	3.017
2.5	32	77.62	0.672	3.804	4.9
3.0	33	76.65	0.513	2.948	3.846
3.5	6	67.01	1.915	4.69	7.001
4.0	3	67.33	2.333	4.041	6.002

In the L2 group that benefited from stimulating feed in the period before lambing, the proportion of sheep that obtained a score higher than 2.5 points was higher by approximately 35% compared to L1. In the case of this batch (L2) the maximum milk production level is obtained from the sheep that obtained BCS = 2.0.
Concretely, the obtained results indicate that the assessment of BCS at the time of lambing offers more valuable indications on the level of milk production in the respective lactation.
Given that BCS is likely to change in a given ewe during pregnancy, it is not too surprising that both Hossamo et al. (1986) reported that in Awassi sheep BCS before calving had a positive effect on milk yield and lactation duration, whereas BCS measured before breeding had no effect.
According to the data obtained from the research carried out, it appears that the stimulating feeding had an influence on the productive level and the information obtained from the evaluation of body condition at the time of weaning provides more relevant indications regarding the specific performances of milk production from the respective lactation. The dynamics of milk production obtained from the ewes that formed the two experimental groups clearly shows that L2 had a higher level of performance for each group based on the BCS determined at weaning of the lambs (Figure 1).

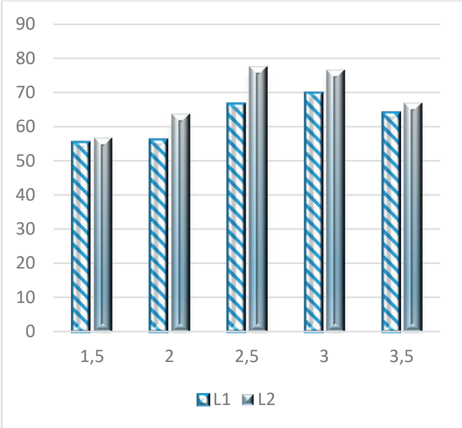

Figure 1. Graphic representation of milk production in relation to BCS (kg)

Contrary to the general picture emerging from these studies, under stimulant feeding the milk production of Sarda ewes with higher BCS in mid-lactation was lower than it was in ewes with lower BCS (Pulina et al., 2012). When feed was restricted, milk production was similarly adversely affected in both groups of BCS ewes. Cannas (2002), cited by Kenyon et al. (2014), questioned whether there is a possibility that ewes achieving a BCS≥4 (very fat) provide lower milk production due to large amounts of visceral fat compressing the rumen, thereby reducing intake. If this is true, it could help explain the results of Pulina et al. (2012).
It could also be assumed that ewes with high BCS in mid-lactation are those that are metabolically programmed not to mobilize accumulated body reserves to be used to

support increased milk production, thus explaining the lower level obtained of milk production.
The nutrition provided in critical periods for the batches that form the core of production has a positive influence on the future productive level. If daily nutritional requirements are not provided at a suboptimal level, metabolic reactions occur that have negative effects not only on daily milk production but also on the total duration of lactation (Jordan & Mayer, 1989; Langlands, 1977).

Table 3. The Difference and its meaning for Milk Yeld (kg)

BCS (points)	BCS (points)	Dif. med.	*P* value	BCS (points)	BCS (points)	Dif. med.	*P* value
L1				L2			
1.5	1.0	1.77	ns	2.0	1.5	7.08	0.01
2.0	1.0	2.53	ns	2.5	1.5	20.96	0.01
2.0	1.5	0.77	ns	2.5	2.0	13.89	0.01
2.5	1.0	13.04	0.01	3.0	1.5	20	0.01
2.5	1.5	11.27	0.01	3.0	2.0	12.92	0.01
2.5	2.0	10.5	0.01	3.0	2.5	0.96	ns
3.0	1.0	16.2	0.01	3.5	1.5	10.35	0.01
3.0	1.5	14.43	0.01	3.5	2.0	3.27	ns
3.0	2.0	13.66	0.01	3.5	2.5	10.62	0.01
3.0	2.5	3.16	0.05	3.5	3.0	9.65	0.01
3.5	1.0	10.42	0.01	4.0	3.0	9.32	0.01
3.5	1.5	8.65	ns	4.0	3.5	0.33	ns
3.5	2.0	7.88	ns	4.0	2.0	3.6	0.05
3.5	2.5	2.62	ns	4.0	2.5	10.29	0.01
3.5	3.0	5.78	0.01	4.0	1.5	10.68	0.01

The comparative analysis of the milk production obtained from the groups based on the BCS evaluation (Table 3) indicates that in L1 the biggest difference was between BCS = 3.0 and BCS = 1.5 being 14.43 kg, being significant for P<0.01. At L2 the biggest difference was found between BCS = 2.5 and BCS = 1.5 significant for P<0.01.

CONCLUSIONS

The results obtained clearly show that the score assigned to the BCS evaluation is an important factor in farm management. The additional feed provided to the sheep in the period preceding the lambing has a double role, i.e. it allows obtaining higher indicators for the reproductive activity, but it will also represent an effective mean of supporting the performances for milk production.
The inclusion of additional feeding among farm management activities to maintain adequate body condition for all ewes will allow appropriate decisions to be made for feed stock allocation.
The final results confirm that both in L1 and L2 the highest milk productions are obtained from ewes with BCS = 2.5 and BCS = 3.0, thus proving that the sheep state of maintenance is an extremely important factor in order to obtain productive performances.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

STREAMLINING ONLINE COMMUNICATION IN AGRICULTURAL HOLDINGS IN TIMIȘ COUNTY. COMPARATIVE STUDY

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Abstract

The study undertaken focuses on a comparative analysis of the online communication of two agricultural structures in Timiș County: Max Agro and Merpano. Following the analyzes carried out, including the SWOT analysis and the multimodal analysis of the discourse of agricultural structures, we proposed a possible effective communication strategies. Since there are a variety of platforms for posting content, the analysis we undertook focuses on the communication tools that the two companies currently use. Thus, the analysis focuses on the websites of agricultural structures, Facebook and Instagram pages. As for the online communication analysis grid that we applied to each agricultural structure, it sums up the following sections: online communication channels: the website, facebook page, the Instagram page, frequency of posts and content of posts.

Key words*: *agricultural holdings, online communication, social media.

INTRODUCTION

Designing communication design as a realistic approach to communication in the online environment conditioned us to identify the functioning mechanism of this analytical system that is geared towards publishing and creating semantic content in the online environment.

The MaxAgro Group is an agricultural operator, with private capital and 100% Romanian, very important at the national level, with its headquarters in Gătaia, Timiș County. It was founded in 1993, by the Zifceak family. A family business that developed gradually, efficiently, healthily and sustainably, from five hectares, to over 15,000 hectares cultivated in Timiș County, performing crops mainly of wheat, rapeseed and corn. It is quality that becomes a company motto, becoming a policy that extends throughout the process, from seeding, to storage and delivery. Also, Maxagro has established a centralized system of 10 modern silos (built to the best market standards for grain storage), with a capacity of over 25.

Another important component is the zootechnical part that the group has successfully developed. The farm of 2,700 cattle, from the Holstein dairy breed, is self-supporting through the performance of its own productions. Milk production, always fresh and of a superior quality, is exclusively dedicated to customers, given the fact that they have 2 milk tanks with a cooler, with a capacity of 65,000 liters. The amount of milk produced per day is 30,000 liters, it is collected accordingly, a high-performance milking room with fifty stalls being made available (https://maxagro.ro/).

Merpano is an agricultural structure with private capital, 100% Romanian, which was born in 2002 and which addresses a varied range of farmers, regardless of the size of the agricultural holding. The main objectives of the company are the supply of agrochemicals, seeds of high genetic value, fertilizers and professional agricultural services. The agricultural structure, based on its own farm (3,000 ha), offers all farmers in the Western Region and not only, integrated customized solutions in order to support the selection

process and the application of the latest and most efficient large crop technologies.
Since its establishment, Merpano has continued to diversify its services and products in the agricultural sector, improving and consolidating its position in the agricultural environment of the Western part of the country. Following a large investment, the agricultural enterprise opened the most efficient seed conditioning station in the western part of the country. It includes more than 250 hectares of seed production of the most established wheat varieties, managing to offer customers more than 1,500 tons of wheat seed, selected, treated and packaged to the highest standards every year (https://merpano.ro/).

MATERIALS AND METHODS

In the current context, the multimodal analysis of a discourse is the subject of research by great communication specialists, in the context of a call for a new way of communicating information from a distance. Authors Kress & Van Leeuwen (2001), but also Baldry et al. (2006), emphasizes the idea that modal texts simultaneously use at least two semiotic systems (linguistic, visual, auditory, etc.) in order to design a product. Thus, we used this analysis in order to examine the online multimodal discourses of two agricultural structures in the Western Region. The second is the graphic method, which consisted in the actual representation in graphic form of the analyzed indicators.
The definition of the SWOT analysis gravitates in two planes, an internal one targeting strengths and weaknesses (Strengths and Weaknesses) and an external one focusing on opportunities and possible threats (Opportunities and Threats). Thus, we used this method in order to project a general, overall vision of the farms.
The conversion rate represents the percentage of people/users who came into contact with a message from a certain agricultural structure (in the present case) and who, after the exposure, took a certain action, showing a certain behavior (either purchasing or requesting a information, or subscribed to the newsletter). The conversion rate is given by the following formula:

RESULTS AND DISCUSSIONS

In what follows, we will proceed with the analysis we undertook, thus exemplifying an analytical perspective of communication in the online environment, where the communication design is reflected very well. The time period in which the study was conducted is April 1, 2022-April 30, 2023.

Maxagro online communication channels
Maxagro uses social media platforms in its online communication strategies: the Facebook page, the Instagram page, the Tik Tok account and, more modestly, the YouTube and LinkedIn (www.linkedin.com/company/maxagro) accounts.
The Instagram page was created on 16.03.2022 (www.instagram.com/maxagro.ro) has 737 followers and a total of 70 posts. Page advertising is organic, with likes fluctuating from post to post based on content. From our point of view, the TikTok account (www.tiktok.com/@maxagro.ro) is the one that brings more followers and increases the visibility of the agricultural structure in the online environment, having at the time of research 9,566 subscribers and just over 84,000 likes. Thus, the first post, which also corresponded to the date the account was created, 16.03.2022, had approximately 6,700 views. The trend was a fluctuating one, but on 06/23/2023 at the last video posted on this network, the number of views was just over 267,000. Apparently, this trend of community growth on Tik Tok continues.
The Maxagro website is a complex one, but at the same time very well structured and friendly, highlighting, through the designed design, traditional family values. It is composed of sections dedicated to the agricultural structure and the products and services it offers. In the last twenty years, the Maxagro group has grown in the Western area, becoming a brand, which since 2022 has managed to lay the foundations for constant communication (Loiro, 2019) in the online environment as well. A friendly

communication was adopted (Biçoku, 2022), a direct and free style, which would facilitate the reduction of the distance between the company and the customers (Ali, 2021). Communicate in the first person plural, as in family (Mainardes, 2019). That is why the Maxagro group aims to be considered a Romanian brand, but which is close to customers, close to employees, close to the county and the region. This emerges from every discourse of the organization, emphasizing uniqueness and collectivity. At the same time, from the company's mission, vision and values, the same strong idea of preserving tradition, of regional development stands out. The mission communicated on the website emphasizes progress: "Our mission is to perform and turn performance into progress. For the community, for Romania". The vision refers to the development of the region: "Maxagro's vision is to become one of the most important and integrated agricultural operators in South-Eastern Europe, to be able to positively influence the region", and the principles underlying this family business are: performance, innovation, trust, quality, sustainability. A particularly important factor is represented by the consistency that defines the distribution of content on the website. The slogan is the only thing that is not highlighted from the first seconds of interaction with the site. Although the "Home" section welcomes you with a brand aphorism "Everything we do, we do it for tomorrow's generations", the slogan "Performance since 1993" is mentioned only in the "Values" section. From the point of view of communication, it is considered necessary to clearly establish the slogan and always use it with the visual elements of the brand, so that consumers associate the presented image with a well-defined slogan. Also, special attention should be paid to the wording itself. Every agricultural enterprise needs communication based on images as well, which play an essential role in delivering trust to customers (Marin et al., 2020). From this point of view, it can be stated that Maxagro has an effective, adequate communication, the images focusing on the fleet of machines and the technology found within the company. For the purpose of illustrating these elements, the site has in its composition images with a high resolution, in well-defined and chosen colors,

The company's Facebook page was launched on April 8, 2021. Maxagro Group has since built a small Facebook community with 2,800 followers and 2,200 page likes. Of the followers, a percentage of about 2.23% interacts with the page, in one or simultaneously several ways: likes, shares or even direct mentions and tags of the page in a post. This percentage also indicates the conversion rate for the Facebook page of the Maxagro Group, in April 2023. It should be noted that the conversion rate has registered an upward trend in recent months, increasing until the year 2023 by approximately 68% (Figure 1).

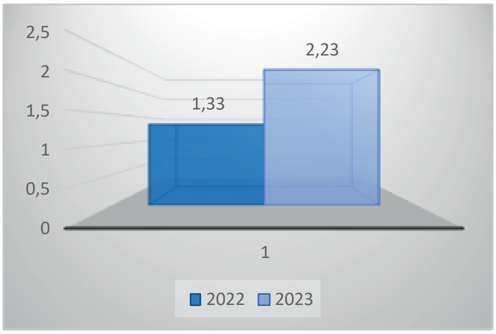

Figure 1. Conversion rate index, in April 2022-April 2023 of the Maxagro Facebook page

The page is very well structured. In addition to the contact information of the structure, information about the products and services offered and the certifications obtained can be identified. All are expressed in the 2nd person plural, in a friendly style that reveals the closeness to the members of the community. The cover page shows the brand visibility elements, the logo and the slogan, which is slightly different from the one posted on the web page. The slogan "30 years of success and performance" conveys a clear message regarding safety and continuity within the company, which is transmitted in a close relationship with the related public. Thus it was possible to plant in the minds of the community members a coherent representation of the agricultural structure, which reflects familiarity and closeness.

In the analyzed period, namely 01.04.2022-30.04.2023, Maxagro does not have a cursive in terms of posts on the Facebook page (www.facebook.com/maxagro.ro). The number of posts per month is fluctuating, we cannot talk about an average of posts per day, but per month, which is around 4, except for the month of January 2023 when nothing was posted on the page. In certain situations, for example: posts from March and April 2023, their number suddenly increased to 10 in March and 33 in April, respectively (Figure 2). Thus, it was desired to increase the visibility, the message of the agricultural structure reaching a considerably larger number of fans of the page, their existence in the online environment not being simultaneous. A higher frequency of posts would direct page activity to a wider segment of the target audience and directly proportional to this, it would also increase the feedback rate.

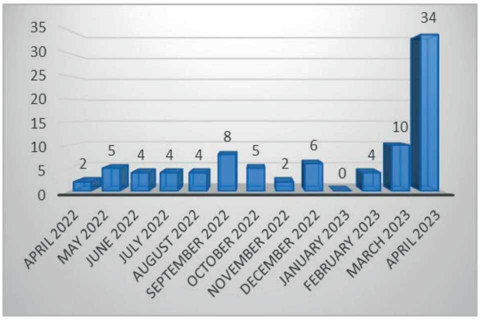

Figure 2. Frequency of posts on the Maxagro page, in the period April 2022-April 2023

The content of the posts is relatable, relevant to the target consumer and presents a stronger balance to user-generated content (Rutherford, 2007; Libaert, 2006), which is a perfect fit for Facebook. Maxagro showcases aspects of the brand and integrates its brand purpose in every post due to the positive flow of consumer engagement. Maxagro also intelligently considered what other aspects of interest their followers would have and effortlessly wove them into the content. With cultivation techniques, farm animals, farming, healthy lifestyle and family life mentioned in almost every post, it offers a balance of content for different demographics. Maxagro does Facebook marketing exactly as they understand how it fits into their audience's lifestyle. We appreciate that one should make the most of the posting times, focusing on a certain number of posts, at least weekly, if not daily, and through the light content it provides, flow the elements of identity in each post visual, in one way or another, in the sense of making sure that its name and logo features are perfectly synchronized in their Facebook feeds, so that users constantly associate the brand with certain visual elements. Following the observation, several types of content were identified in the posts:

- posts related to the services and products sold, accompanied by images and videos of very good quality;
- posts about events organized by Maxagro, either events in which they participated or as sponsors;
- posts in which the Maxagro Group engages in social responsibility and charity activities; posts whose main subject is the employment announcements of the agricultural structure, with the exact statement of available jobs;
- articles that reproduce the positive feedback of the Romanian Maxagro brand from various supporters for the development of products and the support of domestic services.

In the following, the SWOT analysis was carried out in terms of external communication, within the Maxagro agricultural structure in the Western Region, in the online environment, based on the multimodal analysis carried out. As for the strong points, the following can be highlighted: the existence of Facebook, Instagram, Tik Tok pages and Youtube and LinkedIn accounts; the high number of views of posts on the Tik Tok account; a well-structured and complex site; loyal current users; the mission, vision and values interconnect with the practices of the agricultural and public structure on the website; relationships and partnerships developed with local media; making information available through various web-based tools. Weaknesses highlight: lack of direct employee involvement in Facebook advertising strategies; typos creeping into the website; the inconsistency of a design, based on the visual identity elements of the agricultural structure, in social media posts. The following stand out as opportunities: running a campaign on social media; populating the YouTube account; developing strong partnerships to ensure the involvement of all stakeholders; creating new marketing materials; collaboration with influencers from the agricultural sector; user loyalty by creating giveaways (contests with prizes). Aspects such as: competition from other agricultural structures; changes in technology that make your current communication tools obsolete; disruptive events that make it difficult to reach your target audience; data sharing issues and privacy policy.

Merpano online communication channels

Merpano relies in online communication on the following tools: website, Facebook page, Instagram page, and less Youtube. Compared to the previously presented agricultural structure, the Youtube channel, although more populated with videos, has only 6 subscribers, compared to the 500 subscribers and only 9 videos of the Maxagro (www.youtube.com/maxagrochannel). But, in both cases, the YouTube pages are not updated, the attention being focused on the platforms that generate a large number of views in a shorter time. The Merpano Group also has a very popular Instagram page from 25.03.2021, with 981 posts (www.instagram.com/merpanoromania).

Compared to the Maxagro company page, which is newer and has fewer posts, the members who joined the Merpano page are 57.39% less than the Maxagro company followers (Figure 3). Corroborated, although in this case too the advertising is organic, the number of likes is much lower than in the previous case. A very important element highlighted is the consistency and simultaneous and accurate distribution of content and images on all channels used, with the exception of the Youtube channel (www.youtube.com/merpanochannel).

Increasing the number of likes and followers of a page can be increased by initiating the well-known "giveaways", a new way implemented by many of the influential personalities on Instagram.

The Merpano website (www.merpano.ro) is a complex, very well structured web page that emphasizes the company's visual identity elements. It is structured from multiple sections, the first of which is "Home" which highlights the products and services offered, the second section "About" provides information about the history of the agricultural structure, another section is entitled "Product Portfolio" in which the main producers and suppliers of the Merpano group are presented. The "Blog" section fulfills exactly the role of an actual blog where consumers can ask questions characteristic of the field, the answers being elaborated in the form of videos, taken from the company's YouTube account.

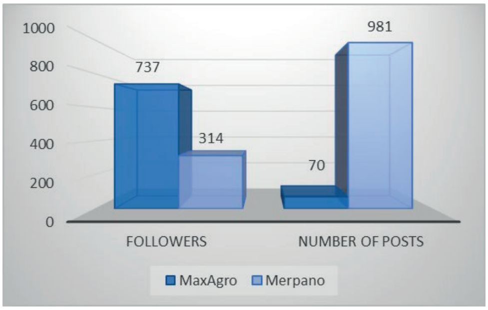

Figure 3. Number of users and likes for Maxagro and Merpano's Facebook pages

The "Why Merpano" section has a demonstrative role regarding the opinion of customers, employees and partners about the choices and experiences with this company (Kuchi, 2006), and in this case the videos are taken from the YouTube platform. Communication on the web page does not have a constancy. There is a combination of styles used, sometimes the informal one, but analyzing in a general approach, the style is impersonal, formal (Caelen, 2002; Dâncu, 2001), the emphasis being in some places on the company. It is communicated in the third person singular, in some places in the first person plural, which determines the establishment of a possible distance between the interlocutors. One can easily identify the

slogan of the company "your partner in agriculture", which always accompanies the logo, so users can identify and associate the company's image very easily. It is very important that the motto is always kept in the same form, there are two variants on the site, one of them previously mentioned, the second being written in capital letters. The essential elements that strengthen the credibility of a company's website are its vision, mission and values, aspects which, moreover, must be reflected in all published content, whether it is online or not. A website display of mission and vision, it is like a succinct definition of the agricultural enterprise, which has an important role in determining the strategic direction of the agricultural society. The company's values, transparency is also highlighted by the publication on the website of the company's values, they represent the own personality of the agricultural entity. The design of the website is essential, from this point of view it can be said that it is an adequate communication, the images used are of good quality, being directly proportional to the products and services offered. From the point of view of communication, it is considered necessary to outline very clearly the company's mission, vision and values, and if they already exist, to be published on the company's website.
The company's Facebook page was created on May 24, 2018 (www.facebook.com/merpano). The Merpano Group has established a community of farmers and beyond on Facebook, with 7,200 followers and 5,700 page likes. Among the followers, in April 2023, a percentage of about 2.5% interacts with the page, in one or simultaneously several ways: likes, shares or even mentions and direct tags of the page in a post. This percentage also indicates the conversion rate for the Merpano Group. The conversion rate in April 2023 increased by 140% compared to the conversion rate in the same month of 2022 (Figure 4).
The conversion rate is higher in the case of the Merpano page, compared to the Maxagro page by 12.1% in the year 2023, although in the year 2022 the situation was reversed, the conversion rate being higher in the case of the Maxagro page with a percentage of 27.9%. This change can be attributed to the adoption by the agricultural structure Merapano of a strategy regarding the loyalty of the public not only through content, but through the consistency of posts.

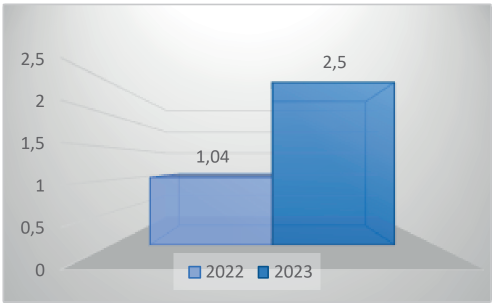

Figure 4. Conversion rate index, in April 2022-April 2023 of the Merpano Facebook page

Unlike Maxagro, which created the page in 2021, Merpano has 157.1% more followers, and 159.01% more likes, all related to the analyzed time period, considering the Merpano company has a advance of 3 years (Figure 5).

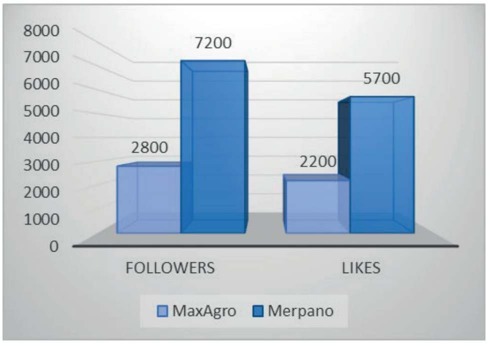

Figure 5. Number of users and likes for Facebook pages of Maxagro and Merpano

The page is very well designed, with complete contact information, a brief presentation of the company, its products and services. The formal style is intertwined with the friendly, familiar one, the expression is made in the third singular. The profile photo contains the company's logo and slogan, which conveys a concrete message regarding farmers, namely that they can rely on a partner in agriculture,

thus establishing a close relationship with the members of the page.
In the analyzed period, namely 01.04.2022-30.04.2023, Merpano has on average 1-2 posts per day. In certain situations, their number varies, increasing to 3, even 4 posts per day.

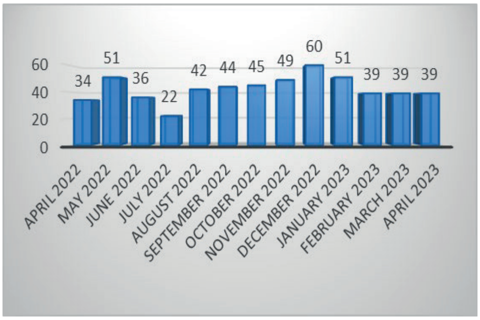

Figure 6. Frequency of posts on the Merpano page, in the period April 2022-April 2023

Thus there is a constant in terms of the number of daily posts, as can be seen in Figure 6. The month of July of the year 2022, was the month with the fewest posts, on average one post every two days, this is due mainly for the summer period, dedicated exclusively to holidays, at the opposite pole most posts were identified in the month of December 2022. The month of May 2022 also has more posts than usual, due to the fact that there was a special event for the enterprise, with double meaning, on the one hand the field and its fruits were celebrated and at the same time the 22 years of existence of the agricultural society were celebrated. The strategy of broadcasting the content at a certain well-established time interval has beneficial effects on the community,
Merpano posts a variety of content on its page. They usually contain information about the company, new products, release dates and promotional offers. They use different forms of media such as photos, GIFs and videos to display their message. They link many of their posts to their official website so customers will have convenient access to connect with them. The Facebook page is used as a customer service tool to address customer concerns.

After analyzing the content of the Facebook page of the Merpano agricultural company, we identified several types of content, as follows:
- posts about the products and services they offer;
- about the events taking place in society;
- referrals to warning campaigns about certain crop pests, thereby wanting to alert farmers to the problems existing in the sector;
- useful advice on topics of common interest between the brand and the community;
- posting of grain exchange information;
- posts with religious themes; posts related to gastronomy and other food;
- questions aimed at engaging the audience in the interactivity created on the basis of the verbal exchange of the conversation, which also generates the most likes and comments.

Also, the SWOT analysis was carried out in terms of external communication, within the Merpano agricultural structure in the Western Region, in the online environment, based on the multimodal analysis carried out. Thus, the strong points can be distinguished: the existence of Facebook pages, Instagram and the YouTube account; positive reviews from the public; loyal current users; a well-structured and complex site; making information available through various web-based tools. The weak points are: lack of online presence on other social media platforms; Interconect's mission, vision and values are not made public on the website; the inconsistency of a design, based on the visual identity elements of the agricultural structure, in social media posts. In terms of opportunities, they focus on: running a social media campaign; populating the YouTube account; developing strong partnerships to ensure the involvement of all stakeholders; development of new marketing materials; expanding online presence on social networks such as Tik Tok, LinkedIn; collaboration with influencers in the agricultural sector and user loyalty by creating giveaways (contests with prizes). The threats are summarized in the aspects that concern: competition from other agricultural enterprises; technological changes that make current communication tools obsolete; disruptive events that make it difficult to reach your target audience; data sharing and privacy policy issues, but also the lack of successful stakeholder engagement.

CONCLUSIONS

The number of likes and shares of posts fluctuates depending on their nature. Posts about owned inputs (equipment, performance techniques used) get over 150 likes, also get the most comments and shares. Posts aimed at engaging participants in a social interactivity, in the form of a conversation-like verbal exchange, get both likes and comments.

Thus we can appreciate the fact that the way of communication in the online environment is quite effective. The statement is supported by several elements: the creation of appropriate content for community members, the degree of receptivity to feedback, the responses to members' requests, the partial achievement of the coherence of the verbal message with the visual one, the coherence and stability of the speech and style, but not least of of the permanently built rapport (enveloped by a permanently positive image - an open, traditional and pleasant agricultural enterprise). We have also identified the use of the identity strategy (emphasizing the idea that it is a Romanian brand, from the Western Region) as an image strategy and at the same time the environmental strategy which is embodied in the simultaneous use and application of several virtual communication tools, interconnected. In this sense, the communication efficiency of the Maxagro agricultural structure is shown by the high degree of visibility in the online environment and at the same time by the generation of multiple search results (94,200 results).

In terms of the number of likes, shares and comments it is high, it fluctuates depending on the nature and content of the posts. In general, users like informative posts, which have the highest number of likes (around 2000 likes).

Thus we can appreciate the fact that the way of communication in the online environment is an effective one. The claim is supported by the constant and frequent way in which content is posted. This method seems to have a high success rate in gaining audience loyalty. At the same time, this agricultural structure also uses the identity strategy as an image strategy, which is highlighted by the simultaneous use and application of several interconnected virtual communication tools. Thus, the effectiveness of the communication of the Merpano agricultural structure is shown by the high degree of visibility in the online environment and at the same time by the generation of search results (17,600 results). The most important factor in online communication is represented by the adaptation of the agricultural enterprise to the environment in which communication is carried out. In this framework, the followers of a page want to receive qualitative information, distributed in a very short period of time, in a common, even familiar language and a friendly style, the adaptation being done at the level of the communication channel. On social networks Facebook, Instagram, it is imperative that the message is accompanied by a visual component, in order to attract more positive feedback, but at the opposite pole is the website where the emphasis should be on the informative part of the agricultural structure. Because the website of any agricultural enterprise is its business card in the online environment, it is a presentation brochure, precisely for this reason it must meet certain standards in terms of content, accessibility and its design. Given that any customer, upon first contact, judges the company by its cover, the first seconds of browsing the site are paramount in order to create customer satisfaction. The chances of losing a customer can increase exponentially, if the site has problems either loading, navigation is not simple or has grammatical errors crept into the content. The increasing popularity of promotion in the online environment attracts the presence of agricultural structures in this environment, and given the ease of access, differentiation is absolutely necessary, since the web page must impress the users. Another tool that is handy and should be developed more and more is the blog. It has recently become an effective tool in virtual communication, due to the fact that it allows the creation of content for the products or services offered, their presentation in a distinct manner and even their humanization. To point out, in this case, is the SEO optimization, which can ensure the increase of the visibility of the blog. The blog, built on the basis of the implementation of a content strategy, can streamline the online communication process of an agricultural enterprise. Blog advertising can be supported by brand ambassadors, who are passionate people, have information about the agricultural sector and are regular users of the products/services delivered by the agricultural company. These, through personal social networks, I can promote the blog, delivering digestible content for potential users. Thus, by appealing to the ethos of communication, the transfer of trust in the company's services/products is also ensured.

The communication campaigns that should be carried out periodically (Șerban et al., 2022) must be different and ingenious to capture the attention of existing and future customers to involve them in all the actions of the company. Thus, social networks must be taken into account, which today are the ones that have the greatest impact on the majority of individuals. In order to make the communication process more efficient, it is imperative to apply an optimal strategy. First of all, it is necessary to monitor information about agricultural structures, already existing in social media. Nowadays, anyone can exercise the right to free speech and create and share content (they can be people who are satisfied or dissatisfied with the products/services offered). Secondly, it is necessary to choose the optimal communication channel, taking into account the audience to which the agricultural society addresses itself. Facebook, Instagram, more recently Tik Tok are very good communication and promotion tools when the agricultural enterprise addresses the final consumer directly. Through these platforms, the relationship with the users (the public) can be strengthened, moving to the next level (it can be humanized). Used correctly, these tools can generate many benefits for any agricultural company: customers and potential customers can be reached more easily; the commitment of already existing customers can be made loyal through different prize campaigns, in this way the consumer remains loyal to the respective agricultural company. Another important aspect to take into account is shaping the humane and positive image of agricultural enterprises, a fact that determines paying special attention to the creation of messages or the subjectivity of the speech, thus strengthening the relationship of closeness with the public, a fact that simultaneously implies the readiness with which the society answers the questions asked, using an appropriate style and language. Starting from the premise of a society overloaded with content, attracting the attention of the public becomes an increasingly difficult process. A new way to surprise the audience is to use celebrities to promote products/services. Even if the communicative design elements were not integrated, completely balanced, the study has its relevance in the meaning-making mechanism, by sedimenting two of the fundamental properties of communication: hypertextuality and interactivity.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

RESEARCH ON THE EFFECT OF RECONSTITUTED MILK IN THE LEARNING OF INFANT LAMBS

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Abstract

The paper aims at the artificial weaning that is currently used in sheep and goat farms. In Romania, the main mating season is autumn, and calving takes place in spring. The price of milk and dairy products is high during this period because the milk produced by sheep and goats is consumed especially by lambs / kids, to be capitalized on the stain only after their weaning. In sheep and goats with very good milk yields, one milking per day can be practiced provided that enough milk remains for sucking lambs. In other cases, the lambs are weaned early and will be raised with milk substitutes and the milked milk is fully exploited on the market. Breeders who want to capitalize on all or part of the milk production produced by the mother sheep as early as possible after farrowing try to find those technological variations of feeding lambs / kids that do not affect their growth. This line also includes the research carried out regarding the effect of using milk substitutes (reconstituted milk) in the feed of lambs / kids by breastfeeding and by incorporating them into the mixture of concentrates.

Key words*: lambs, milk powder, nutrition, Romania, weaning.*

INTRODUCTION

Artificial rearing of lambs is necessary in cases of orphaned and mismothered lambs and is becoming an increasing requirement in the sheep dairy industry. (Caroprese et al., 2016). Traditionally, fulfilment of a lamb's nutrient requirement was considered to depend on milk intake due to negligible solid feed intake in the first few weeks of life (Owen et al., 1970). Consequently, many studies have focused on optimisation of milk composition to improve lamb growth. (Danso et al., 2018).

Early weaning of artificially grown lambs with powdered milk in liquid formula would benefit farmers by shortening the feeding phase, reducing labor costs. In sheep with high milk yields, one milking per day can be practiced if they are higher than the amount of milk that lamb can consume.

This is actually the purpose of our research to find those technological variants that allow us to increase the production of milked milk to be harnessed in the market.

The objective of the study was to investigate the effect of milk replacer on some growth parameters compared to milked whole milk from the mother sheep.

MATERIALS AND METHODS

The researches were carried out at a private farm in the Western Part of Romania, on a herd of 20 lambs of the Turcan breed, coming from twin calving in order to increase the accuracy of the results obtained.

Immediately after calving we individualized the lambs by earmarking and for the formation of batches by marking with paint in the withers region.

In order for the batches to be as homogeneous as possible, the twin lambs born for 48 hours were retained, and subsequently, those whose body mass was too small or too large were eliminated. In order to have as many twin lambs as possible to meet the requirements, the experiment was organized in the middle of the calving period and the maternal sheep herd was large enough to allow the selection of lambs.

The remaining twin lambs were divided into two batches, depending on weight and sex. Each batch consists of 4 males and 5 twin females, according to Table 1.

LM consisted of 9 twin lambs, four males and 5 females, who were artificially breastfed with

milk milked from the mother sheep. These millet come from different mothers
LR had in its composition the brothers and sisters of the lambs from lot 1, also 4 males and 5 females, who were fed with reconstituted milk 200 g of powdered milk / 1 liter of water.
In order not to have an influence on the results obtained, the maintenance of the lambs from the control and experimental group was carried out under the same environmental conditions, in a clean and airy shelter.

Table 1. The mode of organizing the experiment

Specify	LM	LR
n	10.00	10.00
Infant lambs in the age period 7-28 days	Artificial breastfeeding	Artificial breastfeeding
	Milked milk from mother sheep	Reconstituted milk
	+	200 g /1 liter of water
		+
	Mixture of concentrates with 18% PB	Mixture of concentrates with 18 % PB
	Hay alfalfa	Hay alfalfa

Note: LM is the lambs raised with milk from sheep and LR are the lambs raised with reconstituted milk.

The fact that the twin brothers/sisters are in the corresponding group, increases the accuracy of the results obtained. The preparation of the powdered milk replacer was used hot water and the ratio being of 1/4 (g). (Danso et al., 2018).

RESULTS AND DISCUSSIONS

The milk production of the mother sheep was estimated at the beginning of the experimental period. The values obtained are shown in Table 2.

Table 2. Milk production of mother sheep

Curent Number	Milk Production [g]
1	880.00
2	840.00
3	1,210.00
4	970.00
5	1,120.00
6	860.00
Curent Number	Milk Production [g]
7	1,040.00
8	1,010.00
9	970.00
Variability coefficient %	12.41

From the analysis of this table it can be seen that the milk yield of the mother sheep falls within the range of 840 g / day up to 1,210 g/ day. The coefficient of variability is 12.41% which gives us the information that milk production has a medium variability. The average for the 9 mother sheep who have calved twins is 988.89 g/day.
Based on the amount of whole milk (LM) and reconstituted milk (LR) distributed daily and the remaining unconsumed milk, it was possible to calculate the average consumption per lamb and per day. The data obtained are presented in Table 3.

Table 3. Amount of milk consumed by lambs during the experimental period (liters)

Specify	n	LM Milked milk [l]	LR Reconstituted milk[l]
Total quantity	9.00	180,720.00	235.40
Average/ day/group	9.00	8,214.00	10,701.00
Average/ day/lamb	1.00	0.91	1,189.00

We mention that only the consumption of milk until the age of 28 days was taken into account because the increase in weight is constituted on the basis of sucked milk. From the age of two weeks, a mixture of concentrates with 143 g PDIN/1 CFU, respectively alfalfa hay was distributed, but there was no difference in composition between the two batches. According to the consumption of additional fodder for the lambs until the age of the end of the experiment does not have significant influences on the increase, which is why it was not taken into account only the daily consumption (Hutu, 2019).
Analyzing the data contained in Table 3, it can be seen that the milked whole milk and distributed to the lambs was consumed in the amount of 180.72 liters in the 22 experimental days, while the reconstituted milk was consumed in the amount of 235.4 l.
The difference between the two batches is 54.68 liters.
On the lot it is found that, daily, the lambs from the LM group consumed 8.21 liters of whole milk, and those in the LR group consumed more by 2.49 liters and 10.7 liters respectively.

The daily consumption per lamb was 0.91 liters in the batch with milked whole milk and 1.19 liters in the batch with reconstituted milk which means a difference of 0.277 liters per day.
We can conclude that the reconstituted milk is consumed by lambs in the LR group in an amount greater by 30.37% compared to the LM group, whose lambs consume whole milk. The motivation could be that whole milk is more complete and provides all the necessary nutrients for growing lambs, and the state of satiety is satisfied with a smaller amount of milk.
Evolution of body mass and weight gain, at the calving of lambs, with the individualization, a pre-selection of twin lambs was made on the basis of body mass and sex. The data obtained are presented individually in Table 4.

Table 4. Body weight of lambs from the experimental variant

Current number	LM		LR	
	Sex	Weigh [kg]	Sex	Weigh [kg]
1	f	2.80	m	3.30
2	m	3.60	m	3.40
3	f	2.70	m	3.00
4	f	2.60	f	2.50
5	f	2.90	f	2.40
6	m	3.40	f	2.90
7	m	3.10	f	2.60
8	m	2.70	f	2.90
9	f	3.20	m	3.50
Σ		27.00		26.50
X ± Sx		3.00 ± 0.12		2.94 ± 0.13
s		0.35		0.40
Variability coefficient %		11.55		13.49

In the batch of LM lambs fed with whole milk, the body mass was within the weight limits between 2.6 and 3.6 kg with an average of 3.00 ± 0.12 kg, and in the batch of LR lambs fed with reconstituted milk, the body mass was within the range of 2.4-3.4 kg with an average of 2.94 ± 0.12 kg, the difference between the averages of the two lots being only 0.06 kg.
The variability expressed by the coefficient of variability has a value of 11.55% at the LM and 13.49% at LR, respectively.

After the colossal period the, lambs were accustomed to artificial feeding to the nipple until the age of 7 days when they were weighed again.
The data contained in Table 5 was considered the start metrics for the beginning of the experiment.

Table 5. Body weight of lambs from the experimental variant (7 days)

Current number	LM		LR	
	Sex	Weight [kg]	Sex	Weight [kg]
1	f	3.80	m	4.10
2	m	4.50	m	4.30
3	f	3.40	m	3.70
4	f	3.60	f	3.40
5	f	3.70	f	3.20
6	m	4.30	f	3.80
7	m	4.20	f	3.40
8	m	3.80	f	3.90
9	f	3.90	m	4.30
Σ		35.20		34.10
X ± Sx		3.91 ± 0.12		3.79 ± 0.13
s		0.36		0.41
Variability coefficient %		9.08		10.59

At one week, the lambs of the LM group had a body mass in the weight range of 3.4 - 4.5 kg with an average of 3.91 kg. In combination with the body mass at calving, a total increase of 0.91 kg was recorded, which corresponds to an average daily increase of 130 g/day. We mention that during this period the lambs had to adapt with artificial feeding to the bottle, which has negative influences on the increase.
In the LR group, the body mass was classified in the limes 3.2-4.3 kg, with an average of 3.79 kg. Compared to the values recorded at calving, there was a total increase of 0.79 kg and an average daily hope of 113 g, lower by 17 g compared to lambs in the LM group.
The homogeneity of the lots expressed by variability coefficient % increased further, taking into account that for the LM lot the registered value was 9.08% and 10.59%, at LR the difference between them being only 1.15 percentage points.
After 22 experimental days, at the age of the lambs of 28 days, the values for body mass,

presented in Table 6, were recorded and suggestively in Figure 1.
From the analysis of this table it can be found that the body mass of the lambs from the group fed with whole milk (LM) were included in the range of 6.7-8.3 kg with an average of 7.43 kg. In the batch of lambs fed with reconstituted milk body mass had values that fall within the weight limits of 6.4-8 kg with an average of 7.04 kg.

Table 6. Body weight of lambs from the experimental variant

Current Number	LM		LR	
	Sex	Weight [kg]	Sex	Weight [kg]
1	f	6.70	m	6.80
2	m	8.30	m	8.00
3	f	7.10	m	7.30
4	f	7.00	f	6.70
5	f	6.80	f	6.40
6	m	7.80	f	6,6
7	m	8.10	f	6.40
8	m	7.80	f	7.70
9	f	7.30	m	7.50
Σ		66.90		63.40
X ± Sx		7.43±0.19		7.04±0.20
s		0.58		0.59
Variability coefficient %		7.84		8.43

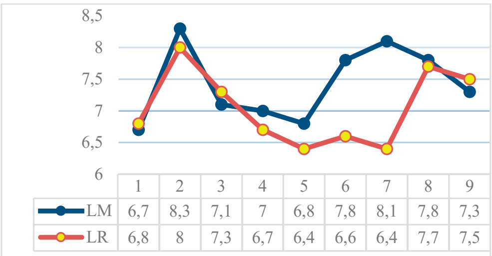

Figure 1. Graphic representation of final body mass

Differentiated feeding with milked whole milk and reconstituted milk had a greater differentiating effect on the LR lot where the reconstituted milk, even if consumed in greater quantity, had the effect of achieving lower body weights that fit into a wider range. In Figure 1 it can be seen that in only 3 couples of lambs (1.3 and 9) the body mass is higher in lambs of the LR group and in 5 couples of lambs (2, 4, 5, 6, 7, 8) it is higher in lambs of the LM group.
As for the sex, the highest body mass is recorded in males regardless of the batch. By the difference between the body mass recorded at the end (28 days) and the beginning of the experiment (7 days), the total individual increase achieved by the lambs of the two batches was calculated, the factorial factor being the composition of the milk. The data obtained are recorded in Table 7.

Table 7.Total gain of suckling lambs during the control period (7-28 days)

Current number	LM		LR	
	Sex	Total weinght [kg]	Sex	Total weinght [kg]
1	f	2.90	m	2.70
2	m	3.80	m	3.70
3	f	3.70	m	3.60
4	f	3.40	f	3.30
5	f	3.10	f	3.20
6	m	3.50	f	2.80
7	m	3.90	f	3.00
8	m	4.00	f	3.80
9	f	3.40	m	3.20
Σ		31.70		29.30
X ± Sx		3.52±0.12		3.26±0.13
s		0.37		0.39
Variability coefficient %		10.41		11.91

Breastfeeding with whole milk, supported in lambs of the LM group a total increase of between 2.9 and 4 kg, with an average of 3.52 kg. On the other hand, breastfeeding with reconstituted milk resulted in lambs in the MRL group of a total increase of between 2.7 and 3.8 kg with an average of 3.26 kg.
As regards the total increase, the coefficient of variability is 10.41% in LM and 11.91% in LR.
By reporting the total increase to 22, which is the number of days in the experimental period, we obtained the average daily increase per lamb. The resulting data are given in Table 8 and Figure 2.

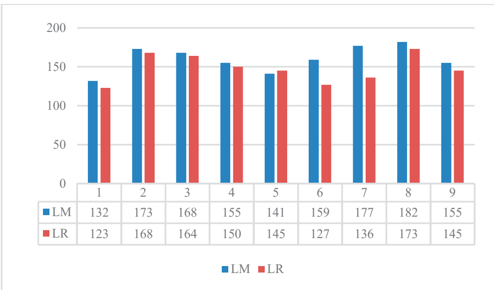

Figure 2. Graphical representation of the average daily increase

Table 8. Average daily gain of suckling lambs during the control period (7-28 days)

Current number	LM		LR	
	Sex	Average daily grain [g]	Sex	Average daily grain [g]
1	f	132.00	m	123.00
2	m	173.00	m	168.00
3	f	168.00	m	164.00
4	f	155.00	f	150.00
5	f	141.00	f	145.00
6	m	159.00	f	127.00
7	m	177.00	f	136.00
8	m	182.00	f	173.00
9	f	155.00	m	145.00
Σ		1,440.91		1,331.82
X ± Sx		160.1 ± 5.56		147.98 ±5.87
s		16.67		17.62
Variability coefficient %		10.41		11.91

From the analysis of this table it can be seen that the highest average daily increase was obtained in the LM group whose lambs were fed whole milk. The values were within the range of 132-182 grams, and the average per batch was 160.1 grams.

In the batch of lambs breastfed with reconstituted milk LR, the data obtained had lower values and were within the limit of 123-173 grams with an average per batch of 147.98 grams. We believe that this increase was achieved only on the basis of milk consumption, and only after the age of 28 days when the milk is no longer enough to support the growth of lambs will the additional distributed feed that lambs have learned to consume since the age of 2 weeks will also enter into account.

To remove the effect of the distribution of this feed on the growth of lambs, its quantities and composition was similar in both batches. Consumption of 10-20 g/day in the first month has an insignificant effect on the growth and development of lambs (Halga et al., 2005).

Just like the total increase, the coefficient of variability has average values and highlights the increasing differentiation of lambs from the batches of twins.

If we analyze Figure 2 it can be seen that in all couples of twins, the average daily increase was higher in the lambs in the breastfed group with whole milk, compared to the reconstituted milk with the exception of the 5th couple, consisting of females in which the lamb from the LR group had an average daily increase of 145 g/day and the one from the LM group a daily average sport of 141 g/day, the difference between them being very small of only 4 g/day. The biggest difference of 41 g/day was recorded in the twin couple number 7, where the male lamb from the LM group had an increase of 177 g/day, and the lamb from the LR lot an increase of only 136 g/day.

The testing of the meanings of differences for body mass and weight gain was carried out using the Mann-Whitney nonparametric test, for a small number of individuals, by using the statistical calculator program 7. The results obtained are shown in Table 9.

Table 9. The significance of the differences for the body mass, the total increase and the average daily increase

Specification	LM Milked milk				LR Reconstituide milk				Differences	Test
	n	x	s	VC%	n	x	s	VC%	L_1-l_2	Mann-Whitney
Initial weight [kg]	9.00	3.91	0.36	9.08	9.00	3.79	0.40	10.59	0.12	0.56 ns
Final weight [kg]	9.00	7.43	0.58	7.84	9.00	7.04	0.59	8.43	0.39	0.13 ns
Total weight [kg]	9.00	3.52	0.37	10.41	9.00	3.26	0.39	11.91	0.26	0.17 ns
Average daily gain[g]	9.00	160.10	16.67	10.41	9.00	147.98	17.62	11.91	12.12	0.15 ns

Note: ns not significant at $p \geq 0.05$ threshold

As can be seen from the analysis of this table, the difference in body mass between the LM and LR group, each consisting of 9 twin individuals, was, at the beginning of the experimental period, only 0.12 kg insignificant at the $p \geq$ threshold of 0.56. At the end of the experiment, the difference for body mass increased to 0.39 kg, which was still considered insignificant at a p-threshold ≥ 0.13.

The total increase recorded in the batch of lambs LM was 0.26 kg higher compared to the LR lot, the difference being insignificant at the p threshold of ≥ 0.17.

Finally, the difference between the calculated average daily increase between the two lots LM and LR was also insignificant ($p \geq 0.15$) and had a value of 12.12 g/day.

CONCLUSIONS

After obtaining these results we can conclude that both breastfeeding with whole milk and reconstituted milk support an average daily increase close and depending on the breeder's option and the economic effect can be applied either of the two growth methods.

The study shows that breastfeeding of twin lambs raised with milked whole milk (LM) and reconstituted face (LR), had an insignificant increase. The final body mass is higher in the lambs of the LM group, on average by 0.39 kg compared to the LR group. This difference disappeared with the approach of weaning, very possibly due to the increased intake of whole milk to the LR group, being on average 2.9 l/day.

The difference between the two lots is insignificant.

This observation agrees with the lack of differences in weaning weight relative to artificially or conventionally grown lambs (Napolitano et al., 2002).

For the farmer, raising lambs with reconstituted milk can be beneficial because it capitalizes on sheep production, but for lambs growing with reconstituted milk can cause negative effects on the behavior of premature separation from the mother being harmful.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

POTENTIALITIES FOR USING CERTAIN MODERN TECHNOLOGIES FOR THE TRACKING AND MONITORING OF FREE-ROAMING HORSES

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Abstract

With reference to the implementation of the areas - `Traditional practices for seasonal grazing of animals` and `Conservation of endangered local breeds`, the interest in free grazing of various farm animals in Bulgaria has been significant in recent years. Horses are particularly suitable for this type of breeding. Pastures are often located in remote areas with limited access, which makes it difficult to visit and inspect the herds and facilities in the pastures on a daily basis. In order to find modern technological solutions to solve these problems and reduce costs and efforts of farmers, we tested several modern devices that are traditionally used in other areas, and their application in animal husbandry in Bulgaria is an innovative approach. These are GPS (Global Positioning System) for tracking animals, photo traps, as well as drones. As a result, we found that they have a successful application in monitoring horses which are raised free grazing, save costs and time, do not cause stress and side effects. These devices can be used to control access to pastures and limit theft, harassment and other encroachments on herds.

***Key words**: behavior, conservation, GPS, sensors, wild horses.*

INTRODUCTION

Karakachan horses are an old autochthonous breed, preserved in relatively the same form in which it existed for centuries (Petrov, 1940; Sabeva, 2009; Popova & Etarska, 2020). In this regard, research on Karakachan horses will give us the opportunity to collect data on a breed that has preserved genes from ancient times.

Today in Bulgaria, this breed is grown predominantely, freely all year round in the typical habitats - pastures in high parts of mountains. In most cases, they inhabit remote pastures, isolated from settlements and buildings. These horses have a hard time accepting rearing in stables and other buildings. This makes it difficult to carry out direct studies on the Karakachan horses, and many of the characteristic features of this resistant breed remain unknown compared to other modern barn-raised breeds allowing detailed studies of the latter.

With the use of modern technologies, we can afford to study horses in remote locations and in their typical habitats. The collection of data on behavioral and defensive responses, social behavior of the horses, such as group dynamics (group sizes and membership), locomotion dynamics, grazing intensity, in addition to allowing for a better knowledge of breed characteristics, will be of benefit also for purely practical applications (White & Garrot, 1990; Anderson & Lindzey, 2003; Cagnacci et al., 2010).

At the same time, the development and application of modern technologies directly in the field will promote among the owners the possibilities of modern animal husbandry and its benefits, as well as the creation and application of information technologies through the creation of databases (Osechas, 2008; Hampson et al., 2010; Bachmann et al., 2014; Burla et al., 2014; Collins et al., 2014; Mann et al., 2014; Radoi et al., 2015; Burov, 2018).

The purpose of the research is to study basic ethological signs and the choice of habitat over a large area by the Karakachan horses from the national gene pool, through the use of GPS trackers and other technologies for monitoring and following horses in ecologically and biocompatible breeding.

MATERIALS AND METHODS

The study was conducted in the areas of the village of Prisadets (on the Bulgarian/Turkish border - within Bulgaria) and the village of Levka, falling within SPA Sakar (BG0002021), which overlaps with SCI (BG0000212), part of the ecological network NATURA 2000 (MOEW, 2013). In biogeographical terms, the area falls into the Southern biogeographical region and, more specifically, according to the biotic basis, it refers to the "Dolnomarishko - Dolnotundzhansky" subregion (Gruev & Kuzmanov, 1999), as Mediterranean influence penetrates the sub-region along the Maritsa and Tundzha rivers. This defines the climate as milder and allows the horses to be kept outdoors all year round.

The object of observation were two herds of horses from the Karakachan breed, which are free-roaming horses all year round. The first herd consists of 29 mares, 1 stallion and 10 foals. The second herd consisted of 1 stallion and 12 mares.

The observations were carried out within 12 months. Twelve (12) and twenty-four (24) hour field observations were conducted.

Ethological studies were carried out - by the method of visual observation as well as tracking the choice of habitat with GPS-trackers, etc.

Five GPS trackers were purchased, 2 of which were placed on the stallions of the 2 herds and the rest were placed on mares from the same herds to attempt to track the sexual behavior of the free range horses.

The frequency of reporting Data from the GPS trackers was set to once every 15 minutes for a period of 12 months.

ArcMap ver.10.0 was used to process the data from the GPS trackers from the *.csv file sent by the transmitters and convert them into a *.shp file. Google Earth Pro ver. 7.3.6.9285. was used to visualize the positions of the horses on a satellite image of the Earth's surface. We used Microsoft Excel for the primary processing of the information.

RESULTS AND DISCUSSIONS

Animal tracking and activity monitoring using wireless sensors provides high-resolution data to retrieve their location and study their behavior. These quantitative measures are useful for better formulating ideas and informing practices in animal ecology, such as resource use, home range, animal dispersal, and population dynamics, which have so far relied on visual observation (Cagnacci et al., 2010).

Dorio stallion herd. The stallion Dorio was marked on 18.09.2021 (Figures 1 and 2).

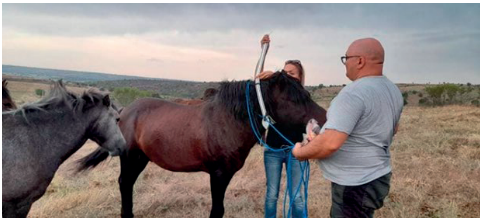

Figure 1. Placing a GPS-tracker on a Dorio stallion

Figure 2. Dorio stallion with tracker attached

Data processed until 31.08.2022 total positions collected from the trackers are from 402 to 32,224, but if we subtract the positions of the mares, from the tracker of the stallion Dorio there are a total of 13,288, of which daily positions (from 06:00 to 21:00) – 1,271, and the night positions (from 21:00 to 06:00) are 966 (Figure 3). The total area of the inhabited territory is 13,659 decares, with a perimeter of 17,590 km.

Interestingly, the Dorio stallion has few positions down in the low by the river, which is due to the episodic range capture. Signals (270 positions) from there were received mostly at dusk and at night, which is very little against the background of all 13,288, and it cannot be said with certainty that they mainly spent the night there and drank water at that time. It is also noticeable that there are not many positions between 11 a.m. and 1 p.m., which may mean that this is when the horses go down to the river to drink water.

There are no clearly specialized places to spend the night, apart from entering the village of

Prisidets, when they are most likely being chased by wolves. There is no mobile operator coverage in the area with fresh running water and rock salt applied. For this reason, it is not possible to model the behavior for the water drinking residence time and the diurnal range. More field observations should be made.
Most positions are on the crests of the ridges (when looking in Google Earth and verifying the positions on the ground) or on the northern slopes. This suggests that in low areas the device loses range.
The old border electrical signaling system serves as a fence (in the West) of the animals' territory, although 4 permanently open portals fall into this area. There is a small clustering of positions (8-10 positions) around the portals apparently on the rare occasion they have passed through them. One portal is near the village and they use it for overnight stays in the village. The other (northernmost) was used once on 17-18.10.21 when staying outside their normal area.
To the East, the newly built border fence and the Tundzha River limit the horses.
In the North, the old border electrical signaling system rests on the Tundzha river and also limits the territory to some extent, despite the open portals, and in the south the terrain is more rugged, but they still have the opportunity to move. On the maps (Figures 3 and 4) it is clearly visible how the old border electrical signaling system limited the territory and the horses went out only through the open portal of the village of Prisadets and through one at the northernmost positions. It can be seen that the remaining positions have been cut off along the border electrical signaling system.
It is noted that there are positions in the old almond plantations in the territory. This means that here, in addition to the mainly open meadows, the old almond orchards are also sporadically used (Figure 5). It can be seen on the map that there is also a reservoir here, which is visited by the herd mainly in the afternoon hours (most often between 14:00-16:30), which is also confirmed by the field observations.
Trackers were placed also on mares from the same herd to try to track sexual behavior. The GPS trackers used have a special feature that tracks more atypical temperature deviations and movements to determine if the mare has been grazed or will be foaled. The results of the observation did not show such activity on the GPS, although the mares foaled and on field observations we observed graze and jumping.

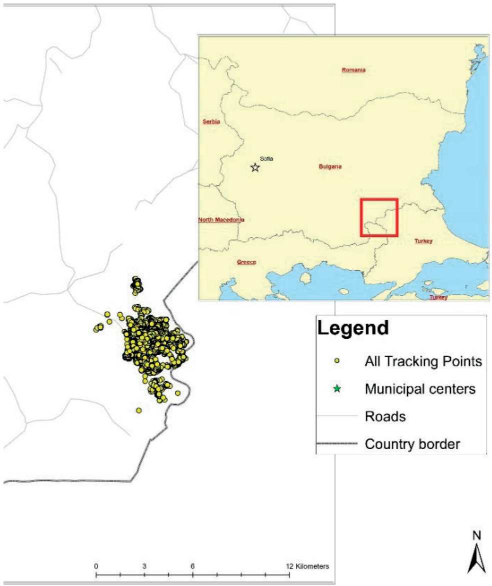

Figure 3. Map of the positions Dorio's herd

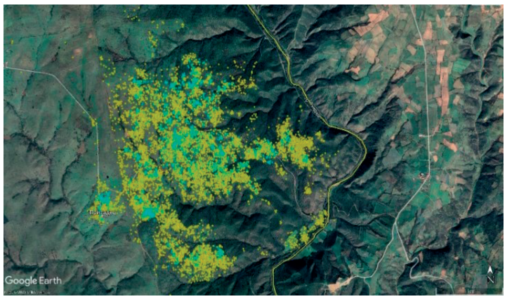

Figure 4. Map of all tracking positions of mare and stallion from the stallion Dorio's herd

Figure 5. Map of the most frequently used positions of the stallion Dorio's herd

This may be due to the loss of range in the area, as well as the fact that the GPS's used did not provide accurate temperature information. Because of the location of the temperature sensors, averaged values were sent between the horses' body temperature and that of the environment.
Data from the mares' trackers was processed from 22.03.2022 - to 27.04.2022 - entirely in the spring period, when the foals and coverings from the stallion were expected. Total positions collected by the trackers are 2,884, of which day positions (from 06:00 to 21:00) – 1474, and night positions (from 21:00 to 06:00) are 1,410 (Figure 6). Apart from 6 evenings, there are no other clearly defined day and night positions in the village.

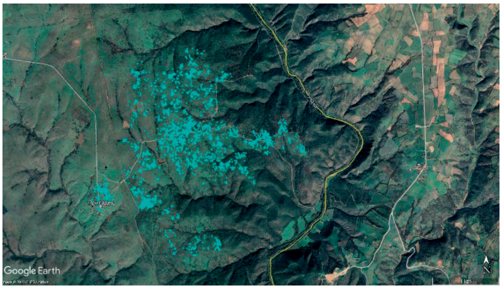

Figure 6. Map of all tracking positions of the mare from the herd of the stallion Dorio in the period from 22.03.2022. until 27.04.2022

When looking at all stallion and mares positions on one map it looks like they are moving together in the same territories, therefore the horses are moving in a herd and not separated. When analyzing the data more thoroughly, when looking at a specific day where there are positions from both GPS trackers at equal intervals, it is observed that the two animals are moving at a certain distance from each other. This distance in the herd can be interpreted as a sign that the animals are calm, not attacked by predators to cluster close together, but graze scattered over the terrain. But it can also be due to the fact that when the GPS trackers are located close to each other, they give some deviation in the coordinates.
Unfortunately, the terrain has deep ravines and there is no range for the transmitters there. Their main watering place and salt blocks are in the deepest part and we cannot ascertain exactly how long they spend there.

From the maps it can be assumed that horses prefer open areas, avoiding scrubby areas and forests. All known positions are only on the higher parts of the terrain 150-215 a.s.l. and there is not enough information on the positions in the lowlands, where there is water and salt - 60 m above sea level, because of the technology limtations.

The herd of stallion Oliver. The pastures occupied by Oliver's herd are fenced with an electric herder, and the pastures are alternated on a rotational basis.
The pastures are alternated using an electric herder, with a minimum of 70-100 acres per horse per year. The annual care (review of the state of health, prevention, individual marking, etc.) and the relocation (Figure 7) between the individual pastures is carried out with the help of volunteers.

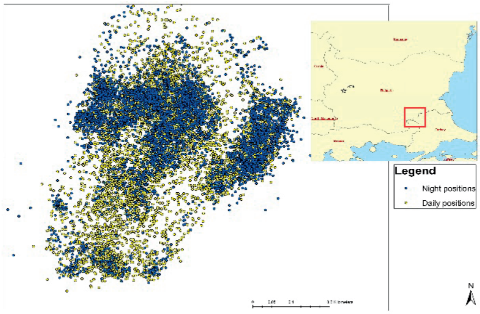

Figure 7. Map of the positions of stallion Oliver's herd until 08.12.2021

Pasture 1 (Figure 8) is used during the autumn (October and November 2021), winter (December 2021, January and February 2022) and spring (from March to May 2022) seasons, and the date of moving to this pasture is 28.10. 2021; date of moving from the pasture – 25.05.2022; collected data – 12,244 positions.
There are clearly marked overnight positions on the highest part of the hill in an open meadow.
The pasture is 331 acres with a perimeter of 2.34 km, with an approximate altitude of 222 to 267m. The pasture is a hill with a sloping top and is a mixture of oak coppice forest and forest glades. Both day and night positions are in the meadows and there are almost none in the forest area.
There is one watering place in the pasture - a probe with a pump that collects water in cisterns.

There is a certain dependence on the watering time, as the horses visit it either around 11-12 hours before lunch or around 16-17 hours in the afternoon, but only once a day.

Figure 8. Pasture 1 – Autumn, Winter and Spring – stallion Oliver's herd

Pasture 2 (Figure 9) was used for a short time as the date of moving to this pasture is 25.05.2022, and the date of moving from the pasture is 09.06.2022; data were collected from 1,775 positions. For daytime positions we take the hours from 5:40 to 21:00 and there are 1,062 in number, and evening positions -703 in number.
The pasture is 357 acres with a circumference of 5.5 km, with an approximate altitude of 147 to 225 m. The pasture is a mixture of overgrown gully and meadows with all the horse positions being in the meadows. Both the day and night positions are there and there are hardly any in the thicket.
There are no clearly defined roosting positions in this pasture. There is one place for the horses to drink water (a micro-dam), but there is no clearly defined time to visit for drinking water.

Figure 9. Pasture 2 – summer season – of stallion Oliver's herd

Pasture 3 (Figure 10) - summer season as the date of moving to this pasture is 9.06.2022, and the date of moving from the pasture is 16.07.2022; a total of 3,193 items were collected.
For daytime positions, we take the time range from 5:40 AM to 9:00 PM, with daytime positions being 2,059 in number and evening positions being 1,134 in number.
The pasture is 223 decares with a perimeter of 3.1 km, with an approximate altitude of 183 to 216 m, with a flat section of about 200 m above sea level prevailing. The pasture is a mixture of forest and woodland meadows, with all horse positions in the meadows. Both the day and night positions are in the open meadows and there are almost none in the wooded area.
In this pasture, there are no clearly defined overnight positions and there is one watering place (micro-dam), but there is also no clearly defined time for visiting a watering hole.

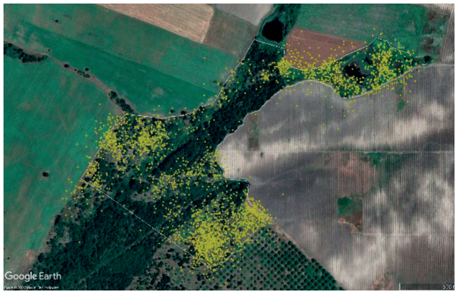

Figure 10. Pasture 3 – summer season – of stallion Oliver's herd

The entry of free-ranging horses into settlements and crops is a major problem. Thanks to the outlined virtual fences, the reason for entering the nearby settlement was established, namely an attack by wolves. As a result, night lighting was spent where the horses would gather when attacked by wolves.
As a result of the study, we can summarize the benefits of using GPS trackers, which are: possibility to outline virtual fences (Figure 11); signals are sent when leaving the fences and immediate measures can be taken; the detection of the horses becomes much faster.
As a result of the conducted research, we found that GPS-trackers facilitated the work related to the selection of suitable pastures according to the number and age of the stallion, rotation of the pastures used, limited the number of victims to predators, limited the entry of free herds into the settlements, by building specially designated

places lit up at night, for the horses to visit in order to escape from predators at night, etc. All this is a prerequisite for reducing the costs of raising herds, for their quick localization if necessary, and timely reaction to incidents. Also, these technologies can provide data on crossing virtual fences, separating certain individuals from the general group, etc.

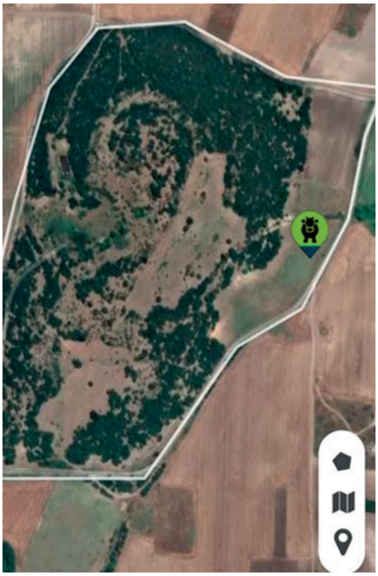

Figure 11. Oliver's virtual herd fence

CONCLUSIONS

The used GPS-tracker model is suitable for tracking the location of free-range horses, which helps to easily find them in hard-to-reach areas and large territories. With enough observations of the herd, certain behavioral responses such as places to hide from predators, water sources, diurnal and nocturnal positions can be learned and tracked. In the event of an attack, the GPS-tracker immediately signals unusual movement or leaving the virtual borders of the herd. The herd's entry into foreign territory can easily be seen and prevented.

Because of the location of the temperature sensors, averaged values were sent between the horses' body temperature and that of the environment. Reproductive indicators as well as clinical indicators (temperature, pulse, etc.) cannot be tracked.

Due to the lack of coverage near the Turkish border, there is still no established watering period and no clearly defined roosting places in the herd of stallion Doryo.

From the maps it can be assumed that horses prefer open areas, avoiding schrubby areas and forests. It follows that forest and shrub areas should not be counted as part of pasture acres.

Despite some difficulties, it is possible to carry out quality monitoring of animals using GPS-trackers. Owners will be able to calmly monitor on their mobile devices where and how much their horses move in the field, in the mountains, they will have the opportunity to identify atypical behavior.

ACKNOWLEDGEMENTS

The study was conducted under research project № 06-21 on topic: "Potentialities for studying the behaviour of free-roaming horses using modern technologies for monitoring and tracking". The project is funded by the Centre for Research, Technology Transfer and Protection of Intellectual Property at the Agricultural University - Plovdiv in partnership with "LIFE for Lesser Kestrel" LIFE19 NAT/BG/001017 implemented with the support of Program LIFE of the European Union.

We would like to acknowledge the translation assistance from Ellie Holden.

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Scientific Papers. Series D. Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

PRELIMINARY RESULTS ON HEALTH ISSUES INCIDENCE EVALUATION IN A FARM WITH ROMANIAN BLACK SPOTTED DAIRY CALVES BREED

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Abstract

Dairy calves are susceptible to a great range of welfare and health issues up to the age of weaning. This study aims to evaluate incidence of the main health disorders affecting dairy calves up to one year of age. The study was carried out at the Experimental Farm of the Research and Development Institute for Bovine Balotesti, Romania, were health data (epidemiological situation of coccidiosis, diarrhea, rickets and respiratory diseases) was collected for two consecutive years, between November 2017 and October 2019, from a number of 176 purebred Romanian Black and White calves. Diarrhea had the highest incidence in un-weaned calves (0-3months old), of 29.55±0.34%, significantly higher (p≤0.001) compared to 3-6 months (2.16±1.24) and 6-12 months age groups (5.88±2.17), respectively. Coccidiosis incidence was on average of 39.20±0.36% in un-weaned calves, 1.44±1.01% in the 3-6 months group and of 5.04 ±2.01% in the 6-12 months group, significantly higher (p≤0.001) in the 0-3 months age group, compared to older calves. The highest number of health affections in our study were attributed to coccidiosis and diarrhea, altogether affecting over two thirds of the calves.

Key words*: animal welfare, cattle health, dairy calves, farm health, Romanian Black Spotted breed.*

INTRODUCTION

Good health is one of the main concerns in animal welfare, given that health traits are reliable indicators of the animals physiological functioning (Fraser et al., 1997; Neamt et al., 2017). Cattle farmers are devoted to good health and nutrition of their livestock, as these approaches result in higher levels of milk yields and growth rates, lower veterinary related costs, and thereby increasing the overall efficiency and profitability of dairy farms (Silva et al., 2023).

Veterinary researchers underlined the importance of promoting good health as a major component of efficient farm management system and welfare practices, as well as its strong relationship with productivity (Spooner et al., 2012). High mortality and morbidity rates are the main animal-based indicators of poor quality when dairy cattle farms efficiency is concerned (Von Keyserlingk et al., 2009).

The current challenge is directed by the dairy calves susceptibility to health issue up to the age of weaning. These risks include high rates of mortality, reduced health, social deprivation, abnormal behaviors and stressful practices (e.g. dam-isolation, vaccinations, dehorning). Furthermore, given the implications of rearing calves practices under current conventional systems on animal welfare, the EU Directive laying down minimum standards for the protection of calves was published and put into place in all European Member States (Council Directive 2008/119/EC).

Health disorders in calves have a major impact on the economic viability of dairy cattle farms, on one hand because of the costs of calf mortality and veterinary treatments, and on the other hand, because of the long-term effects of calves health on the performance efficiency as heifers (e.g. impaired growth and development) (Lorenz et al., 2011). Morbidity and mortality represent reasons of concern in all cattle breeds; however, this is a particular problem in Holstein-Friesian dairy derived breeds, which have a lower organic resistance, due to intense selection for high yields (Mee et al., 2008). The most common causes of dairy calves diseases are parasitological, bacteriological or viral neonatal calf diarrhea (NCD) and bovine respiratory disease (BRD), both having a significant negative impact on welfare and growth rates (Windeyer et al., 2014; Catalina et al., 2018; Sidi et al., 2018). Prevention and control are the main measures that can be taken in order to provide optimal health management in cattle farming, with a positive influence on the overall herd health and production efficiency (Uetake et al., 2013; Dawkins et al., 2017).

In calf-rearing systems, hygiene and biosecurity measures have a significant impact on proper physiological functioning and therefore on welfare (Al Mawly et al., 2015).

Assessment of clinical health, physiology and behavior, requires animal-based measurements, some of which are directly related to management and environmental conditions (Cummins et al., 2016). Clinical health is one of the major aspects of farming system, and can be easily evaluated using visual indicators such as behavior, fecal consistency and respiration rates.

Moreover, calves' robustness is influenced by a series of factors such as genetic information, housing system, environment and nutrition. Ede et al. (2022) observed higher dry matter intake (DMI) and final body weights in group-housed calves, when compared with individually housed calves. When it comes to environmental factors, such as extreme temperatures, limited measures can be taken to alleviate such stressors in animal husbandry (e.g. provide shade and cool water during heat stress exposure).

The aim of the current pilot study was to evaluate incidence of the main health disorders affecting dairy calves up to one year of age.

MATERIALS AND METHODS

1. Farm description

The study was carried out at the Experimental Farm of the Research and Development Institute for Bovine Balotesti (44°36′46″N 26°4′43″E) Romania, were health data was collected for two consecutive years, between November 2017 and October 2019, from a number of 176 purebred Romanian Black and White calves (Holstein Friesian group, national name Bălţată cu Negru Românească), managed under identical conditions (91 males and 85 females, 102 born in the 1^{th} year of study and 74 in the 2^{nd} year, respectively). The data were collected from all the calves born during this period on the experimental farm. The higher standards on this farm were associated with a well-managed farm of a research institute being financed from own resources based on the quality of the products sold. The second reason of maintaining the standards, it is about the status of the farm that belongs to the Romanian government.

2. Calves management

After birth, the calves were ear tagged and separated from their dams and housed in the maternity compartment until the age of 10 days. In the first 3 days of life, calves were fed with 4 kg of colostrum per day, in two equal meals at 12 hours intervals. The following 7 days they received two meals per day consisting of 3 kg of milk replacement (Eurolac 22/16, 125 g/liter of water) per head. Eurolac formula - analyses: Crude protein 21.5%, Crude fat 16.0%, Fat content 9.0%, Crude cellulose 0.8%, Sodium 0.6%; - addition of vitamins/kg: Vitamin A 25,000 I.U.; Vitamin D3 4000 I.U.; Vitamin E 80 mg; Iron, iron sulphate, monohydrate 80 mg; Iodine, calcium iodide 0.8 mg; Copper, copper sulphate, pentahydrate 7 mg; Manganese, manganese sulphate, monohydrate 44 mg; Zinc, zinc sulphate, monohydrate 56 mg; Selenium, sodium selenite 0.2 mg. At 10 days of age, the calves were moved to outdoor individual hutches with deep straw bedding, being fed with 6 kg of milk replacement per day, in two equal meals. The calves diets were supplemented with *ad libitum* starter concentrates and alfalfa hay until the age of 3 months, when the weaning took place, regardless of sex. The concentrates feed contained 18.5% crude protein, 9% fibre, 0.36% methionine, 0.9% lysine, 2.96% calcium, 0.69% phosphorus, 0.9% salt and 1.00% fats, with unrestricted access to clean water.

Transition to weaning took 7 days, with calves receiving half of the milk replacement ration once per day, during the morning feed, in order

to avoid agitation and intense vocalizations during daytime.
Immediately after weaning, calves were moved to a separate barn, and housed in groups of 10-15 individuals, based on their sex, age and body weight. Between 3 to 6 months of age they received a diet consisting of 1.75 kg concentrates, 1.5 kg of alfalfa hay and 3 kg of corn silage. When self- or inter-suckling behaviors were observed, nose rings were fitted to calves (maximum 3 calves/year in the first year of study and 2 calves for the second year of study).
After the age of 6 months, calves were moved to a different barn, where they were kept until the age of 12 months or slaughter age. Animals had free access to outside paddocks, however, they had no access to pasture. Between 6 to 12 months the daily feed rations consisted of 2.2 kg concentrates, 2.5 alfalfa hay and 6 kg of corn silage. The housing was done on deep straw litter, allocating on average 8-10 m^2 per head.

3. Veterinary care

As veterinary prevention, anthrax vaccination was used at the age of two months and vitamin therapy (A, D_3, E) was applied only to the weak and ill calves. The main treatments were applied for symptomatic effects such as diarrhea and respiratory diseases (BRD), these being the most common in the studied calves. Fluid therapy was an important part in diarrhea management (electrolytes solutions). Deworming procedures were made after the age of weaning.
Dehorning was carried out at the age of two months, only on female calves because they remained on farm for replacement, while the male calves were sold for further fattening.
The research activities were performed in accordance with the European Union's Directive for animal experimentation (Directive 2010/63/EU).

4. Data Analysis

The study was carried out on the 4 main issues, recorded from November 2017 to October 2020 by a trained research team with three members, focusing on the targeted conditions (year, affection, calves age categories and mortalities). Data on the epidemiological situation of coccidiosis, diarrhea, rickets and respiratory diseases, were collected from the official register of the farm and included the whole calves population in both years of study. An infected animal is represented by a calf with specific symptoms of each studied affections. For data analysis, descriptive statistics such as incidence of the studied affections, with three different calves age categories tables were calculated. Parameters such as coccidiosis, diarrhea, rickets and respiratory diseases for the entire period were used to calculate the incidence of each affection per age categories in our studied farm. Coccidiosis was defined as a parasitosis caused by the protozoan *Eimeria*, which affect enteric tract mucosa causing diarrhea. Diarrhea was defined as a passage of three or more loose or liquid stools per day (or more frequent passage than is normal for the individual). Rickets was defined as a failure of calcification of osteoid and cartilage in young growing animals, caused by a calcium, phosphorus or vitamin D deficiency. Bovine respiratory disease (BRD) was define as a complex, bacterian or viral infection that causes pneumonia in calves which can be fatal. The number of tested animals was based on official veterinarian of the farm requests for these main issues tacked into account evaluation of the calves in our experimental farm. In order to exclude infections with rotaviruses, coronaviruses, *E. coli* or *Cryptosporidium* from the differential diagnoses, we were using diarrhea test, brand Kerbl (Albert Kerbl, Felizenzell 9, 84428 Buchbach, Germany), for every calf with enteric symptoms. To determine the coccidiosis infestation, Willis method was used. For BRD and rickets diagnostic, we were considering the symptomatology of the calves and the clinic investigations. Frequency of calf assessments was done visually daily, scouring systems or tests applying when clinical signs appear. The evaluation of diarrhea in dairy calves it was used a fecal consistency scoring system, where a score of 1 is considered normal (firm, not hard, original form is distorted slightly after dropping to floor and settling), 2 is soft (does not hold form, piles spreads slightly), 3 is runny (spreads readily to about 6 mm depth), and 4 is watery (liquid consistency, splatters). The evaluation of respiratory diseases it was used scoring system based on clinical signs (UC-Davis), as follows: Cough - 2 points, eye discharge - 2 points, fever (> 39.2ºC) - 2 points, abnormal respiration - 2 points, nasal discharge - 4 points, ear droop or head tilt - 5 points (Maier et al., 2019).

The outcome consisted in the daily checking of calves health status for normality, along symptomatology, treatments employed and differential diagnostic. The sample size was established conform to the calves management system, targeting the 0 to 12 months of age and housing. Collected data for this study was carried up for two years daily all year around.

Decisions about the acceptance or rejection of statistical differences have been made at the 0.05 level of significance. Observation with implausible values were removed and were checked for double entries. At the end of the experiment the Bartlett test was employed to verify whether the experimental data had a normal distribution. Since no assumption could be made, and normality was not fulfilled, a non-parametric test Mann–Whitney (MiniTab18® version 18 Pennsylvania USA) was employed to evaluate the difference between two groups (0-3 month vs. 3-6 months; 0-3 months vs. 6-12 months; 3-6 months vs. 6-12 months). The incidence was calculated using cumulative incidence, as the number of new cases of an affection in a specified time period: Incidence = (new cases)/ (population x specified time period). To calculate the sample size, the following five steps were employed: first the calves population size was defined, and design the margin of error, followed by determination of level of confidence, followed by expected variance prediction and lastly sample size was obtained.

RESULTS AND DISCUSSIONS

Affections with coccidiosis, diarrhea, rickets and respiratory diseases occurred in both studied years from October 2017 and November 2020. Means (± SEM) for issues incidence in Romanian Black Spotted calves from the research farm, based on their age groups are presented in Table 1.

Table 1. Means for disease incidence in Romanian Black Spotted calves

Calves age group	n	*Coccidiosis (%)*	*Diarrhea (%)*	*Rickets (%)*	*Respiratory diseases (%)*
0-3 months	176	39.20±0.36	29.55±0.34	6.25±1.83	3.98±1.48
3-6 months	139	1.44±1.01	2.16±1.24	1.44±1.01	0.71±0.71
6-12 months	119	5.04±2.01	5.88±2.17	4.20±1.85	5.04±2.01
Statistical differences (p value)					
0-3 vs. 3-6		**** (p=0.0000)*	**** (p=0.0000)*	** (p=0.0335)*	*NS (p=0.0688)*
0-3 vs. 6-12		**** (p=0.0000)*	**** (p=0.0000)*	*NS (p=0.4479)*	*NS (p=0.6640)*
3-6 vs. 6-12		*NS (p=0.0972)*	*NS (p=0.1237)*	*NS (p=0.1751)*	** (p=0.0337)*

*NS p>0.05; * p≤0.05; ** p≤0.005; *** p≤0.001.*

Incidence of coccidiosis was significantly higher (p≤0.001) in the 0-3 months age group (39.20±0.36%), compared to the 3-6 months (1.44±1.01%) and 6-12 months (5.04±2.01%) age groups.

Incidence of diarrhea was significantly higher (p≤0.001) in the 0-3 months age group (29.55±0.34%), compared to the 3-6 months (2.16±1.24%) and 6-12 months (5.88±2.17%) age groups. Although, diarrhea incidence was two folded higher in 6-12 month of age calves, there were no statistical differences (p>0.05).

The rickets incidence was most predominant in un-weaned calves (6.25±1.83%), significantly higher (p≤0.05) when compared with the 3-6-month group (1.44±1.01%), and with no inferences (p>0.05) when compared to 6-12 months group (4.20±1.85%).

Respiratory diseases (BRD) incidence fluctuated between age groups, with calves of 0-3 months (3.98±1.48%) being more at risk, compared with the 3-6 months (0.71±0.71%) age group, although there were no statistical inferences (p>0.05), a tendency towards

significance (p=0.068) was observed. BRD incidence was statistically higher (p≤0.05) in 6-12 months age group (5.04±2.01%), compared to the 3-6 months age group.
Mortality rates were not considered for the current study, because during the two years of data collection, only one stillbirth was registered in the experimental herd. This could attribute to the higher level of veterinary care given to animals, compared to commercial dairy farms.
Colibacillosis and haemorrhagic diarrhea was only found in un-weaned calves, up to the age of 3 months, with an average incidence of 8.52±2.11% and 1.13±0.80%, respectively.
Considering that calves had the highest affection incidences during the first 3 months of life, further investigations were conducted, in order to identify the precise weeks with the highest risks for developing health issues. Day 0 for each of the affections was considered the day in which the diagnosis was made.
Coccidiosis incidence in 0-3 months of age calves was predominant in week 1 (23.86±3.22%) and week 2 (17.05±2.84%), and to a lesser extent, affected calves in week 3 (1.70±0.97%) and week 6 (0.56±0.56%) after birth, respectively (Figure 1).
When diarrhea incidence was concerned, up to the age of weaning of calves, the highest risk of developing the affections was on the 1th (19.89±0.30%) and 2nd (7.95±0.20%) weeks after calving. Considerably lower incidences for diarrhea was recorded on weeks 3 to 9 and 11 to 12, with limits ranging between 0.05% and 0.56%/week (Figure 1).

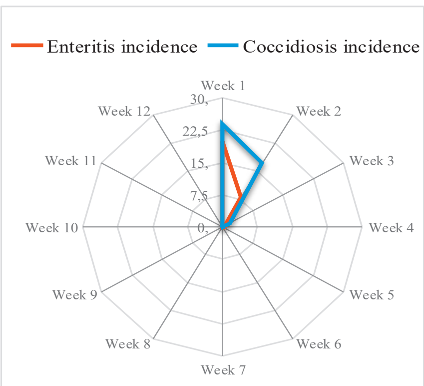

Figure 1. Weekly coccidiosis and diarrhea incidence in 0-3 months of age un-weaned calves

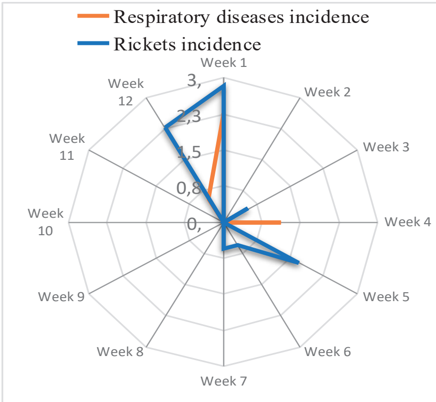

Figure 2. Weekly rickets and respiratory diseases incidence in 0-3 months of age un-weaned calves

Daily weight gains of calves were on average of 621.3±0.61 g, 643.2±2.73 g and 751.0±2.68 g in 0-3, 3-6 and 6-12 months of age groups, respectively.
Dairy calves are susceptible to a great series of affections, particularly during the suckling period, when compared to weaned calves up to the age of 12 months, mainly due to the placental inhibition of immunoglobulins (Ig) transport and protection from foreign antigen *in utero*, calves being born with an immune system that is antigenically immature (Gelsinger et al., 2017). As a result, the adaptive immune cells in newborn calves are incapable of recognizing and fighting foreign antigens until the foreign cells are first recognized, phagocytosed, digested, transported, and the antigens are recognized by innate the immune cells. The immune system in calves is not completely functional until 4 to 5 weeks of age (Nonnecke et al., 2012). Current results are in accordance with previous reports (Mee et al., 2008; Windeyer et al., 2014), which outlined the higher risk rates that un-weaned calves are facing when health and welfare is concerned.
In the present study, the incidence of coccidiosis in new-born calves was lower than previously published data, where incidence of up to 70% were reported (Grandi et al., 2016), with the coccidiosis being reported to have the highest incidence between 1 and 6 months of age. In coccidiosis, adverse environmental rearing conditions play a major role in both parasite pressure and the host susceptibility to

infestation. The calves immune response plays a major role in maintaining low incidence of coccidiosis (Khurram et al., 2023) and is being modulated by stressful environmental conditions, including thermal and humidity extreme ranges. One of the main factors that influence significantly the calves resistance and resilience to pathogens is colostrum management, which in dairy calves represents the only source for passive immunity transfer and the association with calf health has been well documented up-to-date (Furman-Fratczak et al., 2023).

Incidence of coccidiosis in calves was found to widely fluctuate between geographic locations, across herds and different age groups. The highest incidences being reported in dairy farms with poor housing and biosecurity management, especially in those where the colostrum feeding was deficient and no coccidiostatic drugs were included in the concentrates fed. Furthermore, mortality caused by coccidiosis in calves was estimated to be of up to 50% in animals displaying major clinical signs, when no treatment was administrated (Lassen & Ostergaard, 2012).

Diarrhea is considered one of the most common affection in calves, with most of the enteric pathogens being transmitted throughout the fecal route. Environmental risk factors for diarrhea in dairy calves include climatic conditions (Barrington et al., 2002; Gulliksen et al., 2009), housing (Gulliksen et al., 2009; Klein-Jobstl et al., 2014), stocking density (Barrington et al., 2002) and farm hygiene (Klein-Jobstl et al., 2014).

Current results on diarrhea incidence are in accordance with those published by Windeyer et al. (2014), which found a similar pattern for incidence when un-weaned and older calves age groups are compared. Moreover, our data is in accordance with those published by Windeyer et al. (2014), concerning the higher incidence risk for developing diarrhea during the first 14 days after birth.

Housing on uncontaminated bedding, appropriate air quality and ventilation are important for the enteric health management (Barrington et al., 2002). Hanninen et al. (2003) found a significant lower incidence of diarrhea in un-weaned calves housed indoors compared with calves housed outdoors. While Klein-Jobstl et al. (2014) found higher diarrhea incidence in dairy calves kept in groups when compared to calves kept in individual hutches.

Moreover, economic implications of diarrhea were highlighted by Windeyer et al. (2014), with losses estimates ranging between 14.7 $ and 33.4 $ per calf.

Rickets in calves and heifers is manifested by suboptimal growth and occurs in rapidly growing calves fed large amounts of milk or milk substitutes, with no access to quality supplementary feed and a lack of direct sun exposure. The severity of rickets might compromise the future development of calves, with most severe cases leading to an early culling of the animal, since this disease impairs both longevity and fertility, as well as the milk production potential (Dittmer & Thompson, 2011). In our study the rickets incidence was the highest in un-weaned calves, 4 and 1.5 times folded compared to 3-6 and 6-12 months of age, respectively. Thus, special attention to the feeding regime, supplementation with vitamin D and phosphorous of diets and to direct sun exposure, should be given to suckling calves, in order to prevent high incidences of rickets and to mitigate potential negative effects. Regrettably, no previous published articles presenting the incidence of rickets in dairy calves was available, for comparison with current results.

The main risk factors for BRD development identified in calves are the cold climate, mechanical ventilation and drafts. In our study the incidence of BRD is in accordance with data published by Brscic et al. (2012), which investigated BRD calves reared in France, Italy and Netherlands, and found an average incidence of 7% in dairy calves.

Conversely, reports by Wilson et al. (2017) on morbidity attributed to BRD accounted for over 3/4 of the total health problems in calves and the authors found that the associated costs of BRD in dairy calves are ranging between 50$ and 250$ per calf. Furthermore, BRD was the most prevalent calf disease found in Holstein Friesian breed, which lead to a significant reduction in calves survival rates according to North American authors (Stanton et al., 2012; Closs & Dechow, 2017). The differences between our results and those published by North American authors could be attributed to the climatic differences between the two continents, given that our data are comparable to those registered in Central European countries. Thus, highlighting the significant influence of the environment on BRD, considering that in both continents, Holstein cattle are being the dominant dairy breed and, moreover, given the intense use of frozen semen exchanges, the European and North American breeds are closely related.

Calves issues have a detrimental effect on the overall animal welfare and also to the direct farm returns, due to mortality rates, costs associated with veterinary treatments and prevention, and indirectly due to decreased growth performances and feed efficiency, an increase in the number of days on feed and lower market values of the calves. Economically, these negative effects reflect especially on female calves, kept for farm replacement or to be sold for reproduction, and to a lesser extent to dairy male calves, which generally are considered as a by-pass product and have significantly lower market values. However, from the animal welfare point of view, both sexes represent importance, in equal measure.

Housing, feeding regime, ventilation and on-farm biosecurity measures could be used as prediction indicators for dairy calves morbidity (Callan & Garry, 2002). Moreover, the Dairy Calf and Heifer Association published a report containing recommendations on targeted morbidity rates for calves, with the gold standard proposed being less than 25%. Given that in the current study 2/3 of calves were diagnosed with a form of affections, results being in accordance with the literature published on affections incidence in dairy calves, in which affections incidence ranged between roughly 40% and up to 80% under commercial practices, a great deal of efforts and improvements are needed to achieve such welfare and health thresholds.

Future research is needed in order to establish the heritability levels and consequently, the feasibility of inclusion of issues resistance in calves as selection traits in Holstein Friesian breed strains. Thus, incorporating organic resistance of dairy calves and heifers in the estimated breeding values (EBVs) of bulls used for artificial insemination, in order to have an integrated approach on both productivity and animal welfare.

CONCLUSIONS

The highest number of health affections in our study were attributed to coccidiosis and diarrhea, altogether affecting over two thirds of the calves. The calves in first week of life poses the highest risk associated with developing affections, thus health monitoring and veterinary care being crucial at this point.

The results could prove useful for setting-up future alarm thresholds especially for the farm under study, however also for dairy farmers and veterinarians, when rearing un-weaned calves and female replacements are concerned. Good veterinary and health practices should be put in place at farm level in order to mitigate the effects of issues on productivity and animal welfare.

ACKNOWLEDGEMENTS

In The authors would like to thank Dr. Ioana Nicolae for her valuable and constructive suggestions during the English reviewing process.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

COMPOSITION AND PROCESS FOR ADDITIONAL FEEDING AND DEWORMING OF HARES

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Abstract

The study of the composition and process for additional feeding and deworming of hares is an important, fundamental and, especially, applicative issue, because some species serve as definitive hosts in the development cycle and as parasitic vectors, being dangerous for both domestic animals and humans. Parasitosis are the most common diseases in wildlife of the hunting fauna, which results with substantial economic losses. The invention relates to the protection of hunting fauna, namely to a composition and a process for additional feeding and deworming of hares. The composition, according to the invention, comprises, in %: oats 30.0-50.0, wheat 4.0-7.0, barley 2.0-4.0, corn 2.0-4.0, sunflower groats 2.0-4.0, soybean groats 2.0-4.0, bentonite 20.0-30.0, molasses 1.0-2.0, dextrin 2.0-3.0, premix containing vitamins, oligoelements, minerals, coccidiostatic and antioxidant 1.0-2.0, and a preparation containing 20% albendazole 1.0-2.0. The process, according to the invention, provides for the administration to hares of said composition, in a dose of 75 g/hare, in winter, twice with an interval of 14 days, in the form of briquettes, placed at a height of 25-40 cm from the soil.

Key words: *additional feeding, composition, deworming, hare.*

INTRODUCTION

The zoo-veterinary complex occupies an important position in the production structure of the agro-alimentary system in the Republic of Moldova. The further development of agriculture depends on: the economic situation of agricultural enterprises, satisfying the population's demand in high quality food and ensuring the food security of the region. The crucial factors that contributed to a succesful development in this category of national economy are zoo-veterinary actions that aim to ensure health to domestic and wild animals by combating contagious and parazitary diseases (Erhan et al., 2001; Rusu et al., 2020; Erhan, 2020).

Changes that had place in the zootechnical sector in the last three decades, regarding land appropriation are: reorganizing the zootechnical units, founding multiple small farms, redirectioning a large number of animals from complexes to particular households, which lead to major changes of the parasitary fauna.

Bovines that were in the stable, moving to grazing in different anthoprogenic stations, also enter in natural ecosystems, where they can transmit patogens to other wild animals as well (Rusu, 2017; Erhan, 2020; Toderaș et al., 2019).

The set of specific abiotic and biotic conditions, in which the individual lives, the population or a species to a living organism, is named environment, that in parasitic organisms, the extern conditions act indirectly through the host-organism. Therefore, it is common for parasites to be distinguished - 1st order medium (direct environment of the parasite, the host) – the totality of life conditions of the parasite in the host-organism. Life conditions of hosts in external environment serve for parasites as the 2nd order medium (the indirect environment of the parasite, the host's environment) (Hora et al., 2015; Sergi et al., 2018; Didă & Duca, 2002).

Fauna of hunting interest is part of the national hunting heritage. Both the population and the totality of the spectrum of main and complementary species determine the value of this fund. That is why, the parasitary fauna study of wild animals have a big significance (Rusu et al., 2019; Erhan et al., 2001; Zamornea et al., 2002).

The filed hare (*Lepus europaeus* Pallas, 1778) is a herbivorius mammal, medium-sized, with a body length between 48 and 57 cm, plus a tail of 8-9 cm. The ears have the length of 12-17 cm. The body weight, depending on the geographical ecotype within it's distribution area, varies between 3,5 and 6 kg. The long roar limbs have 5 fingers and the short front ones – 4 fingers. The color of the fur varies a lot depending on the place and season, the general shade being grey-reddish with whitish on the abdomen and white on the lower part of the tail. The tip of the ears and tail is black. Phenotipically, the male does not differ from the female. Due to the difference between the hindlimbs and forelimbs, the hare cannot walk, but only jump, the lenth of which can reach 3 m, and running up the hill is even more advantageous.

The hare manifests preferences for agricultural properties from plain areas and low hills, but also in forests with or without undergrowth. In the conditions of our forests, the hare is also adapted in large bodies, forming the so-called "forest ecotype". It avoids swampy places with stagnant water. It shows a high degree of fidelity to the place of living, having individual sectors from 25 to 80 ha, joining a circle with a radius of no more than 1 km. The hare has a predominantly nocturnal activity, primarily after sunset and in the hours until sunrise. During the reproductive period, it is also active during the day, when it is distributed in familiar groups. Hare's enemies are extremely diverse: dogs, stray cats, weasels, foxes and dogs. In addition to these, ravens, black crows and woodpeckers, as well as some day and night birds of prey, are a great danger for chickens. This list of enemies is joined by a series of diseases of various origins (coccidiosis, brucellosis, staphylomycosis, myxomatosis), which contribute negatively to it's development. The natural losses of the population during the year, and especially during the cold period of the year, are between 20.0-35.0%. Researches showed that cca 62.0% oof rabbits die before turning 1 year old, 7.0% reach up to 2 years, 6% reach up to 3 years, and only 3% tend to reach 4-7 years. According to the data obtained at the Demographic Research Institute "MAX PLANK", the hare in nature lives on average 12 years, and in captivity - no more than 6-7 years (Savin & Ciocoi, 2017).

The hare is an important part of the hunting fauna in both Romania and the Republic of Moldova. In Romania, there is conflicting information related to the dynamics of the hare herds, which has increased, according to some authors, from a number of 266,000 specimens in 1950 to a maximum number of 1.330,000 specimens in 1977 on a positive note. In the period 1978-2013, the curve of hares flattened a lot, the effects remaining relatively constant, below 1,200,000 rabbits. A downward tendency in hare numbers has been observed in all Europe, in the last 35 years (Soveri & Valtonen, 1983; Dubinský et al., 2010).

As a result, the dinamics analysis of hares in the agrarian ecosystems of the Republic of Moldova, in the past years, a constant annual numerical increase of 25-40% and, respectively, an increase of the herd in the spring stock has been established, compared to the previous years by 3.9 times (from 42 thousand rabbits - year 2012 to 166 thousand - year 2019).

In the spring, in the agrocenoses of the Republic of Moldova, the hare is found on non-forested areas on about 2.540 thousand per ha and on 329 thousand per ha in forest areas, with a breeding stock of about 4,2 thousand rabbits. In the period of 2018-2020, in the Northern Zone of the Republic of Moldova, the breeding stock of hares counts about 179 thousand with an average density of about 71 rabbits per 1000 ha. In the centre of the republic, the medium density of cca 63 sp./1 thousand per ha has been highlighted, and in the South – with a density over the average value of 80 sp./1 thousand per ha (Yearbook IPM -2019, 2020).

The ecotone areas (vineyards and orchards at the edge of the forests) are very populated - 160-240 sp./1 thousand per ha. Autumn plowing and sowing make up 73.0% of the area of the hunting fund with densities of more than 75 rabbits per 1 thousand per ha. The statistical analysis of the evaluations from past years shows that the autumn population of the hare reaches the quota of over 250 thousand hares with a republican average density of 95 rabbits per 1 thousand ha, thus signaling an annual increase of 6-7%. Herd losses during the dormant period are estimated between 25 and 30% of the autumn herd (Savin & Ciocoi, 2017).

Neutralizing these negative factors and implementing the necessary recommendations will make it possible to organize the hunting farm at an effective hunting level. It must be mentioned that that the management of hunting farms in the countries of Central and Eastern Europe (Czech Republic, Slovakia, Hungary, Romania) have shown that the acclimatization of the hare imported from other regions does not give the desired results, therefore it is necessary to preserve and optimize the density of the local population, by implementing the protection recommendations against various parasitic and infectious diseases, stimulating the reproductive process and the rational exploitation of the species. The most visible changes in the report period are contained in the results of the evaluation number of hunting species and hunting itself (Toderaș et al., 2019).

Hares harbor a wide spectrum of parasites that are of great interest to hunting managers and veterinarians as important sources of zoonotic agents (Erhan et al., 2001; Rusu, 2020; Alibekov, 2010).

The epidemiological implications, the lack of bibliography on the parasitosis of wild rabbits, the increase in sanitary standards for hunting products, as well as the importance given to their state of health, motivate the establishment of the prevalence of gastrointestinal parasite infestation in hares.

In the last decades, once with the intensification of the anthropogenic impact and technogenic factors on natural ecosystems, the study and biodiversity protection in natural ecosystems have became an actual problem that presents a great interest for specialists and public organisations.

The problem of antiparasitic protection of humans and animals, based on the concept of integrated prophylaxis, includes the set of theoretical principles, means and practical methods, of organizational measures to combat parasites in the host's body, as well as to protect the environment from parasitic invasions. For the successful development in the hunting field, and to increase the animal amount it is necessary to continuously improve the maintenance tehnology and to use new biological methods of prophylaxis as well as combating parasiting diseases. It has been observed that in cynegetic households, where parasitic diseases are present, the death rate is growing (Efremov et al., 2017).

The success in combating parasitosis in animals can only be ensured with the active and organized participation of all specialists in the veterinary field. It is known that it is easier to avoid the disease, than treat it. The prevention of parasitic diseases is conditioned, to a large extent, by coordinating the activity of the specialists in the zoo-veterinary sector, fulfilling the technological measures of maintenance and feeding, etc. Complying with the whole set of arrangements is a crucial factor in increasing the amount of animals. Although, the economic factor is not critical, because many parasitic diseases that are found in wild animals are common for humans as well. Therefore, experts from the zooveterinary field are responsible for public health too. Public health, according to The OMS definition, is:

“Science and the art of disease prevention, life extension and promoting health through organized efforts of society” (Frank et al., 2013; Erhan et al., 2020; Rusu et al., 2019; Efremov & Muromtsev, 2016).

MATERIALS AND METHODS

In order to establish the diversity of the most dangerous species of parasitic agents in the hare, during the years 2021-2022, 420 biological samples were collected from various natural and anthropogenic biotopes of the Republic of Moldova. Parasitological researches were done in Laboratory of Parasitology and Helminthology of the Zoology Institute.

In order to identify the parasitic agents, partial parasitological, coproovoscopic (Fulleborn, Darling, repeated washing) and coprolarvoscopic methods were used.
The parasitological evaluation was performed by determining the extensiveness (EI, %) and the intensity of the invasion (II, specimens), using the Novex Holland B series microscope, ob. 20-40 WF 10 x Din/20 mm.
For statistical data processing STATISTICA 12 and MICROSOFT EXCEL 2019 programs were used.

RESULTS AND DISCUSSIONS

The fauna of hunting interest is the component part of the national hunting fund and both the herd and the totality of the spectrum of main and complementary species determine the value of this fund. It is known that parasitic diseases not only restrain the growth and development of the hare, but can lead both directly to their death through the appearance of diseases, and indirectly by weakening or exhausting the body and increasing the possibility of their capture by predators. The multiple measures aimed at the numerical increase of the hare will not be enough, until measures to combat the parasitic fauna will also be taken, which is of particular importance. Along with this, the hare population, in natural winter conditions, will need additional food, when everything around is covered with snow.
Research on the parasitofauna study of hares, carried out by, from various natural biotopes of the Republic of Moldova, where they live, allowed us to highlight a high level of their infestation with various parasitic agents.
As a result of the parasitological research carried out, it was established the infestation of hares with various parasitic agents, from the class *Cestoda* - 1 species, the class *Trematoda* - 2 species, the class *Secernentea* - 8 species and from the class *Gonoidasida* - 7 species (Table 1).
The parasitological examination carried out on coprological samples collected from hares living in various natural and anthropogenic biotopes of the Republic of Moldova, showed that parasitic agents are present in all samples (100% of cases).
To this date, no specific measures or procedures are known for complementary feeding and deworming of hares using lighters. After the obtained result, the closest solution is the method of deworming house rabbits, which consists in deworming them with the use of phenothiazine and piperazine in appropriate doses. The disadvantage of this method lies in the fact that the preparations used in deworming (phenothiazine and piperazine) are very toxic and immunosuppressive on the dewormed animal organism.
The problem solved in the current invention consists in the development of a composition for feeding and deworming hares and an effective, harmless, relatively cheap and simple complex deworming process, which simultaneously ensure complementary feeding and deworming of hares in the cold period of the year (Toderaş et al., 2019).
The composition, according to the invention, contains, in %: oats – 30-50; wheat – 4.0-7.0; barley – 2.0-4.0; corn – 2.0-4.0; sunflower meal – 2.0-4.0; Soybean meal – 2.0-4.0; hunted clay (Bentonite) – 20.0-30.0; molasses – 1.0-2.0; dextrin – 2.0-3.0; complex vitamin-mineral premix for rabbits – 1.0-2.0; antiparasitic preparation Alben granulated – 1.0-2.0.
The procedure of complementary feeding and deworming of hares, according to the invention, provides for the administration during the frosty winter period (December-February) of the mentioned composition dosed per head of animal in the form of briquettes of 75.0 g/rabbit, in two intervals of 12-14 days, suspended with a string passed through the holes at a height of 25-40 cm from the ground.

Table 1. Parasitofauna diversity in *Lepus europaeus* Pallas, 1778 from the Northern Zone of the Republic of Moldova

Invasion		Level of infection	
		EI (%)	II (ex.)
Class CESTODA			
1.	*Cysticercus pisiformis* (Zeder,1803)	25.0	19-21
Class TREMATODA			
2.	*Fasciola hepatica* (Linnaeus, 1758)	3.5	2.8
3.	*Dicrocoelium lanceolatum* (Rudolphi,1919)	7.1	1,7
Class SECERNENTEA			
4	*Trichocephalus leporis* (Frolich, 1789)	13.4	6.0
5.	*Strongyloides papillosus*(Wedl, 1856)	79.1	128
6.	*Trichostrongylus retortaeformis*(Zeder,1800)	4.1	2.0
7.	*Passalurus ambiguus* (Rudolphi, 1819)	14.6	3.0
8.	*Trcihostrongylus probolurus* (Railliet,1896)	15.3	6.0
9.	*Trichuris leporis* (Frölich, 1789)	17.5	8.0
10	*Graphidium strigosum* (Dujardin, 1845)	2.7	1.0
11	*Nematodirus abnormalis* (May, 1920)	4.7	3.0
Class GONOIDASIDA			
12	*Eimeria acervulina*	82.6	98
13	*Eimeria anceris*	76.6	64
14	*Eimeria brunette*	36.2	43
15	*Eimeria necatrix*	21.2	17
16	*Eimeria mitis*	18.4	5.0
17	*Eimeria adenoids*	7.8	6.0
18	*Eimeria meleagrimitis*	6.3	8.0

The anti-parasitic preparation Alben (granules) - with 20% Albendazole active substance (produced and registered in the Republic of Moldova by Agrovetzașcita, Russia). Alben is a broad-spectrum anthelmintic active on mature, immature nematodes, cestodes as well as mature trematodes. It is indicated for combating gastrointestinal nematodes (haemonchosis, bunosomosis, esophagostomosis, nematodirosis, ostetagiosis, habertiosis, cooperiosis, strongyloidosis, trichostrongylosis, giostrongylosis, parascaridosis, ascaridosis, trichocephalosis, toxocarosis, toxoscaridosis, hookworm, uncinariasis, ascaridiosis, heterakidosis). Trematodes (fasciolosis, dicroceliosis, paramphistomatosis). Cestodoses (moniosis, avitelliosis, botryocephalosis, caviosis, liguliosis). Pulmonary nematodes (dictycaulosis, protostrongylosis, muelleriosis, neostrongylosis, cystiocaulosis, metastrongylosis).

The recommended dose for animals with fur is 50-100 per preparate, mixed with food, placed in feeders for a group of 10-100 animals.

The Alben (granules) preparate is well tolerated by fur animals and has no contraindications.

The complete vitamin-mineral premix for rabbits is a product based on vitamins, trace elements, concentrated assimilable and coccidiostatic minerals. Manufacturer and distributor in the Republic of Moldova is Vitafort Zrt. (Hungary). The composition of the complete vitamin-mineral premix for rabbits is represented in Table 2.

The complete vitamin-mineral premix for rabbits does not contain genetically modified organisms. As enzymes, coccidiostatic and antioxidant, Diclazuril and Ethoxyquin preparations are taken. The mixing rate of the premix in the final ration for rabbits is 2%. The coccidiostatic Diclazuril, from the composition of the complete vitamin-mineral premix for rabbits, is a coccidiostatic preparation, with a wide spectrum of use on all species of coccidia in rabbits. The result of the invention consists in deworming animals in natural conditions and the compensation of the deficit in the cold period of the year with vitamins, trace elements, assimilable concentrated minerals, which will allow the effective preservation of healthy hare species and their reproductive potential in the nature.

Table 2. Composition of complete vitamin-mineral premix for rabbits

Vitamins			Microelements		
Vitamin A	UI/kg	400000.0	E6 Zinc (Zinc oxide)	mg/kg	972.0
Vitamin D	UI/kg	78000.0	E1 Iron (Iron sulfate)	mg/kg	3860.0
Vitamin E	mg/kg	725.0	E5 Manganese (Manganese oxide)	mg/kg	341.0
Vitamin K	mg/kg	70.0	E4 Copper (Copper sulfate)	mg/kg	198.0
Vitamin B12	mg/kg	100.0	3b202 Iod (iodat de calciu)	mg/kg	25.52
Acid pantotenic	mg/kg	430.0	3b202 Cobalt (Carbonate)	mg/kg	9.42
Vitamin B6	mg/kg	20.0	Macroelements		
Nicotinic acid	mg/kg	950.0	Calcium	%	11.63
Biotin	mg/kg	2.60	Phosphorus	%	5.28
Choline chloride	mg/kg	30000.0	Sodium	%	7.72
			Enzymes, coccidiostatic, antioxidant		
			Diclazuril	mg/kg	50.0
			Ethoxyguin	mg/kg	385.0

Also, this procedure allows to effectively and economically use both the food, the premix, and the antiparasitic preparation. The technical result obtained is due to the use of the mixture as an antiparasitic product against endoparasites, as well as its administration to hares during the frosty period, when they lack food in nature. Based on the daily norm of a rabbit in the winter period (December - February) of 50 g of grain concentrates, ingredients were taken for 200 rabbits: 10 kg of concentrated feed mixture (oats - 7000 g, wheat - 1,000 g, barley - 500 g, corn - 500 g, sunflower seed - 500 g, soybean meal - 500 g), to which 5 kg of food supplement is added (clay (bentonite) - 4 kg; molasses - 200 g and 400 g dextrin, which has the role of fixing the ingredients on the surface of the seeds and contributes to making all this food taste more attractive and protected until final consumption from atmospheric conditions; premix vitamino - complex mineral for rabbits - 200 g; anti-parasitic preparation - Alben granulated for - 200 g. mix 2 liters of drinking water with the dry ingredients (15 kg). The whole mass consisting of 15 kg concentrates [oats - 7,000 g, wheat - 1,000 g, barley - 500 g, corn - 500 g, sunflower seed - 500 g, soybean meal - 500 g and ingredients 5 kg (Betonite - 4,000 g, Molasses - 200 g, Dextrin - 400 g, Premix complex for rabbits - 200 g, Alben granulated - 200 g)] is briquetted by hand in the form of corncobs in the form of pellets with holes inside, weighing 75.0 g formed from the following calculations: 15 kg of mass: 200 rabbits = 75 g one briquette for one rabbit, consisting of 50 g of cereal and 25 g of ingredients. The obtained briquettes (200 pieces) are dried in the sun or, to speed up the process, in ovens at a temperature of up to 45°C. The briquettes are administered in two rounds at an interval of 14 days, in feeders, suspended with a string passed through the holes at a height of 25-40 cm from the ground.

Based on the total content of the components calculated for 200 rabbits, the quantitative ratio of the components of a lighter was calculated in grams, per animal: Oats – 35.0 g; wheat - 5.0 g; barley - 2.5 g; corn - 2.5 g; sunflower seed - 2.5g; soy sauce - 2.5 g; hunting clay (bentonite) - 20.0 g; molasses - 1.0 g; dextrin - 2.0 g; complex vitamin-mineral premix for rabbits - 1.0 g; antiparasitic preparation - Alben granulated - 1.0 g.

In order to deworm and compensate the physiological needs of the hare's body, during the cold period of the year (December-February), with vitamins, trace elements, assimilable concentrated minerals, which give a new qualitative effect and allow to ensure survival, increase their reproductive potential under natural conditions, as well as to reduce the risk of their capture by predators in two seasonal installments: December and February, 150 g (75 + 75 g) of briquetted complementary food is provided for each rabbit. About 50 rabbits live on one thousand ha of hunting land, which requires 7.5 kg of briquettes (100 briquettes of 75 g each), in both seasons distributed in 5 feeders (10 briquettes in each feeder per season) x 2 seasons = 100 briquettes in total. Deworming the hare under natural conditions and compensating the deficiency of vitamins, trace elements, assimilable concentrated minerals in the cold period of the year allow the preservation of healthy hare herds and their reproductive potential in nature, being administered simultaneously with the preferred supplementary feed of antiparasitic preparations.

Furthermore, this composition allows using food efficiently and economical, the premix, as well as the dewarming preparates. In order to determine the therapeutic effectiveness of the antiparasitic preparation Alben granulated on endoparasites in hares, biological samples were collected from them, establishing the extent of the invasion with initial endoparasites before and after the administration of the preparation. The administration of the Alben granulated preparation to the hare was carried out in identical doses (1.0 g of the preparation included in the briquetted food of 75.0 g for a hare) (Table 3).

Table 3. The effectiveness of the Alben granulated preparation in combating endoparasites in hares

Invasion	Extensiveness of the invasion until treatment, %	Extensiveness of the invasion after treatment, %
Trichocephalus leporis (Frolich, 1789)	13.4	0
Strongyloides papillosus (Wedl, 1856)	79.1	2.6
Trichostrongylus retortaeformis (Zeder,1800)	4.1	0
Passalurus ambiguus (Rudolphi, 1819)	14.6	1.0
Trcihostrongylus probolurus (Railliet,1896)	15.3	1.4
Trichuris leporis (Frölich, 1789)	17.5	1.7
Graphidium strigosum (Dujardin, 1845)	2.7	0
Nematodirus abnormalis (May, 1920)	4.7	0

The obtained results proved that the Alben granulated preparation has a high efficacy on the endoparasites established in the hare. The results of the research showed that the proposed procedure provided the hare with vitamins, trace elements, assimilable concentrated minerals deficient in food from nature during the cold period of the year and to carry out, for curative-prophylactic purposes, their deworming with minimal expenses.

Therefore, the simultaneous deworming and compensation of the body's physiological needs in vitamins, trace elements, assimilable concentrated minerals give a new qualitative effect, which allows to increase the survival and reproductive potential of the hare in natural conditions. The proposed procedure can be used in all areas of the Republic of Moldova, populated by hares. The coccidiostats Diclazuril and Clinacox from the complete vitamin-mineral premix for rabbits are prepared with a broad spectrum of use against all species of coccidia in them. The mixing rate of the premix in the final ration for rabbits is 2%. The effectiveness of these coccidiostats is represented in Table 4.

Table 4. Efficacy of the coccidiostats Diclazuril and Clinacox in the complete vitamin-mineral premix for rabbits in combating eimeriosis in field rabitts

Invasion	Extensiveness of invasion until treatment, %	Extensiveness of invasion after treatment, %
Eimeria acervulina	82.6	4.4
Eimeria anceris	76.6	3.6
Eimeria brunette	36.2	2.2
Eimeria necatrix	21.2	0
Eimeria mitis	18.4	0
Eimeria adenoids	7.8	0
Eimeria meleagrimitis	6.3	0

As a result, the effectiveness of the coccidiostats Diclazuril and Clinacox in the complete vitamin-mineral premix for rabbits possesses a high coccidiostatic efficacy against all species of coccidia detected in the rabbit - in the field. For the experimental control of the proposed composition, 3 variants of briquette mixtures were prepared. The versions of the experiences are presented in Table 5.

Table 5. Experimental variants

Variants / Briquette	Alben granu-lated (gr)	Complete vitamino-mineral premix for rabbits (%)	Oats (%)	Wheat (%)	Barley (%)	Corn (%)	Sunflower groats (%)	Soy bean groats (%)	Ben-tonite (kg)	Molasses (g)	Dextrin (g)
Component 1	0.5	1	50	20	10	10	5	5	3	100	300
Component 2	1.0	2	70	10	5	5	5	5	4	200	400
Component 3	1.5	3	75	5	5	5	5	5	5	300	500

For deworming the hares, it is recommended to use the briquettes with a mixture from the 2nd composition, which has demonstrated optimal consumption results during a daily feeding cycle. The species, the spatial distribution, the herd and the density of the hare on a certain territory are determined and the coprological analysis of the biological samples regarding the presence of parasitic agents is carried out in detail. From the herd of heres, complementary food (briquettes) is prepared with the addition of an anti-parasitic preparation and a complete premix for rabbits with coccidiostats, which is placed in specially designed feeders, installed in advance specifically for the complementary feeding of hares. The hares know very well where the feeders are located, and during the period of lack or insufficiency of food, the reflex brings them closer to these feeders from which they consume the additional food brought by the caretakers. During this period, we recommend to carry out, along with the additional feeding of the hare, their deworming. The method was applied in the frosty months of the year, when everything around is covered with snow, and the hare has a shortage of food. After deworming, in 1-2 weeks, the analysis of the biological samples from the dewormed hares is carried out to establish the effectiveness of the treatment and its repetition in 12-14 days. The deworming results are presented in Table 6.

Table 6. The results of coprological research until and after the application of the anti-parasitic treatment to hare

The place of research	Number of examined samples	% of infestation	
		Until deworming	After deworming
The hunting fund “Ialoveni”	50	endoparasites	endoparasites
		82.6	4.4

The achieved results showed us that the proposed procedure allows the deworming hares and provide them with vitamins, trace elements, assimilable concentrated minerals deficient in food from nature, with minimal expenses.

Therefore, the simultaneous performance of deworming with endoparasites and compensating the physiological needs of the body in vitamins, trace elements, assimilable concentrated minerals give a new qualitative effect, which allows to ensure the survival and increase the reproductive potential of hares in natural conditions, as well as to reduce the risk of their capture by predators.

The proposed procedure can be used in all natural and man-made biotopes in the Republic of Moldova, where the hare is found.

CONCLUSIONS

In hares, from various natural and Anthropogenic biotopes of the Republic of Moldova, an increased level of infestation with various parasitic agents was established: from the *Cestoda* class - one species, the *Trematoda* class - 2 species, the *Secernentea* class - 8 species and from the *Gonoidasida* class - 7 species.

From the total of established species (15 species): 5 species (33.3%) are specific only for rabbits (*Cysticercus pisiformis, Trichuris leporis, Trichocephalus leporis, Passalurus ambiguous, Graphidium strigosum*), 10 species (66.7%) are common and other species of wild and domestic animals (*Strongyloides papillosus, Trcihostrongylus probolurus, Trichostrongylus retortaeformis, Nematodirus* *abnormalis, Fasciola hepatica, Dicrocoelium lanceolatum, Eimeria stiedae, Eimeria leporis, Eimeria exigua, Eimeria intestinalis),* of which 2 species (*Fasciola hepatica, Dicrocoelium lanceolatum*) are also present in humans.

The procedure, according to the invention, provides the administration for hares of the mentioned composition, in a dose of 75 g/rabbit, in winter, twice, with an interval of 14 days, in the form of briquettes, placed at a height of 25-40 cm from the ground. It was established that the simultaneous performance of deworming with endoparasites and compensating the body's physiological needs in vitamins, trace elements, assimilable concentrated minerals give a new qualitative effect, which allows to ensure the survival and increase the reproductive potential of hares in natural conditions as well as to reduce the risk of their capture by predators.

ACKNOWLEDGEMENTS

The investigations were carried out within the State Program 20.80009.7007.12 "The diversity of hematophagous arthropods, of zoo- and phytohelminths, vulnerability, climate tolerance strategies and elaboration of innovative procedures for integrated control of species of socio-economic interest" and within the Postdoctoral Program no. 22.00208.7007.06/PD II "Parasitofauna, the impact of parasitosis on the main species of hunting importance, prophylaxis and treatment".

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

NATURAL REMEDIES USED IN FIGHTING ECTOPARASITES IN GALLINACEOUS BIRDS

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Abstract

The scientific paper elucidates the importance of using remedies of natural origin, compared to those of chemical origin, in combating ectoparasites in gallinaceous birds. For the first time, the antiectoparasitic efficacy of the natural extract Ectogalimol, obtained from the plant raw material, dry aerial parts of the Dalmatian chamomile (Pyretrum cinerariifolium Trev.) which in a concentration of 3%, administered by spraying in two rounds at an interval of 14 days, in a dose of 50 ml per bird, possesses a high therapeutic effectiveness against the various species of ectoparasites (bird lice, fleas and gamasid mites). For comparative purposes, the drug Ivermec-OR is used, which also possesses a high antiparasitic efficacy against various species of bird lice, fleas and gamasid mites in gallinaceous birds, compared to the natural extract Ectogalimol, which does not require restrictions on the consumption of products and by-products from treated birds.

Key words: *antiparasitic drug, ectoparasites, gallinaceous birds, natural remedy.*

INTRODUCTION

The prevention and treatment of parasitosis in animals is generally achieved by dehelminthisation with chemical compounds, which affect not only the parasites, but also the host. In this context, it is very important to identify natural remedies with an antiparasitic effect. The practice has shown that the remedies of plant origin have a number of advantages over synthetic ones: in addition to being produced from accessible and cheap raw materials, they are not toxic to the host, can be used by free-feeding, and the use of products obtained from treated animals have no restrictions in consumption. Herbal medicines, used since ancient times, have not lost their importance even today and are applied in the treatment of various disorders of the cardiovascular, nervous, digestive system and last but not least, as anti-parasitic preparations. Some plant compounds have been found to have medicinal properties with antibacterial, antiparasitic and antifungal effects (Olteanu et. al., 2001; Ciucă, 2002; Erhan, 2020; Rusu et al., 2011; 2020; 2021).

The use of natural insecticides such as pyrethrin extracted from pyrethrum flowers (*Chrysanthemum cinerariaefolium*), is part of the objectives of sustainable agriculture to reduce the incidence of pests and the diseases caused by them without producing any change in the natural balance (Kinz & Kemp, 1994; Schleier & Peterson, 2011; Gupta & Shah, 2013; Campos et al., 2019).

Some authors reveal that the development and production of safer and naturally preferred insecticides is an important objective. Pyrethrins are natural insecticides biosynthesized from the pyrethrum plant [*Chrysanthemum cinerariaefolium* (current species name: *Tanacetum cinerariifolium*)] of the Asteraceae family. Pyrethrum (*Chrysanthemum cinerariaefolium*) is a small perennial plant grown commercially for the extraction of natural pyrethrins used in the manufacture of insecticides. These are known for their rapid destruction and lethal action on a wide range of insect pests (Maciver, 1995; Hitmi et al., 2000; Anadón, 2009; Chen et al., 2018; Markhamet et al., 2020;).

Therefore, pyrethrins are ideal substitutes for synthetic insecticides. Natural pyrethrin insecticides produced from *Tanacetum cinerariifolium* have been shown to have toxicity for mammals and short persistence in the environment, providing an alternative to widely used synthetic insecticides that pose a threat to human health and the environment (Bhat & Menary, 1986; Bodrug, 2004; Freemont, 2016; Lybrand et al., 2020; Jeran et al., 2021).

In 2015, Campbell & Omura were awarded the Nobel Prize in Physiology and Medicine for the development of Ivermectin and the development of "new treatments for diseases caused by nematodes".

Another antiparasitic preparation with a broad spectrum of action, recommended to combat some groups of ecto and endoparasites in birds and mammals, which is part of the group of macrocyclic lactones, is Ivomec. It has the property of irreversibly blocking neuromuscular transmission, acting at the level of synapses, thus stimulating gamma-aminobutyric acid (GABA), which is an inhibitor of nerve stimuli (Cernea et al., 2006).

Avermectins are produced by the soil microorganism *Streptomyces avermitilis*. A complete study of the bacterium's genome was completed in 2003. Avermectins were first isolated and identified in 1976 in Japan. *Streptomyces avermitilis* culture produces four main forms of avermectins - A_1, A_2, B_1, B_2 and each component has two isomeric forms: a and Ivermectin and Abamectin, members of the Avermectin family of compounds, were introduced to the market in the 1980s as a veterinary antiparasitic drug and an agricultural pesticide, respectively. Their acceptance and commercial success were remarkable; both are highly effective and used worldwide (Campbell, 1989).

The avermectin complex, obtained from the mycelium of *Streptomyces avermitilis* VKPM S-1440 containing the group B – 67.8-77.4% was named Avertin N – practically an analogue of Aversectin-C, which differs only in the strain of the producer (Ardelean, 1999) (Table 1).

Table 1. Physical and chemical properties of the antiparasitic drug Ivomec

Chemical properties	
Formula	$C_{48}H_{74}O_{14}$(22,23-dihydroavermectin B = 1a) $C_{47}H_{72}O_{14}$(22,23-dihydroavermectin B = 1b)
Molecular mass	875,10 g / mol (B = 1a) 861,08 g / mol (B = 1b)
Physical properties	
Difusin temperature	155 ° C
Solubility	Insoluble

The antiparasitic drug Ivermec OR, being of chemical origin, has a broad spectrum of action against ecto- and endoparasites in gallinaceous birds. It is active against ticks, bird lice, fleas, bed bugs, feather mites – scaly leg mite (*Cnemidocoptes mutans*), depluming mite (*Cnemidocoptes levis*) and fowl cyst mite (*Laminosioptes cisticola*) with localization in the subcutaneous connective tissue and muscles. The product also acts on parasitic nematodes: *Ascaridia* spp., *Capillaria* spp., *Syngamus tracheae*, *Trichostrongylus* spp., *Heterakis gallinarum*, etc.

One of the primary social problems in the Republic of Moldova is providing the population with dietary food products: meat, milk, eggs, etc. Along with the continuous growth of the population, the consumption of proteins of animal origin also increases (Tomşa & Bondoc, 2014).

The evolution of diseases in humans and animals, in general, but in particular zoonoses, causes incalculable damage through loss of human and animal lives, through the transition to incapacity of work for some people, and low productions and confiscation of carcasses and organs in animals.

Numerous authors reveal the evolution of zoonoses, the importance of natural outbreaks and the role of birds as vectors, especially migratory ones, which can transport parasites and microbes over long distances (Toderaș & Movilă, 2008; Zamornea et al., 2022).
Currently, new ecological solutions are needed to obtain and use ectoparasiticides in the control of ectoparasitoses in animals. The interest of the prophylaxis and treatment of ectoparasitic agents with the help of the use of natural ectoparasiticides does not leave without interest even parasitologists from the Republic of Moldova (Zamornea et al., 2021).

MATERIALS AND METHODS

In the antiparasitic therapy of gallinaceous birds, a new drug of vegetable origin was used in different concentrations - Ectogalimol developed in the Laboratory of Parasitology and Helminthology of the Institute of Zoology in collaboration with the Center of Advanced Biological Technologies within the Institute of Genetics, Physiology and Plant Protection (Rusu Ş. et.al. 2008).
To evaluate the effectiveness of the drug Ectogalimol against bird lices, fleas and gamasid mites, 6 experimental batches of 10 Adler Silver chickens aged 4 months and 6 batches of 10 pheasants (*Phasianus colchicus* L.), aged 4 months, spontaneously infested with ectoparasites were selected. In order to establish the diversity of the parasitic fauna, bird lice, fleas and gamasid mites were collected from live birds, according to a new procedure for collecting the ectoparasites from live birds according to the method described by Rusu Ş. et al. 2021. The collected material was subsequently examined with the help of the MBC-9 stereo microscope (ob. x 4) and the МБИ -3 microscope (ob. x 10). Extensivity of invasion (EI) and intensity of invasion (II) with ectoparasites were determined in chickens and common pheasants.
The drug of vegetable origin - Ectogalimol was used in comparison with one of chemical origin - Ivermec-OR, which is a product registered in the Republic of Moldova and placed on the website of the National Agency for Food Safety (ANSA) in the compartment "Nomenclature of pharmaceutical products for veterinary use" .
Infected birds were divided into groups (chickens and pheasants), which were maintained in separate places with free access to food and water. The naturally infested gallinaceous bird groups were treated with the antiparasitic drugs Ectogalimol and Ivermec-OR. The natural extract obtained from the Dalmatian chamomile (*Pyretrum cinerariifolium* Trev.) - Ectogalimol was used for research in the form of an aqueous solution in concentrations of 1%, 2%, 3%, 4% and 5%. The application of the medication Ectogalimol was carried out by spraying each bird separately in a dose of 50 ml in two rounds at an interval of 14 days. The antiparasitic drug Ivermec-OR was administered orally, 0.4 ml of medicine per 1 kg of weight, in three rounds: two administrations were given at an interval of 24 hours, and the last administration was carried out on the 14th day since the last application.
The drug Ectogalimol is a natural, biologically active extract obtained from the plant raw material, dry aerial parts of Dalmatian chamomile (*Pyretrum cinerariifolium* Trev.), the active substance being Pyrethrin. The medication Ectogalimol was obtained in the laboratory of Parasitology and Helminthology of the Institute of Zoology by the following procedure: 500 g of dry aerial parts of Dalmatian chamomile (*Pyretrum cinerariifolium* Trev.) were subjected to extraction with the alcoholic-aqueous solution of 60% in the ratio 1:4 on the refrigerated circulating baths for 8 hours. The procedure is repeated in three rounds, and the extract obtained after filtration was distilled to dryness in a vacuum evaporator at 50°C. Thus, obtaining 38.7 g of dry residue rich in biologically active substance. The verification of the obtained product was carried out with the help of the chromatograph placed in a thin layer on «Silufol» plates in the solvent system «chloroform:methanol» = 75:25 (v/v).

RESULTS AND DISCUSSIONS

In order to determine the effective dose and the harmlessness of the natural extract Ectogalimol, 6 groups of 4 month old Adler Silver chiks of 10 specimens each, spontaneously infested with various species of ectoparasites were formed: bird lice - *Cuclotogaster heterographus*, *Eomenacanthus stramineus*, *Goniocotes gallinae*, *Goniocotes maculatus*, *Goniodes dissimilis*, *Lipeurus caponis*, *Menopon gallinae*, *Menacanthus cornutus, Menacanthus pallidulus*; fleas - *Ceratophylus gallinae*, *C. hirundinis* and gamasid mites - *Dermanyssus gallinae*, *D. hirundinis*).

In the antiparasitic therapy, the natural extract Ectogalimol was applied, which was used for research in aqueous solutions in concentrations of 1%, 2%, 3%, 4% and 5%.

Group I – control (infested, untreated), Group II – treated with Ectogalimol 1%; Group III - Ectogalimol 2%; Group IV - Ectogalimol 3%; Group V - Ectogalimol 4% and Group VI – Ectogalimol 5%.

The application of the drug Ectogalimol was carried out by spraying each bird with a dose of 50 ml.

The obtained results are presented in Table 2.

Table 2. The effectiveness of the drug Ectogalimol in various doses and periods of time

Group No.	No. of chikens	The concentration of the drug, (%)	The effectivenes of the drug after administration (%)			
			2 hours	12 hours	24 hours	72 hours
I	10	control	-	-	-	-
II	10	1	0	10	30	40
III	10	2	30	40	60	70
IV	10	3	90	100	100	100
V	10	4	100	100	100	100
VI	10	5	100	100	100	100

Each group of birds was kept isolated in separate spaces. The effectiveness of the drug, administered in various doses, was determined over 2, 12, 24 and 72 hours after the treatment. The results of the conducted research demonstrate that, in groups II and III, where Ectogalimol with a concentration of 1% and 2% was used, a low effectiveness of this drug was established (40% and 70%, respectively), and in groups IV, V and VI where a concentration of 3%, 4% and 5% was applied, a maximum therapeutic efficacy (100%) was registered. Since Ectogalimol in concentrations of 3%, 4% and 5% has the same therapeutic efficacy - 100%, to combat ectoparasites in chickens, Ectogalimol in a concentration of 3% is recommended.

It was found that the Ectogalimol 3% administered to chickens has a high therapeutic effectiveness against various species of ectoparasites in chickens: bird lice - *Cuclotogaster heterographus*, *Eomenacanthus stramineus*, *Goniocotes gallinae*, *Goniocotes maculatus*, *Goniodes dissimilis*, *Lipeurus caponis*, *Menopon gallinae*, *Menacanthus cornutus, Menacanthus pallidulus*; fleas - (*Ceratophylus gallinae*, *C. hirundinis)* and gamasid mites - *Dermanyssus gallinae*, *D. hirundinis*). The clinical condition of the chickens after the treatment has improved, the birds calmed down, the appetite increased.

Thus, as a result of the administration of the drug Ectogalimol 3%, there was a considerable decrease of the extensivity of invasion with ectoparasites - up to 100%. The medication Ectogalimol 3% is not toxic, for these reasons the overdose does not produce adverse effects and does not require high costs and a large volume of work. The curative therapy consists in the application by spraying of the aqueous solution of Ectogalimol 3% in two rounds at an interval of 14 days, in a dose of 50 ml per bird. The prophilaxys is carried out by spraying the aqueous solution of Ectogalimol 3% in a dose of 50 ml per bird, in a single dose of clinically healthy birds from contaminated households. It is recommended to desinfect the surface of the shelters, both inside and outside, using the aqueous solution of Ectogalimol 3%, in two doses, at an interval of 14 days, simultaneously with the deparasitation of birds. The obtained results are presented in Figure 1.

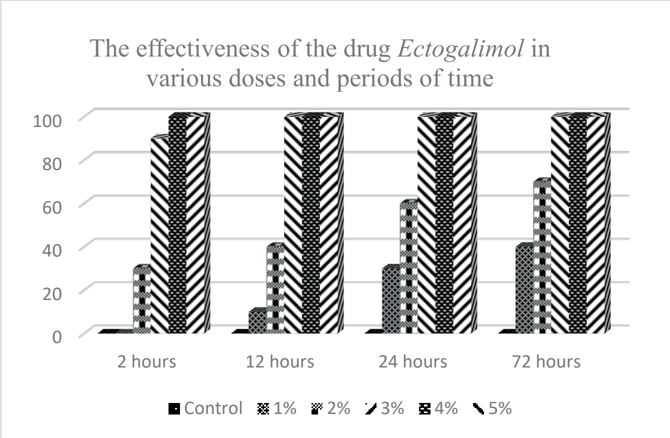

Figure 1. The effectiveness of the drug Ectogalimol in various doses and periods of time

At the same time with the testing of the antiparasitic effectiveness of the natural medication Ectogalimol, the testing of the effectiveness of Ivermec OR in combating ectoparasites in gallinaceous birds was also carried out. For this purpose, 2 groups of birds were formed.

Group 1 consisted of 10 pheasants (aged 4 months) spontaneously infested with bird lice (*Cuclotogaster cinereus, Cuclotogaster heterographus, Goniocotes chrysocephalus, Goniocotes microthorax, Goniodes colchici, Goniodes dissimilsis, Lipeurus caponis, Menacanthus stramineus, Menopon gallinae*), fleas (*Ceratophylus gallinae, Ceratophylus hirundinis*) and gamasid mites (*Dermanyssus gallinae, Dermanyssus hirundinis).*

Group 2 consisted of 10 specimens of 4-month-old Adler Silver chicks spontaneously infested with ectoparasites: bird lice - *Cuclotogaster heterographus*, *Eomenacanthus stramineus*, *Goniocotes gallinae*, *Goniocotes maculatus*, *Goniodes dissimilis*, *Lipeurus caponis*, *Menopon gallinae*, *Menacanthus cornutus, Menacanthus pallidulus*; fleas - (*Ceratophylus gallinae*, *C. hirundinis)* and gamasid mites - *Dermanyssus gallinae*, *D. hirundinis*)

In the antiparasitic therapy, the drug Ivermec OR was applied according to the administration leaflet. Ivermec-OR has a high efficacy against ectoparasites previously detected in gallinaceous birds (Gherman et al., 2003; Rusu et al., 2011).

The obtained results are presented in Table 3.

Table 3. The effectiveness of Ivermec-OR against gallinaceous birds ectoparasites

Species of parasites	pheasants				chickens			
	Before therapy		After therapy		Before therapy		After therapy	
	EI (%)	II (ex.)	EI (%)	II (ex.)	EI (%)	II (ex.)	EI (%)	II (ex.)
Bird lice								
Cuclotogaster cinereus (Nitzsch,1866)	15.3	18.0	2.0	1.0				
Cuclotogaster heterographus (Nitzsch, 1866)	71.9	83.0	-	-				
Goniocotes chrysocephalus (Giebel,1874)	56.9	78.5	-	-				
Goniocotes microthorax (Stephens,1829)	32.3	65.4	-	-				
Goniodes colchici (Denny, 1842)	41.7	96.0	-	-				
Goniodes dissimilsis (Denny, 1842)	11.8	9.0	-	-	5.9	7.0	-	-
Lipeurus caponis (Linné., 1758)	21.2	43.0	-	-	2.7	3.0	-	-
Menacanthus stramineus (Nitzsch, 1818)	74.1	99.0	-	-	14.1	12.0	-	-
Menopon gallinae (Linnaeus, 1758)	32.5	64.0	-	-	45.1	78.0	-	-

Eomenacanthus stramineus (Nitzsch, 1818)					79.5	84.2	2.7	2.0
Goniocotes gallinae (de Geer, 1778)					31.2	17.9	-	-
Goniocotes maculatus (Taschenberg, 1882)					12.3	11.0	-	-
Menacanthus cornutus (Schommer, 1913)					2.9	9.0	-	-
Menacanthus pallidulus (Neumann, 1912)					7.6	5.8	-	-
Fleas								
Ceratophylus gallinae (Schrank, 1803)	14.3	27.0	1.0	1.0	19.4	16.9	-	-
Ceratophylus hirundinis (Curtis, 1826)	23.8	42.1	-	-	12.5	9.7	-	-
Gamasid mites								
Dermanyssus gallinae (Degeer, 1778)	56.9	76.2	2.4	1.0	67.3	81.2	1.9	1.0
Dermanyssus hirundinis (Dugès, 1834)	17.2	32.6	-	-	21.1	26.5	-	-

As a result of the research, there was established that Ivermec-OR has a high antiparasitic action of about 100%, against ectoparasites in the tested gallinaceous birds. At the same time, it is not allowed to administer the product to laying hens and chickens at least 14 days before the eggs are placed in the incubators due to the accumulation of Ivermectin in the eggs. The slaughter is allowed not earlier than 9 days after the last administration of the Ivermec-OR.

CONCLUSIONS

It was found that the natural extract Ectogalimol – a biologically active remedy obtained from the plant raw material, dry aerial parts of Dalmatian chamomile (*Pyretrum cinerariifolium* Trev.) in a concentration of 3%, administered by spraying gallinaceous birds, in two rounds, at an interval of 14 days, in a dose of 50 ml per bird, has a high therapeutic efficacy - approx. 100% against the various species of ectoparasites (bird lice, fleas and gamasid mites).

The drug Ectogalimol 3% is not toxic and therefore the overdose does not produce adverse reactions, it does not require high costs and a large volume of work, and after the administration there are no restrictions on the consumption of products and by-products of the treated birds.

The drug Ivermec-OR has an antiparasitic action of approx. 100% against various species of bird lice, fleas and gamasid mites in gallinaceous birds.

The administration of the Ivermec-OR is forbidden to laying hens at least 14 days before the eggs are laid for incubation due to the accumulation of ivermectin in the eggs.

The slaughter of birds treated with Ivermec-OR is allowed not earlier than 9 days after the last administration. In the case of the forced slaughter of birds before the expiration of the mentioned period, it is allowed to use the meat as food for the fur animals or to transform it into meat and bone meal after heat treatment.

ACKNOWLEDGEMENTS

The investigations were carried out within the State Program 20.80009.7007.12 "The diversity of hematophagous arthropods, of zoo- and phytohelminths, vulnerability, climate tolerance strategies and elaboration of innovative procedures for integrated control of species of socio-economic interest" and within the Postdoctoral Program no. 22.00208.7007.06/PD II "Parasitofauna, the impact of parasitosis on the main species of hunting importance, prophylaxis and treatment".

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

SPATIAL LOCATION OF MILKING EQUIPMENT IN CONNECTION WITH TIME SPENT ON WORKING OPERATIONS

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Abstract

The study of the formalized characteristics' effect of the spatial location of milking equipment, cows and operators in connection with the time spent on working operations is presented. The material for the research was timekeeping of the milking process using different equipment. It has been established that the correlation coefficient between the length of the working area for milking and the duration of the working operation "transition between animals and equipment" is $r=0.330$ ($p<0.001$). The measure of effect size of milking trench's presence/absence factor on the time spent by the operator on milking 1 cow was $\eta^2=0.779$ ($p<0.001$). The time when the machine milking operator stays in a position with a strongly bent torso (over 40°) during milking using a trench is almost 50 times less ($p<0.001$) compared to the corresponding time during milking without using a milking trench. When working in the trench, the operator does not perform working actions in the "squatting" position at all. If milking occurs in stalls, then a small distance between adjacent cows makes it difficult for the operator to access the udder.

Key words: *cow, milking trench, ergonomics, timekeeping, working operation.*

INTRODUCTION

Radical changes are currently taking place in milk production technologies (Kaarlenkaski, 2018). However, to date, farmers still do not sufficiently use fundamentally new, modern approaches to the organization of production processes (Lubega, 2015; Mishra et al., 2020).
This situation is partly due to the fact that the technological processes of milk production are quite complex both in designing and in their implementation (Ivanyos et al., 2020; Gaworski, 2021). This difficulty is explained by the fact that when production is organized, the simultaneous functioning of equipment, animals and people in a unified system should be coordinated. At the same time, each link of this system is connected with others. But a human plays a leading role in this system with the help of active influences on other elements. (Kaarlenkaski, 2018; Ruban et al., 2018).
The interaction between the components of dairy farming is most evident in the machine milking of cows. This production process is key, as it is the core around which the entire technological chain is built.
Milking can also be considered as a complex process of interaction between animals, milking equipment and machine milking operator (Palii et al., 2020).
The milking machine differs from other mechanisms in that during its operation the operator is in close contact with the animal and actively affects one of the most important organs of dairy cows - the udder (Wildridge et al., 2020).
Many different technical and technological solutions can be found on modern farms. Each of these only to some extent corresponds to the biological characteristics of cattle in terms of unlocking the potential for adaptation, health, productivity and longevity (Siewert et al., 2018; Shablia & Tkachova, 2020).
At the same time, milking efficiency is influenced by additional factors. For example, careless operation of the milking machine by the operator, incorrect milking technology, incorrect settings and work with technically defective equipment can cause diseases in cows (Ministry of Agricultural Policy of Ukraine, 2005; Besier & Bruckmaier, 2016; Odorčić et al., 2019).

Milking equipment serves as a means of production for a human, and therefore determines certain labour costs for its operation. Unfortunately, despite the introduction of mechanization of the main technological processes, the work of machine milking operators still remains a type of manual labour with a significant load on the musculoskeletal system (Næss & Bøe, 2011).

In particular, this happens due to the fact that there is insufficient consideration of ergonomic requirements for the organization of operators' workplaces. That is often manifested in the irrational layout of the placement of animals, working units of equipment and control levers in space, as well as in the use of irrational non-mechanized tools (Ulbricht et al., 2014). All this leads to the formation of a high level of difficulty and intensity of work, contributes to a decrease in work capacity (Lubega, 2015).

The disadvantages listed above cause excessive labour costs for the performance of working actions and operations in the milking process, lead to a decrease in the labour productivity of machine milking operators (Næss & Bøe, 2011).

The analysis of the state of study of the problem shows that insufficient consideration of ergonomic requirements in milking technology is caused in a number of cases by the lack of clear, scientifically based knowledge on this issue.

This situation occurred due to the fact that methodical and practical approaches to ergonomic studies, and especially regarding animal husbandry, have only been partially developed.

Accordingly, the laws of influence of certain equipment characteristics, animals, and operators on the effectiveness of their interaction in the process of milking cows have not been sufficiently studied. And the conclusions from the analysis of the technological process of milking are mostly either too general or fragmentary.

In view of this, the study of the effect of formalized characteristics of the spatial location of milking equipment, animals and operators in connection with the time spent on working operations in the milking process is relevant.

MATERIALS AND METHODS

The material for the research was timekeeping observations of the milking processes on equipment of the "DAS-2" type ("Gontarivka" farm), "ADM-100" type ("Stepne" farm and "Mriya" farm), "UDS-2" type ("Agrosvit" farm), type "Herringbone" 2×7 ("Kutuzivka" farm), Kharkiv region and "Parallel" 2×16 type ("Terezino" farm), Kyiv region (Ukraine).

Labour and working operations of the technological processes of milking cows in stalls and in milking parlours were recorded by video recording them on dairy farms. Based on the materials of the video recordings, the timing of the main working operations and working actions was carried out according to the approaches outlined in Shablia (2012; 2018).

Thus, during research, the technological process of milking cows was divided into technological operations and working actions in such a way that each of its elements could be unambiguously attributed to one of the predetermined categories. Separate working actions did not overlap in time. And the same working action cannot be simultaneously attributed to several technological operations.

The duration of the following working operations (seconds) was taken into account as the result characteristics of the efficiency and ease of labour of machine milking operators:

- cows' driving;
- washing the udder;
- wiping the udder;
- preparation, rinsing and wringing of a napkin;
- moving the milking machine;
- transitions between animals and equipment;
- udder massage;
- milking the first streams of milk;
- turning on and off the vacuum;
- putting on milking teatcups;
- after-milking;
- disconnecting and removing the device;
- delivery of milk to the cooling tank;
- post milking teat spraying or dipping;
- manure cleaning;
- distribution of feed;
- observation.

In addition, more generalized characteristics of the time of performing labour actions were established, such as:

1. Time of concentrated observation;
2. The total time the body is in a tilted position, including:
   - torso at an angle of 20-40º from the starting position;

- torso at an angle of 41º or more;
3. Total time spent in the "squatting" position.
4. The total time of the upper arms being in an uncomfortable position - at an angle to the starting position (arms down), including:
- with upper arms at an angle of <90º;
- with upper arms at an angle ≥90º.

For all these indicators, the number, sequence and duration of separate movements were also taken into account.

On the basis of conducted research, databases were created, which included the characteristics of technological operations performed by machine milking operators. In total, milking processes of 412 cows using different milking technologies were studied.

The characteristics of the milking equipment and its spatial location, milk productivity of cows, milk quality, anthropometric and individual characteristics of operators, data on husbandry technologies, cow behaviour, linear evaluation of their exterior type, etc. were taken into account.

An evaluation of the correlations (r) between the studied indicators was carried out, as well as the measure of effect size (η^2) of the main ergonomic factors of the spatial arrangement on the result characteristics was established.

RESULTS AND DISCUSSIONS

In the process of analysing the materials, it was established that it is advisable to consider the ergonomics of the location of milking equipment in space relative to cows and operators in three aspects:
1. From the point of view of the overall dimensions of the working area, where animals, operators and equipment are located during milking.
2. From the point of view of compactness and convenience of location in the space of animals, equipment and operators relative to each other during the performance of basic and additional (accompanying) working operations and working actions.
3. From the point of view of completeness provision of operators and (or) cows with a certain amount of milking and other equipment.

Regarding the dependence of the time of working operations on the total size of working area where milking is carried out, it was established that the correlation coefficient between the length of the working area for milking and the total time spent by the machine milking operator per 1 cow is r = 0.395 ($P < 0.001$).

Accordingly, for an approximate calculation of the total time spent on milking 1 cow, based on the data on the length of working area, you can use the regression equation:

$$T_{m1c} = 1.572 + 0.0211 * L_{wz}$$

where:
T_{m1c} - time spent by machine milking operator per 1 cow, minutes;
1.572 - constant;
L_{wz} - length of the working area for milking, meters;
0.211 - regression coefficient of milking time on the length of the working area (minutes/ meter);

The influence of the working area length on the time spent for milking 1 cow is due primarily to the fact that when the working area length increases, additional time is spent on transitions of the operator between cows, as well as between cows and equipment (Figure 1).

Figure 1. The remote location of the milking equipment, the operator and the cows forces the operator to perform many inefficient, useless and unnecessary labour movements

In particular, the correlation coefficient between the working area length for milking and the duration of the operation "transition between animals and equipment" is r = 0.330 ($P<0.001$). At the same time, transitions are often accompanied by the movement of equipment and tools. These additional actions also cause more operator's fatigue. In researches of Jakob et al. (2012) and Jakob & Liebers (2017) the effect of the working area size on milking efficiency was also established.

In general, the total duration of work operations "transitions between animals and equipment" calculated per 1 cow for milking technologies (Table 1) in the milking parlour (compact placement of animals and equipment) is on the average M = 4.67 ± 0.267 s. And when milking is done in stalls, where cows are kept (stretched placement in space) the total duration of this work operations is M = 23.48 ± 1.690 s.

Table 1. Time spent on milking 1 cow and (including) on performing working operations "transitions between cows and equipment" depending on the place of milking (seconds)

Place of milking	Measures of variability	The time spent by the operator on milking 1 cow	Including the total duration of working operations "transitions between cows and equipment"
In the milking parlour	Number of milkings (N)	263	263
	Mean (M)	66.08	4.67
	Standard error of the mean (SEM)	2.23	0.27
	Standard deviation (SD)	36.22	4.32
	Coefficient of variation (CV), %	54.81	92.55
In places where cattle are kept (in stalls)	Number of milkings (N)	149	149
	Mean (M)	222.11	23.48
	Standard error of the mean (SEM)	5.03	1.69
	Standard deviation (SD)	61.34	20.63
	Coefficient of variation (CV), %	27.62	87.84
In general	Number of milkings (N)	412	412
	Mean (M)	122.51	11.47
	Standard error of the mean (SEM)	4.36	0.77
	Standard deviation (SD)	88.46	15.72
	Coefficient of variation (CV), %	72.21	137.02

The measure of effect size of the factor "place of milking" (milking parlour/in stalls) on the duration of operator's transitions is $\eta^2 = 0.332$ ($P<0.001$), and on the time spent by the operator on milking 1 cow – $\eta^2 = 0.720$ ($P<0.001$).
If we consider the compactness and convenience of the location of animals, equipment and operators in the space relative to each other (Figure 2), then the key influencing factor is the presence of an equipped milking trench as an element of equipment that significantly brings the object of labour (udder) closer to the milking machine and to the operator's arms (Jakob et al., 2012; Jakob & Liebers, 2017).
In particular, factor of "milking trench presence/absence" significantly affects the duration of most work operations in the process of milking cows (Table 2). Thus, the measure of effect size of this factor on the time spent by the operator on milking 1 cow was $\eta^2 = 0.779$ ($P<0.001$).

Figure 2. Compact and convenient arrangement of the animals and equipment in the space using the milking trench enables operator to perform working operations in a comfortable body position

The time for washing the udder during milking with the use of a milking trench is most prominently allocated for the better. It is significantly ($p < 0.001$) 2.5 times smaller compared to washing in the case when the trench was not used.
In addition, the operator's work with the location of his workplace in the milking trench has a beneficial effect on his energy expenditure and fatigue (Cockburn, 2015; Hwang et al., 2010). In particular, in our studies, it was established that significantly less time was spent on performing the majority strenuous working operations in a trench compared to performing the same operations without the use of a trench (Table 3). For example, it should be noted the complete absence of working actions in the "squatting" position when the operator works in the milking trench.
In addition, the advantage of milking with the use of a milking trench is very considerable and significant when evaluating the time spent by the operator with a very bent torso (more than 40°). So, this time is almost 50 times shorter compared to the time spent in a very bent position when milking is done without using a milking trench. And working with a bent torso is one of the most difficult options (Oliveira et al., 2018).

The use of a milking trench also has the effect of reducing the time of labour operations with an inconvenient position of the arms. At the same time, the completeness and quality of execution of working operations by the operator also improves. This happens, in particular, due to less muscle load and weariness of the operator (Németh et al., 1990; Jakob et al., 2012; Jakob & Liebers, 2017).

Table 2. Time spent on working operations when milking cows is done with and without the use of a milking trench

Presence of a milking trench during milking	Measures of variability	Duration of working operations per milking 1 cow, seconds:						
		washing the udder	preparation of napkins	wiping the udder	milking of the first streams of milk	putting on milking teatcups	post milking teat spraying or dipping	cows' driving
Without the use of a milking trench (there is no milking trench)	Number of milkings (N)	185	185	185	185	185	185	185
	Mean (M)	17.36	7.52	3.95	6.02	11.01	2.75	5.46
	Standard error of the mean (SEM)	0.94	0.52	0.4	0.35	0.56	0.2	1.01
	Standard deviation (SD)	12.72	7.08	5.45	4.76	7.64	2.75	13.68
	Coefficient of variation (CV), %	73.27	94.15	137.97	79.07	69.39	100.00	250.55
With the use of a milking trench (there is a milking trench)	Number of milkings (N)	227	227	227	227	227	227	227
	Mean (M)	6.88	3.28	4.95	5	7.63	1.8	8.81
	Standard error of the mean (SEM)	0.43	0.18	0.23	0.22	0.2	0.12	0.34
	Standard deviation (SD)	6.42	2.77	3.41	3.33	3.06	1.78	5.12
	Coefficient of variation (CV), %	93.31	84.45	68.89	66.60	40.10	98.89	58.12
In general	Number of milkings (N)	412	412	412	412	412	412	412
	Mean (M)	11.58	5.19	4.5	5.46	9.15	2.23	7.31
	Standard error of the mean (SEM)	0.54	0.27	0.22	0.2	0.29	0.11	0.5
	Standard deviation (SD)	11.06	5.58	4.46	4.06	5.84	2.32	10.05
	Coefficient of variation (CV), %	95.51	107.51	99.11	74.36	63.83	104.04	137.48
Measure of effect size, η^2		0.223	0.143	0.012	0.015	0.083	0.042	0.027
Significance level, P		<0.001	<0.001	0.023	0.012	<0.001	<0.001	<0.001

If milking occurs in places where cows are kept (in stalls), when the cows are tied, and the milking trench is not used, then the close distance between the cows causes a number of negative consequences due to the difficult access of the operator to the cow's udder and to some elements of equipment. In particular, the insufficient distance between adjacent cows for the passage of the operator (Figure 3) forces the operator to change the position of the cows in the stall to ensure the space of the workplace.

Table 3. The duration of the machine milking operator's stay in uncomfortable body positions when using and without using the milking trench, per cow (seconds)

Presence of a milking trench during milking	Measures of variability	Uncomfortable body positions:						
		torso being in an uncomfor table position	including with a torso bent at an angle of 20–40°	including with a torso bent at an angle of 41° or more	in the "squatting" position	upper arm being in an uncom-fortable (raised) position	including with upper arm raised at an angle of <90°	including with upper arm raised at an angle of ≥90°
Without the use of a milking trench (there is no milking trench)	Number of milkings	185	185	185	185	185	185	185
	Mean	105.83	19.37	63.66	22.81	108.82	103.44	5.37
	Standard error of the mean (SEM)	5.1	3.41	3.17	2.57	5.17	5.1	0.21
	Standard deviation (SD)	69.31	46.36	43.07	34.95	70.26	69.38	2.9
	Coefficient of variation (CV), %	65.49	239.34	67.66	153.22	64.57	67.07	54.00
With the use of a milking trench (there is a milking trench)	Number of milkings	227	227	227	227	227	227	227
	Mean	29.18	27.87	1.28	0	33.06	31.03	1.99
	Standard error of the mean (SEM)	0.66	0.66	0.16	0	0.69	0.7	0.05
	Standard deviation (SD)	9.88	9.92	2.43	0	10.43	10.53	0.83
	Coefficient of variation (CV), %	33.86	35.59	189.84	-	31.55	33.93	41.71
In general	Number of milkings	412	412	412	412	412	412	412
	Mean	63.6	24.05	29.29	10.24	67.08	63.54	3.51
	Standard error of the mean (SEM)	2.98	1.58	2.09	1.28	2.99	2.92	0.13
	Standard deviation (SD)	60.51	32.16	42.41	25.99	60.77	59.3	2.64
	Coefficient of variation (CV), %	95.14	133.72	144.79	253.81	90.59	93.33	75.21
Measure of effect size, η^2		0.398	0.017	0.536	0.191	0.385	0.37	0.407
Significance level, P		<0.001	0.007	<0.001	<0.001	<0.001	<0.001	<0.001

At the same time, the machine milking operator performs additional movements and working actions that are not related to the performance of the main technological operations (Maia & Rodrigues, 2012).

As a result, the total number, duration and intensity of labour movements increases, time spent on preparatory and final milking operations increases, as well as operator's weariness (Jaejin Hwang et al., 2010). Instead, the completeness and quality of execution of working operations deteriorates.

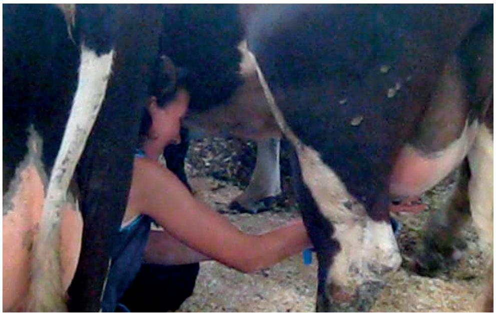

Figure 3. A small distance between cows during milking in stalls makes working operations difficult, forces the operator to make unnecessary movements to move the cows in order to ensure a comfortable working area

CONCLUSIONS

The compact placement of milking equipment elements and animals in space assists to reduce time spent on transitions between equipment elements and animals. In particular, the correlation coefficient between the length of the working area for milking and the duration of the labour operation "transition between animals and equipment" is r = 0.330 (p<0.001).
The factor of milking trench's presence / absence significantly affects the duration of most working operations in the process of milking cows. The measure of effect size of this factor on the time spent by the operator on milking 1 cow was $\eta^2 = 0.779$ (p<0.001).
The time when the operator stays in a position with a strongly bent torso (over 40°) during milking using a trench is almost 50 times less (p<0.001) compared to the corresponding time during milking without using a milking trench. In addition, when working in the trench, the machine milking operator does not perform working actions in the "squatting" position at all. If milking occurs in places where cows are kept (in stalls), then a small distance between adjacent cows makes it difficult for the operator to access the udder and to some elements of equipment, as a result of which the operator has to perform a number of additional and redundant labour action to arrange his workplace.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

THE USE OF AGRIVOLTAIC SYSTEMS, AN ALTERNATIVE FOR ROMANIAN FARMERS

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Abstract

The climate changes of recent years require more and more the finding of alternative solutions for the provision of electricity at the farm level. More farmers from countries such as Germany, France, Italy or USA use agri/agrovoltaic systems. Such a system used in livestock farms and placed directly on the grassland, allows, in addition to obtaining the necessary electricity for the operation of essential consumers (e.g. watering system, electric fence or electric tractor), providing shaded spaces for species such as sheep, cows or rabbit. Research carried out over the years has demonstrated the effectiveness of such systems, especially in the case of shade-tolerant fodder crops (clover, alfalfa). At the EU level, legislative solutions are being wanted that can be easily implemented in the member states and that can support farmers. This study reviews and analyzes the existing legislative solutions for Romanian farmers and opportunity to use agrivoltaic systems on the lands intended for grazing and the cultivation of fodder plants.

Key words*: agrivoltaic farming, climate change, Farm 5.0, solar grazing.*

INTRODUCTION

The extreme climatic phenomena (prolonged droughts, floods, extreme temperatures for several days, etc.) present more and more frequently in the last decades in countries where they appeared at large intervals of years, have attracted the attention of farmers and researchers from all over the world. The reduction of greenhouse gases is currently an international priority to prevent these extreme phenomena.

The use of renewable energy sources is imperative and also represents an extremely beneficial option for farmers, where local sources can be used (solar energy, thermal energy, wind energy, biomass, biogas, etc.). In this context, one of the first actions in the European Green Deal is the 2030 Climate Target Plan, aiming to reduce 55% of the greenhouse gases by 2030 (European Green Deal, 2020). Among the challenges of the last few years, the most often talked about are land use conflicts for agricultural purposes or for the installation of equipment for the conversion of renewable sources (Brohm & Nguyen, 2018). Food and energy systems have a profound impact on society, economies and the environment, making them central to meeting multiple Sustainable Development Goals (IRENA-FAO 2021).

As is knows, solar photovoltaic energy is one of the cheapest sources of electricity available (Vartiainen et al., 2019). In this context, the German Fraunhofer-Institute for Solar Energy Systems (Fh-ISE) in Freiburg has been a pioneer for solar dual use research in the early 1980's. Researchers from this institute have tried to find a solution for the dual use of the land, both for obtaining solar energy and for growing plants, using solar panels mounted at height and not at ground level. This type of installation allows the activities specific to agriculture, horticulture or aquaculture to be carried out under these panels (Frauenhofer, 2022).

The simultaneous use of the same area of land for photovoltaic and agricultural production (which includes aquaculture also) is known as agrivoltaic, agrovoltaic or agrophotovoltaic systems (abbreviated AV or APV). Many studies, carried out in specialized research institutes or universities worldwide, have discussed international best practice in solar energy dual use applications in different regions of the world. As previously stated, a frequently discussed problem in countries where the production of electricity at the level of photovoltaic parks is in full development is represented by the conflict of land use, respectively mono-use versus food production. (Trommsdorff et al., 2022). A variant proposed by researchers and accepted by farmers in several countries is represented by the integration of solar energy and grazing in one place. This solution offers benefits to both renewable energy operators and animal breeders. Thus, the productive use of agricultural land can be maximized, especially pastures or more degraded land, but also to reduce the operating costs of the photovoltaic park (Clean Energy Council, 2021).

After Germany, Japan is the second “birthplace” of the solar dual-use concept (being called “solar sharing”). A guideline for dual-use solar applications on agricultural land has been introduced in this country by the Japanese Ministry of Agriculture, Forestry and Fisheries (MAFF) since 2013 and 2014 (https://www.maff.go.jp/e/pdf). Sheep grazing on solar farms (‘solar grazing’) has been introduced and employed in the past decade across many countries across Europe, Americas and Oceania (especially Australia and New Zealand) (ASGA, 2019).

The Innovative Solar Practices Integrated with Rural Economies and Ecosystems (InSPIRE) program, managed by the National Renewable Energy Laboratory (NREL), seeks in this context to improve the mutual benefits of solar, agriculture, and native landscapes in USA (U.S. Department of Energy, 2022). In additions to sheep, other species (e.g. cattle, goats, horses, rabbits, chickens or geese) have been proposed and tested for solar grazing. Depending on the chosen mounting system of the AV panels (height from the ground, way of orientation, fixing of the supporting frame, etc.) they were considered in the case of certain species unsuitable for large-scale solar farms, for example due to the size (in the case of cattle) or their behavior (in the case of goats) (Stepanek Shiflett, 2021). In the case of rabbits, researchers from the Michigan Technological University tested a new way to integrate the agrovoltaic system in micro farms with the assessment of the life cycle of these animals. (Pascaris et al., 2021).

It must be stated that the obtained electricity on a ‘solar pasture’ it can be used to run the farm with electric tractors and precision equipment or to provide electricity for irrigation installations with reduced consumption and losses. Depending on the daily consumption, the surplus energy can be stored in battery banks, for use during the night or in bad weather, or it can be sent to the grid for use by other farmers (Nealon, 2023).

In countries with a tradition of sheep grazing, good practice guides have been developed in recent years to inform farmers of the advantages of agrivoltaic systems. For example, in 2015 the first Australian solar farm that started grazing sheep and implemented agrisolar practice was Royalla Solar Farm (Stepanek Shiflett, 2021). With the growing interest in Solar Grazing in 2016 in the US, the American Association was established to connect solar farm advocates and agricultural businesses (ASGA, 2019). In many cases it has been shown that animals that graze around solar fields offer several benefits. Besides the fact that their manure enriches the soil, a big advantage is the fact that the consumption of the vegetation around the panels keeps plants from growing too tall and by this to shade the panels. Therefore, by grazing in the area of solar panels, lower vegetation maintenance costs are obtained, largely replacing lawnmowers or other landscaping (Freehill–Maye, 2020). Field-scale arrays of ground-mounted PV modules, or “solar farms”, were seen in Britain only since 2011. The general design and construction philosophy appears to be to minimize any changes to the ecosystem and to maintain agricultural production. To help promote the dual use of land the British Renewable Energy Trust has in July 2014 produced the Agricultural Good Practices Guide which details various agricultural uses such as sheep, geese, chickens and hay (Fletcher & Lewis, 2014).

For sheep or cattle’s farmers, in the context of solar grazing, vertical bifacial solar panels can replace farm fences, thus improving animal welfare, protecting grazing areas and providing protection against predators (Fookes, 2020).

In the year 2020 in Baden-Württemberg (Germany) was put into use the Donaueschingen-Aasen solar park. This is the largest agrivoltaic system in Europe. The 14 ha total area is use for hay and silage (Next2sun

2022). Also, previous experiments carried out on different types of crops have shown that agrovoltaic systems work well with both shade-tolerant and shade-intolerant plants (Sekiyama & Nagashima, 2019).
Fish farming is particularly a sensitive sector in terms of environmental impacts (www.maff.go.jp). In the case of aquaculture in recent years, some applications have appeared for the integration of solar energy in this sector as well. In Asian countries (for example, Thailand or Vietnam), where there is a tradition of eating fish and seafood, photovoltaic power plants have been built over shrimp farms and fish farms on land. They ensure, in addition to the necessary shade for certain periods of the day or for different species, the production of electricity used to operate pumps, aerators or food distributors (Fraunhofer, 2020).
A special attention was paid at a solar pasture, to the mixture of native or sown honey plants, which can provide a habitat for bees and other pollinators (Burns, 2019).
Supporting biodiversity and maintaining or restoring native vegetation in solar farms represented another objective proposed and pursued both in European countries and in the USA or Australia. The aim is thus to provide a protected habitat for wildlife and beekeeping (Horowitz et al., 2020). Thus, the first and most important step when thinking of combining both activities (livestock and energy production) will be analyzing the different crops and livestock options also the legislation in the field of renewable sources for every type land and countries.

MATERIALS AND METHODS

In the next years investments in the solar sector will definitely answer the rising demand for electricity in the all countries. Yet the most likely from an ethical point of view, it must be ensured that the production of food always takes priority, instead of removing the land from agricultural use and installing solar panels on these surfaces. Thus, an agrivoltaic system presents a number of advantages compared to traditional ground-based photovoltaic systems: higher electricity yield (especially in the case of bifacial vertical systems), harvest yield (systems that can self-orient during the day) and savings of water (for washing the panels) compared to usual practices (Campana, 2022). When an agrivoltaic project is proposed, there are usually two or more parties whose interests may be different. On the one hand, the farmer or the land administrator, and on the other, the company developing the solar park. In some cases, the opinion and acceptance of the local community of the development and implementation of such a project is also taken into account (Dreves, 2022).

Agrivoltaic systems

By definition the Agrivoltaic systems refers to the way of farming parallel to generating solar power by installing solar panel arrays on arable land and cultivating crops on the ground, beneath or between the panel (Kim et al., 2021). As is known at the present time there are two types of agrivoltaic systems: a) systems involving agricultural activities on available land in pre-existing PV facilities, and b) systems intentionally designed and installed for the co-production of agricultural crops and PV power (Kumpanalaisatit et al., 2022). There are several possibilities for mounting agrivoltaic systems. They can be installed like a traditional solar plant, with the panels in close rows slightly raised from the ground or spaced and elevated to provide more light to the plants and to allow livestock and farm equipment to move between and under them (Dreves, 2022). In recent years several forms of Agrivoltaic have been developed around the world, the innovative approaches emerging depending on the specifics of the area or countries, the legislation regarding agricultural land, use (for horticultural crops or animal husbandry) etc. (Clean Energy Council, 2021). Typically, agrovoltaic systems are broadly classified according to different criteria (Figure 1). Thus one can take into account: the type of system (closed or open), the type of structure (interspaced PV, overhead PV, integrated PV greenhouses), the inclination of the modules (fixed, one-axis tracking, two-axis tracking) and the type of application (grassland farming, arable farming, horticulture and aquaculture) (Gorjian et al., 2022).
As previously stated, agrivoltaic applications are diverse and include, depending on the country: the production of crops and food

(vegetables, cereals, hay or vines), solar greenhouses, animal production and ecosystem services through vegetation management (Figure 2) (Macknick et al., 2022)

Many studies carried out up to now had as their objectives the finding of optimal technical solutions, from the point of view of the materials used in the manufacture of solar cells, (e.g. modules based on c-Si, a-Si, CIGS, and CdTe) the way of mounting the solar panels and their orientation during the day (Victor du John et al., 2020). The degree of opacity of the photovoltaic panels was also studied, establishing its influence on the development of plants in the area shaded by them and the rate of water evaporation (Yano et al., 2014, Pulli et al., 2020). Dupraz et al. proposed since 2011 a combination of PV panels and food crops to maximize land use. An indicator initially introduced to determine the productivity of a certain land and used to evaluate the value of mixed crop systems is the LER (Land Equivalent Ratio) (Dupraz et al., 2011). Because it combines two types of production on the same land area, the concept of LER can be successfully applied to the agrivoltaic system (dual-use solar system)

So according to the definition, the LER of a dual-use agrivoltaic/solar system is defined as follows:

LER = (Yieldcrop in dual use / Yieldmonocrop) + (Yieldelectricity in dual use / YieldelectricityPV)

Marrou et al. (2013) and Adeh et al. (2018) proposed another coefficient, namely the water usage efficiency (WUE) that quantifies the impact of the PV design on agricultural production.

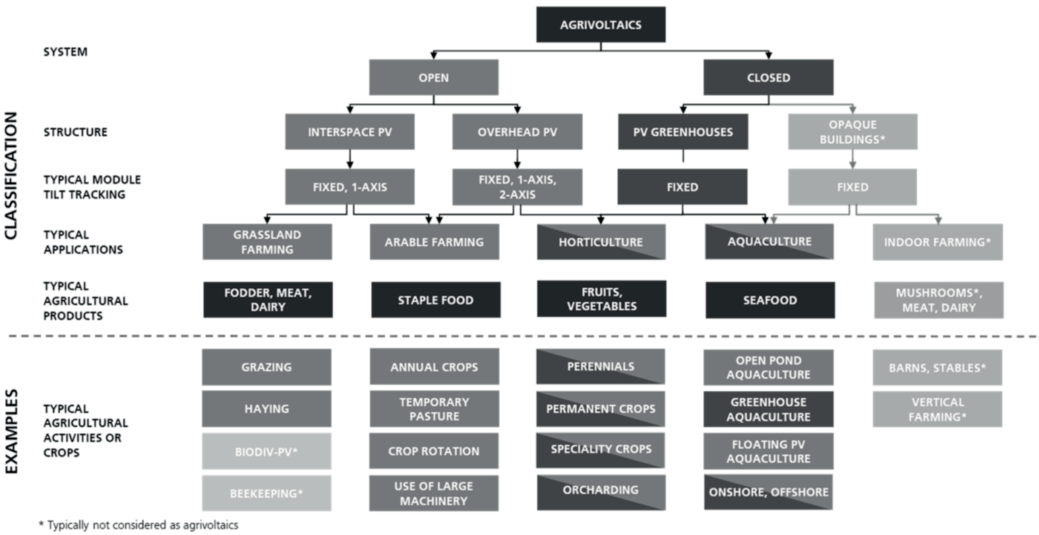

Figure 1. Classification of agrivoltaic systems (Gorijan et al., 2022)

Water use efficiency (WUE) is defined as *the amount of carbon assimilated as biomass or grain produced per unit of water used by the crop* (Hatfield & Dold, 2019).

According to *The Green Grid* (Watkins, 2013), water consumption includes, in the case of MW solar farms, water used for equipment cooling, humidity regulation and on-site electricity generation. The photosynthetic process and the transpiration of the crop represent two elements with major influence on the yield of the crop. It has been demonstrated through numerous studies that the solar radiation of the soil represents an influencing factor of these processes, being a parameter that can change under the conditions of agrivoltaic systems. Also, the important factor for crop growth is the wavelengths between 400 nm and 700 nm of the solar spectrum that is called the photosynthetically active radiation (PAR) (Willockx et al., 2020).

Usually, the design and evaluation of agrivoltaics must open new perspectives, this being possible if the system is approached as a three-dimensional model (Toledo & Scognamiglio, 2021). As shown in the Figure 3, such a model is characterized by a certain degree of randomness, both in the horizontal and vertical arrangement of the modules.

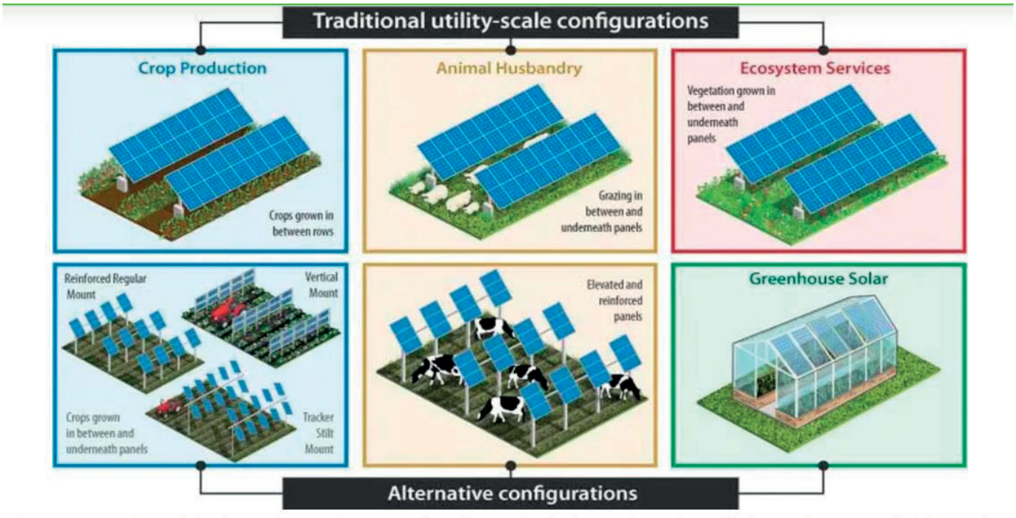

Figure 2. Different types of agrivoltaics systems (Macknick et al., 2022)

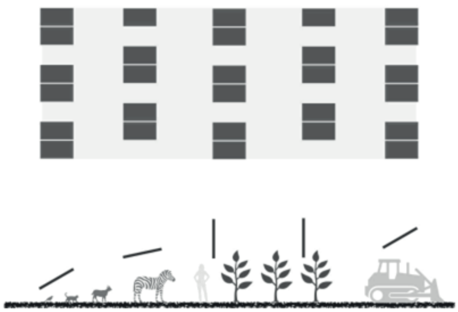

Figure 3. The design of agrivoltaics
(Toledo C. and Scognamiglio A., 2021)

For technical barriers, there are already commercial solutions that combine mobile panels that are automatically controlled by software (Toledo & Scognamiglio, 2021). These can be integrated with a specific agricultural crop, processing data on temperature, soil moisture, weather forecasts, etc., to ensure optimal performance and a better plant protection (Weselek et al., 2019).

The type of farming activity that is possible and its success can be determined by the type of racking system. The construction type and the installation of the panels can affect the availability of the land for vegetation, crops and animal activities (Macknick et al., 2022).

From an agricultural point of view, an agrivoltaic system can reduce water evaporation, protect crops from certain weather conditions and generate new agricultural business models, by being able to incorporate new varieties of shade-tolerant plants, especially for areas intended for grazing (Chalgynbayeva et al., 2023).

In most cases, the photovoltaic panels are positioned approximately 5 m from the ground, free to rotate around 1 or 2 orthogonal axes. At such a height it is possible to use, for example, machines for harvesting fodder or those for meadow maintenance.

Next2Sun Company (Germany) has developed and experimented a series of installations vertical, installations with bifacial photovoltaic modules facing east and west and with a distance about 10 m between the rows, which lends itself very well to activities in agriculture (https://www.next2sun.de).

Bifacial modules are characterized by the fact that both sides have a glass surface, so can both generate electricity. At the moment, bifacial n-type glass-glass modules with an output of approximately 400 watts and efficiency of around 20% are generally used. A vertical fence with a southern orientation produces only around 5% lower electricity yield than an east-west orientation (if neither are shaded (https://next2sun.com/en/solar-fence/solar-fence-the-pv-innovation/).

Already, worldwide, AV systems are used on a GW scale. We can definitely say that an AV system can increase the efficiency of the land, through the massive expansion of photovoltaic energy in the farm area. At the same time, fertile soils are preserved for agriculture or grazing, and in the case of lean soils, biotopes rich in different species can be created (Wirth, 2021).

In recent years, with the decrease in the costs of solar PV technology, it has been demonstrated worldwide that the development of the dual-use application can be successful. Romania can develop its own agrivoltaic good practice projects, taking as an example the countries that have successfully implemented them, this approach being a viable option for Romanian farmers to avoid or reduce agricultural land use conflicts.

RESULTS AND DISCUSSIONS

The concept *Farm 5.0* describes *sustainable agriculture*. The aim of this is firstly to reduce CO_2 emissions, but also for preserve biodiversity and restore natural cycles (Goldbeck Solar GmbH, 2023). PV modules on agricultural land produce electricity used to operate electric agricultural machines (water pumps, tractors, robots) but also for consumers in production buildings (e.g. animal shelters or greenhouses). The farmers' current goal is for the entire farm to become CO_2 neutral (Goldbeck Solar GmbH, 2023). At the same time, an AV system can integrate into the landscape without causing additional damage. Agrivoltaic systems developed for livestock include grazing and livestock management under, around and directly adjacent to solar infrastructure (Macknick et al., 2022). Very important is the way the animals are directed/housed in the area of the AV modules. They can be on site year-round, seasonally or as needed. This is established by the farmer or together with the solar site administrator.

Sheep

From the experience of farmers from countries with an old tradition in raising sheep it has been demonstrated that hair sheep breeds are popular choices into most solar grazing farms (Hartman, 2022). Other breeders prefer the Merino breed that has a reputation for keeping their heads down and not jumping on equipment (Shiflett, 2021).

In any farm, access to water for animals is essential. The Australian experience indicates that having multiple water sources is all the more ideal for housing sheep in a solar farm. In this way, the grazing of the plots can be ensured and at the same time the risk of the animals consuming too much energy at large distances from these sources are eliminated. Another advantage is the reduced risk of dust formation and thus increased contamination of the PV panels (Clean Energy Council, 2021). It is recommended that the grazing plan can be designed such that sheep are rotated from paddock to paddock, preferable every one to four days (Agrivoltaic Solutions, 2020).

In the case of sheep that graze near adjustable panels (with shaft-tracking systems), a problem that must be avoided is the entanglement of the wool in the moving parts of the motors and joints of the adjustment devices (Clean Energy Council, 2021). For the safety of the equipment and the animals, the protective casings of the electric motors and the emergency stop buttons must also be checked periodically. The method of fixing the electrical cables is also very important. They should be fixed tightly behind the modules, reducing the risk of interference with the wiring of the modules and also eliminating the risk of the sheep's horns getting tangled. A very serious accident that can occur in the case of fallen or improperly fixed cables is strangulation (Clean Energy Council, 2021).

Another aspect that any farmer must take into account is the expenses of operating the livestock. They, also known as variable costs generally vary according to: volume of production (e.g cost with sheep expenses, labor expenses), number of grazed sheep or contracted area (Shiflett, 2021).

Sheep grazing around agrivoltaic systems can be a profitable practice offering local, grass-fed lamb, solar grazing in protected areas and free from polluting agents. This technique brings

important incomes to local sheep producers and can help to regenerate damaged land by cultivating specific plant species (Kochendoerfer et al., 2018; Shiflett, 2021; Hartman, 2022).
It is very important that in the case of grazing in the areas of the photovoltaic systems there is a contract between the farmer and the operations manager of the site. This implies collaboration and permanent communication between the farmer and the weekly or monthly maintenance staff. Most of the time, according to local legislation, the farmer can be compensated for grazing under these panels, because grazing significantly reduces the costs of removing excessively high vegetation that can block the orientation systems or cover the surface of the panels. American Solar Grazing Association provides farmers with various sample contracts (Hartman, 2022).

Horses and Catlles

In the case of large animals, such as horses and cattle, the problems that arise are due to the risk of dislocation of the standard mounting systems, the animals having enough strength and weight to destroy them (Scurlock, 2014). In the case of dairy cows raised on pastures provided with AV systems, studies conducted in recent years have demonstrated that in most cases, during the summer, the intensity of heats stress can be reduced and the well-being of the cows can be increased. At the same time, the efficiency of land use also increases (Sharpe et al., 2021). The shadow effect produced by AV modules and the influence on milk production (by reducing heats stress) is a current and future concern of researchers and farmers in areas with solar potential and with herds of dairy cows kept on pasture (Sharpe et al., 2021).
In other case, in a New Zealand farm with Angus bull yearlings was built an array that had panels set at 2.4 meters off the ground. It was observed that the 15-month-old bulls particularly love to scratch on it. The high cost of installation (an extra 80% compared to one suitable for sheep) come from the use of more robust materials to withstand the higher winds found at height, the heavier animals rubbing against them, and added construction costs at height (Delwyn, 2022).

Goats

It is known that the most important characteristic of photovoltaic systems is the production of direct current. The studies carried out in the case of goats, shows some problems due to the fact that they sometimes jump on panels and chew wires (Freehill-Maye, 2020). This behaviour can lead to problems in the operation of an AV system, which generates continuous electricity as long as the surface of the panels is illuminated. Therefore, they cannot be stopped so easily. Due to the fact that sometimes the wires are partially destroyed or the connectors between the panels becomes loose, the flow of current is not interrupted immediately, and the appearance of an electric arc can cause either electrocution of animals or the outbreak of a fire, especially when there is dry vegetation (Wirth, 2021).

Rabbits

In the case of rabbits growth, Michigan Technological University researcher have conducted a life-cycle assessment for a new possibility to integrate the AV system in such a farm. The objectives pursued were the reduction of CO_2 emissions and the consumption of fossil fuels, compared to classic maintenance systems. Three experimental variants were analyzed and compared: an agrivoltaic project based on pasture-fed rabbits, solar photovoltaic generation combined with conventional rabbit farming, and a conventional electricity generation business combined with rabbit farming. In the case of the developed new agrivoltaic concept the results showed a 69.3% reduction in CO_2 emissions and an 82.9% reduction in fossil fuel consumption, compared to non-integrated production (Pascaris et al., 2021). In another study by Lytle et al., in 2020, it was found that the integration of some AV systems in rabbit farms may have enough benefits to lean towards their widespread use. For example, economic gains can be recorded (income either from the sale of rabbits or from land rent), cost savings for maintenance (e.g. by using the ground support for the panels, as a support for the installation of protective fences) and a better animals welfare (through protection against of the sun or precipitation and last but not least of the predators from the air) (Lytle et al., 2020).

Honeybee

The problem of natural pollinators, especially bees, is also a fairly current and intensively discussed topic. The dangers, to which bees are exposed, due to the excessive use in agriculture of various chemical substances as well as due to urban pollution, have been a topic of worldwide interest for many years. A solution that can help both the breeder and the local bee population can be the use of areas with potential for the growth of honey plants for a double purpose: the integration of AV systems on these surfaces and the cultivation of some plant species without the risk of being contaminated.

At the same time, the success of a solar beekeeping venture depends on a solid relationship between the beekeeper and the solar project manager. The best practice guidelines established after the studies carried out by in the New York State in the field of solar beekeeping recommend, among other things, the establishment of positive working relationships between a commercial beekeeper and a solar site (MacKenzie, 2021). It must be taken into account that beekeepers prefer unrestricted access to their apiary locations. Another requirement of the beekeepers is related to the confidentiality of the location of the hives, to ensure the security of the bee colonies. But, at the same time, those who maintain the panels must be warned not to destroy the hives by mistake and worse, not to be attacked by bees. Another aspect that should not be omitted is the bees' access to water, for drinking and moderate temperature in the hive, the source being preferably located near the hive (MacKenzie, 2021).

Fish

The integration of solar infrastructure with aquaculture activities constitutes another area of interest, being an application that is starting to be promoted and developed especially in Asian countries (Vo et al. 2021). Even if in some countries the use of these systems in intensive fish farms are not yet recognized as agrivoltaic systems, with climate change they can be successfully integrated to ensure, in addition to the production of energy necessary for the operation of various equipment (pumps, filters, aerators, food distributors, etc.) better conditions for the growth of species less tolerant to light or high water temperature.

Agrivoltaic in Romania

Due to its geographical position, Romania has a fairly extensive solar potential. The country benefits from approximately 210 sunny days per year in the south-east, west, center and east, which are the best places to locate a PV park (Vrînceanu et al., 2019). In 2021, approximately 7% of the energy generated in Romania was from solar PV sources (International Trade Administration, 2022). The government of Romania announced plans to add around 7 GW of new renewable capacity comprising around 3.7 GW of solar energy by 2030 (Mordorintelligence, 2022). In the year 2020 about 38.75 % of the arable area of Romania utilised for agriculture and horticulture (Data Worldbank Romania). The concept of "prosumer" introduced in Romanian legislation starting in 2018, allows people to produce green energy for their own consumption and deliver any surplus to the distribution grid (RWEA 2021).

The biggest problem for the symbiosis of agriculture and energy in is the ban on the use of concrete constructions on agricultural land. Until now, the process of building SRE in agricultural areas in Romania has been quite complicated and confusing. First of all, the land must be prepared, the status changed from "agricultural" to "for construction purposes", so that the construction activities of SRE plants can start on it. The fees for this transformation in Romania are not small at all and are paid before there is any certainty regarding the final success of the project (European Commission, 2020). According to Emergency Ordinance 34/2013 regarding the organization, administration and exploitation of permanent pasture and for the amendment and completion of the Land Fund Law no. 18/1991 ("Pasture Ordinance"), permanent pasture located outside the village can only be used for animal grazing and fodder production (Nyerges, 2022; Bellini, 2022).

By way of exception, the permanent pasture located outside the village can be used for

certain objectives, including the establishment of new renewable energy production capacities.
A practical solution to ensure the coexistence of solar projects and grasslands is to install taller poles to allow livestock grazing and forage production under the panels (Nyerges, 2022). By Law no. 21/2023, the new changes are implemented that favor the acceleration of the development process of photovoltaic parks in Romania and of green energy in Romania with a significant period of time. (Official Monitor - Law no. 21 of January 9, 2023)
The new rules also set lower permit fees for PV projects in case of dual system applications. (Mărgărit et al., 2023).
The new concepts of agrivoltaic use developed on the plan worldwide can be represent land sharing solutions without interfering with different environmental policies.

CONCLUSIONS

Different national legislations forbid the fixing of photovoltaic panels to the ground using concrete, which limits the technical possibilities of installation. An Agrivoltaic system defuses the land use conflict and offers farmers additional sources of income. With this, farmers can supplement or even replace their electricity consumption from conventional sources and at the same time improve the conditions for raising animals and fodder plants. The numerous studies carried out in recent years in countries on all continents, show that an AV power plant is not incompatible with the grazing activity or other agricultural activities carried out on the land where it is locate. And unlike classic PV constructions, it has a minimal impact on the environment. Installing solar panels on farmland, slightly elevated to allow sheep or other animals to graze underneath, is an easy way to combine food production and electricity production on the same land. As long as there is enough sun for grass to grow, sheep provide the advantage of controlling vegetation, thus avoiding overgrowth and fire hazards. Farmers hope that the new amendments to the Land Fund Law will open a new stage in the development of agrivoltaic systems in Romania.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

REDUCTION OF THE GREENHOUSE GAS EMISSIONS FROM THE PIG MANURE USING INORGANIC SUBSTANCES

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Abstract

Pig farming is one of the most productive and early maturing branches of animal husbandry, which plays an important role in the meat balance of the state. Simultaneously with the production of the main products a significant amount of by-products of animal origin accumulates. A large number of pollutants of various origins are released into the atmosphere during the decomposition, including greenhouse gases, which causes pollution of the soil, surface and underground waters. Therefore, the basis of the planned research was to find out the emission of the greenhouse gases from the pig manure under the influence of different doses of inorganic substances (natural sorbents). According to the results of the conducted research, it was established that the level of release of the studied gases – methane (CH_4), carbon dioxide (CO_2) and nitrogen oxide (NO) from the pig manure (in vitro) when using different doses of natural sorbents – vermiculite and saponite with a simultaneous shift of the pH to the acidic side respectively, was 5.31 and 5.67, against 6.2–6.5 in the control. In a comparative assessment of the effect of the investigated sorbents on the emission of greenhouse gases from the pig manure, it should be noted that the level of release of CH_4 from the substrate in the variants with vermiculite decreases to 18.6%, CO_2 to 37.5%, and NO to 25.7%, and with saponite, respectively up to 7.2%, 20.2 and 22.3%. Therefore, the obtained data indicates the expediency of the usage of the studied sorbents, in particular vermiculite, to reduce the emission of greenhouse gases during the storage of the manure in the storage facilities (lagoons) and directly in the premises, which will make it possible to minimize the negative impact of intensive pig farming on the state of the environment.

Key words: *carbon dioxide, methane, natural sorbents, nitrogen oxide, pig manure.*

INTRODUCTION

Animal husbandry is an important branch in the formation of food security and independence of the state, as its share in the food structure is more than 45% (Myhalko, 2021; Lykhach et al., 2021). In the structure of the production of livestock products, the leading role belongs to the pig industry, which provides food products in a relatively short period of time, which have a stable and wide demand on the consumer market (Lozynska, 2014). In the structure of meat production, pig farming is the second largest after poultry farming (Nykyforuk & Zhukorskyi, 2014). The share of pork in total meat consumption is more than 20%. Today, the consumption of this product in our country is lower than the recommended norms. In particular, the state provides the needs of the domestic market in meat consumption – at the level of 52 kg per person per year, which is 28 kg less than the rational norm which is 35%. In the structure of the world meat balance, the first place is taken by pork (about 37.6%), the second place is poultry meat (35%), the third one is beef (22.8%) (Myhalko, 2021). However, the increase in food supply, both for the population of our country and for export demand, is accompanied by the accumulation of a much larger amount of by-products of animal origin, especially of waste. Thus, during the production of 1 kg of pork (for the growth of 600 g per day), 11 kg of waste is obtained, which is a source of gases entering the environment – methane, carbon dioxide, nitrous and nitrogen oxide (Monteny et al.,

2001; Nykyforuk & Zhukorskyi, 2014; Marszałek et al., 2018).
According to the Food and Agriculture Organization of the United Nations (FAO), in the agriculture, forestry and fisheries sector, greenhouse gas emissions have doubled over the past 50 years, and their further increase is predicted until 2050 by 30% (Tubiello et al., 2014). According to the evaluations of foreign scientists, animal husbandry causes emissions – 44% of anthropogenic methane, 53% of nitrous oxide and 5% of anthropogenic carbon dioxide, which provokes acidification and eutrophication of ecosystems and contributes to global warming process (Gerber et al., 2013). An analysis of a number of literary sources shows that methane has a 21-34 times higher global warming potential than carbon dioxide and is able to accumulate in the atmosphere for up to 12 years (Herrero et al., 2013, Marszałek et al., 2018, Caro, 2019). The climate potential of such a greenhouse gas as nitrous oxide is 265-310 times higher than CO_2 (Dennehy et al., 2017; Marszałek et al., 2018; Caro, 2019). The latter also causes the destruction of the stratospheric ozone layer, which protects living or-anisms from the sun's ultraviolet radiation. The accumulation of greenhouse gases in the atmosphere contributes to the increase in the average annual temperature, the appearance of acid rain, droughts, floods, the formation of atmospheric aerosol and the reduction of drinking water supplies, etc. (Monteny et al., 2001; Khodorchuk et al., 2014; Caro, 2019).
In the structure of animal husbandry pig farming ranks second in the level of greenhouse gas emissions (methane and nitrous oxide), after cattle breeding, and first in terms of their excretion from the manure (Nykyforuk & Zhukorskyi, 2014). Thus, according to the data of V.V. Herman (2009), methane emissions from intestinal fermentation of pigs with the amount of 1.5 kg/head/year and from the manure decomposition – 3.19 kg/head/year (Herman, 2009). According to the National Cadastre of anthropogenic emissions of greenhouse gases the second place in terms of the volume of their formation in Ukraine belongs to animal excrements and pig farming accounts for 46% of all branches of animal husbandry. Thus, the priority tasks in pig farming are not only the need to ensure the growing demand for pork, but also to significantly reduce the impact of the production of this product on the environment.
A large number of studies, both of domestic and foreign scientists, are devoted to the prevention of environmental pollution as a result of the activities of agricultural enterprises. However, in literary sources, organic materials or acids are mainly used to reduce gas emissions from waste, he last ones which are dangerous and harmful to use (Cicek et al., 2004; Shah & Kolar, 2012; Petersen et al., 2014; Misselbrook et al., 2016; Maurer et al., 2017; Dalby et al., 2022). Also, in the studies of many scientists the attention is focused on the adsorption of moisture from organic waste and reducing the level of ammonia or hydrogen sulfide release (Alvarado et al., 2015; Broshchak et al., 2018). At the same time, the problem of the negative impact on the environment of emissions from by-products of animal origin of the set of the greenhouse gases – CH_4, CO_2, NO – remains relevant and poorly studied. Thus, the search for the effective ways and means to reduce the level of environmental pollution with greenhouse gases from the waste products (manure) of pigs has both scientific and practical importance, which will thereby increase the efficiency of pig farming.
The aim of the study was to find out the effectiveness of different doses of natural sorbents – vermiculite and saponite on the emission of greenhouse gases (CH_4, CO_2, NO) from the pig manure (*in vitro*).

MATERIALS AND METHODS

The research on establishing the influence of different doses of natural sorbents on the level of greenhouse gas emissions from pig manure was carried out using laboratory, statistical and analytical methods. To carry out the experiment, samples of pig manure were taken from the State Enterprise "Experimental farm Radekhivske" Institute of agriculture of the Carpathian Region of the National Academy of Agrarian Sciences of Ukraine.
The study was conducted *in vitro* (Skliar et al., 2019). Hermetically sealed containers were used in the experiment, ensuring anaerobic conditions. For the stability of the biofermentation

process, the humidity of the substrate was 92%. During the experiment, the temperature was within the psychrophilic regime.

To reduce the emission of greenhouse gases after passing through the stages of hydrolysis, oxidation, acetogenesis, natural sorbents were added to the fermented pig manure for 11 days in different doses, presented in the following options: Option I – control (without adding substances); II – vermiculite, 2.5%; III – vermiculite, 5%; VI – vermiculite, 10%; V – saponite, 2.5%; VI – saponite, 5%; Option VII – saponite, 10%. Each variant of the experiment was repeated three times.

In the control and experimental variants, the level of emissions of gases – CH_4, CO_2 and NO was measured one day after the introduction of the substances, and thereafter every three days until the end of the research. Determination of the amount of greenhouse gas emissions during anaerobic fermentation of pig manure *(in vitro)* was carried out with the Dozor S-M-5 gas detector-analyzer (certificate of verification of legally regulated measuring equipment No. 84709/92 and certificate of conformity No. UA.TR.002. CB.1234-19). In the experiment, acidity (pH) was determined at the beginning and at the end of the experiment with a Tur N5170 pH meter. During the research, mixing of the fermented mass was carried out by shaking the containers. The conditions of the biofermentation process were similar in all variants, both in the control one, where anaerobic fermentation of the substrate took place due to the natural microflora of manure, and in the experimental analogues with the introduction of the investigated natural sorbents in different doses.

Statistical analysis of research results was carried out using the methods of variational statistics with the help of *Microsoft* Excel and *AtteStat* programs using the Student's t-test. A simple avarage (M) and its errors (±m) were calculated. Differences between simple averages were considered probable according to: * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

RESULTS AND DISCUSSIONS

During the research, it was established that the introduction of natural sorbents into the pig manure contributed to the inhibition of fermentation processes and is confirmed by the shift of the pH indicator (Figure 1) to the acidic side. In particular, in the versions with natural sorbents – vermiculite and saponite, a lower pH level of 5.31 and 5.67 respectively, was observed, which indicates the inhibition of the vital activity of microorganisms due to a higher concentration of H+ ions. In the control (without introduction of the substances), the pH of the studied substrate *(in vitro)* throughout the experiment, both at the beginning and after its completion, was in the range of 6.2-6.5.

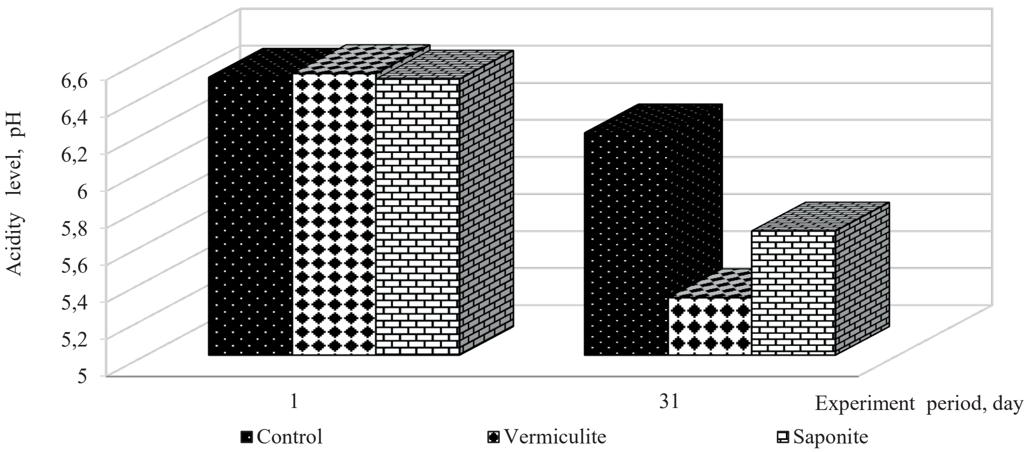

Figure 1. Level of acidity pig manure using natural sorbents

On the basis of the obtained experimental data, it was established that the studied natural sorbents, at the same time as the pH of pig manure decreases, cause a decrease in the emission of greenhouse gases – CH_4, CO_2, and NO.

The analysis of research results shows that the most effective effect on reducing the level of greenhouse gas emissions from pig manure *(in vitro)* when using natural sorbents – vermiculite, saponite – is observed on the fourth day. At the same time, the prolongation of the positive effect on the reduction of the emission of the investigated gases from the fermented substrate with the introduction of sorbents gradually decreased up to 31st day of the research.

The obtained experimental data indicates that the process of emission of greenhouse gases from the pig manure depends on the duration of its storage.

In the course of the experiment, it was established that the addition of the natural sorbent vermiculite in doses of 2.5%; 5 and 10% contributes to a lower level of CH_4 release (Figure 2) from pig manure *(in vitro)*, compared to the control, namely: first day – by 11.8% ($P<0.05$); 15.1 ($P<0.001$) and 14.6% ($P<0.01$), with an increase in the effectiveness of exposure on the 4th day, respectively – 14.8% ($P<0.01$); 18.6 ($P<0.001$) and 16.3% ($P<0.01$), and on the last day of the experiment – by 2.4-5.5% ($P<0.05$). The introduction of saponite in the above-mentioned quantities into the fermented researched substrate leads to a decrease in methane emissions, depending on the day of the experiment, respectively, by 2.5%; 6.5 ($P<0.01$) and 5.3% – on the first day, by 2.8%; 7.2 ($P<0.01$) and 5.8% ($P<0.05$) – on the fourth day, by 0.3-0.4% – on the 31st day of research, in relation to the control analogue.

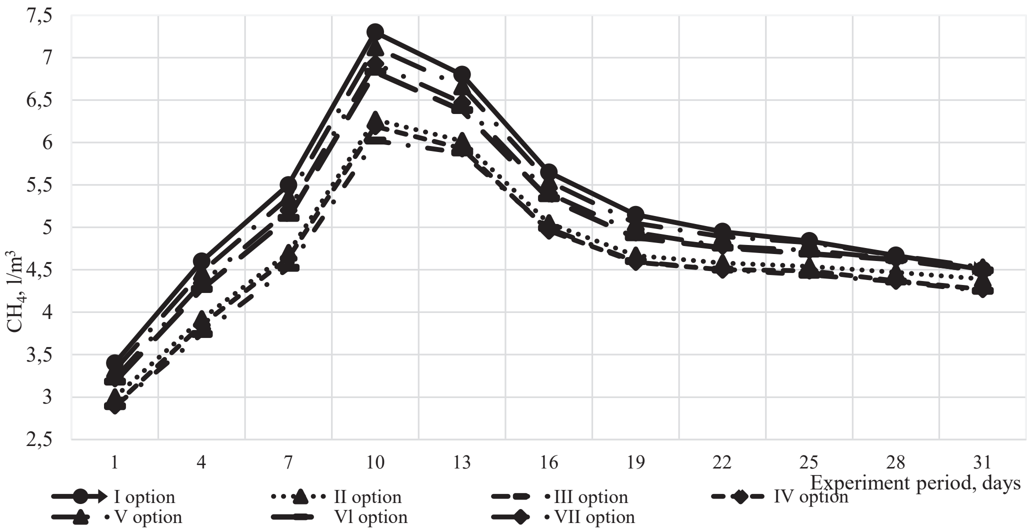

Figure 2. The level of CH_4, emission in variants with the use of different doses of natural sorbents – vermiculite and saponite

The use of different doses of the studied natural sorbents – vermiculite and saponite – also has a positive effect on reducing the level of carbon dioxide release (Figure 3) from pig manure. In the variants with the addition of vermiculite, there is a decrease in CO_2 emission from the investigated substrate in the process of anaerobic fermentation *(in vitro)*, depending on the quantities – 2.5%; 5 and 10%, respectively, 1st day – by 25.4%; 33.2 and 29.5%, 4th day – by 29.7% ($P<0.01$); 37.5 ($P<0.01$) and 32.5% ($P<0.01$), and on the last day of the experiment – by 7.5%; 8.9 and 6.5%, compared to the control. The obtained research results show that adding saponite to pig manure in similar doses contributes to lower release of carbon dioxide, compared to the control, by 4.5%; 16.6 and 10.2% – 1st day, by 6.7%; 20.2 ($P<0.05$) and 12.7% – 4th day, by 0.8-5.3% – 31st day, respectively.

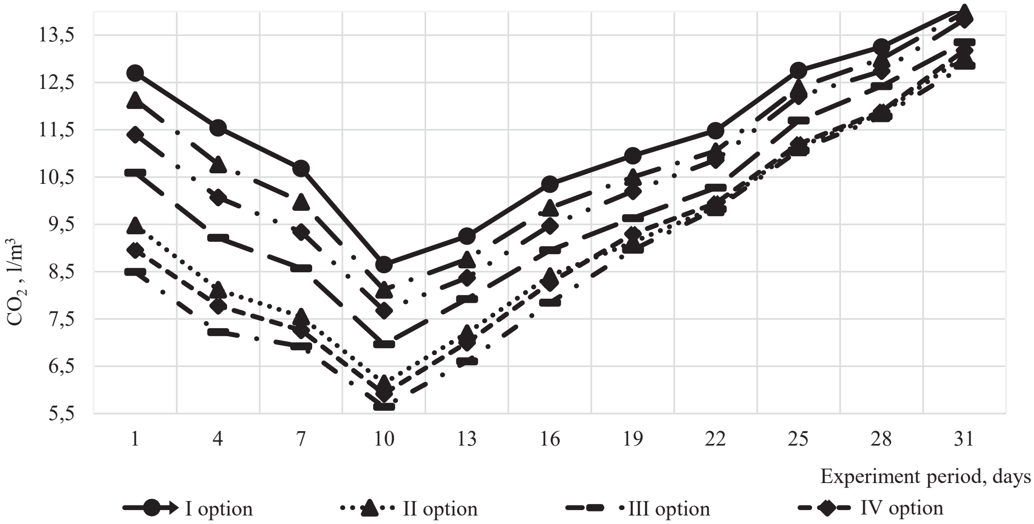

Figure 3. The level of CO_2 emission in variants with the use of different doses of natural sorbents – vermiculite and saponite

The emission level of nitrogen oxide from pig manure *(in vitro)* using the natural sorbent vermiculite in doses is 2.5%; 5 and 10% decreases, depending on the day of the experiment, by 14.3% ($P<0.05$); 20.2 ($P<0.05$) and 18.1% ($P<0.05$) – 1st day, by 18.8% ($P<0.05$); 25.7 ($P<0.01$) and 21.3% ($P<0.05$) – 4th day and 5.9-7.1% – the last day of the experiment, compared to control. The use of saponite in similar quantities leads to a decrease in the release of NO from the fermented researched substrate, respectively: by 6.1%; 17.7 ($P<0.05$) and 7.6% – 1st day, by 13.2% ($P<0.05$); 22.3 ($P<0.01$) and 8.6% – 4th day, by 1.5-6.1% – 31st day of the experiment.

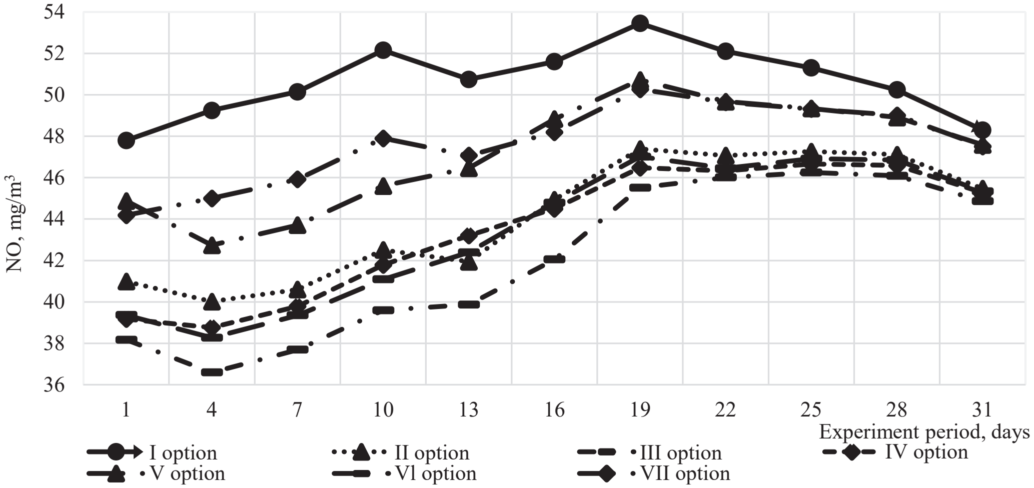

Figure 4. The level of NO emission in variants with the use of different doses of natural sorbents – vermiculite and saponite

Therefore, based on the analysis of the obtained research results, it should be noted the positive effect of different doses of the investigated natural sorbents – vermiculite and saponite during anaerobic fermentation of the pig manure *(in vitro)* on the reduction of the greenhouse gas emissions – CH_4, CO_2 and NO. It was experimentally established that the most effective impact on the reducing of the level of greenhouse gas emissions from the studied substrate was observed both when using vermiculite and saponite in the amount of 5%, while increasing the dose of natural sorbents did not show a better result.

Summarizing the data obtained in the course of the research, it should be noted that the best results for reducing the emission of investigated greenhouse gases from pig manure *(in vitro)* from natural sorbents (vermiculite, saponite) were obtained in variants with vermiculite (by 18.6-37.5%) in the optimal amount (5%), while saponite in a similar dose causes a lower level of gases compared to the control – by 7.2-22.3%. Thus, vermiculite has a more effective impact on reducing the emission of the gases – CH_4, CO_2 and NO from the investigated substrate – by 3.4-17.3%, compared to perlite.

Therefore, the results of the research indicate the prospects of using the studied natural sorbents to reduce the level of greenhouse gas emissions, both in the premises where pigs are kept, and in the manure storage facilities (lagoons) when storing by-products of animal origin.

CONCLUSIONS

The positive effect on the reduction of the emission of greenhouse gases – CH_4, CO_2 and NO from pig manure during anaerobic fermentation *(in vitro)* has been experimentally confirmed of the investigated natural sorbents in the various doses. It is experimentally proven that the use of vermiculite and saponite is most effective in a dose of 5% and causes a lower level of gas release – CH_4, CO_2 and NO from the waste products (manure) of pigs by 18.6-37.5% and 7.2-37.5%, respectively.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

ANIMAL WASTE AS A SOURCE OF GREENHOUSE GAS EMISSIONS AND A FACTOR OF CLIMATE CHANGE

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Abstract

The anthropogenic impact of agricultural production on the atmosphere occurs as a result of the release of decomposition products of organic waste into it – greenhouse gases, which contribute to global warming and climate change, and thus to a decrease in the efficiency of agriculture. Livestock waste due to mass accumulation is not only a valuable organic fertilizer, but also a producer of environmental pollution, as it causes a significant ecological load on soils, surface and underground waters. The article examines the problem of environmental pollution by greenhouse gases in the livestock industry, the main trends of climate change, causes and consequences. The harmful impact on the natural environment of the activities of large livestock complexes is analyzed. The livestock sector has a significant contribution to the total greenhouse gases emissions, which reach 18% and exceed emissions from transport (14%). The main greenhouse gases are characterized and their role in creating the greenhouse effect is revealed. Certain promising directions for solving the considered problems have been outlined, which will make it possible to minimize the negative impact of livestock farming on the environment.

Key words: *animal husbandry, climate change, greenhouse gases, pollution, waste.*

INTRODUCTION

Solving the issue of increasing the greening of livestock production is given priority in modern global approaches to reducing the negative impact of agricultural production on the environment (Smith et al., 2008; Tubiello et al., 2014; Caro, 2019). Agricultural production is closely related to environmental conditions, availability and exploitation of natural resources – land, water, forests, flora and fauna (Smith et al., 2008; Demchuk et al., 2010; Mykhailova, 2016).

The future of the country is impossible without a powerful agricultural sector of the economy, the success of which requires the stable development of animal husbandry. The development of this industry remains an important condition for food security, a stable socio-economic state of the state, and a significant reserve for the export of agricultural products (Demchuk et al., 2010; Binkovska and Shanina, 2016; Furdychko et al., 2019). At the same time, the activity of agricultural enterprises leads to an increase in the anthropogenic load on the environment, due to the accumulation, simultaneously with the main products (milk, meat, eggs, etc.) of a significant amount of by-products of animal origin, in particular waste (Johnson et al., 2007; Herrero et al., 2013; MacLeod et al., 2013). Due to the accumulation of manure, the formation and emission of greenhouse gases into the surrounding natural environment – methane (CH_4), carbon dioxide (CO_2), oxide (NO) and nitrous oxide (N_2O), etc., which act as moving factors of climate change (Khodorchuk et al., 2014; Caro, 2019; Pinchuk and Borodai, 2019). Protection of the natural environment is considered one of the most important tasks, because without scientifically based and purposeful work in this direction, effective management of the livestock industry will be impossible.

Greenhouse gases, entering the atmosphere, absorb heat and retain thermal radiation, thereby contributing to the increase in the average annual temperature of the Earth's surface, which causes the rise in the level of the oceans, the frequency and power of natural disasters and cataclysms (desertification, landslides, hurricanes, droughts, floods), the occurrence of acid rains, the formation of atmospheric aerosol and the reduction of drinking water supplies (Monteny et al., 2001; Johnson et al., 2007; Binkovska & Shanina, 2016).

Climate change on the planet is one of the most urgent environmental problems today in the context of the development and implementation of strategies to reduce greenhouse gas emissions and the gradual transition to low-carbon development of all sectors of the economy and components of human life (Khodorchuk et al., 2014; Mykhailova, 2016; Caro, 2019). Thus, taking into account the danger of the occurrence of the greenhouse effect at the global level, great efforts are currently being made to reduce greenhouse gas emissions into the environment.

Ways of solving this issue are reflected in a number of successively concluded international agreements (Kholod, 2009; Udova et al., 2014; Pinchuk, 2015). In particular, the United Nations Framework Convention on Climate Change was adopted in 1992. Five years later, the Kyoto Protocol was adopted in the Japanese city of Kyoto – an international agreement on limiting emissions of greenhouse gases into the atmosphere (Kholod, 2009; Udova et al., 2014). Given that with the help of the tools provided by the first two agreements, it was not possible to achieve a significant reduction in greenhouse gas emissions, the Paris Agreement was concluded, which is aimed at reducing the level of greenhouse gas emissions and limiting the increase in air temperature to 1.5ºC from the pre-industrial level. In the Paris Agreement, Ukraine undertook to limit greenhouse gas emissions to a level that would not exceed 60% of the 1990 level by 2030 (Mykhaylova, 2016; Tymoshchuk et al., 2022).

An objective assessment of climate change is periodically provided by the Intergovernmental Panel on Climate Change (IPCC). In 2007, the IPCC in the IV Report on the assessment of climate change notes that the concentration of CO_2, compared to the pre-industrial era, has increased by a third, and there has also been an increase in the level of methane and nitrous oxide. Over the 100-year period (1906-2005), the temperature increase according to the linear trend is equal to 0.74°C. (Mykhaylova, 2016). In 2018, the IPCC in its V report, which became the scientific basis for the Paris climate agreement, emphasized that human activities have caused 1.0°C of global warming above pre-industrial levels, with a likely range of 0.8°C to 1.2°C. The VI Climate Change Assessment Report (2021) emphasizes that human activity, accompanied by emissions of greenhouse gases and aerosols, has led to an increase in temperature in the climate system at rates unprecedented in at least the last 2,000 years. The increase in temperature occurs more over land (by 1.6°C) than over the sea (0.9°C). It is believed that the critical limit of the increase in the average annual temperature is 2-2.5°C, since its further increase will lead to a catastrophic state of the biosphere. The document states that the concentration of carbon dioxide has increased by 47% since 1750, and that of methane by 156% (Timoshchuk et al., 2022).

Considering the above, it is necessary to use the potential of agriculture under the conditions of minimizing the negative consequences of global warming. In order to ensure a sustainable ecological situation on our planet, maintaining a balance between production and cost-effective ways of disposing of organic waste is an extremely important condition in the livestock industry.

The purpose of the article is to highlight the problems of environmental pollution with greenhouse gases in the livestock industry.

MATERIALS AND METHODS

Data from scientific literature, periodicals of foreign and domestic scientists, regulatory documents, systematic and complex approaches to the level of environmental pollution due to the activity of livestock complexes served as the theoretical and methodological basis of the analysis. The following methods were used to process information: empirical-theoretical (collection, anamnesis and synthesis of scientific information), theoretical (definition, description, interpretation) and abstract-logical (logical approach to the formation of conclusions and proposals), etc.

RESULTS AND DISCUSSIONS

Agricultural enterprises are powerful sources of environmental pollution because they produce much more waste than can be disposed of in their surrounding areas (Smith et al., 2008; Demchuk et al., 2010; Binkovska and Shanina, 2013). Millions of tons of organic waste from livestock farms, processing enterprises, etc. are accumulated annually in our country, that leads to certain problems with their storage and further use (Aneja et al., 2008; Burlaka et al., 2016; Korbych, 2021). It is known that in Ukraine, more than 52 million tons of manure is produced from cattle, pigs and poultry kept by enterprises of the agro-industrial complex (Buzovsky et al., 2008). In particular, on large livestock complexes, the amount of manure per year can be: on pig complexes with a livestock of 12,000 heads – up to 36,000 m^3, on complexes for beef production with a herd of 10,000 heads – 95 thousand m^3, on large farms for milk production with a herd of 800 cows – 16 thousand m^3 (Burlaka et al., 2016). A typical 100,000 pig farm produces about 1,000 m^3/day (up to 365,000 m^3/year) of manure (Zakharchenko, 2017).

Most manure is produced in cattle breeding (44%), pig farming (39%) and poultry farming (17%) (Pinchuk and Boroday, 2019). For example, for each liter of milk (with a productivity of 5 thousand liters per year), 4 kg of excrement are produced, for 1 kg of pork (for live weight gains of 600 g per day) – 11 kg, for 1 kg of beef – 30 kg (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 the water and litter that gets into the manure (Buzovsky et al., 2008). It is also known that the production of 8,000 eggs is accompanied by the accumulation of 277 kg of droppings. In particular, one laying hen produces 40-65 kg of droppings with a moisture content of 65-75% per year, which is three times more than the resulting egg mass, which is 15-18 kg, and the latter exceeds the hen's own weight by 5 times (Zhukov, 2016). It should be noted that in order to ensure food security, domestic producers must produce 8,230,000 tons of milk, 135,000 tons of beef, 600,000 tons of pork, and 1,620,000 tons of poultry meat. and eggs 14,100 million pieces. Taking into account the above, the priority tasks are not only the need to provide the population with the optimal amount of livestock products, but also the corresponding quality, as well as to minimize the negative impact on the environment of the by-products obtained at the same time, to guarantee the welfare of animals and at the same time the profitability of the industry. Therefore, large livestock complexes require special attention. According to Clause 23 of the Resolution of the Cabinet of Ministers of Ukraine No. 808 dated August 28, 2013 "On approval of the list of activities and facilities that pose an increased environmental hazard", livestock complexes for raising pigs in the amount of 5 thousand heads or more, cattle livestock – from 2,000, fur animals – from 3,000, poultry – from 60,000 laying hens and 85,000 broilers, as well as meat plants and meat processing enterprises, productions for processing and disposal of animal waste origin, in particular, poultry farming, fish farming and leather tanning operations belong to objects of increased ecological danger (Palapa et al., 2016).

Livestock waste is characterized by a high content of nutrients. However, they are not only a valuable fertilizer, but also represent a significant ecological burden on the air environment, soils, water reservoirs and groundwater, contribute to the spread of pathogenic microorganisms, weed seeds, eggs and larvae of helminths, flies, etc. (Melnyk, 2009; Demchuk et al., 2010; Iashchenko, 2010). Thus, violation of the technology of keeping animals and storing waste causes nitrogen, phosphorus and other substances to enter surface waters, which pollute them and harm wetlands and coastal ecosystems. Enrichment of a reservoir with biogenic elements causes its eutrophication, i.e. rapid development of algae and an increase in the number of zooplankton, resulting in the death of algae, the formation of phenols and hydrogen sulfide, which leads to the poisoning of all living organisms in the reservoir (Palapa et al., 2016; Korbych, 2021).

When a large amount of waste is accumulated, in particular manure, as a result of the decomposition of organic matter, both in aerobic and anaerobic conditions, under the action of microorganisms, the formation of volatile decomposition products occurs, as a result of which a significant number of gaseous air pollutants of various origins are released into the atmosphere, including greenhouse gases (Monteny et al, 2001; Moller et al, 2004). According to the National Cadastre of anthropogenic emissions of greenhouse gases, the second place in terms of the volume of their formation in Ukraine belongs to animal excrements: pig farming accounts for 46%, cattle breeding – 30%, poultry farming – 20%, other types of animals – 4% (Vovk, 2021; National inventory of anthropogenic emissions). Only one pig farm for 100,000 pigs or a cattle complex for 35,000 animals pollutes the environment as much as a large industrial center with a population of 400-500,000 people (Maksishko and Malyk, 2012; Zakharchenko, 2017). Methane emissions from cattle are about 90 million tons per year, or almost 16% of the annual global emissions of this greenhouse gas (Pinchuk, 2015). Violations of manure and litter storage technologies cause air emissions of 7% of the total volume of nitrous oxide emissions (Palapa et al., 2016). During the 15 months of its growth, the calf gains about 500 kg of weight and at the same time emits into the atmosphere the amount of methane equivalent, from the point of view of the greenhouse effect, to 75 thousand km of mileage of an average car, which is 5 times more than the car travels in the same period of time (Binkovska and Shanina, 2013). Poultry droppings accumulated throughout the year in poultry farms, where up to 400,000 laying hens are grown, decompose and release into the atmosphere: up to 700 tons of biogas, of which 208 tons of carbon dioxide or 30%, 462 tons of methane, i.e. 66% and up to 5% – other gases and compounds – hydrogen sulfide, skatole, indole, ammonia, hydrogen, etc. (Dutka, 2018). Thus, the average anthropogenic load on the territory of Ukraine from poultry waste is 0.22 million tons/km^2 that for every thousand of population is 3000 tons (Iashchenko, 2010).

The exhaust ventilation system in complexes with livestock of 10-40 thousand pigs emits up to 6.05 kg of dust, up to 14.4 kg of ammonia, up to 5 kg of hydrogen sulfide and up to 83.4 billion microbial bodies into the atmospheric air within an hour (Krychkovska et al., 2013; Zakharchenko, 2017; Vovk, 2021). 8.7 billion microbial bodies, 0.75 kg of dust, 4.8 kg of ammonia and 2,058 kg of moisture in the form of aerosols are released per hour from a farm with a capacity of 2,000 cows. In a complex for 10,000 calves, 103.9 billion microbial bodies, 6.2 kg of dust and 23 kg of ammonia are released into the air in one hour in winter. A farm with 720,000 poultry emits up to 41.1 kg of dust, up to 13.3 kg of ammonia, up to 1490 m^3 of carbon dioxide and up to 174.8 billion bacteria into the air in one hour (Krychkovska et al., 2013). The amount of ventilation emissions from one typical poultry house for keeping laying hens or raising broilers is 10-50 thousand m^3/h in winter, and 200-500 thousand m^3/h of polluted air in summer. In a poultry house, an average of 25 mg of ammonia, 15 mg of hydrogen sulfide, and 8 mg of carbon dioxide are released per hour from one m^2 of litter surface, and from each m^2 of litter-free litter of natural moisture – 8 mg/h, respectively; 5 and 5 mg/h. The emission of these gases increases significantly with an increase in the humidity of the waste and non-compliance with the regulatory parameters of the microclimate (Melnyk, 2009).

The harmful effect of pollution can be more significant when livestock complexes are located near settlements or water resources (Smith et al., 2008; Demchuk et al., 2010). It should be noted that as a result of waste storage, unsanitary conditions are created not only directly on the territory of this farm, but also at a considerable distance from it. Thus, the spread of a specific smell from a complex for growing and fattening 10,000 heads of cattle occurs at a distance of up to three kilometers. At the same time, air pollution and smell spread from the pig complex for 108,000 heads up to 5 km (Dubin & Vasylenko, 2014).

It should be noted that greenhouse gases are not ordinary pollutants with a direct local effect, they act cumulatively, indirectly, globally, therefore the cumulative effect (greenhouse effect) determines the need to monitor emissions (Khodorchuk et al., 2014). According to the National Inventory of Anthropogenic Emissions from Sources and Adsorption of Greenhouse Gas Absorption in Ukraine, the inventory covers emissions of 6 greenhouse gases of direct action: carbon dioxide, methane, nitrous oxide, hydrofluorocarbons, perfluorocarbons, and sulfur hexafluoride (Mykhaylova, 2016, Korbych, 2021).

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According to estimates by the World Food and Agriculture Organization, the livestock sector emits 18% of all greenhouse gas emissions, which is more than emissions from transport (14%) (Tubiello et al., 2014; Palapa et al., 2016; Korbych, 2021). If deforestation is also taken into account, the share of agriculture in total greenhouse gas emissions increases to 30% (Demyanenko and Butko, 2012). On the other hand, agriculture is a key sector that, along with forestry, with effective management can slow down and stabilize the process of greenhouse gases entering the atmosphere (Udova et al., 2014; Mykhailova, 2016). In particular, forests in the world absorb up to 25 billion tons of carbon dioxide annually, but they are unable to fully reduce the concentration of greenhouse gas emissions into the environment (Mykhaylova, 2016).

The livestock sector accounts for the formation of about 9% of global emissions of carbon dioxide (retention time in the atmosphere is 50-200 years), 37% of anthropogenic methane, 64% of ammonium emissions, and 65% of nitrous oxide, that is, the industry affects atmospheric air and is potentially an important additional source of emissions directly related to climate change (Gerber et al., 2013; Binkovska & Shanina, 2016; Vovk, 2021). Greenhouse gases have different physical and chemical properties and can remain in the atmosphere for different periods of time (from a few days to centuries), that is, they have a certain greenhouse potential. Therefore, for each gas, an indicator is determined as the potential of global warming, which is calculated as the ratio of one kilogram of greenhouse gas that entered the atmosphere to one kilogram of CO_2 over a certain period of time (100 years) (Tymoschuk et al., 2022). So, for carbon dioxide, the global warming potential is one. CO_2 flows between the atmosphere and ecosystems are regulated by absorption through plant photosynthesis and release during respiration and decomposition of organic matter (Symbirskyi, 2013). The potential impact of methane on the planet's climate is 21-34 times higher than that of CO_2, and this gas can persist in the atmosphere for up to 12 years (Koneswaran & Nierenberg, 2008; Asgedom & Kebreab, 2011; Herrero et al., 2013). Methane is released in the process of methanogenesis in anaerobic conditions in soils and manure storages, in the process of enteral fermentation, and in the case of incomplete combustion of organic matter (Symbirskyi, 2013; Binkovska & Shanina, 2016). Ammonium emissions significantly contribute to acid rain and acidification of ecosystems (Binkovska & Shanina, 2016). The role and potential of N_2O in the process of global warming is on average 265-310 times greater than that of CO_2 (Koneswaran & Nierenberg, 2008; Asgedom & Kebreab, 2011; Caro, 2019). The main sources of oxide emissions are industries engaged in animal husbandry, storage and processing of manure, including the process of applying it to the soil (Simbirskyi, 2013; Palapa et al., 2016). Nitrous oxide contributes to the destruction of the stratospheric ozone layer, which protects living beings from the sun's harmful ultraviolet radiation (Binkovska & Shanina, 2016). An indirect source of potential greenhouse gas N_2O

is ammonia, which is released mainly during the formation of manure on fields, when it is stored in lagoons and applied to fields. This gas settles in surface waters and can cause their eutrophication. Ammonia emissions lead to acid rain, which damages crops and natural ecosystems, causes soil acidification, reduces the nitrogen content, and therefore the value of manure as a fertilizer. Ammonia emissions are associated with the formation of aerosols that can pose a health hazard to people (Blunden & Aneja, 2008; Korbych, 2021). An air pollutant such as hydrogen sulfide is considered one of the most toxic gases with an unpleasant smell, among the by-products of manure decomposition. It is formed due to bacterial reduction of sulfate and decomposition of sulfur-containing organic compounds in manure. Hydrogen sulfide is one of the factors in the occurrence of acid rain, and therefore in climate change (Aneja et al., 2008; Blunden & Aneja, 2008). According to the data of the European Union, more than 80% of the ammonia that pollutes the atmosphere and 10% of the methane that destroys the ozone layer comes from manure when it is untimely buried in the soil, when it is stored in open storage facilities (lagoons) (Maksishko & Malyk, 2012).

According to the latest data, the concentration of greenhouse gases in the atmosphere has increased significantly. In particular, the amount of carbon dioxide over the last 100 years has increased on average by more than 40%, which is the highest level in the last 650 thousand years, while the concentration of methane, relative to the pre-industrial period, has increased by 2.4 times, and nitrous oxide – by 20% (Udova et al., 2014). The analysis of literary sources shows that the concentration of methane in the air increases annually by 1.2-1.5%, the level of N_2O increases by 0.3%, and other gases by 4%. Thus, in general, by the middle of the 21st century, the greenhouse effect of CH_4 and N_2O may be equal to the effect of doubling the concentration of CO_2 in the atmosphere (Palapa et al., 2016). It is predicted that emissions of the main greenhouse gases will increase by 25-90% by 2030, if a number of measures to improve the situation are not adopted, relative to the indicators of 2000 (Smith et al. 2008; Udova et al., 2014). According to the forecast of IPCC experts at the United Nations, by 2050 climate changes may make a large part of the territory of Africa and Asia uninhabitable, which will contribute to migration processes.

In the agriculture, forestry and fisheries sector, according to the Food and Agriculture Organization of the United Nations, greenhouse gas emissions have doubled over the past 50 years (Gerber et al., 2013; Tubiello et al., 2014). Thus, agriculture in general, and animal husbandry in particular, which often suffers from climate change, is simultaneously a significant source of greenhouse gas emissions into the atmosphere, that is, one of the causes of this change (Vovk, 2021). Since human production activity causes disruption of the natural environment, society has to take care of restoration of its properties and protection from further degradation. Therefore, prevention of environmental pollution by greenhouse gases formed in the natural environment from by-products of animal origin remains an urgent issue and is an important aspect in the functioning of enterprises of the agro-industrial complex. Promising ways to solve the problem of disposal and use of livestock waste are its storage in storage facilities (lagoons) with possible further composting or anaerobic fermentation of biomass, which ensures zero waste production (Boldrin et al., 2009; Demchuk et al., 2010; Binkovska & Shanina, 2016). Processing of organic waste is an important component of any biologically oriented system. It is known that 1 gol. of cattle produces 45 kg of manure per day on average, from which 2.5 m^3 of biogas can be produced, the output of manure and gas from 1 head. pigs – 6.5 kg and 0.3 m^3, poultry – 0.137 kg and 0.02 m^3, respectively (Vovk, 2021). Analysis of literary sources shows that on average 52 m^3 of biogas is released from one ton of manure, 60% of which is methane, which is a greenhouse gas. In particular, the processing of animal waste products by the method of biological fermentation is of great ecological importance, since all chemical substances are fully utilized, as well as pathogenic microflora and weed seeds are destroyed, that is, the properties of manure as fertilizers are improved, thereby reducing to a minimum pollution of water, air and soil (Demchuk et al., 2010; Korbych, 2021). Thus, thanks to the introduction of waste-free technologies in animal husbandry, the problem of waste disposal is not only eliminated, but also the protein-mineral-vitamin feed base increases, the share of renewable energy increases, and there is a resource for increasing and restoring soil fertility due to the return of biomass nutrients in the most readily available form. Therefore, the use of this technology will allow to transform organic livestock waste from harmful to the environment into a profitable and useful product and ensure high competitiveness and profitability of the industry. Solving the problems of effective and rational disposal of waste in animal husbandry will contribute to increasing the productivity of animals, obtaining good-quality ecologically safe products and will ensure the proper ecological condition in the area of operations of farms, thereby increasing the efficiency of the industry.

CONCLUSIONS

It is theoretically substantiated and confirmed that the livestock industry causes significant damage to the environment. During the production of livestock products, the external environment must be reliably protected from pollution by its waste, as an integral part of the technological process. Thus, it is advisable to use the technology of waste processing and utilization, in which manure is considered not only as an object that creates an environmental hazard, but is also taken into account as a raw material for obtaining additional products in the form of biogas and biomass – with their subsequent use in various industries.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

INFLUENCE OF PARATYPICAL FACTORS ON MILK PRODUCTION IN UKRAINE

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Abstract

The article presents data on the influence of paratypical factors on the milk productivity of dairy cows in different regions of Ukraine on farms with different methods of keeping animals - tethered and loose ones. In order to more accurately determine the impact, a multi-criteria analysis was conducted by 10 indicators. When comparing untethered and tethered methods of keeping dairy cows, the advantage of the loose method was revealed; its objective function according to the considered criteria was the smallest one of 0.1391. This indicator appeared to be 1.1553–5.3394 times worse for the tethered method. Also, to establish the correlation between paratypical factors - daily yield of standardized milk, diet overall nutrition value, crude protein content, undegradable protein content, daily ambient temperature and air humidity, mathematical models were developed and analyzed: linear, incomplete quadratic and full quadratic ones.

***Key words**: mathematical model, method of animal keeping, milking cows, multi-criteria analysis, paratypical factors.*

INTRODUCTION

Ukraine's integration into the EU and the WTO encourages the production of dairy and meat products that are competitive and at the same time safe for the life and health of the population.
This is an impetus for the improvement of production technologies in accordance with international standards in the direction of reducing the impact of negative factors on the animal production level, stress resistance to technological and natural factors, and resistance to diseases.
Milk productivity of cows depends not only on genetic factors, physiological state, but also on environmental conditions. Most scientists rightly believe that when working with dairy cattle populations, it is necessary to take into account the influence of both genotypic and paratypical factors in specific economic conditions (Sklyarenko, 2018; Voitenko et al., 2019; Vedmedenko, 2019).
Of the latter, feeding and housing conditions are the most influential.
Feeding is a factor that determines the vital activity of animals. The productivity level, reproductive qualities, health and ultimately the economic and breeding value of livestock directly depend on the level and completeness of feeding. The use of innovative methods of preparing fodder for feeding allows not only to increase the productivity of cows, but also improves ruminal digestion, has a positive effect on their health and productive longevity (NRC, 2001; Popkov et al., 2018; Podobied et al., 2020; Erickson & Kalscheur, 2020).
The conditions and methods of keeping have no less effect on milk productivity. Loose keeping is considered more progressive, but in Germany almost 30% of dairy cows are kept on a tether, in the USA almost 60% of dairy farms had cowsheds with a tethered stall (Popescu et al., 2013). Very often this is caused by economic considerations, lack of space, equipment, convenience of service, especially in small and medium-sized operations. At the same time, when cows are kept loose, there are fewer leg, neck, and skin injuries (Beaver et al., 2021), as well as better fertility (Sawa & Bogucki, 2011).

MATERIALS AND METHODS

The objective of the research was to determine the paratypical factors on the milk productivity of dairy cows in different regions of Ukraine on farms with different methods of keeping animals - tethered and loose ones.

The research was conducted on a number of experimental farms incorporated in the system of the National Academy of Agrilcutltural Sciences of Ukraine (NAAS): State Experimental Farm Gontarivka of the Institute of Animal Science of the NAAS, Kharkiv region; State Experimental Farm Shevchenkivske, Kyiv region; State Experimental Farm Askaniyske, Kherson region (tethered and loose keeping); State Experimental Farm Ivanivka, Chernihiv region, State Experimental Farm Named After Decembrists, Poltava region, as well as on Private Agricultural Enterprise Pechenizke, Kharkiv region.

With the tethered keeping, cows were walked daily on the farm grounds on all the farms.

During the experiments, the following parameters were taken into account:

- the actual chemical composition and nutritional value of feed determined according to standard methods in the Laboratory for Evaluation of Animal Feed and Products of the Institute of Animal Science of the NAAS;
- actual feed consumption determined every ten days, with control feedings being applied during two consequent days, by determining the difference between the amount of feed given and feed remained for each group;
- level of cow milk yield determined monthly by conducting control milkings with further milk sampling to determine its quality;
- results of milk analysis performed to determine chemical composition, nutritional and energy values, physical and technological properties using the Bentley-150 infrared milk analyzer;
- ration cost;
- ambient temperature and air humidity determined every ten days during two consequent days;
- statistical processing of research results carried out by biometric methods.

Diets were balanced by all limited organic and mineral nutrients according to Ukrainian detailed feeding allowances (Bohdanov, 2013) according to cow milk yield taking into account actual feed chemical composition and nutritional value.

The research was conducted in the winter period on farms with different methods of cow keeping to determine the following values: daily ration costs per cow in Ukrainian hryvnias; daily milk yield per cow, kg; diet total nutrition value, MJ; feed consumption per kg of milk, MJ; diet crude protein content, g; diet undegradable protein content, g; milk protein percentage; milk fat percentage; costs per liter of milk, Ukrainian hryvnias (UAH); profit gained per cow, UAH.

Methodological approaches of multi-criteria analysis involve obtaining an estimate of the distance-to-target integral criterion under the influence of paratypical factors in the production of livestock products. The distance-to-target integral criterion is obtained using the approach of collapsing all values of paratypical factors through normalization and obtaining one value of the integral criterion (Piskun et al., 2020).

Using the MATLAB program, models of correlation between paratypical factors and standardized milk yield surface of response model were developed.

RESULTS AND DISCUSSIONS

The obtained research results are presented in Table 1.

When comparing loose and tethered methods of keeping dairy cows using the multi-criteria analysis, the advantage of the loose method was revealed; its objective function according to the considered criteria was the smallest one of 0.1391 (Table 2).

This value for the tethered method appeared to be 1.4486 times worse in State Experimental Farm Askaniyske, 1.1553 times worse in Shevchenkivske, 1.4537 times worse in State Experimental Farm Gontarivka, 5.3394 times worse in State Experimental Farm Ivanivka, 1.5112 times worse in State Experimental Farm named after Decembrists and 1.6499 times worse in Private Farm Pechenizke.

Table 1. Data to determine dairy cow milk productivity for different methods of livestock keeping

Indicator	Name of the farm						
	Askaniiske	Askaniiske	Shevchenkivske	Gontarivka	Ivanivka	named after Decembrists	Pechenizke
	Method of keeping animals						
	loose	tethered					
Ration costs per cow/day, UAH	98.01	98.01	103.52	87.03	66.64	98.06	90.21
Diet total nutritional value, MJ	225.60	225.60	229.70	223.90	157.00	219.20	245.00
Feed consumption per kg of milk, MJ	8.21	10.48	8.27	8.64	13.1	11.39	11.89
Crude protein, g	3144	3144	3461	3216	2290	3267	3505
Undegradable protein, g	765	765	848	717	550	825	981.6
Daily milk yield per cow/day, kg	27.78	21.52	27.76	25.9	12	19.25	20.62
Protein percentage	3.15	3.15	2.9	2.87	2.95	3.21	3.11
Fat percentage	3.62	4.13	3.61	3.99	3.75	3.82	3.93
Cost of 1 liter of milk, UAH	4.24	4.80	6.22	6.99	8.05	5.67	6.12
Profit gained per cow, UAH	168.2	149.58	185.3	162.5	56.49	200.63	123.98

Table 2. Multi-criteria analysis of dairy cow productivity according to different methods of livestock keeping

Indicator	Name of the farm						
	Askaniiske	Askaniiske	Shevchenkivske	Gontarivka	Ivanivka	named after Decembrists	Pechenizke
	Method of keeping animals						
	loose	tethered					
Ration costs per cow/day, UAH	1.5535	1.4708	1.5535	1.3060	1	1.4715	1.3537
Diet total nutritional value, MJ	1.0860	1.0860	1.0666	1.0943	1.5605	1.1177	1
Feed consumption per kg of milk, MJ	1	1.2765	1.0073	1.0524	1.5957	1.3874	1.4483
Crude protein, g	1.1149	1.1149	1.0128	1.0899	1.5306	1.0729	1
Undegradable protein, g	1.2832	1.2832	1.1576	1.3691	1.7848	1.1899	1
Daily milk yield per cow/day, kg	1	1.2909	1.008	1.0726	2.3150	1.4432	1.3473
Protein percentage	1.0191	1.0191	1.1069	1.1185	1.0882	1	1.0322
Fat percentage	1.1409	1	1.1441	1.0351	1.1014	1.0812	1.0509

Table 2 (continued)

Indicator	Name of the farm						
	Askaniiske	Askaniiske	Shevchenkivske	Gontarivka	Ivanivka	named after Decembrists	Pechenizke
	Method of keeping animals						
	loose	tethered					
Cost of 1 liter of milk, UAH	1	1.1321	1.4670	1.6486	1.8986	1.3373	1.4434
Profit gained per cow, UAH	1.1928	1.3413	1.0828	1.2347	3.5516	1	1.6183
$\sum U_k$	11.3904	12.0148	11.6066	12.0212	17.4264	12.1011	12.2941
$N(C_k)$	0.1391	0.2015	0.1607	0.2021	0.7426	0.2101	0.2294
Times	-	1.4486	1.1553	1.4537	5.3394	1.5112	1.6499

The conducted multi-criteria analysis showed (Table 3) that the best results were obtained in State Experimental Farm Shevchenkivske with the tethered method of keeping dairy cows, where the objective function according to the considered criteria was the smallest and was equal to 0.1420. This value was 1.3191 times worse in State Experimental Farm Askaniyske (tethered method), 0.0401 times worse in State Experimental Farm Gontarivka, 5.0648 times worse in State Experimental Farm Ivanivka, 1.3620 times worse in State Experimental Farm Named after Decembrists, 1.4895 times worse in Private Farm Pechenizke.

Table 3. Multi-criteria analysis of the productivity of dairy cows under the tethered method of keeping them

Indicator	Name of the farm					
	Askaniiske	Shevchenkivske	Gontarivka	Ivanivka	named after Decembrists	Pechenizke
Ration costs per cow/day, UAH	98.01	103.52	87.03	66.64	98.06	90.21
Diet total nutritional value, MJ	225.60	229.70	223.90	157.00	219.20	245.00
Feed consumption per kg of milk, MJ	10.48	8.27	8.64	13.1	11.39	11.89
Crude protein, g	3144	3461	3216	2290	3267	3505
Undegradable protein, g	765	848	717	550	825	981.6
Daily milk yield per cow/day, kg	21.52	27.76	25.9	12	19.25	20.62
Protein percentage	3.15	2.9	2.87	2.95	3.21	3.11
Fat percentage	4.13	3.61	3.99	3.75	3.82	3.93
Cost of 1 liter of milk, UAH	4.8	6.22	6.99	8.05	5.67	6.12
Profit gained per cow, UAH	149.58	185.3	162.5	56.49	200.63	123.98
$\sum U_k$	11.8726	11.4198	11.8206	17.1917	11.9339	12.1142
$N(C_k)$	0.1873	0.1420	0.1821	0.7192	0.1934	0.2115
Times	1.3191	-	0.0401	5.0648	1.3620	1.4895

Also to establish the correlation between paratypical factors of daily standardized milk yield, kg (Y) and total nutrition value of the diet, MJ (X_1); crude protein content, g (X_2); undegradable protein content, g (X_3); daily ambient temperature, °C (X_4); air humidity, % (X_5) mathematical models were developed and analyzed: linear, incomplete quadratic and full quadratic ones.

The linear model has the following form:

$Y = -2.38687+0.21867*X_1 + 0.00772*X_2 – 0.04820*X_3 – 0.17269*X_4 – 0.07093*X_5$

Sample variance D = 13.35906

Figure 1 shows the standardized milk yield surface of response, kg (Y) to the total nutrition value of the diet, MJ (X_1) and the average daily ambient temperature, degrees C (X_4).

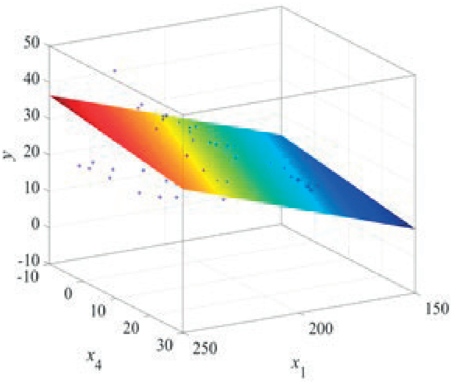

Figure 1. The standardized milk yield surface of response, kg (Y) to the total nutrition value of the diet, MJ (X_1) and the average daily ambient temperature, °C (X_4) (linear model)

The incomplete quadratic model has the following form:

$Y = -323.82746 + 1.23571*X_1 + 0.28351*X_2 - 0.46145*X_3 - 0.24459*X_4 - 0.39289*X_5 - 0.00249*X_1^2 - 0.00005*X_2^2 + 0.00029*X_3^2 + 0.00350*X_4^2 + 0.00228*X_5^2$

Sample variance D = 8.92430.

Figure 2 shows the standardized milk yield surface of response, kg (Y) to the total nutrition value of the diet, MJ (X_1) and the average daily ambient temperature, °C (X_4).

The complete quadratic model has the following form:

$$Y = -237.09235+1.26345*X_1 – 0.18096*X_2+0.93135*X_3+ 2.73535*X_4+ 1.34315*X_5 +0.02508*X_1^2+ 0.00001*X_2^2+ 0.00104*X_3^2+ 0.01052*X_4^2+ 0.00036*X_5^2 – 0.00004*X_1*X_2 –0.01418*X_1*X_3 – 0.04518*X_1*X_4 –0.01968*X_1*X^5+ 0.00015*X_2*X_3 +0.00081*X_2*X_4+ 0.00052*X_2*X_5+ 0.00516*X_3*X_4+ 0.00150*X_3*X_5+0.00438*X_4*X_5$$

Sample variance D = 2.78920.

Figure 3 shows the standardized milk yield surface of response, kg (Y) to the crude protein content (X_2) and the average daily ambient temperature, °C (X_4);

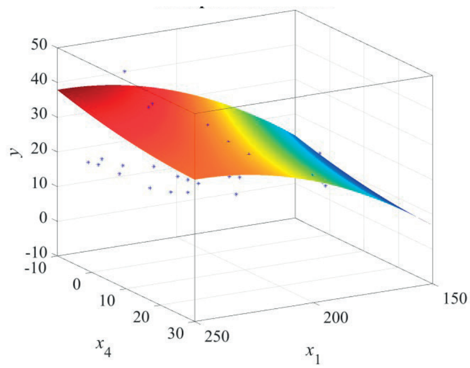

Figure 2. The standardized milk yield surface of response, kg (Y) to the total nutrition value of the diet, MJ (X_1) and the average daily ambient temperature, ºC (X_4) (incomplete quadratic model)

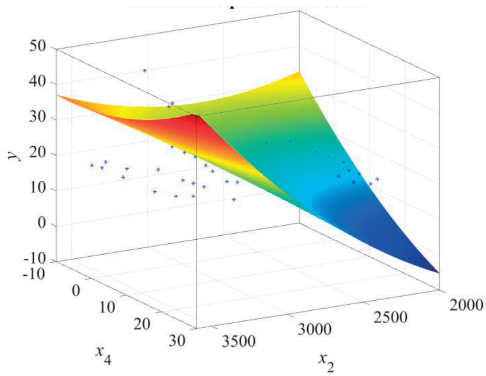

Figure 3. The standardized milk yield surface of response, kg (Y) to crude protein content (X_2) and average daily ambient temperature, °C (X_4) (full quadratic model)

The analysis of the obtained models shows that the sampling variance of the full quadratic model is the smallest, i.e., this model most adequately describes the correlation between paratypical factors.

CONCLUSIONS

According to the results of the multi-criteria analysis, the advantage of the untethered method of keeping dairy cows was established as the objective function according to the considered criteria was the smallest one of 0.1391. Other options were 1.1553-5.3394 times worse.

Mathematical models of the effect of paratypical factors that included diet total nutrition value, crude protein content, undegradable in the rumen protein content, daily ambient temperature and air humidity on the standardized milk daily yield were developed. The full quadratic model most adequately describes the relationship between paratypical factors.

ACKNOWLEDGEMENTS

The work was carried out within the scope of the R&D project "Study of biological features of the productive potential of animals under the influence of various paratypical factors" (state registration number 0116U003201.

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TECHNOLOGIES
OF THE AGRO FOOD
PRODUCTS PROCESSING

Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

EVALUATION OF MICROORGANISMS AND MOLECULAR VARIABILITY OF SOME OLD VARIETIES OF *Malus domestica* L.

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Abstract

Even if the Romanian market has been overrun in recent years by non-indigenous hybrids, the high degree of adaptability of the Romanian varieties helps them to persist in the struggle for survival. The demands of organic consumption have determined a segment of the country's population to refocus on the consumption of indigenous apples or originating from other areas, but grown for a very long time in our country.
The interest for these varieties with tasty fruits and special flavors led us to make a microbiological and molecular assessment. Molecular variability was investigated using ISSR (Inter Single Sequence Repeats) and SCoT (Start Codon Targeted) markers. The microbiological methodology is based on techniques for the isolation and determination of mesophilic bacteria, molds, yeasts, the presence of faecal bacteria and Escherichia coli, Salmonella and Shigella species. Escherichia coli was observed in some samples and enterococci were highlighted in only one sample. The results indicate a variation of the microbial groups in the varieties analyzed and the absence of species that can disturb serious the digestive tract (for example Salmonella and Shigella).

Key words: *Escherichia coli, Malus domestica L., microorganisms, molecular variability.*

INTRODUCTION

The period 2016-2025 has been called the "decade of nutrition", as in this time the implementation of government measures will be followed to eliminate all forms of malnutrition in emerging or developing region. According to the available data, one person out of three suffers from "malnutrition, micronutrient deficiency and obesity". In response, measures have been proposed to support sustainable food systems and healthy food (Goncearov et al., 2004; Petcu et al., 2007). This is mainly possible by stimulating and sustaining the agriculture and horticulture sectors (United Nations 2016-2025/https://www.un.org/nutrition/sites/).
In this context, the UN (United Nations) has declared 2021 as the "International Year of Fruits and Vegetables", to emphasize their importance in human nutrition and health. It is known that fruits are a source of vitamins, minerals, antioxidant compounds and fibers (Slavin & Lloyd, 2012). Thus, the contribution of fruits and vegetables in solving the above-mentioned problem is very significant. FAO (2015) recommended 400 grams of fruit per day/person. According to statistics, in 2019, in Romania, food spending in each household represented 61.0% of total spending, including 6.1% for fruit. Fruit consumption for each person was 366.3 grams per year in 2019 (Dobre-Baron, 2020).
Also, recent studies have shown that there is a correlation between non-communicable diseases and diets poor in fruits and vegetables (Jhee et al., 2019). According to WHO data (2019), in such cases, the incidence of mortality is 41 million persons per year. The consumption of fruits and vegetables is beneficial and promoted all over the world, but the availability and quality of fruits depends on

their interaction with microorganisms. Therefore, knowledge of the diversity of microorganisms on fruits is essential, as it influences the health of consumers (https://www.fao.org/).

Knowledge of the microbial load and their diversity can give us a better idea of their sustainability over time and help us improve food security, because research has shown that microbial degradation (Janisiewicz & Korsten, 2002; Savu & Petcu, 2002) and contamination (Petcu et al., 2019) of agrifood products is a global problem. The consequences of microbial spoilage are: food waste (Snyder & Worobo, 2018) and the negative effect on the social and economic environment (Jeswani et al., 2021).

Over the past few years, the "eco" and "organic" fruit and produce market has diversified.Unfortunately, Romania has turned from an exporting country to a consumer market for fruit from abroad (Ministry of Agriculture and Rural Development (2017), especially from the EU. However, there are still old varieties with special aromas and tastes. These varieties are not chemically treated and can be found in family households or orchards. According to data from the National Institute of Statistics (INS) (cited by Dobre-Baron, 2020) plum, apple and cherry plantations are dominant in Romania.

The apple originates from Central Asia and has been present in Europe and Asia since antiquity. *Malus domestica* L. grows in temperate zones and is part of the genus *Malus,* family *Rosaceae,* class *Magnoliopsida,* division *Manoliophyta* (Li et al., 2022). The Malus genus includes over 7500 varieties (Koseoğlu & Al-Taie, 2022). Apples are most commonly consumed in Romania (Dobre-Baron, 2020) and around the world (Wassermann et al., 2019).

Experts in the field appreciate that apples have a health benefit because they are sources of flavonoids, procyanidines and pectins (Shoji & Miura, 2014; Samout et al., 2016; Shtriker et al., 2018). It is known that flavonoids and procyanidins have antioxidant and antitumor potential (He & Liu, 2007; Shoji & Miura, 2014; Ribeiro, 2014; Zielinska, 2019; Azizah et al., 2020).

In Romania, in rural households or orchards in more isolated areas, there are genotypes of apples, grown for a very long time, adapted to local environmental conditions, the origin of which is uncertain. It may be considered that some of them originate from known varieties because of specific traits.

For this reason the present research aimed to assess the degree of relatedness between these forms, cultivated for a very long time in the same geographical region. In order to assess the genetic diversity between the apple genotypes studied, molecular markers were used to establish genetic fingerprints. Inter Simple Sequence repeats (ISSR) markers that amplify regions between adjacent opposite microsatellite sequences were used (Pradeep et al., 2002), because they do not require prior knowledge of the sequences analyzed. Besides, we used Start Codon Targeted (SCoT) markers, which amplify sequences in the gene region, the used primers having sequences complementary to the start codons (Colard et al., 2009). These markers are widely used for genetic evaluation in various plant species, including apple (Stepanov et al., 2021; Yao, 2022, Raja et al., 2022).

Microbial diversity in fruits and vegetables (Wassermann, 2019) is not widely known, because most studies have focused on food safety, food-borne pathogen germs and food poisoning (WHO, 2015) and relatively few with the study of the apple microbiome.

In this context, our studies have focused on apple varieties, cultivated for a long period in Romanian households, which meet the requirements regarding aroma, taste, but also the category "organic apple".

Our research focused on the study of microbiological load (in several ecophysiological groups), starting from the assumption that a "healthy apple" should also be safe from a hygienic and health point of view.

MATERIALS AND METHODS

Microbiological analysis of apple varieties

The microbiological determinations were performed on 6 varieties of apples, grown in the territory of our country during a very long time, from orchards located in the county of Hunedoara (varieties 1-5), respectively Bihor (variety 6).

The apples were harvested from the soil and were transported to the Microbiology laboratory, within the University of Life Sciences "King Mihai I" from Timisoara, in October. From each variety, four apples were chosen. The varieties studied were: Biotype similar with Starkinson originally from the United States of America, named Bot de iepure (Rabbit snout), 2 - Poinic, known as an ancient autochthonous variety, 3 - Biotype similar with Jonathan originally from the United States of America, 4 - Piros, an old variety with unknown origin; 5 - Biotype similar with Basil, an old variety originally from France, named Rosu busuioc (Red Basil) and 6 - Variety Golden Delicious, originally from the Netherlands.

Most of the microorganisms found in fresh fruit are saprophytes, such as: lactic bacteria, sporulant microorganisms, coliforms, micrococcis and pseudomonades, derived from soil, air and water. Due to the acidity of raw fruit, the primary spoilage microorganisms are fungi, predominantly molds and yeasts, such as *Sacharomyces cerevisiae, Aspergillus niger, Penicillum* spp., *Byssochlamys fulva, B. nivea, Coletotrichum gloesporoides,* but also sporulated and non-sporulated bacteria (Alzamora et al., 2000).

We were interested to identify the microorganisms that are represented by the faecal contamination indicators, belonging to the enterobacteria family – total coliforms, *Escherichia coli* and possibly *Salmonella, Shigella* and *Enterococcus faecalis* respectively, since the fruits tested were also harvested from the soil.

For this reason, we analyzed epicarp and mesocarp separately in terms of contamination by coliform microorganisms and fecal streptococci.

The isolation and determination of microorganisms was carried out using conventional group-specific methods (Misca 2011; Jeddi et al., 2014).

All phases were carried out under sterile conditions.The microbial groups were isolated using the successive dilution method.Three serial dilutions were prepared. For the epicarp, the 10^{-3} dilution was inoculated on media specific to each individual microbial group. For the mesocarp, the dilution of 10^{-1} was inoculated. MacConkey medium was used for coliform isolation, and bile-esculin-azide (BEA) agar was used as isolation medium for enterococci.To confirm the pathogenic species, the so-called faecal contamination indicators were used concomitantly and the chromogenic media together with the biochemical culture media for confirmation.Thus, for coliform bacteria, as biochemical confirmation media, we simultaneously use Triple Sugar Indol (TSI), SIM and Citrate Simmons (CS) media.

For the isolation of mesophilic bacteria, the 10^{-3} dilution corresponding to the epicarp was inoculated on nutrient medium Plate Count Agar (PCA) (ISO 4833-1:2013), respectively on Sabouraud+Chlormphenicol (SC), for the isolation of molds and yeasts.The SC medium replaced the Sabouraud dextrose agar medium (Jeddi et al., 2014).Cultivation conditions varied depending on the microbial group, the mesophilic bacteria, molds and yeasts were incubated at a temperature of 28°C, for 24-48 hours for the bacteria, respectively for 5 days for the last two microbial groups.

Molecular analysis

DNA extraction was performed on young leaves collected in early spring using the modified CTAB method (Doyle et al., 1987). 10 ScoT and 9 ISSR primers were initially used for amplification, from which 6 SCoTand 2 ISSR primers were selected, generating the most complex and clear fingerprints (Table 1).

Table 1 The primers sequences

Primer name	Primer sequence
SCoT 1	5'CAACAATGGCTACCACCA3'
SCoT 14	5'ACGACATGGCGACCACGC3'
SCoT 24	5'CACCATGGCTACCACCAT3'
SCoT 34	5'ACCATGGCTACCACCGCA3'
SCoT 35	5'CATGGCTACCACCGGCCC3'
SCoT 36	5'GCAACAATGGCTACCACC3'
UBC808	5'$(AG)_8$C3'
UBC811	5'(GA)8C3'

The amplification was carried out in 25 µl with the following components: 150 ng DNA, GoTaq® Green Master Mix 1x (Promega, USA), 20 pmol primer, 10pmol supplementary $MgCl_2$. The amplifying program followed the known steps, the annealing temperature being 55ºC. The amplification products were separated by electrophoresis in 1.8% agarose gel. The results obtained were evaluated using Vison Works software and statistically interpreted with UPGMA software (unweighted pair group method with arithmetic mean) (http://genomes.urv.cat/UPGMA/index.php).

RESULTS AND DISCUSSIONS

Evaluation of the microbial load and faecal contaminants

In quantitative terms, we found a very large difference between the microbiological load of the epicarp and the microbiological load of the mesocarp. Both coliform and *E. coli* bacteria and *Enterococcus fae*calis species were absent in the mesocarp while on the epicarp, a consistent load of total coliforms between 4.5x10^3 and 95x10^3 germs/10 grams of product was identified, and for *Enterococcus faecalis*, 3x10^3/10 grams of product, only in the Piros variety.

From a qualitative point of view, we identified the presence of the species *E. coli, Proteus* sp., *Klebsiella* sp. and *Enterococcus faecalis*. We present the appearance of each species on chromogenic and Mac Conkey culture media respectively (Figure 1). *Salmonella* and *Shigella* species were absent.

Figure 1. Coliforms and enterococci isolated on selective nutrient media

On the basis of these results, we observe that the microorganisms of faecal origin are concentrated on the surface of the fruit without penetrating the mesocarp, which is beneficial, because their titre may be significantly reduced by washing them before consumption. It can also be said that the source of contamination may be telluric, as the apples were also harvested from the soil.

Transportation conditions, harvesting and storage methods, soil, water, animals and feces can lead to contamination of fruit (Lopez-Camelo, 2007; NM ISO 6888-1., 2008; Sperber and Doyle, 2009; Ofor et al., 2009). It is obvious that at the time of harvest, the fruits harvested from the tree must be separated from those harvested from the soil, in order to avoid contamination of the production. But at the same time, it is necessary to consider the integrity of the epicarp, to prevent the entry of pathogenic microorganisms into the mesocarp, in order to prevent the consumption of contaminated fruit, which could be the basis of food poisoning.

These measures are also recommended in other studies that examine the status of faecal germs in fruits and vegetables (Erahioui et al., 2021).

According to our research, the same authors report that the level of fecal contamination is variable. The species emphasized by them include *Escherichia coli* and *Staphylococcus aureus*, however, *Salmonella* and *Shigella* have not been isolated, similar to the case we presented.

Some research shows that in the microbiome of unprocessed apples, the orders *Betaproteobacteriales* and *Enterobacteriales* dominate (following molecular analyses, the values were 51.3% and 20.4%, respectively) (Wicaksono et al, 2022b), but some authors have also shown that a substantial fraction of the apple microbiome is beneficial (Abdelfattah et al., 2021).

The statistical evaluation of our data emphasized the relation between different types of detected microorganisms. The number of coliforms was correlated with the number of Yeast (Figure 2).

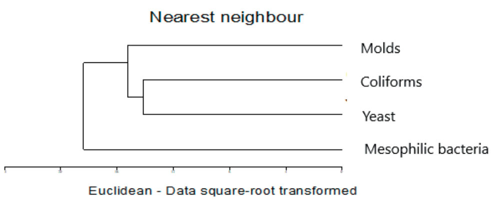

Figure 2. Cluster analysis based on Euclidian Distances

The results regarding the load of microorganisms isolated from the epicarp of the 6 varieties of apples are presented in the Figure 3.

The prevalence of the most important mesophilic bacterial load on apples was on the epicarp of the Poinic and Rosu busuioc (Red Basil) varieties, followed in descending order by the Bot de iepure (Rabbit snout) >Piros> Biotype Jonathan > Golden varieties.

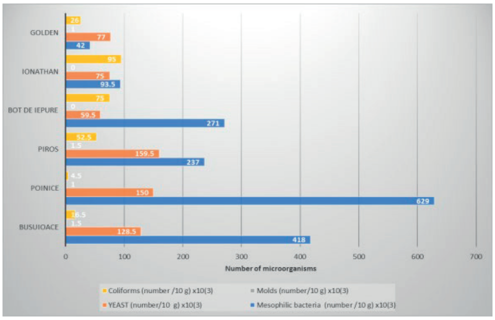

Figure 3. Abundance of microorganisms on the apples epicarp

The high bacterial abundance was also proven by other authors (Wicaksono et al., 2022b). Research shows that microbial abundance and diversity are dependent on the growth system (wild plantations, conventional systems, fruit trees in the garden) and the area. The same authors state that the microbial load is higher in natural plantations and on fruits from the garden (Wicaksono et al., 2022a). The difference between microbial diversity in the conventional and organic system is also confirmed by others (Lupatini, 2017; Wassermann et al., 2019). The abundance can also be explained by its origin, because it is known that the microorganisms present on fruits, plants and flowers come from the soil, an ecosystem that is a microbial reservoir (Zarraonaindia et al., 2015; Massoni et al., 2021). The soil increases its microbial diversity through organic fertilization.

In our study, there were differences according to the area, such as the Golden variety, which comes from a conventional plantation and a different zone, compared to other varieties, has a lower microbial load (Figure 4).

Yeast levels were high in the Piros, Poinic and Rosu busuioc (Red Basil) varieties. The lowest number of yeasts was isolated from the epicarp of the Bot de iepure (Rabbit snout) variety. In general, the load of bacteria and yeasts was high in the Rosu busuioc (Red Basil), Poinic and Piros varieties (Figure 5).

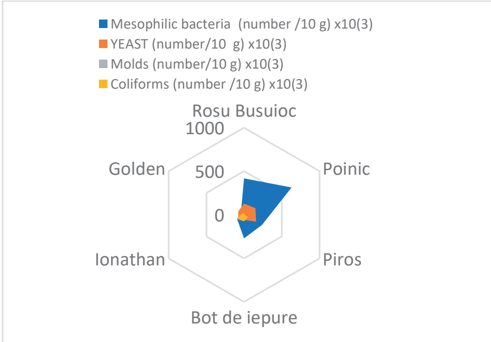

Figure 4. Apple Microorganisms Chart

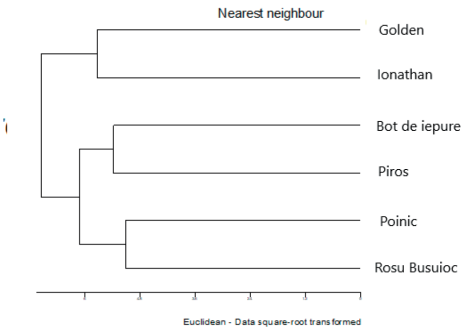

Figure 5. Cluster analysis of apple varieties based on microorganisms number

Compared to other authors who observed a significant growth of molds (1.6x105) on apples (Wicaksono et al., 2022a), our research demonstrated that molds have a lower growth (1.5x103). Their growth was absent in the Bot de iepure (Rabbit snout) and Biotype Jonathan varieties.

Molecular analysis

For biotypes originated from the same region (Hunedoara County), genetic studies were conducted to assess their degree of relatedness using ScoT and ISSR molecular markers.

Once the primers that produced the clearest fingerprints were selected, they were used to amplify the 5 DNA samples extracted and purified. Agarose gel electrophoresis analysis of the amplification products with primers ScoT 34, SCoT 35 and SCoT 36 is shown in Figure 6.

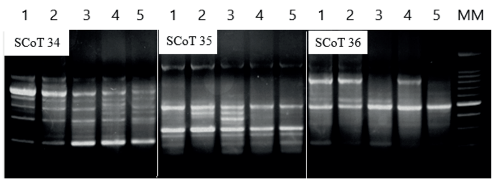

Figure 6. The results of the amplification with ScoT 34, SCoT 35 and SCoT 36 markers
Legend: 1- Piros; 2- Rosu busuioc (Red Basil); 3- Bot de iepure (Rabbit snout); 4- Poinic; 5-Ionathan biotype

A total of 55 alleles were amplified (6.9 alleles/primer) with the selected primers of which 42 were polymorphic (76.4%). Analysis of the amplified fragments with UPGMA software allowed the establishment of similarity indices based on Jaccard coefficients (Table 2). Based on the similarity coefficients, a dendrogram was established showing the degree of relatedness between the genotypes analyzed (Figure 7).

Table 2 Similarity Matrix computed with Jaccard coefficient

Genotype	1	2	3	4	5
1	1.000	0.745	0.696	0.680	0.640
2		1.000	0.814	0.750	0.673
3			1.000	0.702	0.696
4				1.000	0.714
5					1.000

Legend: Bot de iepure (Rabbit Snout); 2-Poinic; 3-Ionathan biotype; 4- Piros; 5- Rosu busuioc (Red Basil)

The average similarity index between the genotypes studied was high (0.711). The highest similarity index was found for the Poinic and Ionathan varieties (0.814). In addition to these two genotypes in the same cluster was the Piros biotype.
The lowest similarity index was found between genotype Bot de iepure (Rabbit Snout) and Rosu busuioc (Red Basil) (0.640).
It appears that genotypes of American origin and those with unknown origin were located in a common cluster, whereas the genotype Rosu Busuioc (Red Basil) of French origin differed from them, having the lowest degree of similarity.

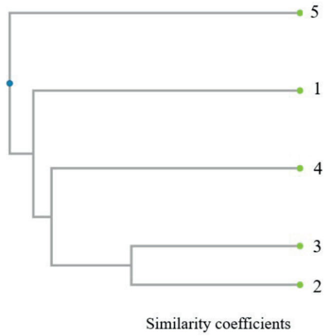

Figure 7. Dendrogram of the relatedness degree based on ISSR and SCoT analysis
Legend: 1- Bot de iepure (Rabbit snout); 2-Poinic; 3-Ionathan biotype; 4- Piros; 5- Rosu busuioc (Red Basil)

CONCLUSIONS

In conclusion, indicators of fecal contamination, belonging to the *Enterobacteriaceae* family, were highlighted on the epicarp of the analyzed apple varieties. Faecal enterococci were observed only on apples belonging to the Piros variety. *Escherichia coli* was isolated from some apple varieties, while *Salmonella and Shigella* were absent. The source of the contamination is telluric, because the apples were harvested from the soil, so compliance and promotion of hygienic-sanitary measures are essential in these cases. The load of mesophilic bacteria and yeasts remained high, especially in the varieties Rosu busuioc (Red Basil), Poinic and Piros, on the other hand, the number of molds was reduced or even absent.
From a genetic point of view, the genotypes analyzed had a high similarity index, differing according to their origin. The Red Basil genotype, with French origin, showed the highest degree of diversity compared to genotypes with USA or unknown origin.
Taking into account the importance and high consumption of apples, but also the limited research on the microbiome of these fruits. Future studies will assess the microbiome using methods for harvesting, handling and storing apples.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

SENSORIAL CHARACTERIZATION OF MUTTON PRODUCTS IN MEMBRANE MADE IN THE MEAT PROCESSING

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Abstract

The paper aimed to produce four different types of sheep meat products with heterogeneous structures in the meat processing workshop of the University of Life Sciences Iasi, which presented as variation factors the type of membrane and the type of product (salami/sausage, imprinted by the membrane used). Two varieties of salami (in collagen and polyamide membrane) and two varieties of sausages (in natural pork and sheep membrane) served as the four samples. The four samples obtained were subjected to sensory analysis, carried out in two stages: the first stage consisted of assessing the products based on the main sensory attributes (appearance, aroma, taste, texture, overall acceptability), and the second stage aimed at describing the products using specific sensory terms included in the CATA (Check-All-That-Apply) test from the perspective of consumer perception. The results obtained revealed sensory attributes characteristic of membrane products with heterogeneous structure, with the CATA test describing the products through positive attributes (colour, aroma, texture), with the differentiation of a firmer, harder texture in the case of natural membrane products, superior juiciness in the case of the S3MC sample, and a slightly brittle texture in the S4MP sample. In terms of the results of the hedonic scale, the sausage samples received a higher score for section appearance (7.91 ± 0.831 for C1MP and 8.00 ± 0.775 for C2MO), compared to the salami samples (7.73 ± 1.104 for S3MC and 7.18 ± 1.168 for S4MP). The overall acceptability was highest for C1MP, which received 8.36 ± 0.674 points, being followed in descending order by C2MO (8.55 ± 0.688), S3MC (8.18 ± 0.751) and S4MP (7.45 ± 0.522).

Key words*: CATA, mutton, natural / sintetic membranes, sensory evaluation.*

INTRODUCTION

Sensory evaluation is used in the food industry to meet various purposes, the most important of which is to control the quality of the sensory properties of a product by checking compliance with established parameters and design criteria (specifications). Thus, sensory testing is an essential tool to ensure that food products meet the required quality standards, helping to identify defects, unfavourable flavours and other quality issues that may affect the overall acceptability of a product (Lawless & Heymann, 2010; Saint-Denis, 2018). Hence, the sensory analysis examines the properties (texture, aroma, taste, appearance, smell, etc.) of a food product through the senses (sight, smell, taste, touch and hearing) of the panellists. This type of analysis has been used for centuries to accept or reject food products (Nederkoorn et al., 2015; Issanchou, 2018). Technological advances in recent years have led to the development of new sensory analysis techniques, such as electronic noses and tongues, which use sensors to detect and analyze the chemical composition of food (Di Rosa et al., 2020).

In addition, since, for many products, sensory properties deteriorate before microbial quality, sensory testing can be used to determine shelf life and product variability along the supply chain in tandem with microbial testing (Kemp et al., 2011).

The meat we eat is an integral component of the human diet. It contains essential nutrients that help maintain normal physiological functions, improve immunity and prevent certain diseases, including malnutrition (Biswas & Mandal, 2020).

In sheep meat, as in meat in general, the nutritional value, as well as the quality, is determined by the chemical composition, especially the essential amino acids, vitamins and mineral salts contained in it. The complex but at the same time balanced chemical composition of sheepmeat, mainly due to its protein, vitamin and mineral salt content, determines the nutritional and biological value of this product.

The influence of chemical composition on the sensory quality of meat mainly involves the lipid components as variations in these affect sensory attributes such as taste, flavour, juiciness and texture. Sheep meat, in particular lamb meat, is recognised as a good source of Omega-3 polyunsaturated fatty acids, with more than 60 mg/100 g of meat. Moreover, it also contains significant amounts of conjugated linoleic acid, with multiple benefits for the body, found in proportions between 0.2 - 2% of total fat (Ponnampalam et al., 2016).

In meat processing to obtain unstructured products, membranes are used as fillers. These are natural, semi-synthetic or synthetic coatings used to introduce the meat composition, give it a certain size and shape, reduce weight loss and protect the product against the harmful action of microorganisms in the external environment (Georgescu et al., 2000; Wenther, 2003).

Considering the wide variety of meat products on the market, the paper aims to conduct a sensory evaluation of some assortments of salami and sausages made of mutton in four different membranes, products manufactured in the Meat Processing Workshop of USV Iasi.

MATERIALS AND METHODS

To characterize the sensory properties of the products an experimental protocol has been designed, including the assortment manufacturing technology and the sensory evaluation questionnaire. Therefore, the sausage assortments were placed in natural pork (Ø 28-30 mm) and sheep (Ø 20-22 mm) membranes and formulated according to Table 1.

The salami assortments were placed in collagen and polyamide membranes, their manufacture following the ingredients in Table 1 and the heat treatment parameters presented in Table 2.

Table 1. Formulations to prepare the experimental batches

Batch code	Membrane type	Ingredients (%)							
		1st QSM	2nd QSM	Salt	Black pepper	Garlic	Sweet paprika	Spicy paprika	Chili
C1MP	pork intestine	35	65	2	0.3	0.5	2	0.2	0.2
C2MO	sheep intestine								
S3MC	collagen								
S4MP	polyamide								

1st QSM - first quality mutton; 2nd QSM - second quality mutton; C1MP - sausages in pork natural membrane; C2MO - sausages in sheep natural membrane; S3MC - salami in collagen membrane; S4MP - salami in polyamide membrane.

The manufacturing process involved specific steps for membrane meat products:
- salting of the raw meat material in the mixers, followed by a maturation period of at least 24 hours at a temperature of 2-4°C in the frigorific maturation room;
- granulated mincing of the meat, with a grinding machine (GRINDER WP - 105), through an 8 mm sieve for salami and 6 mm for sausages;
- the bradt is formed by weighing 30% of the total mass of ground meat and finely mincing without exceeding a temperature of 13°C;
- preparing the raw and auxiliary materials according to the technological sheets of the four batches;
- mixing the raw materials with the condiments for approximately 10-20 minutes until the composition is completely homogenised;
- filling the membranes corresponding to each batch, forming the sausage sticks and twisting the sausages to form individual pieces of 15 cm each.

The heat treatment involved four stages (drying, smoking, boiling and hot air drying / high-temperature cooking) carried out at different parameters, considering the types of membranes used, as shown in Table 2. After the heat treatment stage, the product was cooled to room temperature for a maximum of 60 minutes, packed, labelled and stored until sensory evaluation.

The samples were prepared before sensory evaluation by cutting them into identical size pieces, coding and distribution to the evaluators.

Sensory analysis was carried out in the Sensory Analysis Laboratory of Iasi University of Life Sciences and involved sensory evaluation by a group of 11 trained evaluators from the Food Science and Technology Department. The evaluators assessed 6 parameters: surface appearance, section appearance, aroma intensity, taste, texture, and overall acceptability, scoring the samples on a 9-point scale.

The second stage of the sensory evaluation involved applying the CATA (Check-All-That-Apply) test to a group of 52 consumers for the four samples of sheep meat products in membranes. The descriptive terms (20) that composed the CATA test were: uniform colour, red colour, colour intensity, mosaic appearance, characteristic aroma, intense meat flavour, strong lamb flavour, rancid flavour, salty taste, tasteless, perfect seasoned, bitter taste, acid taste, juicy texture, firm texture, elastic texture, brittle texture, hard, dry, fatty.

The chemical composition (moisture, fat, protein, and collagen) was determined using a FoodCheck analyzer (Bruins Instruments, OmegAnalyzer), a spectrophotometer that uses infrared light rays.

The results obtained after applying the first stage of sensory evaluation were subjected to analysis of variance (ANOVA) followed by Tukey's test at a 5% significance level ($p < 0.05$) to compare the mean values. CATA test results were expressed by analysing the citation frequency for each sensory term of each sample using XLStat software (Addinsoft version, 2022).

Table 2. Heat treatment scheme of the batches

<table>
<tr><th rowspan="2">Batch code</th><th colspan="5">Heat treatment</th></tr>
<tr><th>Drying</th><th>Smoking</th><th>Boiling</th><th>Hot air drying</th><th>High temp. cooking</th></tr>
<tr><td>C1MP</td><td rowspan="2">t = 30 min.; U = 10%; T = 50°C (4°C inside the product)</td><td rowspan="2">t = 30 min.; U = 10%; T = 60°C (50°C inside the product)</td><td rowspan="2">T = 72°C (69°C inside the product); U = 99%</td><td rowspan="2">-</td><td rowspan="2">t = 25 min.; U = 10%; T = 80-85°C (69°C inside the product)</td></tr>
<tr><td>C2MO</td></tr>
<tr><td>S3MC</td><td rowspan="2">t = 30 min.; U = 22%; T = 60°C (50°C inside the product)</td><td rowspan="2">t = 35 min.; U = 22%; T = 65°C (55°C inside the product)</td><td rowspan="2">t = 60 min.; U = 99%; T = 72°C (69°C inside the product)</td><td rowspan="2">t = 20 min.; U = 22%; T = 80°C (69°C inside the product)</td><td rowspan="2">-</td></tr>
<tr><td>S4MP</td></tr>
</table>

t - time; U - humidity; T - temperature

RESULTS AND DISCUSSIONS

The results from the sensory evaluation of the salami and sausage samples by the scale method were assessed by the mean on each parameter (mean ± SD). The specific values including statistical evaluation are presented in Table 3.

The highest scores for all 6 attributes were given to the sausage in sheep natural membrane (C2MO). Therefore, in the classification of the experimental samples in descending order of the scores given by the sensory evaluation, it can be observed that the natural membrane preparations (sausage samples) were scored higher, followed with close scores by the salami in collagen membrane (S3MC) and the salami in polyamide membrane (S4MP, Figure 1) samples.

Regarding surface and section appearance, the mean scores ranged from a minimum of 7.18 ± 1.168 (S4MP) to a maximum of 8.36 ± 0.674 (C2MO). The samples received relatively high scores (Table 3); insignificant differences were observed for the appearance attributes between samples of sausage in pork membrane (C1MP) and salami in collagen membrane (S3MC), possibly due to the similarity of the products in terms of diameter and external appearance.

Taste and aroma intensity attributes were superior in the sausage samples, the two assortments being close in mean scores. These results can be explained by the fact that natural membranes behave differently to heat treatment, it has high permeability so that flavour substances during smoking are deposited and absorbed more efficiently by the product.

The sensory attributes of texture and overall acceptability received the highest scores of the sensory characteristics evaluated (Figure 1). The most appreciated sample in terms of texture was identified in the C2MO product (8.73 ± 0.467), which may be due to the smaller diameter of the membrane type and the fact that the heat treatment was more intense on this product, the degree of drying being higher. Significant differences ($p < 0.05$) were particularly evident for C2MO and S4MP samples (6.82 ± 0.751). At the acceptability level, the sausage samples also received the highest scores, not being significantly different ($p > 0.05$).

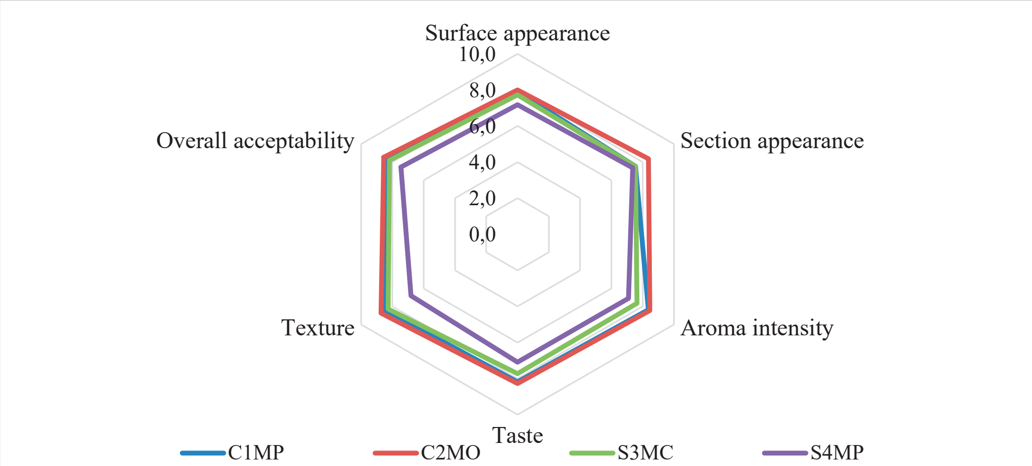

Figure 1. The results of sensory evaluation of sausages and salami samples

The statistical analysis of the mean results using the ANOVA test revealed by p-values (Table 3) the non-significant influence of product type and membrane on the sensory attributes related to appearance (external and section). Regarding the texture and aroma intensity attributes, both factors of variation (product type and membrane) imprinted distinctly significant differences ($p < 0.001$) on the samples, while the differences identified for the taste attribute were more evident between product types (highly significant differences, $p < 0.001$) and less due to membrane type (significant differences, $p < 0.05$).

Table 3. Sensory analyses of sausages and salami samples

Sensory attributes	Sausages samples		Salami samples		p-value	
	C1MP	C2MO	S3MC	S4MP	Membrane type	Product type
Surface appearance	$7.91^{ab} \pm 0.831$	$8.00^{a} \pm 0.775$	$7.73^{b} \pm 1.104$	$7.18^{c} \pm 1.168$	0.223^{ns}	0.098^{ns}
Section appearance	$7.55^{b} \pm 1.214$	$8.36^{a} \pm 0.674$	$7.55^{b} \pm 0.820$	$7.36^{c} \pm 0.809$	0.058^{ns}	0.082^{ns}
Aroma intensity	$8.36^{a} \pm 0.809$	$8.45^{a} \pm 0.522$	$7.64^{b} \pm 0.674$	$7.11^{c} \pm 0.831$	0.00013^{***}	$<0.0001^{***}$
Taste	$8.18^{a} \pm 0.751$	$8.27^{a} \pm 0.647$	$7.73^{b} \pm 0.647$	$7.09^{c} \pm 0.701$	0.001^{*}	0.0004^{***}
Texture	$8.45^{b} \pm 0.688$	$8.73^{a} \pm 0.467$	$8.27^{b} \pm 0.786$	$6.82^{c} \pm 0.751$	$<0.0001^{***}$	0.0002^{***}
Overall acceptability	$8.36^{a} \pm 0.674$	$8.55^{a} \pm 0.688$	$8.18^{b} \pm 0.751$	$7.45^{c} \pm 0.522$	0.002^{**}	0.004^{**}

Means presented with the same letter on the same row show that there was no significant difference ($p < 0.05$) in the Tukey test; ns = $p > 0.05$; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$

The overall acceptability of the samples was distinctly significantly ($p < 0.01$) influenced by both product type ($p = 0.004$) and membrane type ($p = 0.002$).

The CATA test was used to describe the sensory characteristics of the products by assigning specific terms to each sample by a group of 52 consumers.

The results of the CATA test were statistically analysed to highlight the sensory terms checked by the evaluators for each of the four samples. Therefore, a symmetric plot (Figure 2) was used to design the distribution of the four products evaluated on the F1 and F2 axes, which explains the variation between the samples, as well as a distribution of the descriptive terms according to the evaluators' responses.
Hence, the symmetric plot places the positive attributes mainly in the right-hand quadrants. By grouping the terms in this manner, it is noticeable that the majority of the evaluators described the samples as having a uniform, red and intense colour, showing the characteristic mosaic of products with a heterogeneous structure, with a characteristic, intense meaty and appropriate seasoned flavour.
The terms describing possible negative attributes of the samples were represented in Figure 2 in the left quadrant, thus, it was noted that the evaluators did not identify rancid flavour, bitter or sour taste, intense sheep flavour, or bland taste in the products.
By placing the samples closer to the respective attributes in the graph, it is observed that the sausage samples (C1MP and C2MO) were described as having a firm texture, slightly higher elasticity and a more intense salty taste identified by consumers compared to the salami samples. Moreover, the natural membrane samples were also described by some consumers as dry and hard, explained by the smaller diameter of the products (compared to salami assortments), the permeability of the membrane allowing for higher water loss and a higher degree of dryness, and the possibility to apply a more intense heat treatment.

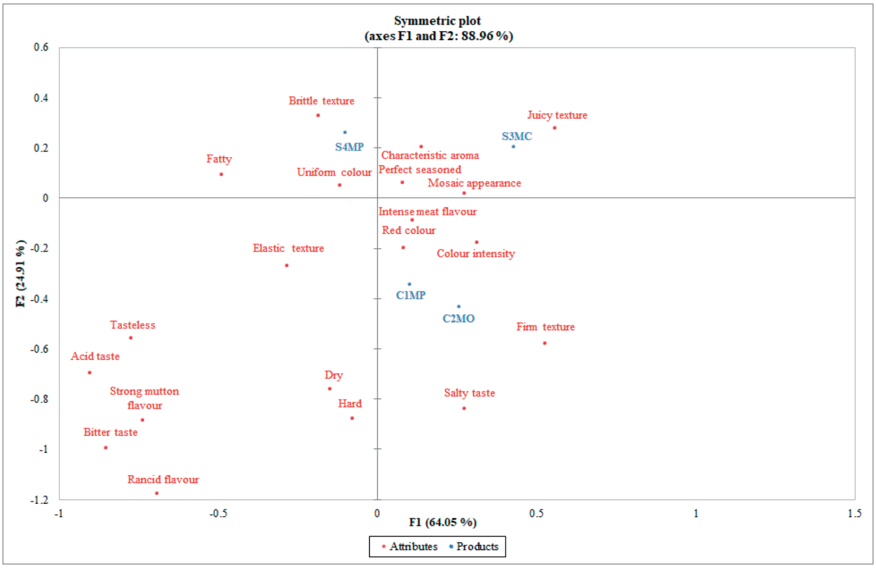

Figure 2. Multiple factor analysis (MFA) of membrane mutton products evaluated by means of the CATA test

The salami samples (S3MC and S4MP) were located at the top of the diagram. Sample S3MC, in collagen membrane, was described as displaying a juicy texture in addition to the positive attributes identified in all samples. The presence of juiciness in the salami sample in the collagen membrane can be explained by the higher diameter compared to the sausage samples, correlated with the lower permeability of the membrane compared to the natural ones.
At the opposite pole, the sample in polyamide membrane was described as having a brittle texture, a characteristic explained by the melting of the fat in the product structure and its elimination to the outside, accumulating between the product and the membrane, since due to the impermeability no exchange of substances was possible.

The results for the chemical composition are shown in Table 4.
The water content in the four batches varied in the interval 61.12±0.21% (for C2MO) – 62.88±0.35% (for S4MP), with the products in natural membrane showing a higher humidity, although no significant differences were found between bathes ($p > 0.05$).

The lipid content in the sausage and salami batches did not differ significantly ($p > 0.05$), thus showing an insignificant influence determined by the type of membrane.
The protein content varied within strict limits (19.11±0.09% for S3MC and 19.88±0.25% for C2MO) as all samples were obtained with the same quantities of raw materials.

Table 4. Analysis of the proximate composition of the batches

Parameters	C1MP	C2MO	S3MC	S4MP	p-value
Moisture (%)	61.29 ± 0.14	61.12 ± 0.21	62.22 ± 0.08	62.88 ± 0.35	0.072^{ns}
Lipid (%)	16.64 ± 0.19	16.88 ± 0.22	16.41 ± 0.28	16.02 ± 0.41	0.161^{ns}
Protein (%)	19.27 ± 0.23	19.88 ± 0.25	19.11 ± 0.09	19.35 ± 0.18	0.093^{ns}
Collagen (%)	16.80 ± 0.40	17.10 ± 0.29	16.40 ± 0.54	16.80 ± 0.62	0.055^{ns}

Means followed by standard deviation. ANOVA Tukey test: ns = $p > 0.05$; *** = $p < 0.001$

CONCLUSIONS

The sensory characterisation of four different types of mutton products showed that, although differentiated by membrane type (different in diameter, colour, and properties), the samples did not differ significantly ($p > 0.5$) in the 9-point scale sensory evaluation of appearance characteristics (external and per section). Sensory attributes of aroma, taste and texture were significantly influenced by the two factors of variation determined by membrane type and product type. The highest sensory acceptability was observed for sheep and pork natural membrane products, with mean scores around 8.5, and the lowest acceptability was observed for polyamide membrane products, which obtained a mean score of 7.45.
Through the CATA test, the samples were described with positive sensory attributes, with small differences between the products in terms of texture attributes due to the characteristics of the membranes used. It was observed that consumer perception was positive for all products, without being influenced by the form of presentation given by the type of membrane. The differences in perception were rather attributed to the way the product performed during the production process (specifically heat treatment), considering that the heat treatments were differentiated according to the origin of the membrane (natural/artificial).

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

THE EFFECTIVENESS OF HEAT TREATMENT PROCESSES APPLIED TO SOUR CREAM FOR THE PRODUCTION OF BUTTER, VALIDATED BY ENZYMATIC METHODS

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Abstract

The purpose of this article was to evaluate the efficacy of heat treatment procedures used on sour cream, a raw material used to make butter. To investigate the safety of sour cream, 25 samples of unpasteurized sour cream, 25 samples of pasteurised sour cream, and 25 samples of butter were evaluated. The samples were then subjected to enzymatic and biochemical analysis. The titratable acidity of unpasteurized sour cream resulted was 19.84±0.10 °T. All 25 samples tested positive for peroxidase activity. The titratable acidity of pasteurised sour cream was 20.24°T, although the peroxidase activity was negative. Using the reductase test with methylene blue, the samples of unpasteurized sour cream ranged in the second quality class, with a discoloration interval of samples substrate till 289.60±3.49 minutes. The titratable acidity of the butter was 2.59±0.04°T, and the peroxidase activity was negative in all 25 samples. The sour cream heat treatment techniques have been validated, with the examination of the two dairy products yielding good findings in compliance with standards.

Key words*: butter, dairy products, enzymatic methods, heat treatment, sour cream.*

INTRODUCTION

Milk has an important role in the human diet, as drinking milk, fresh, or kept for a longer period of time through the application of sterilization methods, either in the form of dairy products. Cattle are the primary source of milk in the world's most important regions, with the exception of the Indian subcontinent and Egypt, Mediterranean regions, parts of the Middle East and some African regions where buffalos, sheep and goats contribute significantly to native milk production (Davidescu et al., 2020; Grădinaru et al., 2015).
A specific category of dairy products, favoured by consumers, lies the dairy products with higher fat content, for example –sour cream and butter.
For all milk products, in a separate measure, must be respected as a basic condition, for consumers safety (Banu, 2009; Stănescu, 2010). This condition is observance of the safety throughout production process of dairy products. To reduce the risk of contamination of product, shall apply the heat treatment processes to raw material. The peroxidase is an important enzyme, whose presence has been tested on sour cream, raw material for butter. This enzyme can be identified and in butter, whether the pasteurization of sour cream has not been made properly (Fox & Kelly, 2006; Kosikowski, 2006).
The aim of this paper was to investigate the effectiveness of heat treatment processes applied to sour cream by using enzymatic methods.

MATERIALS AND METHODS

For each test sample, respectively, pasteurized sour cream (PSC), unpasteurized sour cream (USC) and butter (B) were harvested work samples and analyses were made to determine the freshness and degree of microbial load of the sour cream raw material and butter. In the second stage, were made enzymatic analysis for the validation of the effectiveness of the pasteurization sour cream (PSC), a process which is considered to be carried out at a temperature of > 90°C. For each analysis, we applied 25 replicates. In the first stage, as work samples, used sour cream, random harvested, up to a volume of 2 liters, in sterilized containers. The harvesting was made in the dairy factory, after centrifugal separation of fat, from the milk. In the second stage of research, was harvested pasteurized sour cream, in quantities of 100 milliliters in 5 consecutive rounds.

For each analysis method, we applied 25 replicates, the same as unpasteurized sour cream. In the third stage of research, was analysed the butter with a content of 65% fat, up to a volume of one kilo. For determine the freshness of the unpasteurized sour cream and pasteurized sour cream, raw material for butter, has been measured the titratable acidity, by titrimetric analysis, respectively Thorner method, which consist in neutralizing the acids from a batch of 100 milliliters sour cream, using NaOH (n/10) and phenolphthalein as a color indicator.

The enzymatic analysis that were carried out for the appreciation of status hygiene of sour cream, involved reductase test using methylene blue and resazurine (Fernandes, 2012). Reductase enzyme is a microbial-origin enzyme and has the ability to reduce certain colored substrates. The principle of the method is based on the fact that the reductase enzyme reduces the amount of 5 mL of methylene blue to colorless substances called leuco-derivatives. Depending on the decolorization interval of the sample substrate, the degree of microbial load is assessed. Classifying samples in the two quality classes (corresponding/inadequate) was done in accordance with the resazurin method which is based on the action of the reductase in the samples, which, in the presence of resazurin, produces color changes (William, 2007).

In the case of butter, the freshness was appreciated by Kreis reaction, identifying, degree of lipids oxidation (Fernandes, 2012.

In validation of heat treatment processes applied to pasteurized sour cream we applied peroxidase test, using hydrogen peroxide and benzidine, method which help to determine effectiveness of the pasteurization processes applied to sour cream (Sakkas et al., 2014; Stănescu, 2010). Was used module of statistical calculation, MS Excel and we determine for each analysis, the parameters: arithmetic mean ($\bar{x}$), standard deviation (s), standard error of the mean (±) and coefficient of variation (C.V.%).

RESULTS AND DISCUSSIONS

Milk contains a large number of native enzymes with varying functions, processing stability, impact on dairy products, and consumer safety significance (e.g., antimicrobial enzymes). Some enzymes are of interest for their beneficial activity (e.g., lactoperoxidase), some for use as processing indices (e.g., alkaline phosphatase) and some for effects on the quality of dairy products (e.g., plasmine, lipoprotein lipase), which may be positive or negative for various products (Kelly & Fox, 2006; Rankin et al., 2010; Savu, 2008).

The investigation for unpasteurized sour cream were carried out in a number of 25 samples. Following Thorner's analysis, the analytical range of acidity was between a minimum of 19.20°T and a maximum of 20.80°T, with a calculated mean of 19.84 ± 0.10°T. The coefficient of variation presented a value of 2.54%, suggesting that the homogeneity of the analyzed samples, as well as the accuracy of the method application, was sufficiently good. The obtained value is close to the one specified by the butter production standard regarding the quality of the raw material used, SR ISO 6092:2008 (max. 20°T) (Figure 1).

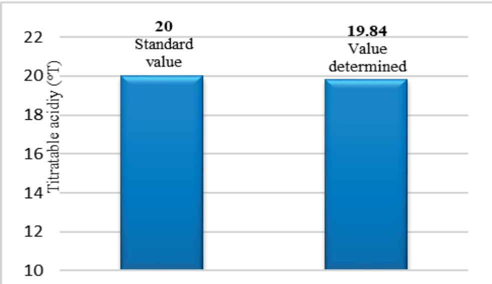

Figure 1. The titratable acidity of unpasteurized sour cream, compared to maximum permissible value of the standard

Appreciation of hygienic status for the unpasteurized sour cream, was effected through reductase test using methylene blue. We noted the length of time until the complete discoloration of samples, after incubation at 37°C. The rate of observation it is been every 20 minutes. For the 25 of samples analyzed, the minimum discoloration interval of samples was 260 minutes and the maximum discoloration amount was 320 minutes, with an average of 289.60±3.49 minutes (Figure 2). According to scale of assessment, it follows that the samples of unpasteurized sour cream ranged in second quality class (satisfactory rating).

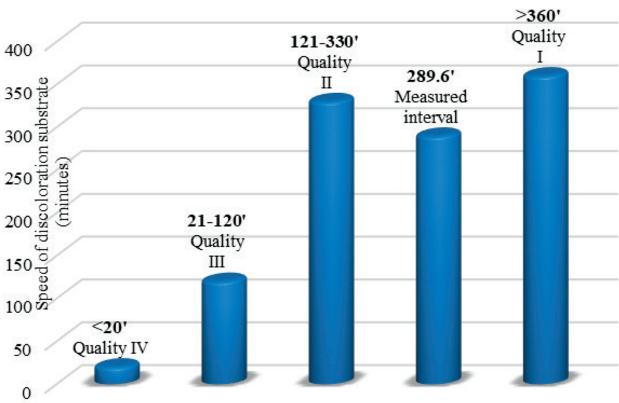

Figure 2. The hygienic quality of unpasteurized sour cream, appreciated by reductase test using methylene blue

According to the obtained results by carrying out the reductase test using resazurine: no samples doesn´t ranged in first quality class and the fourth quality class but 16 samples (64% of the total) ranged in second quality class and 9 samples (36% of the total) ranged in third quality class (Figure 3).

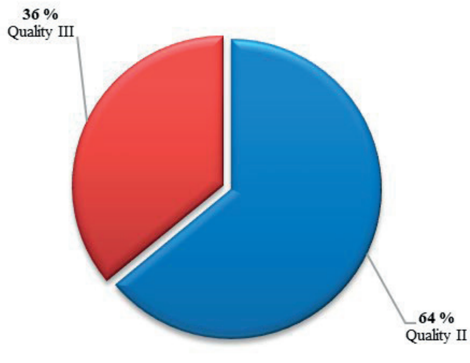

Figure 3. The distribution of unpasteurized sour cream samples by quality class (reductase test using resazurine)

To have a reference value was made the peroxidase test for unpasteurized sour cream, the reaction was positive (color in blue-green), for all 25 samples. The values obtained for measurement of titratable acidity for pasteurized sour cream ranged between 19.39°T and 21.42°T, with an average of 20.24°T±0.1°T (Figure 4). And in this case, the measured value was close to the standard value (SR ISO 6092:2008).

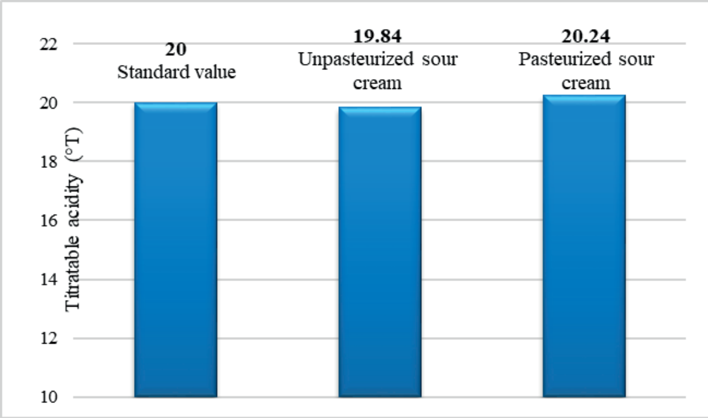

Figure 4. The titratable acidity of pasteurized sour cream, compared to value for titratable acidity of unpasteurized sour cream and with the maximum permissible value of the standard

In the case of pasteurized sour cream samples, was made the peroxidase test to establish the efficacy of the high pasteurization. The results were negative for all 25 samples, after the benzidine are added, the sour cream´s color remained unchanged, contrary to the case of unpasteurized sour cream, when the color changed in blue-green. Therefore, it is considered that pasteurization of sour cream, raw material for butter, was made at an appropriate level at temperatures above 90°C, which distorted the marker enzyme and destroyed the microorganisms.

The acidity of butter with 65% fat content, was measured by titrimetric method (Thorner). The values obtained ranged between 2.4°T and 2.8°T with an average of 2.59±0.04 °T, value close to the standard value (SR ISO 1740:2008), which is 2.8°T (Figure 5). Interpretation of results was carried out in accordance with specific literature (Ramesh, 2011; Usturoi, 2020).

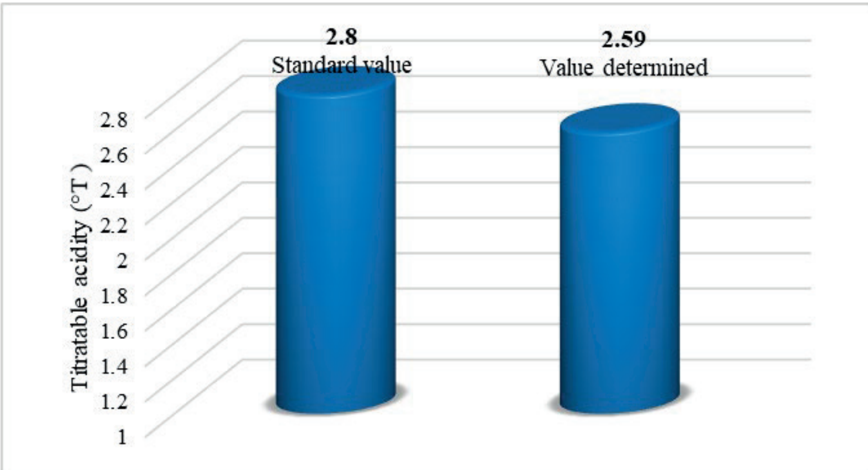

Figure 5. The distribution of unpasteurized sour cream samples by quality class (reductase test using resazurine)

For the titratable acidity expressed in grams of oleic acid (%), the values obtained ranged between 0.91 g oleic acid (%) and 1.11 g oleic acid (%) with an average of 1.02±0.02 g oleic acid (%), value close to standard value which is 1 g oleic acid (%) (Figure 6).

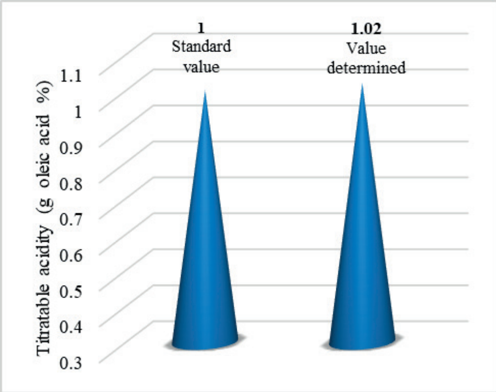

Figure 6. The acidity of butter expressed by grams oleic acid (%), compared to standard value

According to the results of the Kreis reaction, the samples of butter which have been analysed have presented variable colors, from yellowish white, serous or opalescent to yellow serous, according to Figure 7. The shades of white were the most common and the shades of yellow were the least common.

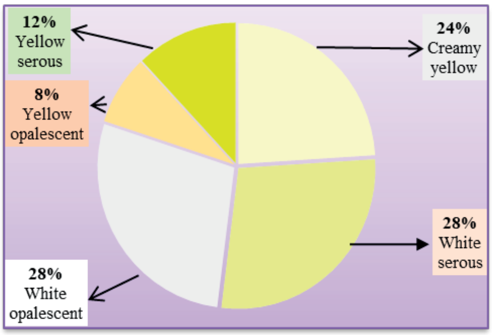

Figure 7. The frequency of colors butter determined by Kreis reaction

So, the results obtained in laboratory are representative of fresh butter (yellowish white color, of different shades), therefore, the peroxidase test has been validated. The peroxidase test applied to butter was negative, for all 25 samples, the color of butter has remained unchanged.

CONCLUSIONS

The research performed to determine the freshness and safety of sour cream, raw material for butter and freshness of butter, finished product, have generated satisfactory results, close to optimal values, in accordance with the specific standards. So, the heat treatment processes applied to sour cream have been validated. The quality of butter is heavily influenced by the quality of raw material, in this case, sour cream. Thus, the main objective of research was the investigation effectiveness of heat treatment processes applied to sour cream, by enzymatic methods. A few recommendations on improve the quality of researched dairy products, in this study, related to the analyses that have been made in laboratory, are: increase the level of automation analytical of laboratories, of the equipment, improving of the food safety by maintaining comply of hygienic status, monitor activity in laboratory and control of product traceability from the producer to consumer.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

STUDY ON THE DYNAMICS OF CATTLE LIVESTOCK, MILK PRODUCTION AND FRESH DAIRY PRODUCTS IN ROMANIA BETWEEN 2016-2020

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Abstract

Farmers receive subsidies from the state and an assessment of the living standard in the respective nation can be made based on the dynamics of cattle livestock and the development of dairy products. The EU Council receives data on these issues from EU nations each year.The data processed in the study were collected from the Annual Statistical Surveys. The data came from about 600 economic operators but summative data organization and presentation techniques (descriptive statistics) were used for processing. In Romania, during the analyzed period, even though the cattle herd decreased by 8.01%, milk production increased by 15.91% from 2016 to 2020, as farmers were interested in exploiting genetically valuable specimens. Butter production followed a sinusoidal trajectory, increasing in 2017 (177 tons), decreasing by 12 percent until 2019, and then increasing by 14.33 percent in 2020. Cheese production followed an upward trend ranging from 1 to 5.68 percent, with the highest determined increase (5 127 tons) in cheese production for 2018 compared to 2017.

Key words*: butter and cheese production, cattle livestock, dairy, development regions, fresh dairy.*

INTRODUCTION

In Romania, milk and dairy products are frequently consumed food products. This is also reflected in the farmer's interest to increase productivity and in the diversification of the dairy products range by processors. (Constantin, 2009).
Over the last 30 years, milk production has increased by 64% worldwide and by 225% in Asia (https://www.fao.org). A statistical study conducted by *Our World in Data* ranked the countries with the highest milk production worldwide from 1961 to 2018. From 1998 to 2018, India ranks first, with a production about three times higher compared to US, which ranks second. Milk production depends mainly on the productive and reproductive performance of cattle (Rajiv Baliram et al., 2018).
The world's largest milk producer is India. It accounts for almost 20% of global production (Mishra, P. et al, 2020). The United States, China, Pakistan and Brazil also make a significant contribution to global milk production (FAO).
The Food Outlook (June 2022) estimates world milk production at 937 million tonnes in 2022, an increase of 1% compared to the previous year.
Globally, a fairly high proportion (16.9%) of milk consumed by humans comes from different species other than cattle (Faye & Konuspayeva, 2012).
Milk is an important component of the human diet (Kapaj & Kapaj, 2021), dairy products are vital sources of nutrition (*OECD-FAO, 2022*) with implications for health (Bleasdale, 2021), it also indicates the development level of a country (Vidu, 2002).

MATERIALS AND METHODS

Development regions are administrative divisions at a certain level. Funds (national and European) are allocated at the level of these regions. A series of statistical studies are carried out at the regional level in various fields. In Romania there are 8 development regions: North-East, South-East, South-Muntenia/South, South-West Oltenia/ South-

West, West, North-West and Center, Bucharest-Ilfov, Figure 1.

Figure 1. Development regions in Romania
Source: https://www.mediafax.ro

Data (from about 600 economic operators) were collected from the Annual Statistical Surveys of the National Institute of Statistics in order to track the trend in cattle herds, milk production and dairy products at the national level and by development region. The working methodology for these surveys is in accordance with the Council Directive no.96/16/EC on the milk production and fresh dairy products from 10.03.1996. This Directive is designed to allow short and medium-term monitoring of the EU market for dairy and fresh dairy products. The Directive specifies deadlines according to which EU countries must send the results of various surveys to the European Commission (EUROSTAT). Another source for data collection (animals livestock registered in the official production control) was the National Agency for Animal Husbandry. Statistical processing was carried out using techniques and procedures designed to organize and present data in a summative way by graphically representing the frequencies of the analyzed variables categories (Defta, 2021).

RESULTS AND DISCUSSIONS

Cattle livestock dynamics over the period under analysis reveal a decrease of 166631 heads, percentage representing a decrease of approximately 8.01%. from 2016 to 2020. The largest decline was in 2017 compared to 2016 (68949 heads or 3.31 percentage points).

The lowest cattle population of the last 10 years was recorded in 2015 (Shahbandeh, 2021). A statistical study elaborated by Our World in Data indicates that in 2018, India was the country with a livestock of 185 million cattle heads. The same source (https://ourworldindata.org) states that in Europe, countries with cattle livestock between 10 - 50 million heads were established in: Russia (18.29 million heads), France (18.55 million heads) and Germany (11.95 million heads).

Analysing the cattle livestock distribution by development regions in the period 2016 - 2020, it was found that the only region with an increase (4202 heads, i.e. 1.26%) of cattle livestock in 2020 (338158 heads) compared to 2016 (333956 heads) was the Center region, Table 1.

For the North-East and South-West areas, a decrease in the herd was observed every year, and this reached 7.91% (2018 compared to 2017 in the South-West region). The Bucharest-Ilfov area has a small number of cattle livestock compared to the other development regions. This area also registered the largest decline in cattle number (34.62% less in 2020 compared to 2019).

Even though cattle livestock were decreasing during 2016-2020, milk production increased by 15.91% (180055 tonnes). This increase is due to the exploitation of animals with a high productive potential, with special attention being paid to welfare status and nutrition in order to boost genetic potential.

Comparing the quantity of raw cow's milk collected by processing units from livestock holdings and collection centers for the mentioned period, the milk production dynamics are as follows:

- in 2017 there was an increase of 75878 tons of milk (7.38%) compared to 2016. For 2017 milk production was 1027830 tons;
- in 2018 milk production was 92583 tons of milk (8.26%) more than what was collected in 2017;
- for 2019 there was an increase of 4887 tons (0.43%) of milk collected compared to the previous year;

Table 1. Cattle livestock dynamics during 2016-2020

Year	NE	SE	SM	SV	V	NV	C	BI	**Total**
2016	537717	240571	233222	203503	156877	368640	333956	6747	**2081233**
2017	512175	229814	221490	193566	158010	364909	325516	6804	**2012284**
2018	502939	224585	223338	178254	154938	372814	338472	6679	**2002019**
2019	486041	225321	214741	171654	153385	365055	340005	6371	**1962573**
2020	467771	216263	210676	160571	151961	365037	338158	4165	**1914602**
Mean									
	501328.6	227310.8	220693.4	181509.6	155034.2	367291	335221.4	6153.2	**1994542.2**
Standard error of the mean (SEM)									
	11840.981	3972.040	3875.394	7665.582	1105.101	1550.443	2625.536	502.642	27657.217

Note: NE - North-East; SE - South-East; S - South-Muntenia; SV - South-West Oltenia; W - West; NW - North-West; C - Center; BI - Bucharest-Ilfov.

- compared to 2019, in 2020 there was an increase in milk production of 6,707 tons (0.59 percentage points).

Even though its share varies only between 4.77% (2017) and 6.90% (2020) for the total annual production, the West region is the only one for which an upward trend in milk production has been observed.

The situation was different for the South-East region, determining a downward trajectory from 2016 (64428 tons, Chart 1A) to 2020 (48308 tons, Figure 2A), which represents a 2.5% decrease in the share of total annual production. The largest year-on-year difference was 6687 tons in 2019 compared to 2018 (11.23%), Figure 2B.

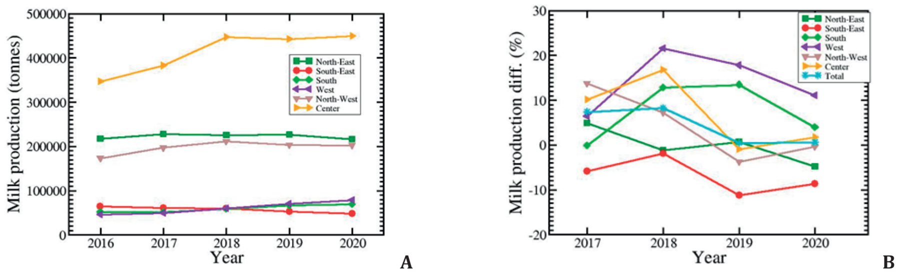

Figure 2. The dynamics of milk production in the period 2016-2020, by development regions

For the South-Muntenia/South region, milk production has been increasing from one year to the next, except 2017 when production slightly decreased (64 tons, Figure 1A; 0.12%, Figure 2B) compared to 2016.

The Centre, North-East and North-West regions have the highest contribution to the total annual production in all years included in the study. For the Center region, the only year when production decreased was 2019 (442368 tonnes, Figure 1A), with 1% difference (Figure 1B) compared to 2018 (446743 tons).

This region has the highest contribution to total annual production ranging from 36.48% (347314 tons, 2016) to 39.87% (449801 tons, 2018).

The Central, North-East, and North-West regions contributed 75% to the annual production in 2018.

The results of the research carried out in Romania regarding the consumption of fresh dairy products are consistent with the statistical processing of the present study, which shows that during 2016-2020 the quantity of fresh dairy products increased by 25736 tons, which represents an increase in production of about 9.60% (Figure 3). In 2018 and 2020 production showed a lower increasing compared to 2017 and 2019. For example, in 2017, production increased by 9829 tons (3.66%) and in 2019 by 9293 tons (3.29%), whereas in 2018 production increased by only 4497 tons (1.61%) and in 2020 by 2117 tons (0.72%).

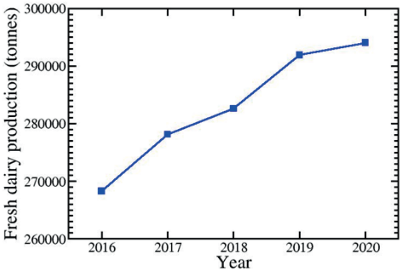

Figure 3. Dynamics of fresh dairy production in the period 2016-2020 in Romania

Comparatively, among the eight development regions, the largest increase in production of fresh dairy products during the period under review was reported for the North-West region (+9386 tons).

This region is the only one where fresh dairy production is on an upward trend (Figure 4A). For the Center region, production was 1880 tons less than the region mentioned above.

The two development regions, North-West and Center contribute each year with the highest shares to the total production in the country: the Center region with almost twice as much as the North-West.

For the South-Muntenia/South region, a decrease of about 6612 tons (27%) was reported from 2016 to 2020.

The largest percentage increase, year-on-year, was determined for the South-West region, i.e., an increase of 16.50% in 2017 compared to 2016 (Figure 4B).

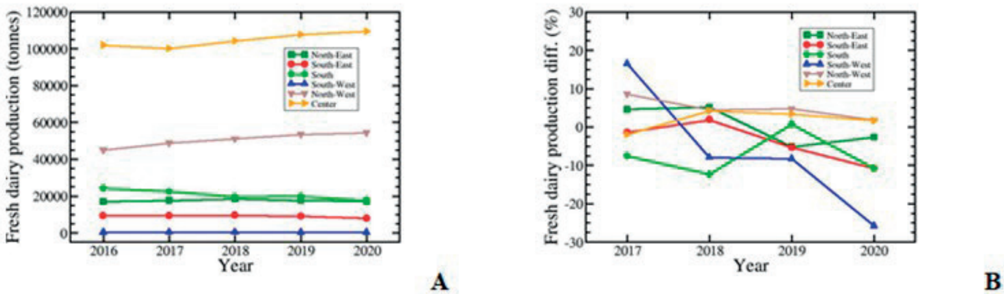

Figure 4. Fresh dairy production (2016-202) by development regions

Annual butter production follows a sinusoidal trajectory, increasing by 177 tons in 2017 compared to 2016, then decreasing by approx. 10.21% in 2018 and by approx. 2 percent in 2019, before increasing by 1526 tons (14.33 percent) in 2020 (Figure 5).

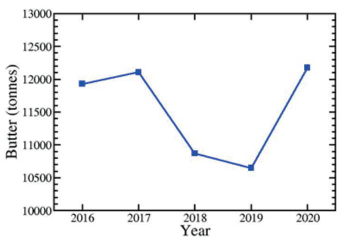

Figure 5. The dynamics of butter production in the period 2016-2020 in Romania

National butter production was mainly reported in the Center region (about half each year); those in South East and South regions reported only 1-3.5%.

In 2018 production increased to 359 tons (Figure 5A), which is about 60.27 % higher than the previous year.

For the North-East region, 2017 was the only year with a reported increase in butter production: +4.65% compared to 2016 production. In the following years (2016-2020), production was decreasing, with 23.47% total (Figure 6A and 6B).

Figure 6B shows the differences from one year to the next by development region.

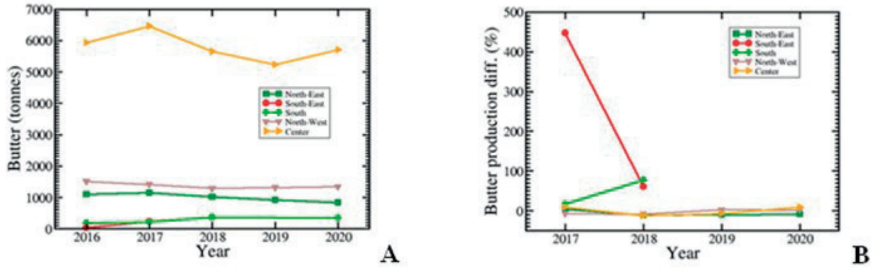

Figure 6. Butter production (2016-2020) by development regions

Cheese is another dairy product appreciated by Romanians. A continuous increase in the production of cheese (including curd) was observed for the studied period. Each year, the increased production varied between 1 and 5.68%, with the highest increase in 2018 (5127 tons) compared to 2017 (Figure 7).

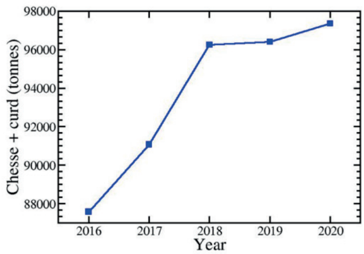

Figure 7. Cheese production (including curd) in Romania (2016-2020)

For the analysed period, it was observed that in five of eight development regions (North-East, West, North-West, Center and Bucharest-Ilfov) production increased at the end of 2020 compared to 2016, with fluctuations in cheese production during this period.

The highest production was recorded in the Central region and the lowest in the Bucharest-Ilfov region (47 tons, 2016 - 65 tons, 2020). For the Center, the dynamics of cheese production followed an upward path from 2016 (31136 tons) to 2020 (40575 tons), with an increase of 9436 tons in production and 30.31% respectively. It is the only area where cheese production (including curd) increased year-on-year (Figure 8A).

The graphical representation of cheese production in the North-East region shows a sinusoidal production trajectory: it increased by 1335 tons in 2017 (Figure 8A), decreased by 828 tons in 2018, and increased again by 1385 tons in 2017 (6.27%, Figure 8B), with a small decrease in the following year (135 tons). Figure 8B shows the percentage differences from one year to the another.

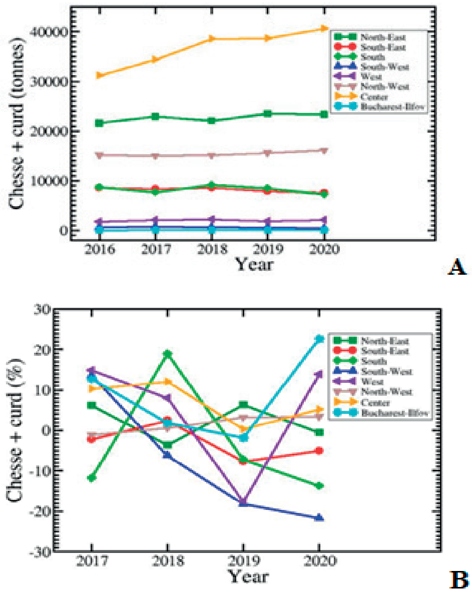

Figure 8. Cheese production, including curd (2016-2020) by development regions (A, B)

CONCLUSIONS

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 during the period under review, milk production has increased, as farmers are interested in exploiting animals with high production potential and ensuring optimal conditions for animal welfare.

ACKNOWLEDGEMENTS

This research work was carried out with the support of University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Animal Productions Engineering and Management.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

THE USE OF *Moringa oleifera* AS VALUE-ADDED INGREDIENT IN BAKERY INDUSTRY

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Abstract

In this study, the nutritional, physicochemical, phytochemical and sensory potential of Moringa flour, composite Moringa/wheat flours in different proportions, and bread obtained from these flours were examined. The nutritional analysis revealed an increase in the mineral content of the bread with the addition of 10% Moringa flour, as well as an increase in protein and fat content. The results obtained show that the maximum mineral and fat contents were recorded in the case of bread with 10% Moringa flour and are 3.89% and 4.93%, respectively. They also show that the addition of moringa flour results in an abundance of micro- and macro-nutrients in composite flour samples and breads depending on the percentage added. The bread containing 2.5% of Moringa flour has values close to the control bread in terms of physical properties: 83.88%, 250.57 cm^3/100 g, 93.66% and 0.717 respectively for porosity, volume, elasticity and H/D ratio. The total polyphenol content was between 191.87 and 279.83 mg/100 g and flavonoids between 6.18 and 9.76 mg EQ/100 g. The sensory analysis showed a reluctance of consumers to bread with more than 2.5% of moringa flour. This study reveals that the addition of moringa leaf flour up to 2.5% allows obtaining an elastic bread, acceptable from an organoleptic point of view while improving the nutritional quality without negative effects on the physicochemical characteristics.

***Key words**: flavonoids, Moringa, nutrition, organoleptic, polyphenols.*

INTRODUCTION

From ancient times to the present day, people around the world have relied on the major cereal grains of wheat, rice, and maize for their diets. The lack of dietary diversity justifies the current state of global nutrition (Petcu et al., 2019; Matthews & Ghanem, 2020), which poses a threat to nutritional security. To alleviate this, diverse food crops as well as underutilized or orphan crops rich in protein, dietary fiber, phenolic antioxidants, and micronutrients improving nutritional quality should be part of the diet (Savu & Petcu, 2002; Goncearov et al., 2004; FAO, 2010; Khoury et al., 2014).
Indeed, *Moringa oleífera* or tree of life is one of the plants containing many bioactive compounds and which strengthens the immune system (Oyeyinka & Oyeyinka, 2016). It is a miracle plant in the sense that all its parts (leaves, seeds, flowers, etc.) can be used as a food supplement, medicine, water purifier and animal fodder (Daba, 2016).
Moringa leaves contain significant amounts of micro and macronutrients including calcium, potassium, magnesium, iron etc. also they are an important source of protein, β-carotene, and vitamins A, B1, C and E (Sanchez-Machado et al., 2010; Hekmat et al., 2015; Gonzalez-Burgos et al., 2021). A study by Manzo et al. in 2016 on the composition of *Moringa oleifera* dry leaf powder in three regions of Niger shows that Moringa dry leaf powder produced in Niger was found to be rich in protein with an average of 24.8% with micronutrient values ranging from 21.58% and 28.72%. Depending on the region, the composition varies for iron between 51.9 and 55.12 mg/100 g; 0.45 and 1.58 mg/100 g for zinc, 1192.5 and 1957.5 mg/100 g for calcium, 414.37 and 714.37 mg/100 g for magnesium, 1587 and 2037 mg/100 g for potassium, 207.75 and 326.25 mg/100 g for sodium, 32 and 61 mg/100 g for phosphorus Several authors have reported the antioxidant properties of *Moringa oleifera* leaves as well as a good level of polyphenol and flavonoids (Siddhuraju &

Becker 2003; Sreelatha & Padma 2009; Belhi et al., 2018).

Several scientific studies have shown that supplementation of dried Moringa leaves to staple foods such as bread, cheese, yogurt and cookies improves their nutritional properties (Dachana et al., 2010; Hekmat et al., 2015; Salem et al., 2013; Sengev et al., 2013; Hedhili et al., 2021). Indeed, in their study on the effect of *Moringa oleifera* leaf powder supplementation on some quality characteristics of wheat bread in 2013, Sengev et al, revealed that addition of Moringa leaf powder significantly increased fiber (2.10% to 3.28%), ash (1.10% to 1.65%), protein (9.07% to 13.97%), and ether extract (1.51% to 2.59%), while decreasing moisture content (35.20% to 27.65%). Also, this study reveals that there was a significant increase in magnesium (Mg), calcium (Ca) and beta-carotene contents from 0.76 to 1.27 mg/100 g, 3.67 to 6.07 mg/100 g and 0.02 to 3.27 mg/100 g, respectively.

Not only does moringa have effects on the sanitary quality of the products to which it is added, but it also acts on the textural quality of the product (Sengev et al., 2013; Hernandez-Aguilar et al., 2021). In 2013 the studies of Sengev et al. showed that supplementation with moringa leaf powder significantly decreased the volume, weight and height of bread. The specific volume of the bread decreased from 796.70 to 496.70 cm 3, and the height from 7.00 to 5.83 cm. Studies by Hernandez-Aguilar et al on photoacoustic characterization of wheat bread mixed with Moringa oleifera revealed that the addition of moringa in bread making slows down textural changes (hardness, elasticity, cohesion, resilience, and chewing) during storage.

Although there are several studies on the addition of moringa in bread making, none of these studies made a comparison between the nutritional, organoleptic and physicochemical parameters of the composite flours to the finished products. All these studies only addressed the characteristics of the final products.

The objective of this paper is to study the nutritional, physicochemical and organoleptic potential of Moringa flour and Moringa/wheat composite flour in different proportions. Thus, breads with different proportions of Moringa flour will be characterized from a nutritional, physicochemical and organoleptic point of view.

MATERIALS AND METHODS

Preparation of composite flours

Moringa flour (MF) was purchased in Benin, and wheat flour (WF) type 650 at Profi supermarket, Timisoara (Romania). Four types of composite moringa flours were made: MWF 1 (2.5% moringa flour and 97.5% wheat flour); MWF 2 (5% moringa flour and 95% wheat flour); MWF 3 (7.5% moringa flour and 92.5% wheat flour); and MWF 4 (10% moringa flour and 90% wheat flour).

Sample preparation

The breads were prepared according to Hernandez-Aguilar C. et al, (2021) and Plustea L. et al (2022) with some modifications. All ingredients (honey, wheat flour type 650, salt, oil and Pakamaya yeast) used to produce the bread apart from Moringa flour were purchased from local supermarkets Profi and Auchan, Timisoara, Romania. Four experimental breads (MWB1, MWB2, MWB3 and MWB4) were prepared by supplementing a control bread (WB) with different amounts of MF (WB - wheat bread, MWB 1 - Moringa/wheat bread 2,5%, MWB 2 - Moringa/wheat bread 5%, MWB 3 - Moringa/wheat bread 7.5% and MWB 4 - Moringa/wheat bread 10%).

The breads obtained from this study are presented in Figure 1.

Figure 1. Final products. WB - wheat bread, MWB1 – Moringa/wheat bread 2.5%, MWB 2 - Moringa/wheat bread 5%, MWB3 - Moringa/wheat bread 7.5% and MWB 3 - Moringa/wheat bread 10%

Table 1. Recipe for bread with composite Moringa flours

Samples	Ingredients						
	Moringa flour (g)	Wheat flour type 650 (g)	Yeast (g)	Salt (g)	Honey (g)	Oil (mL)	Water (mL)
WB	-	1000	50	20	30	80	800
MWB1	25	975	50	20	30	80	800
MWB2	50	950	50	20	30	80	800
MWB3	75	925	50	20	30	80	800
MWB4	100	900	50	20	30	80	800

After its fermentation in 800 g of warm water (30°C), the yeast was incorporated to the mixture of flour, honey and salt. They were then mixed for 5 minutes, in a mixer with a spiral hook at the first speed. At the second speed, which also lasted 5 minutes, the oil was added gradually. The obtained dough is then placed in a covered plastic bowl for 1 hour at a temperature of 20°C. Then the dough was kneaded and placed in a greased bread pan, where it increased its volume for 30 min at a temperature of 35°C. The doughs were simultaneously baked in an electric oven preheated to 230°C, for 24 min. After baking, the loaves will be left to cool at room temperature for 24 h and then cut into slices with a knife. The technological scheme for obtaining bread is presented in Figure 2.

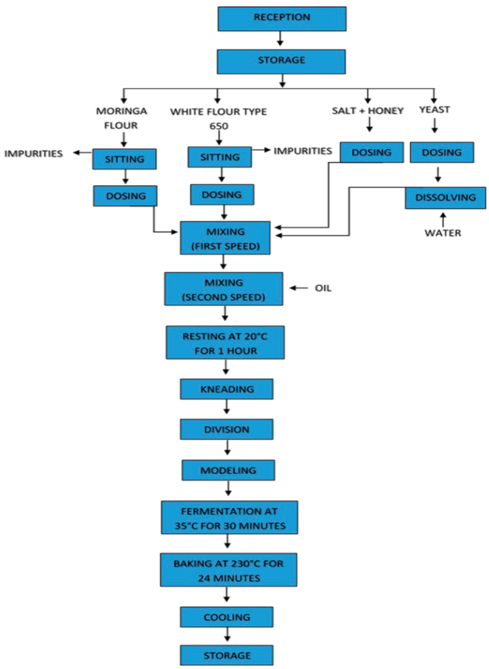

Figure 2. The technological scheme for obtaining bread

Determination of mineral content

3 g of each sample (weighed on an analytical balance: KERN ALT 220-4NM, series WL 074130, Germany) in a porcelain capsule were calcined for 6 to 8 hours in a calcination kiln (Naberthem GmbH, series 190945, Lilienthai/Bremen, Germany) heated to 550°C and then cooled down and weighed as soon as it reached room temperature. The ash content is calculated with the relation:

Ash (%) = $[(m_2-m_0)/(m_1-m_0)] \times 100$,

where: m_2 - mass of the crucible after calcination, in (g); m_1 - mass of the crucible containing the sample before calcination, in (g); m_0 - mass of the empty crucible (g).

Determination of lipid content

The determination of the lipid content was carried out in a practical way, by hot extraction using the Soxhlet apparatus (Raypa Espinar, S.L., series 31454, mode SX-6). The fat content is calculated using the formula:

$$\text{Fat} = \frac{m}{m_1} 100\ (\%)$$

where:

m - is the amount of fat extracted; m_1- is the amount of sample taken.

Determination of carbohydrate content

The carbohydrate content is obtained by subtracting 100 from the sum of the protein, fat, moisture and ash contents of the sample.

Determination of protein content

The protein composition was determined following SR EN ISO 8968-1:2014.

Determination of the energy value

The energy value was calculated by adding the caloric intake of fats, carbohydrates and proteins, taking into account the following composition:

1 g of fat = 9 kcal,
1 g of protein = 4 kcal,
1 g of carbohydrates = 4 kcal

Determination of moisture content
5 g of each sample was weighed with an analytical balance (KERN ALT 220-4NM, series WL 074130, Germany) to the nearest 0.0002 g into a glass vial with a lid. The vial was then placed in an oven (Binder GmbH, Tuttingen/Germany) at 105°C where it was held for 1 hour, then removed and allowed to cool. Then it was weighed. The moisture content was calculated using the formula:

$$\text{Moisture (\%)} = \frac{m_1 - m_2}{m_1} \times 100$$

where: m_1 - mass of the sample before drying, in (g); m_2 - mass of the sample after drying (g).

Macro and microelements
Determination of the content of macro- and microelements was determined by atomic absorption spectroscopy (AAS) using the Varian 220 FAA equipment according to the method described by Plustea et al. (2022). The results were expressed in ppm.

Physico-chemical properties
For each type of bread, the following physicochemical characteristics were used to assess quality: volume, porosity, core elasticity, height/diameter ratio, moisture and acidity. All analytical methods used were in accordance with STAS 91/83. The results are the arithmetic mean of three parallel determinations.

Evaluation of the total phenolic content
The methods used here are those of Folin-Ciocâlteu with some modifications according Obistioiu et al. (2021). The total phenolic content of MF, WF, composite flours and bread with different percentages of Moringa flour was analyzed. The absorbance of the samples was read at 750 nm with the Specord 205 spectrophotometer (Analytik Jena AG, Jena, Germany) using ethanol (Sigma-Aldrich; Merck KgaA, Darmstadt, Germany) as a blank sample. Results were expressed as mg gallic acid equivalent (GAE) per 100 g sample based on the calibration curve (concentration range: 2.5-250 μg/mL). All the determinations were performed in triplicate.

Sensory analysis
The panel consisted of 36 ISO 6658:2017 trained raters, aged 21-56 years, non-smokers, with no known cases of food allergies. A 1 cm thick slice of each type of bread was presented on cardboard plates, coded with two numbers and served randomly, under normal lighting conditions and at room temperature. To assess consumer acceptance, a five-point hedonic scale was used [ISO 6658:2017], with the following rates: 1 = extremely disliked; 2 = slightly disliked; 3 = neither liked nor disliked; 4 = slightly liked; 5 = extremely liked.
Each panelist was asked to rate the sensory attributes of each sample: appearance, color, texture, flavor, taste, and overall acceptability. The score and acceptability level ranges were as follows: 1.00-1.49 = Not Acceptable; 1.5- 2.49 = Slightly Acceptable (SA); 2.50-3.49 = Moderately Acceptable; 3.5-4.49 = Acceptable; 4.5-5.00 = Very Acceptable.

Statistical analysis
All determinations were made in triplicate and the results are reported as mean values ± standard deviation (SD). Differences between means were analyzed by t-test (two-sample assuming equal variances) using Microsoft Excel 365. Differences were considered significant when p-values < 0.05.

RESULTS AND DISCUSSIONS

Proximate composition
The results presented in Table 2 illustrate the nutritional characteristics of the wheat/Moringa composite flours as well as the breads made from these flours.

Table 2. Proximate composition of Moringa composite flours and breads

Samples	Nutritional parameters					
	Moisture (%)	Ash (%)	Protein (%)	Lipids (%)	Carbohydrates [g/100 g]	Energy value [kcal/ 100 g]
COMPOSITE FLOURS						
WF	10.80 ± 0.04^{a}	0.33 ± 0.03^{a}	11.26 ± 0.02^{a}	1.33 ± 0.03^{a}	76.28 ± 0.04	362.13 ± 0.16
MF	9.79 ± 0.01^{b}	9.97 ± 0.10^{b}	37.55 ± 0.3^{b}	3.31 ± 0.03^{b}	39.38 ± 0.35	337.51 ± 0.53
MWF1	10.59 ± 0.04^{c}	0.79 ± 0.08^{c}	12.28 ± 0.2^{c}	$1.40 \pm 0.05^{a,c}$	74.94 ± 0.32	361.48 ± 0.74
MWF2	$10.40 \pm 0.13^{c,d}$	1.34 ± 0.02^{d}	12.97 ± 0.05^{c}	$1.60 \pm 0.15^{c,d}$	73.69 ± 0.08	361.04 ± 1.32
MWF3	$10.20 \pm 0.13^{d,e}$	1.84 ± 0.01^{e}	13.77 ± 0.07^{d}	$1.77 \pm 0.05^{d,e}$	72.42 ± 0.17	360.69 ± 0.76
MWF4	10.05 ± 0.14^{e}	2.32 ± 0.04^{f}	14.35 ± 0.08^{e}	1.96 ± 0.06^{e}	71.32 ± 0.18	360.32 ± 0.57
COMPOSITE BREAD						
BW	35.65 ± 0.03^{a}	1.33 ± 0.02^{a}	10.87 ± 0.02^{a}	3.19 ± 0.01^{a}	48.97 ± 0.01	268.09 ± 0.16
MWB1	34.89 ± 0.05^{b}	2.94 ± 0.04^{b}	11.47 ± 0.09^{b}	3.76 ± 0.06^{b}	46.98 ± 0.1	267.65 ± 0.44
MWB2	$34.67 \pm 0.09^{b,c}$	3.62 ± 0.05^{c}	11.78 ± 0.07^{b}	4.15 ± 0.18^{c}	45.78 ± 0.17	267.59 ± 1.09
MWB3	$34.35 \pm 0.02^{c,d}$	4.63 ± 0.02^{d}	12.83 ± 0.03^{c}	4.61 ± 0.04^{d}	43.59 ± 0.04	267.16 ± 0.32
MWB4	34.22 ± 0.03^{d}	5.34 ± 0.02^{e}	13.82 ± 0.03^{e}	4.93 ± 0.02^{e}	41.68 ± 0.08	266.41 ± 0.1

$^{a-e}$data within the same column sharing different superscripts are significantly different ($p < 0.05$) according to t-test.

The results of Table 2 show that MF is a good source of minerals (9.97 ± 0.10%), proteins (37.55 ± 0.3%) and lipids (3.31 ± 0.03%). These results are close to those obtained through the metanalysis made by Witt in 2013 on 49 scientific articles on the nutritional composition of dried Moringa leaves (Witt, 2013). In this study, on 100 g of dried Moringa leaves, 24 ± 5.8 g of protein, and 6 ± 2.5 g of lipids are found. These results are also close to those of Manzo et al. (2013), where the protein content of moringa leaves ranged from 19.34- 30.24% and Ndong et al. (2007), where the protein concentration was 39.69%.

These results also show that MF is more than twice as concentrated in protein as WF (37.55 ± 0.3% against 11.26 ± 0.02% for WF). On the other hand, WF is about 2 times richer in carbohydrates than MF (76.28 ± 0.04% against 39.38 ± 0.35% for MF). These results are similar to those of Sengev et al. (2013), in their studies on the effect of Moringa oleifera leaf powder supplementation on some quality characteristics of wheat bread. In their studies, MF had a protein concentration of 27.82 ± 0.06 % against 11.20 ± 0.04% for WF. Concerning carbohydrates MF had a concentration of 38.20 ± 0.02% against 69.70 ± 0.04% for WF.

WF provides more energy than MF (362.13 ± 0.16% against 337.51 ± 0.53% for MF). The same thing was observed for the breads obtained. Thus, the more the quantity of MF increases in the composition of the breads, the more the rate of carbohydrates and energy values decrease. For the carbohydrate rate we have respectively 48.97 ± 0.01%; 46.98 ± 0.1%; 45.78 ± 0.17%; 43.59 ± 0.04% and 41.68 ± 0.08% for WB, MWB1, MWB2, MBW3 and MBW4. As for the energy value of 267.65 ± 0.44 kcal/ 100 g for MWB1, it increased to 266.41 ± 0.1 kcal/ 100 g for MWB4.

MF being more than 30 times more concentrated in mineral substances than WF, the more its quantity increases in the composition, the more the quantity of mineral substances increases in both flours and breads. We have respectively for the flours 0.33 ± 0.03%; 9.97 ± 0.10%; 0.79 ± 0.08%; 1.34 ± 0.02%, 1.84 ± 0.01% and 2.32 ± 0.04 for WB, MWB1, MWB2, MBW3 and MBW4 For breads the mineral content increased from 2.94 ± 0.04% (MWB1) to 5.34 ± 0.02% (MWB4). The same was observed for lipids and proteins. That is, the higher the amount of MF, the higher the lipid or protein content in the composite flours and in the breads.

Significant differences ($p < 0.05$) according to t-test were recorded between the samples for most of all analysed parameters.

These results show that the partial substitution of wheat flour by moringa would increase the level of minerals, proteins and lipids in both the composite flours and the breads produced with these flours. There is a significant difference between the values obtained for the control samples (WF for flours and WB for breads) and the other samples for each of the parameters studied (moisture, ash, proteins and lipids.

Macro and microelements

Table 3 shows the macro- and micro-nutrient content of the moringa flour composites and breads. The experimental results obtained show that the addition of moringa flour leads to an increase in the contribution of micro and macro elements in both composite flour samples and breads depending on the percentage added. The same finding was made in the studies of Sengev et al. (2013) on the effect of *Moringa oleifera* Leaf Powder Supplementation on Some Quality Characteristics of Wheat Bread. In this study, as the amount of MF increased, the micro and macronutrients (Fe, Mg, Ca and Cu) were more abundant. The most abundant macronutrient in the analyzed samples is calcium (Ca). It varied between 2875.58± 20.61 and 475.44±1.15 mg/kg in the flour samples while in the bread samples it varied between 128.65±0.38 and 386.75±0.49 mg/kg. Similar results were obtained in the studies of Sengev et al. (2013), where calcium was the most abundant macroelement.

Statistically significant differences were found both in terms of Ca content in moringa, wheat and composite flours, but also in the resulting bread.

Table 3. Macro and micro-elements content of moringa flour composites and bread

Samples	Macro- and Microelements Content (mg/kg)							
	Cu	Ni	Zn	Fe	Mn	Ca	Mg	K
Composite flours								
MF	5.42± 0.02 ^{a}	0.76±0.006 ^{a}	29.58± 0.01 ^{a}	93.553± 0.02 ^{a}	32.89± 0.01 ^{a}	2875.58± 20.61 ^{a}	742.06±1.14 ^{a}	975.31±0.95 ^{a}
WF	2.40± 0.002 ^{b}	nd	11.40± 0.01 ^{b}	12.13±0.03 ^{b}	8.69±0.02 ^{b}	166.22±0.99 ^{b}	273.15±1.28 ^{b}	65.29±0.09 ^{b}
MWF1	2.79± 0.04 ^{b,c}	0.103± 0.005 ^{b}	12.26± 0.04 ^{c}	14.23±0.04 ^{c}	8.87±0.03 ^{b,c}	228.30±0.90 ^{c}	280.47±1.31 ^{c}	114.61± 0.51 ^{c}
MWF2	2.93± 0.02 ^{c,d}	0.257± 0.003 ^{c}	12.43± 0.02 ^{c}	16.96±0.01 ^{d}	9.01±0.02 ^{c}	325.43±0.74 ^{d}	286.81±0.85 ^{c,d}	144.98± 0.68 ^{d}
MWF3	3.00± 0.01 ^{d}	0.366± 0.005 ^{d}	13.04± 0.04 ^{d}	19.49±0.04 ^{e}	9.31±0.06 ^{c,d}	391.93±0.56 ^{e}	289.20±0.68 ^{d,e}	172.18± 1.15 ^{e}
MWF4	3.10± 0.05 ^{d}	0.430± 0.026 ^{e}	14.63± 0.07 ^{d}	21.21±0.02 ^{f}	10.01± 0.01 ^{d}	475.44±1.15 ^{f}	292.38±1.01 ^{e}	198.08± 0.75 ^{f}
Composite Breads								
WB	1.85± 0.02 ^{a}	0.014± 0.005 ^{a}	4.08±0.05 ^{a}	11.21±0.04 ^{a}	2.97±0.05 ^{a}	128.65±0.38 ^{a}	244.77± 0.68 ^{a}	62.31±0.91 ^{a}
MWB1	2.12± 0.03 ^{b}	0.087± 0.003 ^{b}	5.64±0.02 ^{b}	13.80±0.05 ^{b}	4.08±0.03 ^{b}	220.22±1.35 ^{b}	266.27± 0.95 ^{b}	102.03± 1.12 ^{b}
MWB2	2.41± 0.01 ^{c}	0.136± 0.004 ^{c}	6.09±0.02 ^{c}	14.71±0.01 ^{c}	4.65±0.03 ^{b}	275.61±0.66 ^{c}	271.47± 1.20 ^{b}	167.21± 1.52 ^{c}
MWB3	2.65± 0.02 ^{c,d}	0.173± 0.002 ^{c,d}	6.40±0.06 ^{c}	15.49±0.03 ^{d}	5.62±0.01 ^{c}	357.38±0.82 ^{d}	286.54± 2.52 ^{c}	174.91± 0.89 ^{c,d}
MWB4	2.87± 0.01 ^{d}	0.205± 0.005 ^{d}	7.60±0.06 ^{d}	16.85±0.03 ^{e}	6.21±0.02 ^{d}	386.75±0.49 ^{e}	296.47± 1.47 ^{d}	182.22± 0.38 ^{d}

$^{a–f}$ data within the same column sharing different superscripts are significantly different ($p < 0.05$) according to t-test

It should be noted that calcium is a mineral that plays an essential role in the optimal functioning of the organism, particularly in the growth of the skeleton during childhood and ensures the necessary bone mineral mass during adult life; it is also involved in cell permeability, neuromuscular excitability, cardiac function and in blood coagulation. These different functions of calcium explain why the needs are rigorously covered in young growing children (Dansou et al., 2000). The FAO and WHO, quoted by Dansou et al. (2000), recommend that the daily calcium intake of the healthy population should be between 500 and 800 mg per day.

Potassium is the second most abundant element and increases with the amount of MF added. Thus, in both composite flours and breads, it is more abundant in the sample with 10% moringa.

We have respectively 198.08± 0.75 mg/kg for MWF4 (flour) and 182.22± 0.38 mg/kg (bread). There is a significant difference not only between the different composite flour samples but also between the bread samples.
The magnesium (Mg) content of the composite flour samples MWF1 (280.47±1.31 mg/kg), MWF2 (286.81±0.85 mg/kg), MWF3 (289.20±0.68 mg/kg) and MWF4 (292.38±1.01 mg/kg) showed significant differences from WF (273.15±1.28 mg/kg). MWF recorded the highest Mg content (742.06±1.14 mg/kg). The same was true for the bread samples, which means that there was a significant difference between WB and other bread samples containing moringa. The WB content was 244.77± 0.68 mg/kg, lower than the other bread samples which recorded values between 266.27± 0.95 mg/kg (MWB1) and 296.47± 1.47 mg/kg (MWB4).
The content of iron (Fe) varies between 12.13±0.03 mg/kg (WF) and 21.21±0.02 mg/kg (MWF4) for the composite flour samples and between 11.21±0.04 mg/kg (WB) and 16.85±0.03 mg/kg (MWB4) for bread samples with significant difference between them. MF recorded the highest Fe content (93.553± 0.02 mg/kg). Also, as in the case of the previous nutrients, the higher the amount of MF the more abundant the iron content is. This observation will remain the same for the other nutrients (Cu, Ni and Zn). Also, for each of these nutrients MF has the highest rate. Finally, there is a significant difference between the values obtained for the control samples (WF for flours and WB for breads) and the other samples for each of the remaining nutrients (Cu, Ni and Zn). These different findings are in agreement with the results obtained by Sengev et al. in 2013.

Bread properties

For each bread samples it was determined according to STAS 91/1983, the volume, porosity, elasticity, height/diameter ratio (H/D) and moisture. The results obtained are presented in Table 4.

Table 4. Bread quality indicators for WB, MWB1, MWB2, MWB3 and MWB4

Indicator	M.U.	WB	MWF1	MWF2	MWF3	MWF4
Volume	cm^3/100 g	251.09±0.05	250.57±0.01	234.96±0.02	231.05±0.01	230.32±0.33
Porosity	%	85.67±0.06	83.88±0.02	83.15±0.05	81.77±0.06	80.81±0.01
Elasticity	%	91.67±1.15	93.33±0.58	93.67±1.15	94±1.00	94.33±1.53
H/D	-	0.752±0.002	0.717±0.003	0.702±0.009	0.653±0.001	0.598±0.002
Acidity	Acidity/ 100 g	3.33±0.02	6.33±0.01	7±0.4	7.66±0.2	8.33±0.05

From the analysis of the data obtained from the Table 4, it can be seen that the volume of the control sample (251.09±0.05 cm^3/100 g) is higher compared to the other bread samples with different proportions of Moringa flour (between 250.57 and 230.32). However, the products were not excessively flattened (Table 3). Thus, as the amount of moringa increases in the bread composition, the volume decreases. The same observation was made by Sengev et al. in 2013 where the volume decreased from 4.70 ± 0.03 cm^3/g for the control sample to 2.65 ± 0.09 cm^3/g for the sample with 5% MF.
The control sample (wheat flour, without addition of moringa) recorded the highest values in terms of volume (251.09±0.05 cm^3/100 g), H/D ratio (0.752), porosity (85.67%) compared to the samples with addition of moringa, which recorded lower values for these different parameters analyzed. On the other hand, the more the quantity of Moringa increases in the recipe, the more the elasticity of the bread increases. Thus, MWB4 recorded the highest percentage in terms of elasticity (80.81%). These results are consistent with Bourekoua et al. (2018), who revealed that the addition of moringa in the bread formulation decreases its volume but increases its elasticity.
MWB1 has a good presentation and well smooth with a volume of 250.57±0.01 cm^3/100 g, soft and flat pores for a porosity of 83.88±0.02%, with 94.33±1.53% elasticity, the H/D ratio is 0.717±0.003. MWB1 recorded the best scores closest to the control sample compared to other breads containing Moringa.
As far as acidity is concerned, it is observed that it increases with the addition of moringa flour in the composition. From 3.33 grades of

acidity/100 g of bread in the standard bread (WB), it increases to 6.33 grades of acidity/ 100 g of bread for MWB1, to 7 grades of acidity/100 g of bread for MWB2, to 7.33 grades of acidity/100g of bread for MWB3 and to 3 grades of acidity per 100 g of bread for MWB4. We can therefore conclude that Moringa powder has a higher acidity than wheat flour. This parameter must then be taken into consideration in the production of bread with Moringa flour in order to have bread with better physicochemical properties.

Phytochemical proprieties

The following figures show the polyphenol content for the different flours (Figure 3) and for the different breads (Figure 4).

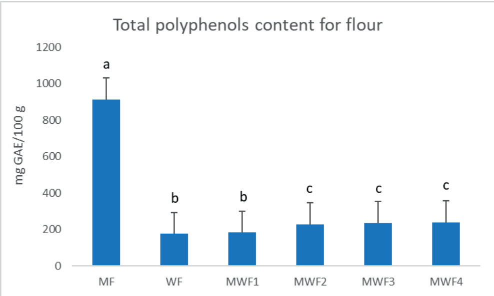

Figure 3. Polyphenol content for the different flours
[a–e]data within the same column sharing different superscripts are significantly different ($p < 0.05$) according to t-test

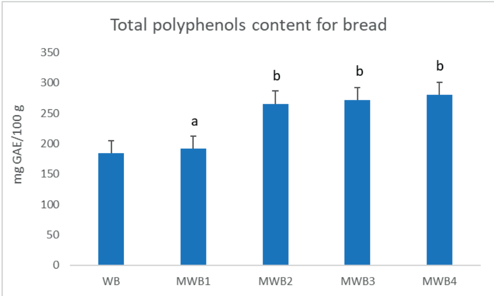

Figure 4. Polyphenol content for the different bread
[a–e]data within the same column sharing different superscripts are significantly different ($p < 0.05$) according to t-test

The analysis of the data obtained shows that the polyphenol content in moringa flour is 5 times higher than in wheat flour. Thus, it can be seen that the polyphenol content increases with the increase of the moringa flour content in the sample. Then, in the case of flour samples, it is found that the lowest PT content is observed in the control sample WF (176.70±0.693 mg GAE/100 g), while the highest value is observed for the control sample MWF4 (239.40±0.346 mg GAE/100 g). This is due to the high polyphenol content in moringa leaves (Siddhuraju & Becker, 2003; Sreelatha & Padma, 2009; Belhi et al., 2018).

It is also found that the TP content of the bread samples is higher than the TP content of the flour. This observation would be due to the presence of polyphenols in the honey used as an ingredient to obtain the bread samples (Dico et al., 2019). Thus, as for the fava samples, the higher the amount of moringa powder, the higher the polyphenol content in the bread samples. We have 183.800±6.059 mg GAE/ 100 g; 191.870±1.198 mg GAE/100 g; 265.503±3.593 mg GAE/100 g; 271.580±1.920 mg GAE/100 g and 279.833±9.469 mg GAE/100 g, respectively for the samples WB, MWB1, MWB2, MWB3 and MWB4. Similar results were found by Hayat et al, in 2017 in their studies on the evaluation of physical, sensory and antioxidant properties of gluten-free bread enriched with *Moringa oleifera* leaf powder. In this study with the same proportions of moringa (2.5%, 5%, 7.5% and 10%) the polyphenol content varied between 212 and 239 mg GAE/100 g. On the other hand, the level of polyphenols for dry moringa leaves was 331.3 mg GAE/100 g against 912.7 mg GAE/100 g in our study. This difference would probably be due to the fact that the composition of moringa leaf varies from one region to another (Manzo et al., 2016). As such, the polyphenol level was 236.5 mg GAE/100 g for moringa leaf powder in the study conducted by Belhi et al. in 2018 on the chemical properties and anti-nutritional factors of *Moringa oleifera*.

Table 5 presents the flavonoid composition of the composite flours as well as the breads resulting from these flours.

Table 5: Flavonoid composition of the composite flours as well as the breads resulting from these flours

Sample		Flavonoids (mg QE/100 g)	
Flours composites		Breads composite	
WF	1.72±0.08 [a]	WB	2.90±0.05 [a]
MF	12.40±0.07 [b]	-	-
MWF1	4.28±0.05 [c]	MWB1	6.18±0.22 [b]
MWF2	4.78±0.03 [c,d]	MWB2	7.95±0.00 [c]
MWF3	5.16±0.16 [d]	MWB3	9.72±0.01 [d]
MWF4	5.91±0.00 [e]	MWB4	9.76±0.01 [d]

[a–e]data within the same column sharing different superscripts are significantly different ($p < 0.05$) according to t-test

From the analysis of the results in this table, it can be seen that moringa flour is richer in flavonoid than wheat flour (12.40 ±0.07 mg EQ/100 g versus 1.72 ±0.08 mg EQ/100 g). Approximately similar results were obtained by Sulastri et al. in 2018 in a study on total phenolic, total flavonoid, quercetin content and antioxidant activity of the standardized extract of *Moringa oleifera* leaf from regions at different altitudes. In this study, the values found for flavonoids varied between 8.9 and 9.6 mg EQ/100 g.
Also, we notice the increase of the flavonoid rate with the increase of the Moringa flour (4.28 mg EQ/100 g for MWF1 and 5.91 mg EQ/100 g for MWF4). This same observation was made in the composition in flavonoids of the obtained breads where we have respectively 6.18 mg EQ/100 g; 7.95 mg EQ/100 g; 9.72 mg EQ/100 g and 9.76 mg EQ/100 g for MWB1, MWB2, MWB3 and MWB4. It should be noted here that the concentration of flavonoids in breads is higher than in flours. This finding would be due to the presence of flavonoid in the honey used as an ingredient to obtain the breads (Silva et al., 2021).

Sensory analysis
Consumer acceptability of the bread samples with different proportions of moringa and the standard bread was performed by sensory evaluation with a panel of 36 raters using a five-point hedonic scale. Figure 5 shows the mean scores for the sensory attributes (appearance, color, texture, taste, aroma and overall acceptability) of the different breads. Recall here that WB, is the control wheat bread; MWB1, the one with 2.5% moringa flour; MWB2; MBWB3 and MBWB4 respectively the breads with 5%; 7.5% and 10% moringa flour.
The control sample (WB) had the highest scores regardless of the evaluation criteria (color: 4.61±0.80; taste: 4.56±0.80; appearance: 4.47±0.94; texture: 4.72±0.74; flavor: 4.47±0.84 and overall acceptability: 4.53±0.77).

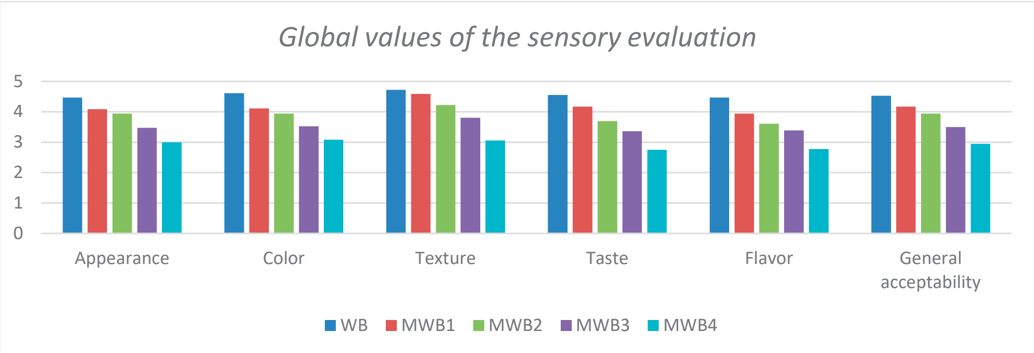

Figure 5: Global values of the sensory evaluation (consumer acceptance) of bread with Moringa (MWB1, MWB2, MWB3 and MWB4; WB (control bread) by using 5-point hedonic scale (n = 36)

Among the bread samples with different proportions of Moringa flour (MWB1, MWB2, MWB3 and MWB4), MWB1 was the most liked by the raters followed by MWB2, MBWB3 and MWB4 respectively. Not only was MWB1 the most appreciated among the Moringa composite flour samples, it also scored close to the control sample (WB). For texture, it was very acceptable having obtained a score of 4.53±0.55. It scored 4.08±0.97; 4.11±0.95; 4.17±0.85; 3.94±0.89 and 4.17±0.70 for appearance, color, taste, flavor and overall acceptability respectively. These different scores being in the range of 3.5 to 4.49 then these criteria are classified in the acceptable level by the consumers. Concerning the sample MWB2 all the scores being between 3.5 and 4.49 (3.94; 3.94; 4.22; 3.69; 3.61 and 3.94 respectively for the aspect, the color, the texture, the taste, the flavor and the overall acceptability), we retain that they are classified acceptable by the consumers. Concerning the two other samples (MWB3 and MWB4), similar to the two previous ones, the higher the quantity of moringa, the lower the acceptability level of the products. The results of this evaluation show us that the addition of a significant amount (more than 2.5%) negatively affects the acceptability of bread by consumers (Bourekoua et al., 2018).

CONCLUSIONS

In this study, it was discussed the production of bread with different levels of substitution of wheat flour by moringa leaf flour in order to evaluate the nutritional, physicochemical and organoleptic potential of the flours and finished products.

The results indicate that the incorporation of MF increased significantly the nutritional quality of the composite flours and breads obtained. On the other hand, the addition of moringa in the bread formulation affected its physicochemical characteristics: its volume was decreased but its elasticity was increased. As for the acidity, it is observed that it increases with the addition of moringa flour in the composition. These parameters must be taken into account in the production of bread with Moringa flour in order to have a bread with better physicochemical characteristics.

The results of these studies also reveal that the addition of MF significantly increased the total polyphenols and flavonoids of the bread obtained. The results of the organoleptic evaluation indicate that the addition of a significant amount (more than 2.5%) negatively affects the acceptability of the bread by consumers (Hayat et al., 2017).

Finally, our results allow us to conclude that substitution with a small amount of moringa flour in bread allows to obtain a smooth bread with soft and flat pores, an elastic core, acceptable from the organoleptic point of view while improving the nutritional quality without negative effects on the technological and physicochemical characteristics. MF is therefore, in small quantities, an excellent product for enriching wheat flour bread.

ACKNOWLEDGEMENTS

This study was performed during the doctoral studies of the PhD Dossa Sylvestre, University of Life Sciences “King Mihai I” from Timisoara, PhD coordinator: Prof.Adrian Rivis.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

INNOVATIVE PASTA FORMULATION BASED ON BARLEY/OAT FLOUR FORTIFIED WITH SEA BUCKTHORN POWDER

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Abstract

This study aimed to design innovative pasta formulations with improved functional properties starting from a basic matrix consisting of a mixture of barley and oat flour which was fortified by including sea buckthorn powder in the recipe. For this purpose, in the novel pasta formulations, the barley/oat flour was replaced with sea buckthorn powder in proportions of 5, 10, 15, 20, and 25% (w/w). After preparation, pasta formulations were assessed in respect of proximate composition, total phenolic content, total flavonoid content and antioxidant activity expressed by ferric reducing antioxidant power (FRAP) value and the inhibition percentage of 2,2-diphenyl-1-picrylhydrazyl (DPPH). The results showed that the content of analyzed bioactive compounds and antioxidant properties of innovative pasta formulations increased significantly (p < 0.05) by using the sea buckthorn powder in the recipe. The highest increase for all investigated parameters was recorded for pasta in which barley/oat flour was substituted with 25% of the sea buckthorn powder. The obtained results reveal the desirability of using sea buckthorn to develop novel pasta formulas with enhanced functionality.

Key words: *antioxidant properties, barley/oat, flour sea buckthorn, proximate composition.*

INTRODUCTION

Food industry manufacturers are increasingly encouraged to seek out non-conventional raw materials for improving the nutritional quality of certain foods, due to the huge and growing demand from public decision-makers, scientists and consumers for healthy foods with improved nutritional characteristics (Romano et al., 2021). Cereal-based products such as pasta are a staple food that make up a significant part of the daily diet of millions of people around the world, making them an ideal matrix for enrichment with functional ingredients (Patel et al., 2011; Vignola et al., 2018). Pasta is mostly prepared with wheat flour or refined flour, despite its high starch digestibility and gluten content. On top of this, it contains a low nutritional profile, notably a low quantity of minerals, vitamins and bioactive constituents. Because of this state of affairs, several researchers have been working on the partial or total substitution of wheat by other cereals, either for their nutritionally rich and bioactive sources or to reduce or prevent allergenicity and/or gluten intolerance (Kumari & Gupta, 2022; Valle et al., 2021; Romano et al., 2021; Nilusha et al., 2019).

Whole grains, including barley (*Hordeum vulgare* L.), are important crops for human consumption, animal feed and industrial applications. Barley is an excellent source of phytochemicals. As such, it can be an excellent ingredient in the production of functional foods, including pasta and bakery products. It is therefore readily available and relatively cheap (Verardo et al., 2011, Suriano et al., 2018, Marconi et al., 2000). Barley is considered to

have higher antioxidant activity than oats, wheat and rye. This high antioxidant activity of barley makes it a useful natural means of preventing the development and progression of diabetes and obesity (Zielinski & Kozlowska, 2000). The majority of free phenolic compounds present in barley are flavanols and tocopherols, while bound phenolic compounds are mainly phenolic acids (Holtekjølen et al., 2006). In a study carried out by Robles-Ramírez et al. in 2020 entitled Barley bread with improved sensory and antioxidant properties, barley flour had a higher fat, ash, protein and dietary fibre content than wheat flour. The dietary fiber and ash content of barley flour was around five times higher than that of wheat flour. Total phenolic compounds and antioxidant activity increased when wheat flour was replaced by barley flour in the bread formulation. The addition of barley flour to bread formulations, for example, is known to dilute gluten proteins and break its chains during mixing (Gill et al., 2002).
Oats (*Avena sativa* L.) are of whole-grain cereals that contain higher concentrations of certain nutrients and phytochemical compounds (Rasane et al., 2015). Welch reported in 2016 in his study entitled: Nutritional composition and nutritional quality of oats and comparisons with other cereals, that oats contain bioactive phytochemical compounds such as vitamins, and phenolic acids. It is known for its nutritional benefits due to its high composition not only of soluble fiber but also of essential amino acids and lipids, particularly unsaturated fatty acids. Oats are also rich in minerals, vitamins and avenathramide, an antioxidant found only in oats (Rousta et al., 2022; Sandhu et al., 2017; Youssef et al., 2016; Sangwan et al., 2014). Daily consumption of a 90 g/day portion of oats in the form of hot cereal, for example, provides 13-15% of dietary energy (Welch, 2016).
Sea buckthorn (*Hippophae rhamnoides* L.), native to the Caucasus region, Central Asia and Western Siberia, belongs to the *Elaeagnaceae* family. It is a thorny deciduous shrub and is considered to have high ecological and economic value (Sytařová et al., 2020; Zeb, 2004; Yang & Kallio, 2001). Sea buckthorn offers several beneficial effects as it contains several bioactive substances characterized by antioxidant effects (Dupakb et al., 2022). It is safe and can be used as a valuable source of important bioactive compounds to create new enriched bread products for patients with cardiovascular disorders (Serban et al., 2019). It is rich in a variety of bioactive constituents and nutrients. The most abundant compounds found in sea buckthorn are lipophilic antioxidants, carotenoids, hydrophilic compounds such as phenolic acids, flavonoids and ascorbic acid. It is also rich in proteins, lipids, carbohydrates and minerals (Jaśniewska & Diowksz, 2021; Ciesarová et al., 2020; Tkacz et al., 2019).
The present study aims to design innovative pasta formulations with improved functional properties from a base matrix consisting of a mixture of barley and oat flour, enriched by adding sea buckthorn powder to the recipe.

MATERIALS AND METHODS

Preparation of pasta based on Barley/oat fortified with sea buckthorn powder

Preparation of composite flours

Table 1. Recipes for production of pasta samples

	Quantity [g]					
Ingredient	P0	P1	P2	P3	P4	P5
Barley/oat Flour	100	95	90	85	80	75
Sea buckthorn powder	-	5	10	15	20	25
Salt	1	1	1	1	1	1
Eggs (pcs)	1	1	1	1	1	1

Barley and oat flours were purchased from Pronat Timisoara, Romania. Sea buckthorn was purchased from local producers in the western region of Romania. In order to avoid the degradation of bioactive compounds in the sea buckthorn fruits, they were conditioned by drying in a dehydrator (Froilabo AC60/France, 1000 W) for 16 hours at 60°C. Dried dehydrated sea buckthorn fruits were ground with a Grindomix GM 2000 laboratory mill (Retsch GmbH, Haan, Germany) until they were transformed into a fine powder passed through a 60-mesh sieve. This powder was used to obtain the pasta according to the recipes presented in Table 1.

Six (6) types of composite barley/oat flours with sea buckthorn were made to produce the following 6 types of pasta:

- P0 (Pasta with 100% Barley/oat flour);
- P1 (Pasta with 95% barley/oat flour and 5% sea buckthorn);
- P2 (Pasta with 90% barley/oat flour and 10% sea buckthorn);
- P3 (Pasta with 85% barley/oat flour and 15% sea buckthorn);
- P4 (Pasta with 80% barley/oat flour and 20% sea buckthorn) and
- P5 (Pasta with 75% barley/oat flour and 25% sea buckthorn).

For the preparation of pasta, the raw materials are mixed after being weighed. This process results in a homogeneous dough. The pasta is dried in an airy space. After drying, the product is packed in bags and labelled (Figure 1).

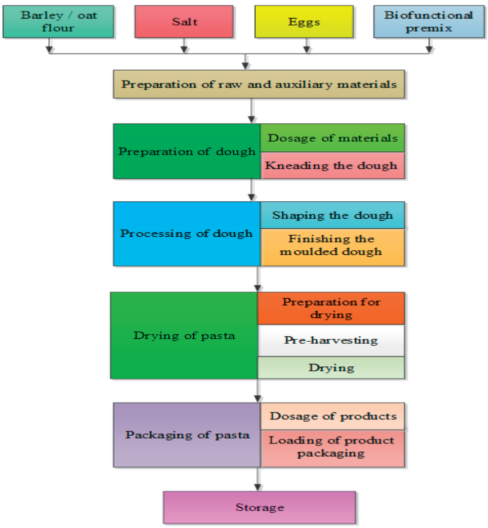

Figure 1. The technological scheme for obtaining pasta

Proximate composition

The proximate composition was determined in accordance with the following ISO Methods:

- Moisture (%) SR 91/2007 pct.10;
- Ash (%) SR ISO 2171/2010;
- Protein (%) SR EN ISO 8968-1:2014 ;
- Lipid (%) SR 91:2007 pct.14.4.

The carbohydrate content is obtained by subtracting 100 from the sum of the sample's protein, fat, moisture and ash contents.

The Energy value (Kcal/100 g) was determined according to the following equation:

Energy value (kcal/100 g) = (lipids x 9) + (carbohydrates x 4) + (proteins x 4)

Phytochemical profile

The total polyphenol content (TPC) was determined by the method of Folin-Ciocâlteu (Plustea et al., 2022) with some modifications. It is expressed in mg GAE/kg. Concerning the total flavonoid content (TFC) it is expressed in mg QUE/kg and was analysed according to the method described by (Plustea et al., 2022) with some modifications. The antioxidant activity is analysed by the FRAP method according to the method described by Cocan et al. (2022). It is expressed in μM Fe^{2+}/g.

All determinations were made in triplicate and the results are presented as mean values ± standard deviation (SD). Differences between means were analysed by a t-test (two samples assuming equal variances) using Microsoft Excell 365. Differences were considered significant when the p-values were less than 0.05.

RESULTS AND DISCUSSIONS

The pasta obtained in this study is shown in Figure 2.

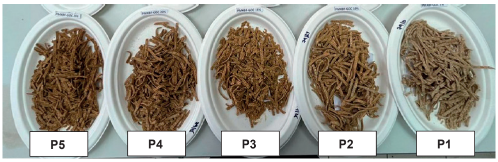

Figure 2. Pasta samples obtained

Proximate composition

The different types of pasta obtained were subjected to different analyses in order to establish the nutritional profile of each of them. The results of these analyses are presented in the following Table 2.

Table 2. Proximate composition of the pasta fortified with sea buckthorn

Sample	Moisture (g/100 g)	Ash (g/100 g)	Protein (g/100 g)	Lipids (g/100 g)	NaCl (g/100 g)	Carbo-hydrates (g/100 g)	Sugar (g/100 g)	Energy value [kcal/100 g]
P0	35.07 ± 1.14	1.28 ± 0.04	14.01 ± 0.46	5.16 ± 0.15	0.31 ± 0.01	44.17	0.14 ± 0.01	284.98
P1	41.14 ± 1.42	1.26 ± 0.04	13.56 ± 0.44	5.09 ± 0.15	0.31 ± 0.01	38.65	0.39 ± 0.01	259.83
P2	43.05 ± 1.48	1.24 ± 0.04	13.11 ± 0.42	5.02 ± 0.14	0.31 ± 0.01	37.28	0.63 ± 0.02	251.73
P3	44.96 ± 1.52	1.23 ± 0.03	12.65 ± 0.40	4.94 ± 0.13	0.30 ± 0.01	35.92	0.88 ± 0.02	243.63
P4	46.86 ± 1.59	1.21 ± 0.03	12.20 ± 0.37	4.87 ± 0.13	0.30 ± 0.01	34.55	1.13 ± 0.04	235.54
P5	48.77 ± 1.66	1.19 ± 0.03	11.75 ± 0.35	4.80 ± 0.12	0.30 ± 0.01	33.18	1.38 ± 0.04	227.44

Analysis of the results from this table shows that the moisture content changes with the addition of sea buckthorn powder to the pasta composition. From 41.14 g/100 g for the sample with 5% sea buckthorn powder, the moisture content rose to 48.77 g/100 g for the sample with 25% sea buckthorn. The Sea Buckthorn powder would therefore have a higher moisture content than the barley/oat mixture.

Our results also show that the amount of minerals in the pasta decreases as the amount of sea buckthorn increases. Mineral concentrations were respectively as follows: 1.28; 1.26; 1.24; 1.23; 1.21 and 1.19 g/100 g for P0, P1, P2, P3, P4 and P5. The barley/oat mixture is richer in minerals than sea buckthorn. This is justified by the fact that the ash content of barley and oats is higher than that of sea buckthorn. Sea buckthorn would therefore provide fewer minerals and proteins than the barley/oat mixture. Rivera-Dommarco's study of the 2001 Food Composition and Nutrition Table shows that the ash content of sea buckthorn berries is 0.3 g/100 g, while the mineral content of oats and barley is 2 g/100 g (Rousta et al., 2022) and 3.38 g/100 g (Zheng et al., 2022) respectively. The mineral content of each of our different samples is higher than that obtained for durum semolina dough (0.83 ± 0.009 g/100 g) by Hidalgo et al., 2020. This confirms that the different flours used for pasta formulation in our study are good sources of minerals.

As with the minerals, the increase in sea buckthorn content in the various samples is synonymous with an increase in protein content. From 14.01 g/100 g in the control sample, the protein concentration dropped to 13.56 g/100 g in the sample with 5% sea buckthorn. The sample with 25% sea buckthorn had 11.75 g/100 g protein. This suggests that sea buckthorn is responsible for the drop in protein levels in pasta. Sea buckthorn, therefore, has a lower protein content than barley and oats. Protein levels are respectively 0.6 to 2.5 g/100 g (Tan et al., 2018; Liang et al., 2011); 11.3 g/100 g (Aly et al., 2021) and 11.1 (Rousta et al., 2022) for sea buckthorn, barley and oats. Pasta obtained from durum wheat semolina was less rich in protein (between 11.3± 0.1 and 11.4 ± 0.05 g/100g) than our pasta (Hidalgo et al., 2020; Panghal et al., 2019).

The NaCl content of the compounds remained virtually unchanged from sample to sample. Lipid and carbohydrate content followed the same pattern as mineral and protein content. For each of these parameters, the higher the quantity of sea buckthorn, the lower they became. For lipids, we have respectively 5.16; 5.09; 5.02; 4.94; 4.87 and 4.80 g/100 g for P0, P1, P2, P3, P4 and P5. Carbohydrates fell from 44.17 g/100 g in the control sample to 33.18 g/100 g. Our samples have a higher lipid content than that obtained for durum wheat semolina dough by Hidalgo et al. in 2020, which was 1.69± 0.05 g/100 g. In our study, the addition of sea buckthorn would be responsible for the lower carbohydrate and lipid content of pasta. As far as lipids are concerned, they were 2.04 g/100 g (Zheng et al. 2022) and 7.7 g/100 g (Rousta et al., 2022) in barley and oats respectively, whereas sea buckthorn fruit had a lipid content of between 0.8 and 5.9 g/100 g (Tan et al., 2018; Liang et al., 2011). The same was true of carbohydrate content, which was lower in sea buckthorn (between 9.5 and 17.9 g/100 g) than in barley (69.3 g/100 g) and oats (55.7 and 59.9 g/100 g) (Aly et al., 2021; Tan et al., 2018; Sandhu et al., 2015; Liang et al., 2011).

Sugar levels in pasta increased by 1.24 g/ 100 g between the control sample (P0) and the sample containing the highest sea buckthorn content (P5). This could be explained by the high sugar content of sea buckthorn fruit. Sugar levels in sea buckthorn fruit ranged from 3.4 to 6.7 g/100 g in the studies by Tan et al. in 2018 and Liang et al. in 2011. According to our results, although sea buckthorn is a source of sugar, it reduces the energy intake in pasta. There was a significant decrease in energy intake from one sample to another when sea buckthorn was added. The control sample (P0) had a kcal intake of 284.98 per 100 g, compared with 227.44 kcal/100 g for the sample with 25% sea buckthorn.

Total polyphenols content (TPC)
Total polyphenol content was determined for each sample. The results are shown in the Figure 3.
The results show that the polyphenol content of the pasta obtained was in the range 701.26 and 2211.24 mg GAE/kg. Furthermore, as the amount of sea buckthorn in the pasta increased, the polyphenol content increased significantly.

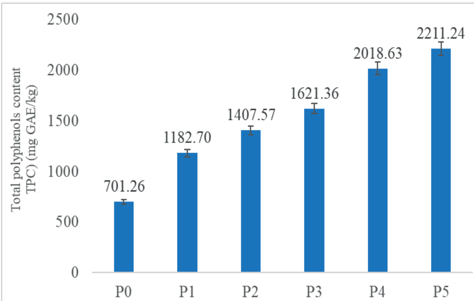

Figure 3. Total polyphenol content for the pasta samples supplemented with sea buckthorn

Between the sample with 5% (P1) sea buckthorn and that with 25% (P5), there was an increase in total polyphenol content of 1028.54 mg GAE/kg. Sea buckthorn is believed to be responsible for the increase in total polyphenol content of the pasta. In the work of Guo et al. on the comparative evaluation of phytochemical profiles, antioxidant and antiproliferative activities of sea buckthorn berries (*Hippophaë rhamnoides* L.), total polyphenol content ranged from 27.6 ± 1.9 to 38.7 ± 1.3 mg GAE/g. On the other hand, in the studies by Bei et al. the total polyphenol content was 0.44 ± 0.02 in oats, and 2.22 ± 0.192 in barley in the studies by Suriano et al. on the phenolic acid profile, nutritional and phytochemical compounds and antioxidant properties of coloured barley grown in southern Italy.

Total flavonoids content (TFC)
Once the total polyphenol content of the various samples had been determined, they were then tested for flavonoid content. The Figure 4 below shows the flavonoid content of the various pastes.

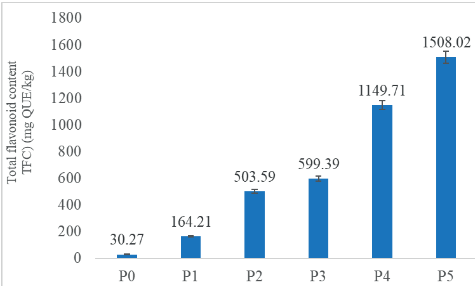

Figure 4. Total flavonoid content for pasta samples supplemented with sea buckthorn

Flavonoids are compounds of real importance to the human organism, reducing cholesterol and triglycerides in the blood. They are the main active substances in herbal medicines for the treatment of cardiovascular disease. Flavonoids have a significant purifying effect on blood tartar, old blood cells and waste products in the blood. They reduce capillary permeability, improve blood vessel flexibility, prevent and improve angina pectoris and cardiac function (Chen et al., 2023). In our study, the results show that from P0 to P5 there is a significant increase in flavonoid content. Thus, the increase in sea buckthorn powder in the recipe corresponds to the increase in flavonoid content. There were 30,266; 164,207; 503,593; 599,393; 1149,713 and 1508,020 mg QUE/kg for P0, P1, P2, P3, P4 and P5 respectively. The total flavonoid content (TFC) of oat cultivar flour varied significantly from 433 to 612 µg EC/g in the work of Sandhu et al. on Effect of heating on the physical, functional and antioxidant properties of oat cultivar flour (*Avena sativa* L.).

Antioxidants activity (AA)
As with total polyphenol content and flavonoid content, antioxidant activities increased

proportionally with increasing sea buckthorn content (Figure 5).

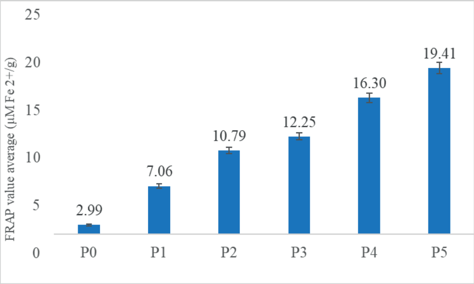

Figure 5. AA (FRAP) of the pasta samples supplemented with sea buckthorn

The antioxidant activity of the different pastes obtained was in the range of 2.988 and 19.411 µM Fe2+/g, i.e. an increase in antioxidant activity of 16 µM Fe2+/g. This increase would be due to the better antioxidant activity of sea buckthorn compared to the barley/oat mixture.

CONCLUSIONS

The aim of this study is to meet current consumer dietary needs. The current trend is to formulate food products with a high nutritional and phytochemical profile, not forgetting the physical aspect. With this in mind, functional pastes based on barley and oats have been formulated and enriched at different substitution levels with sea buckthorn powder. The greatest increase in all the parameters studied was recorded for doughs in which barley/oat flour was replaced by 25% sea buckthorn powder. The results obtained show that it is desirable to use sea buckthorn to develop new pasta formulas with improved functionality.
At the end of this study, the phytochemical power of sea buckthorn was determined. It was also found that the mixture of barley and oats could undoubtedly form the basis for the production of food pastes to replace wheat.

ACKNOWLEDGEMENTS

Studies carried out within the project: POR/824/1/1/ Increasing innovation in companies by supporting innovation entities and technological transfer in areas of intelligent specialization, title: *Realization of technological transfer in order to obtain innovative functional foods enriched in bioactive compounds* - CTTU 2020 SMIS 140030, beneficiary University of Life Sciences "King Mihai I" from Timisoara, project director Prof. Isidora Radulov.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

FUNCTIONALITY AND APPLICATION OF DIETARY FIBER IN FOOD PRODUCTS - REVIEW

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Abstract

Dietary fibers are components of plant material, extremely important in the diet, because they resist enzymatic digestion in the digestive tract, with a role in improving intestinal transit. Dietary fibers are mostly carbohydrates such as cellulose, hemicellulose, pectic substances, gums, and mucilages, but also non-carbohydrate. The presence in the daily diet of whole grains, nuts, vegetables, fruits and seeds positively affects health, because their consumption has been linked to the decrease in the incidence of several diseases. From a technological point of view, dietary fiber is added as a functional food ingredient in food products to provide water-holding capacity, viscosity, gel-forming capacity and fat-binding capacity to food products. These beneficial characteristics of dietary fiber components can enhance the image of products as healthy and functional foods. This article reviews the concept and definition of dietary fibers used in food production and their functional characteristics and health benefits.

***Key words**: dietary fiber, food product, functional property, processing.*

INTRODUCTION

Concern for the health and a healthy diet of the population had an extremely distorted trajectory throughout history. But, in the last decades, easier access to information, the increase in the degree of awareness, the increase in income and the standard of living, pandemic periods caused a greater concern regarding lifestyle and health, and the term dietary fiber entered the daily diet of consumers. Knowing the role and benefits of these dietary fibers on the body greatly stimulated the imagination of the industry and the actors working in the field of nutrition, so they are currently "on the wave". The same, Prosky (2000) mentioned that dietary fiber can be considered a functional food when it gives a special function to that food aside from the normal expected function and similarly when the dietary fiber is used as an additive to foods. For example, dietary fiber brings many benefits, e.g., contributes to colonic health, bifidobacterial or lactobacillus stimulation in the gut, coronary artery health, cholesterol reduction, glucose metabolism, etc. (Prosky, 2000). In conclusion, functional foods are ingredients that offer health benefits that extend beyond their nutritional value and the DF fulfills this role in some cases.

According to a study conducted by Grand View Research, the global market size of the dietary fiber market is estimated to grow at a CAGR of 9.2% from 2022 to 2030. The food & beverage segment is anticipated to continue with the growth of consumer awareness of the importance of dietary fiber in the daily diet (grandviewresearch.com).

Dietary fibers (DF) are components of plant material, extremely important in the diet. They are not digested in the small intestine because humans do not produce enzymes capable of hydrolyzing them into constituent monomers (Turner et al., 2011), but are benefice role in improving intestinal transit. DF is considered to be a non-caloric nutrient that does not contribute calories to our diet because it reaches the colon intact. However, in the colon, these are available for fermentation by some bacteria and the released metabolites can be used to meet some of the body's energy requirements (Cronin et al, 2021).

In the last decades, DF has been extremely promoted for its nutritional and functional effects, with DF being used over time precisely for their beneficial role in body health.

However, looking back over time, DF have always been present in people's daily diets and promoted their beneficial effects.

DIETARY FIBER DEFINITION

Since ancient times, DF have been omnipresent in the diet of the population. There is evidence that in a period of ancient Greece, it was known that bran cereals helped prevent constipation, as well as promote well-being and health. In the 1930s, Kellogg confirmed wheat bran's positive effects on patients with constipation and colitis. But, the actual name of fibers was only mentioned in 1953, by Dr. Eben Hipsley in an article on pregnancy toxaemia (Hipsley, 1953). Concern for the effects and functions of DF on health continued, so that in the 70s, the first definition of DF appeared, which sounded like this: "*Dietary fiber consists of the remnants of edible plant cells, polysaccharides, lignin, and associated substances resistant to digestion by the alimentary enzymes of humans*" (Trowell, 1974).

In the course of time, different definitions were given to them. Such as in 2000, the American Association of Cereal Chemists (AACC) defined "*Dietary fiber is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine*" (AACC Report, 2001). In 2002, the panel on the definition of DF constituted by the National Academy of Science defined "*total fiber*" as the combination of "*dietary*" and "*functional" fiber*". DF is the edible, nondigestible component of carbohydrates and lignin naturally found in plant food. Some foods with DF include cereal bran, sweet potatoes, legumes, and onions. Functional fiber refers to those fiber sources that are shown to have similar health benefits as DF, but are isolated or extracted from natural sources (Institute of Medicine, 2005).

At present, not there is a harmonized definition, and there are still debates in different profile groups regarding a clear definition for DF (Jones JM., 2014). The issuance by *Codex Alimentarius* of an international definition of DF in 2009 marked an important step forward for fiber research and nutrition. The CODEX definition has been adopted or reaffirmed by many national authorities, but it did not completely solve the issue of international harmonization, because a footnote to the definition allows national authorities to choose whether or not to include nondigestible polymers that are shorter than 10 sugar units [degree of polymerization (DP)≤10]. Thus, the DP issue could impair international harmonization if some countries decide not to accept these short-chain oligomers as DF.

In Regulation (EU) No 1169/2011 is mentioned that 'fiber' means carbohydrate polymers with three or more monomeric units, which are neither digested nor absorbed in the human small intestine and belong to the following categories:

- edible carbohydrate polymers naturally occurring in the food as consumed,
- edible carbohydrate polymers which have been obtained from food raw material by physical, enzymatic or chemical means and which have a beneficial physiological effect demonstrated by generally accepted scientific evidence,
- edible synthetic carbohydrate polymers that have a beneficial physiological effect demonstrated by generally accepted scientific evidence.

RECOMMENDATION INTAKE

Intake of DF recommendations is not the same in all countries because of different eating habits and lifestyles (Mehta et al., 1992). The European Food Safety Authority recommends an intake of 25 g per day for adults. This recommendation is based on the role of DF in bowel function. However, the benefit of diets, that are rich in foods containing fiber and provide fiber intakes greater than 25 g per day, has also been noted.

On a national level, dietary recommendations vary with most countries generally recommending daily intakes between 25-35 g for adults. According to a list published by Akbar & Shreenath (2022), the current daily recommended DF consumption ranges between 14-20 g for children, 22-30 g for adolescents, and 25-38 g for the elderly.

The EU Regulation (EC) No 1924/2006 on nutrition and health claims regulation helps consumers identify foods containing fiber and their benefits. In accordance with this regulation:

- "*High in fiber*" claims are permitted on foods that contain at least 6 g of fiber per 100g or at least 3g of fiber per 100 kcal;
- "*Source of fiber*" claims are permitted on foods that contain at least 3 g of fiber per 100g or at least 1.5 g per 100 kcal.

OVERVIEW OF DIETARY FIBER CHEMISTRY

DF are composed of indigestible carbohydrates found inherent to and intact in plants. These carbohydrates include the remains of edible plant cells, polysaccharides, cellulose, hemicellulose, lignin, and other compounds that are resistant to digestion by human digestive tract enzymes but can be soluble or partially soluble in the digestive tract e.g., pectin, mucilage, resistant starch, gums, and other related plant substances (Tabel 1). The composition and nature of fiber in the diet will depend on the age, species, and anatomical source of the plant material (Yangilar, 2013).
The classification of DF is based on the solubility of DFs in water (Figure 1) and is divided into two types, each with different characteristics: soluble fibers and insoluble fibers (Ibrahim & Menkovska, 2022).
Soluble fibers, such as β-glucans, inulin, fructo-oligosaccharides, xyloglucans, mucilage, pectin, psyllium and gums, are water-soluble fibers generated from the inner flesh of plants. Soluble fibers also extend the feeling of fullness due to their property in delaying gastric emptying, causing the feeling of fullness, and they can improve digestion and lower blood glucose (Carlson et al., 2018; Nicholson, 2016). They produce a sticky gel in the colon, where bacteria digest them into gases and by-products such as short-chain fatty acids. For example, foods with soluble fiber include oatmeal, chia seeds, nuts, beans, lentils, apples, and blueberries, oats, barley, fruits, peas, beans, other legumes, and most root vegetables.
Insoluble fibers are present naturally in plants such as vegetables, fruits, and whole grains. These nondigestible fibers such as cellulose, hemicellulose, lignin, and resistant starch, are found in whole-wheat bread, brown rice, seeds and skin of fruits and are not soluble in water or gastrointestinal fluids, remaining more or less unchanged as they move through the digestive tract (Lissoni et al., 2020; Ibrahim & Menkovska, 2022).
Insoluble fibers play an important role in controlling weight and improving bowl health by preventing constipation and colon cancer. Foods with insoluble fibers include whole wheat products (especially wheat bran), quinoa, brown rice, legumes, leafy greens like kale, almonds, walnuts, seeds, and fruits with edible skins like pears and apples.

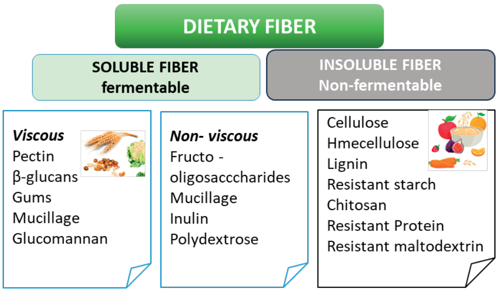

Figure 1. Classification of dietary fibers based on their physicochemical characteristics (Arranz et al., 2012; Ranganathan & Anteyi, 2022)

DF is an essential part of a healthy diet and is essential for keeping the gut healthy and reducing the risk of chronic health conditions.

Table 1. Classification of dietary fiber

Fiber type	Description	Sources	References
Cellulose	The main structural component of the plant cell wall. Insoluble in concentrated alkali; soluble in concentrated acid.	Plant cell walls, especially plants with a rigid structure , in fruits, vegetables (plants), cereals, and various brans.	Dhingra et al, 2012
Hemicellulose	Cell wall polysaccharides contain the backbone of β-1,4 glucosidic linkages. Vary in degree of branching and uronic acid content. Insoluble in water but soluble in dilute alkali.	Cereals, bran, timber, legumes, rice husk, wheat straw	Gírio et al., 2010 Huang et al., 2021 Penfield et al., 1990; Dhingra et al, 2012
Pectic substances	Pectin is an amorphous polysaccharide present in ripe plants and fruits, especially in the primary cell walls and middle lamella of fruits and which varies in methyl ester content. Pectic substances are water-soluble and gel-forming.	In the skin of fruits (apples, plums, peel, quinces and pulp of citrus fruits), vegetables	Nasrollahzadeh et al., 2021 Gawkowska et al., 2018
Mucilages	Synthesized by some specialized secretory cells of the plant and its function is to prevent excessive dehydration of seed endosperm. Mucilage is a water-soluble viscous material characterized by a light color, which is part of the fiber. Food industry use, hydrophilic, stabilizer, emulsifiers, thickening or gelling agents, and viscosity modifiers e.g., guar gum	Leguminous seed plants (guar, locust bean),	Calle et al., 2021 Cakmak et al., 2023 Dhingra et al, 2012
Gums	Gums are secreted at the site of plant injury by specialized secretory cells that appear only in response to injury (wound, mechanical pressure, attack of microorganisms or insects, and physiological disturbances such as water stress or unfavorable environment). Use in the food and pharmaceutical industry, e.g. Karaya gum, Arabic gum, acacia gum	Plant extracts (gum acacia, gum karaya, gum tragacanth) Seaweeds, barley bran, some tree saps and seeds	Shedletzky & Fahn, 1985 Dhingra et al, 2012
Algal polysaccharides	Derived from algae and seaweed. Marine algae contain many polysaccharides including mucopolysaccharides, cell wall structure, and storage polysaccharides. Use in the food and pharmaceutical industry, e.g. carrageenan, agar, alginate.	Seaweed extracts (carrageenan, alginates)	Usman et al., 2017
Lignin	Non-carbohidrate cell wall component. Lignin is a highly cross-linked complex polymer of phenylpropane units. Lignin is insoluble in most solvents and cannot be broken down into monomeric units. Resistant to degradation by most microorganisms.	Plant cell walls, especially xylem (nutrient-transporting) cells. Fruit stones, vegetables (filaments of the garden bean), cereals, soft and hardwood	Popoola-Akinola et al., 2022 Asp, 1987 Liu et al., 2018
Non-digestible oligosaccharides (the prebiotic fibers)	Fibre solubile derivate din vegetale, dar sunt izolate sau modificate într-o formă concentrată care se adaugă la alimente sau suplimente cu fibre.	Non-digestible oligosaccharides (the prebiotic fibers) like inulin, fructo- and galactooligosaccharides	Solange et al., 2007 Atul Rajkumar et al., 2023
Polydextrose	Polydextrose is a complex carbohydrate made from glucose. It's made in a lab and is not digested by the body. Polydextrose is often used as a prebiotic. Polydextrose does not get digested by the human body, but it's digested by good bacteria found in the colon.	Synthesized from dextrose (combined with citric acid and sorbitol), used as a starch replacer in commercial food products	do Carmo et al., 2016 Raza et al., 2017
Resistant starches	Resistant starch: RS1: physically inaccessible; RS2: resistant granules; RS3: retrograded starch, RS4: chemically modified starch	Seeds, legumes, whole grains, potato, corn, green bananas (especially if these foods are cooked then cooled)	Magallanes-Cruz et al., 2017 Mohamed, I. O., 2021
Substances associated with non-starch polysaccharides	Waxes Cutin and suberin are complex polymers present in plant cell walls. These are the polyesters of hydroxy fatty acids. Cutin and suberin are water-insoluble in nature and they come under the category of insoluble dietary fiber.	These occur in plant cells with associated waxes comprising the hydrocarbon chain.	Tungland & Meyer, 2002
Animal origin fibers	Chitosan Chitin, collagen, chondroitin	Fungi, yeasts, invertebrates	Borderías et al., 2005 Tungland & Meyer, 2002

TECHNOLOGICAL FUNCTIONALITY OF DIETARY FIBRE

DF is a complex polysaccharide that shows a wide range of functionalities such as water holding capacity, emulsification, fat replacement, gel-forming, cryoprotectant, thickener, and stabilizer (Figure 2). These technical functionalities are highly influenced by the physicochemical properties of DFs, such as water-holding & binding capacity, oil binding capacity, solubility & viscosity which are discussed (Nayak et al., 2000).

Water holding capacity

Water-holding capacity of DF is usually considered as the amount of water held but the manner in which water is held by the fiber matrix may be more relevant in understanding the role of fiber in technology and nutrition. So, by hydrating a fiber with water, the water enters the pores of the fiber and increases the volume of the fiber, this translates into increasing cooking yields, and probably reducing the caloric content of meat products or similar. Moreover, a high-water holding capacity can control the migration of moisture and the formation of ice crystals, so as to increase the stability during the freezing and thawing process (Gelroth & Ranhotra, 2001). Length, particle size, and porosity of DF components are important because may affect the water-holding capacity and these can contribute to the mouthfeel of the final products (Gelroth & Ranhorta, 2001; Tungland & Meyer, 2002). Longer fibers have an increased capacity to retain water and can lead to changes in texture depending on the fiber level (Yue-yue et al., 2017).

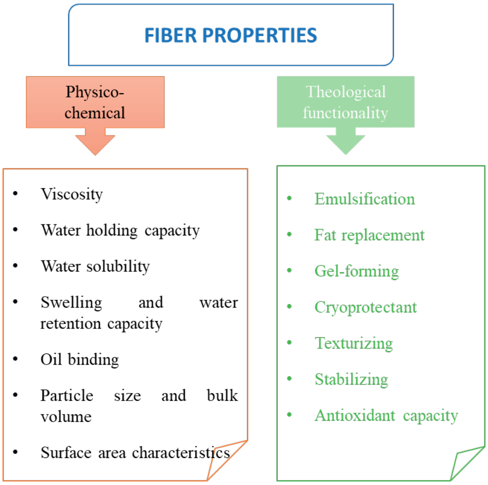

Figure 2. Dietary fiber properties

Swelling and water retention capacity

Fibers are also a great tool for extending freshness because of their water-regulating capacity.

Water solubility

Solubility means the ability of DFs to dissolve in water and the DF solubility depends on several different intern factors structure of fibers but also external factors such as temperature and pH value. Many DF while categorized as being "soluble" are actually poorly soluble in water, and can either aggregate or phase-separate over time. Within the soluble DF, there are known to be substantial differences in their fermentability, with many of them promoting the proliferation of health-promoting bacterial species such as *Bifidobacterium*, *Lactobacillus*, and *Eubacterium*.

Viscosity

Viscosity is a physicochemical property associated with DFs, particularly soluble DFs (Dikeman & Fahey, 2006). Viscosity is an important role of DF, providing rheological properties in the food system including meat products. A high molecular weight or chain length of the fiber increases, and the viscosity of fiber in solution increases. Long-chain polymers, such as β-glucan and gums, exhibit high viscosity in the solution and these are used as thickening agents at low concentrations. Highly soluble fibers that have low viscosity, such as gum arabic, inulins, and oligosaccharides, are generally used to modify texture.

Texturizing

Some fibers can mimic the texture and mouthfeel of fats yet provide less energy, averaging just 2 kcal per gram (as opposed to 9 kcal for fat). Besides acting as a bulking agent and fat replacer, fibers are also used as thickeners to improve the texture and stability of foods (particularly in low-fat and low-kcal products).

Fat holding capacity

Dietary fiber single or in combination with other DF ingredients can be used to reduce fat content special in meat products (Decker & Park, 2010; Mehta et al., 2015). Because they are fat-dispersible and have the capacity to bind, the addition of DF can successfully replace some portion of fat in meat products.

Gel-forming capacity

The gel-forming ability of DF ingredients can contribute to increasing the thickness or viscosity of products. Gelation (gel transition) is the formation of a gel from a system with polymers to form a gel network with a firm three-dimensional structure (Tanaka, 2011; Tungland & Meyer, 2002). This structure may stabilize or modify the physical structure of food products thus helping to minimize shrinkage and improve product density.

Antioxidant capacity

Antioxidants have some health benefits, but up to now, there was less attention paid to the antioxidant properties of DF. The antioxidant effect of DF is based on the polyphenol compounds bound to polysaccharide complexes, which are released in the gut and function as antioxidants (Mézes & Erdélyi, 2018). These polyphenols can be released during digestion or released in the colon after fiber fermentation, which contributes to benefit intestinal health or are excreted in the feces (González-Aguilar et al., 2017).

There are different polyphenol compounds in the antioxidant DF in the plants, therefore their antioxidant capacity varies, but it is approximately equivalent to 50-100 mg DL-α-tocopherol per gram. This antioxidant capacity is considerable and would be suitable for the prevention of some, oxidative stress-related diseases, such as atherosclerosis or other cardiovascular diseases, and colorectal carcinoma (Angulo-López et al., 2023).

It is known that DF can reduce the bioavailability of macronutrients, especially fat, and some minerals and trace elements in the human diet. Because it was demonstrated in humans that pectin strongly decreased the bioavailability of β-carotene (Rock & Swendseid, 1992), DF is probable to also affect the absorption of other carotenoids and probably that of α-tocopherol and polyphenols compounds (Adams et al., 2018; Palafox-Carlos et al., 2011).

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APPLICATION OF DIETARY FIBER IN FUNCTIONAL FOODS

Fibers are very important in food processing because can change the consistency, texture, rheological behavior, and sensory characteristics of the end products. The emergence of novel sources of fibers has been offering new opportunities for their use in food products.

DF holds all the characteristics required to be considered as an important ingredient in the formulation of functional foods, due to its beneficial health effects. DFs used in the food industry are primarily carbohydrate polymers from plant cell walls, such as cellulose, hemicellulose, lignin, and pectin, but several types of DFs can be extracted from waste from cereal, fruit, and vegetable processing (Galanakis, 2012).

APPLICATIONS OF DIETARY FIBERS IN MEAT PRODUCTS

Changing consumer demands and increasing global competition are driving innovation in meat processing. The long-standing positive consumer perception that meat and its products are very important food, is gradually giving way to a more negative view created by media and the new currents regarding food diets (Verbeke et al., 2010). Meat and its products are devoid of DF and the presence of saturated fat (Kausar et al., 2019). It is necessary to improve the total nutrition of meat as a whole by introducing non-meat ingredients that can be incorporated into the meat to boost its functional properties and nutritional value.

Manufactured meat products include a variety of non-meat ingredients that serve various purposes during production or in the finished product. Meat extenders and meat analogues are produced by extrusion of vegetable proteins, resulting in products that have an appearance and texture similar to the fibrilar structure of meat (Fellows, 2017). These extenders often allow for reduced formulation costs but while changing the finished product properties such as water-holding, texture, appearance, and palatability.

The addition of fiber in meat and/or meat products is more common nowadays, as its addition can efficiently provide longer shelf life, and higher quality, as well as improve various processing characteristics. The deficiency of DF can be improved by supplementation of dietary fiber-rich vegetative substances like cereal and pulse flour, vegetable and fruit pulp etc. Fibers such as cellulose, pectin, or fiber extracted from rice, maize, wheat, and beetroot can be used to improve the texture of various meat products including salami and sausages. Are suitable to be used in the preparation of low-fat meat products, such as ‘dietetic hamburgers’. Since DFs are also capable of elevating hydration properties; their inclusion in meat can contribute to rich juiciness.

For example, inulin has been added in different types of meat products including sausages, meatballs and restructured products showing good performance as a fat substitute (Álvarez & Barbut, 2013; Bis et al., 2019) matching its technological and physiological properties improving the stability of emulsions and imprinting characteristics similar to fat.

Resistant starch is suitable in reduced-fat meat emulsion because have the capacity to retain water, decreasing cooking losses and performing a neutral flavor. The same color, texture, and sensorial parameters were not influenced by the replacement of up to 60% fat. However, the research shows that when combined with β-glucan, resistant starch showed to be a suitable ingredient to produce prebiotic sausage (Sarteshnizi et al., 2015, Totosaus, 2009).

Dietary fiber can effectively be incorporated in processed meat products as binders, extenders and fillers they can use as successful alternatives for unhealthy fat components from the products and might increase acceptability by improving nutritional components, pH, water holding capacity, emulsion stability, sensory characteristics, etc. of finished products. Addition of DF in meat products can increase the cooking yield therefore economic gain as well.

Oat fiber can be used as an appropriate fat replacement in ground pork and beef sausage-based products owing to their high-water retention and ability to improve texture and color (Verma & Banerjee, 2010)

Pea hull flour, gram hull flour, apple pulp, and bottle gourd can be used to produce high-fiber functional chicken nuggets with low fat and low salt.

Fortification of DF in meat emulsion can improve emulsion stability and viscosity and decrease cooking loss; moreover, DF may also be associated with the rheological attributes of meat emulsion. Efficient protection against lipid oxidation was attained by the addition of rice bran in frankfurters (Frankfurt sausage) and their antioxidant activity was preserved till 14 days of storage.

APPLICATIONS OF DIETARY FIBERS IN DAIRY PRODUCTS

Milk is free in DF content, and some soluble fibers such as pectin, carboxymethyl-cellulose, inulin, and guar gum are being used as functional ingredients in dairy products such as milk, yogurt, cheese and ice cream.

In cheese products, syneresis is a problem of serious concern, and the addition of guar gum prevents syneresis by water phase management and improves the texture with an addition of up to 3% of the total weight. Guar gum in soft cheeses enhances the yield of curd solids and gives a softer curve with separated whey. The same, guar gum, pectins, and inulin are also added during cheese processing to decrease its percent of fat without losing its organoleptic characteristics, such as texture and flavour (Murtaza et al., 2017)

Numerous studies have proved that the addition of variable amounts of DF in yogurt not only improves its nutritive value, but is also able to influence its texture, rheological characteristics, consistency, and overall consumer acceptability.

Addition of variable amounts of DF in yogurt not only improves its nutritive value, but is also able to influence its texture, rheological characteristics, consistency, and overall consumer acceptability. Yogurt fortified with DF obtained from lemon and orange has exhibited good overall acceptability. The same, Staffolo et al. (2004) reported that yogurt enriched with 1.3% inulin (from wheat and bamboo) and fibers (from apple) is a promising possibility for elevated fiber intake, which also gained increased consumer acceptability (Po Kip & Renger, 2006).

In probiotic products, these fibers can be used as a multi-functional additive because of their satisfactory stability in dairy products. The stabilizer effect can change in dairy products (e.g., ice cream, beverage) with variable composition/formulation.

β-glucan and inulin-type fructans develop textural or rheological properties of dairy products that have relatively more standard composition (such as yogurt, and cheese), at varying degrees depending on the proportion.

The composition and the soluble/insoluble ratio are critical parameters for the exhibited functionality of the added fibres. High content in insoluble fibers led to a reinforcement of the viscosity and thixotropy of ice cream mixes.

Additives used to increase the stability of foods or to extend their shelf life are compounds that are beneficial for health, their usage areas should be increased, and their different potential effects should be known.

APPLICATIONS OF DIETARY FIBERS IN BAKERY PRODUCTS

DFs are common in the manufacture of bread since the flour used can have a low degree of refinement and a high bran content. But for some products, the use of whole meal flour can negatively influence the color, texture, stability and volume of the product. For this reason, food fibers are used to fortify the flour or are added directly in the recipe of biscuits, pasta, etc.

DFs have a high capacity to absorb and retain water, and in the dough preparation process, they compete with gluten, which is also water-loving but with a slower water absorption capacity. This results in low-volume products, because the fibers make the structure of the core difficult, and the gluten network, not being very stable, retains small amounts of gas. The presence of DF influences texture and color, but the overall effect of adding DF e.g., cellulose was smaller compared to cereal bran.

DF derived from pulp or peel of fruits or vegetables is an accessible version adopted by producers who are in search of ingredients with high nutritional value. The baking industry uses a high percentage of insoluble fiber derived from cereals consisting of cellulose fiber, groats, or meal of cereals - especially those from wheat fiber from the structure of the cell walls of soybeans, peas, carrots, citrus and a lower percentage of soluble fiber - gums.

APPLICATIONS OF DIETARY FIBERS IN BEVERAGE

Addition of DF can increase the stability and viscosity of beverages and drinks. Soluble DF is often used due to its higher dispersible properties in water as compared to insoluble fiber.

Pectin, cellulose, and β-glucans are other soluble fibers that gain potential applications. Oat fiber has been supplemented into fruit and vegetable juices, instant beverages (breakfast drinks, milkshakes, sports drinks, iced tea, wine), and other snack products.

DFs can also be incorporated into beverages prepared for people with special needs for weight loss.

Fortification of fiber ingredients such as cellulose gels, guar gums, and alginates can be a good substitute for fat, which is also envisaged to improve emulsion, viscosity, and foam, reduce syneresis, control melting properties, and stimulate the formation of ice crystals. Gums are excellent ingredients used in beverage formulation because don't influence taste or smell when formulated correctly. They can provide many functions, such as stabilising emulsions in drinks, providing a pleasant mouthfeel, and good suspension. New hydrocolloid sources have been developed in recent years which have been used as soluble fiber in beverages, e.g. fructose polymers inulin and oligofructans (Gallo-Torres & O´Donnell, 2003), and polydextrose (Brooks, 2003).

CONCLUSIONS

DFs are valuable ingredients in the recipes of some food products. Water solubility of DF, fiber composition, and the ratio of soluble/insoluble DF in an ingredient are critical parameters for the exhibited functionality of the added fibers.

They can be successfully formulated into different types of food products to improve nutritional values by increasing fiber and reducing sugar or fat and increasing consumer acceptance. DF shows specificity and behavior in different products and for this reason, it is important to know very well the chemical and behavioral properties to help us in food processing. DF are also considered fibers with a functional role both in food and in the body.

ACKNOWLEDGEMENTS

This research was funded by University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania within the internal project “Obtaining an innovative preparation of minced beef, with the addition of fibers from local sources” - FiberBeef, 1066/15.06.2022.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

A MEAT PRODUCTS ALTERNATIVE: VEGAN CASHEW PARISER – ANTIOXIDANT, NUTRITIONAL AND SENSORY CHARACTERISTICS

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Abstract

Since the beginning of the third millennium, globally, there has been a continuous increase of the flexitarian, vegetarian and vegan diet people number, which has led to a higher market demand for plant-based alternatives to meat products. The work first goal was to obtain a cashew nuts pariser, in two assortments: VCP1 and VCP2, the difference between them being that in VCP2 was added red beet juice as natural coloring. Another aim of this paper was to analyze the two finished products concerning total polyphenol content (TPC), antioxidant activity by Cupric Ion Reducing Antioxidant Capacity (CUPRAC) and by 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity (RSA), proximate composition, energy value and sensory characteristics (5 points hedonic scale). Because, compared to cashews, red beet juice had a TPC (9.46±0.14 mg gallic acid/g) more than twice as high, a CUPRAC (68.72±0.18 mg Trolox/g) more than 5 times higher, a stronger RSA, the addition of this juice to VCP2 determined a higher TPC and a better antioxidant activity compared to VCP1. Both finished products were well appreciated by tasters for all organoleptic characteristics.

Key words: *antioxidant activity, cashew, polyphenols, red beet, vegan pariser.*

INTRODUCTION

Today, to be a flexitarian (omnivorous diet, but with a very low consumption of meat and animal products), vegetarian (a plant-based diet without meat and meat products, but with dairy, and eggs) or vegan (only plant-based products diet), for many people, it means more than a fad, it practically represents a lifestyle (Raphaely & Marinova, 2014; Bedin et al., 2018; Sijtsema et al., 2021). Those who have given up eating meat and meat products do so for religious or ethical reasons, related to the environment, their own state of health or from desire to lose pounds (Bedin et al., 2018; Mihai et al., 2021; Gonciarov et. al., 2015). Following the increase in the number of people who choose a diet without meat and meat products has led in the last two decades to a growing market demand for plant-based products to replace conventional meat ones (Savu & Petcu, 2002; Petcu, 2013; Joshi & Kumar, 2015; Bakhsh et al., 2021). There are several producers who make such vegan meat analogues using soy, peas, wheat gluten, rice, mushrooms, nuts as raw materials and who want to convince consumers not only through special organoleptic properties, very close to those of conventional meat products, but also through high nutritional quality (Joshi & Kumar, 2015; Joshi et al., 2016).

Cashew nuts are the main product of the cashew tree (*Anacardium occidentale*), belonging to the *Anacardiaceae* family. These nuts, also called "wonder nuts" are a rich source of polyunsaturated fatty acids (especially linoleic acid), protein, carbohydrates, calcium, phosphorus and iron, also having an important content of phenolic compounds and a good antioxidant activity

(Soares et al., 2013; Sajeev & Saroj, 2015). The kernels of cashew nuts can be consumed both raw, fried and salted or as additions to various confectionery, pastry and bakery products. As a result of the rich protein content, cashews and cashew protein isolates have recently started to be used in vegan products to replace cheeses and even in vegan alternatives for meat products (Ogunwolu et al., 2009; Liu et al., 2018; Chen et al., 2020; Lima et al., 2021; Short et al., 2021; Maciel et al., 2022).
Red beet (*Beta vulgaris* L.), belonging to the *Chenopodiaceae* family, is a rich source of fiber, potassium, manganese, carotenoids, B-vitamins (B1, B2, B3, B6, B9 and B12), as well as vitamin C, zinc, copper, iron and inorganic nitrate (Babarykin et al., 2019; Ceclu & Nistor, 2020; Mirmiran et al., 2020). Recently, more and more researchers have focused their attention on the high content of polyphenolic antioxidants (phenolic acids, phenolic acid esters, flavonoids) in beetroot, but also on the high concentration of betalains - natural water-soluble pigments that contain nitrogen, proving to have good antioxidant and free radical scavenging activity as well as anticancer, antimicrobial and antiviral action (Kavalcová et al., 2015; Masih et al., 2019; Šlosár et al., 2020). Beetroot juice can be used as such in the food industry for the coloring of certain preparations, and also, only the betalains can be extracted from it to be used as food dyes (in powder form) (Ben et al., 2014; Masih et al., 2019).
The first goal of this work was to obtain a plant-based pariser made from cashew nuts in two assortments: one without the addition of dye and one using red beet juice as a natural dye. Another aim of the work was to analyze the finished products in terms of total polyphenols content, antioxidant and free radical scavenging activity, proximate composition, energy value, as well as sensory characteristics.

MATERIALS AND METHODS

Vegan cashews pariser preparation

Were prepared two types of cashew nuts pariser: VCP1 and VCP2, using raw and auxiliary materials from the Romanian market, according to the recipes presented in Table 1.

Table 1. Recipes for the two cashew pariser preparation

Pariser type / Materials (%)	VCP1	VCP2
Cashew nuts (raw)	18.60	18.60
Garlic (powder)	1.90	1.90
Lemon juice (fresh)	5.60	5.60
Coconut oil	1.90	1.90
Sweet paprika (powder)	0.90	0.90
Inactive yeast flakes	0.60	0.60
Dehydrated oregano	0.90	0.90
Dehydrated thyme	0.90	0.90
Salt	0.90	0.90
Pepper	0.90	0.90
Water	65.00	59,40
Agar - agar	1.90	1.90
Red beet juice (fresh)	-	5.60

To obtain the two varieties of vegan pariser, the raw cashew nuts were weighed and then hydrated in water for 4 hours. Separately, fresh juice was obtained from the peeled red beet, using a Tefal ZE 585H38 Easy Fruit centrifugal juicer, and the amount required to obtain VCP2 was measured. Then all the other ingredients were weighed and together with the hydrated cashew they were put into a blender (minus the agar-agar and 1/4 of the amount of water) and blended until a fine paste was obtained. The agar-agar gel formed by boiling with water was later added to this paste and the whole mixture was again subjected to the blending operation. The obtained paste was poured into cylindrical shapes previously greased with a little coconut oil, after which the products were cooled for 6 hours at 4°C. After cooling and solidifying the composition, samples were taken from each type of pariser (VCP1 and VCP2), as well as cashew nuts and red beet juice, in order to determine the total polyphenol content, antioxidant and free radical scavenging activity.

VCP1 VCP2

Figure 1. The two types of cashews pariser

Assessment of the total polyphenol content

The evaluation of the total polyphenols content (TPC) in the finished products as well as in cashew nuts and beetroot juice was carried out by the Folin-Ciocâlteu assay, which is based on the reducing properties of polyphenols against hexavalent molybdenum from the poly-phosphomolybdate contained in the Folin-Ciocâlteu reagent (Folin & Ciocâlteu, 1927). The higher TPC in the sample, the more hexavalent molybdenum will be reduced to lower oxidation states (+4, +5) with the formation, in a basic environment, of a more intense blue color (absorption bands at 750 nm) (Huang et al., 2005; Prior et al., 2005). The same working method was used as that presented by Dumbrava et. al., (2020) and the results were expressed in mg gallic acid/g of sample.

Assessment of the antioxidant activity

The antioxidant activity was determined for the same samples as in the case of TPC, using the cupric ion reducing antioxidant capacity method (CUPRAC), this being able to highlight both water-soluble and fat-soluble antioxidants in the analyzed samples. (Apak et al., 2007). The work methodology was identical to that described by Dumbrava et al., (2020) and the results were expressed in mg Trolox/g of sample.

Assessment of the free radical scavenging activity

The free radical scavenging activity (RSA) was determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method (Hue et al., 2020), both for the cashew nuts and for the beetroot juice as well as for the finished products. For this purpose, 2 g of each sample were weighed and subjected to extraction for 2 hours, at 25°C, under continuous stirring, with 20 ml of 70% ethanol. After the extraction was completed, the samples were filtered through a Whatman no. 1 filter. From each filtrate, 1 ml was measured, which was then treated with 2.5 ml of a 0.1 mM solution of DPPH in 70% (v/v) ethanol. The mixture was well homogenized and incubated at room temperature and in the dark for 30 minutes. For each sample thus prepared, the absorbances were read at 517 nm, using 70% ethanol as a reference. A control sample was also prepared using 1 ml of 70% ethanol and 2.5 ml of 0.1 mM DPPH solution. The following relationship was used to calculate the free radical scavenging activity (RSA):

$$\text{RSA (\%)} = \frac{Ac - As}{Ac} \times 100$$

where: Ac is the control sample absorbance,

As - absorbance in the presence of the sample.

Statistical analysis

The determinations of TPC, antioxidant activity and RSA were carried out in triplicate and the results were expressed as a mean values ± standard deviation (SD). For statistical data processing was used Microsoft Excel 2010.

Assessment of the proximate composition and energy value

In order to determine the proximate composition and energy value for the two finished products (VCP1 and VCP2), the USDA Nutritional Data Base was used.

Sensory evaluation

The obtained vegan pariser assortments (VCP1 and VCP2) were organoleptically analyzed by a group of 36 untrained panelists, men and women, aged between 20 and 54 years, using the hedonic scoring scale from 1 to 5, appreciating: section appearance, texture, color, taste and smell. The obtained vegan pariser assortments (PV1 and PV2) were organoleptically analyzed by a group of 36 untrained panelists, men and women, aged between 20 and 54 years, using the hedonic scoring scale from 1 to 5, appreciating -the appearance in the section, the texture, the color, the taste and the smell, the way of working, the level of acceptability and the interpretation of the score ranges being exactly the same as those presented by Dumbrava et al. (2020).

RESULTS AND DISCUSSIONS

Total polyphenols content

For cashew nuts, red beet juice and for finished products total polyphenols content (TPC) determined using the Folin-Ciocâlteu method led to the results shown in Table 2.

The experimental results presented in Table 2 show that the fresh red beet juice had a TPC more than twice as high (9.46±0.14 mg gallic acid/g) as the cashew nuts (4.02±0.09 mg gallic acid/g), this being also reflected in the concentration of these compounds in the vegan pariser with the addition of red beet juice (VCP2) which had a higher content (3.67±0.07 mg gallic acid/g) than VCP1 (2.76±0.04 mg gallic acid/g).

Table 2. TPC in cashew nuts, red beet juice and finished products

Sample	Total polyphenols (mg gallic acid/g)
Cashews	4.02±0.09
Red beet juice	9.46±0.14
VCP1	2.76±0.04
VCP2	3.67±0.07

Soares et al. (2013) reported for conventional and organic cashew nuts, TPC values of 3.26 mg GAE/g, respectively 3.46 mg GAE/g, these being slightly lower than that found in this work (4.02±0.09 mg gallic acid/g). For beetroot, Ben Haj Koubaier et al. (2014) found total polyphenol content values for a raw beetroot extract of 14 mg gallic acid/g, while Ninfali & Angelino (2013) reported values between 0.720 to 1.276 mg/g.

Antioxidant activity

From the data on the antioxidant activity presented in Table 3 results that the red beet juice had a cupric ion reducing antioxidant capacity more than five times higher (68.72±0.18 mg Trolox/g) than that of cashew nuts (12.64±0.06 mg Trolox/g), which determined that the VCP2 also had an antioxidant activity with over 50% higher (13.48±0.07 mg Trolox/g) than VCP1 (8,96±0.02 mg Trolox/g).

Table 3. Antioxidant activity of cashews, red beet juice and finished products

Sample	Antioxidant activity (mg Trolox/g)
Cashews	12.64±0.06
Red beet juice	68.72±0.18
VCP1	8.96±0.02
VCP2	13.48±0.07

For conventional and organic cashews, Soares et al. (2013) found values of antioxidant activity of 6.77 respectively 7.02 mg Trolox/g, while Slavov et al. (2013) determined an antioxidant activity for red beet juice, through a modified ORAC method, of only 2.38 mg Trolox Equivalents/g.

Free radical scavenging activity

The determinations regarding the free radical scavenging activity (RSA) carried out by the DPPH free radical method, on the ethanolic extracts obtained from cashew nuts, red beet juice and the finished products, presented in Table 4, showed that all extracts had a high RSA. Red beet juice had the highest DPPH radical inhibition percentage value (95.74±0.47%), and of the two finished products, the pariser with the addition of beetroot juice (89.72±0.35%).

Table 4. Free radical scavenging activity (RSA) of cashews, red beet juice and finished products

Sample	RSA (%)
Cashews	89.64±0.32
Red beet juice	95.74±0.47
VCP1	86.47±0.21
VCP2	89.72±0.35

Proximate composition and energy value of finished products

The results obtained by calculation for the proximate composition and energy value of the two types of vegan pariser are presented in Figures 2 and 3.

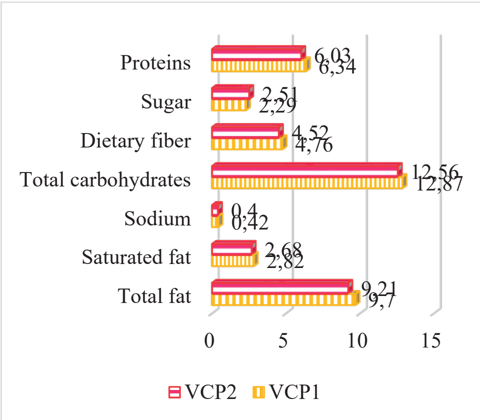

Figure 2. Proximate composition of finished products (g/100 g)

There are no big differences between the two finished products in terms of proximate composition and energy value, the vegan pariser with added beetroot juice (VCP2) being, however, less caloric (142.04 kcal/ 100 g) than VCP1 (148.14 kcal/100g), VCP2 being slightly lower in fat (9.21 g/100 g total fat of which 2.68 g/100 g saturated fat), total carbohydrates (12.56 g/100 g), dietary fiber (4.52 g/100g), protein (6.03 g/100g), but slightly richer in sugars (2.51 g/100g). Both products had a low sodium content (0.4 g/ 100 g and 0.41 g/100 g respectively) and no cholesterol, unlike the meat-based versions of pariser.

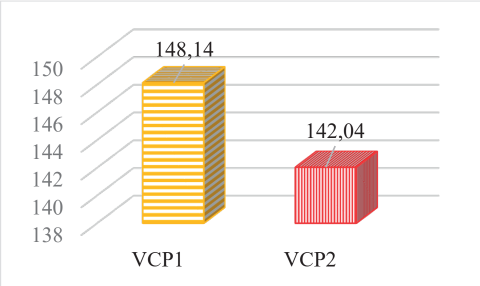

Figure 3. Energy value of finished products (kcal/100 g)

Sensory analysis

The organoleptic properties analysis of the two varieties of vegan pariser VCP1 and VCP2, using the hedonic scoring scale method from 1 to 5, led to the results presented in figure 4.

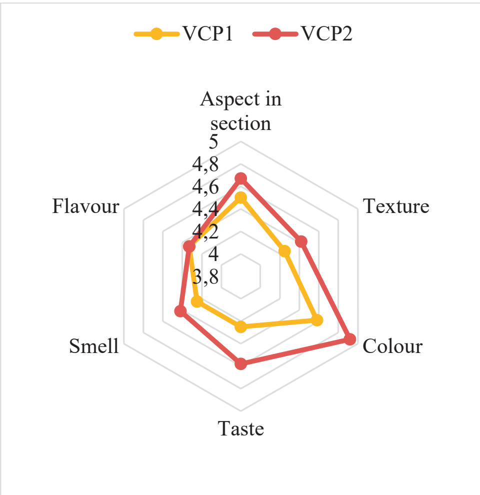

Figure 4. Global values of the sensory evaluation of finished products using a 5-point hedonic scale

Both types of vegan pariser were appreciated with average scores above 4 (good) for all the evaluated sensory characteristics, the highest score (very high level of acceptability) meeting for the appearance in the section (4.50 for VCP1 and 4.67 for VCP2), color (4.58 for VCP1 and 4.92 for VCP2) and taste (4.58 for VCP2). VCP2 scored higher than VCP1 in all sensory characteristics, except for aroma, where both products obtained 4.33 points.

CONCLUSIONS

Meat products, a category of which pariser is a part, are basic foods in the daily diet of many consumers. Since more and more consumers want or are forced, for medical or other reasons, to reduce or even give up the consumption of meat and meat products, there is a growing need on the market for plant-based products that can replace meat and which should be appropriate both nutritionally and organoleptically.

The current work aimed to obtain a plant-based alternative to the meat-based pariser: vegan cashew pariser in two versions: with and without the addition of red beet juice as a natural coloring. The obtaining technology was simple and fast.

The red beet juice, with a content of total polyphenols more than twice as high as cashew nuts, as well as with stronger antioxidant and antiradical activity, had the effect of increasing the values of these characteristics in the pariser assortment in which it was added.

Regarding the proximate composition, both variants of pariser had very close values of the content of proteins, carbohydrates, lipids, sodium. The energy value of the cashew vegan pariser variant with added red beet juice was slightly lower than that of the VCP1 variant. Unlike the varieties of meat pariser, these vegan alternatives are lower in calories, do not contain cholesterol, instead they have dietary fiber.

From organoleptic point of view, both assortments of pariser were well appreciated by the tasters, obtaining scores above 4 for all the analyzed sensory characteristics, the best appreciated being the vegan cashew pariser with red beet juice addition.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

EVALUATION OF CONSUMER KNOWLEDGE, ATTITUDES AND PERCEPTIONS REGARDING ANTIOXIDANTS AND THEIR CONSUMPTION THROUGH MEAT PRODUCTS

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Abstract

As of recent times, consumers are starting to be more and more aware of the impact nutrition has over their health. The present study is based on the use of antioxidants and their consumption through meat products. In this sense, the study focused on using a survey to evaluate the consumers' understanding of what antioxidants are and their potential health benefits, their perceptions of the taste and quality of meat products that contain antioxidants, as well as their willingness to purchase such products. The survey showed that most consumers are aware of the potential harmful effect of long-time consumption of synthetic antioxidants. The surveyed people also stated that they consume meat and meat products almost on a daily basis; however most stated that they would not repurchase a meat product if the used natural antioxidant would cause changes in the product's colour and taste. As shown in the study, research methods such as surveys can provide valuable insights into consumers` attitudes and purchasing habits.

Key words: *antioxidants, meat, survey.*

INTRODUCTION

The use of preservatives in meat products, either natural or synthetic, represents a very important issue in the context of consumers´ demand for safer and healthier products.
In this context, the use of plant-based antioxidants became more and more popular due to the fact that the consumers want to eat less processed food.
Previous studies have evaluated responses in different countries concerning food ingredients or additives (Aoki et al., 2010; Shim et al., 2011; Varela & Fiszman, 2013), chemicals in foods (Dickson-Spillmann et al., 2011) and food safety topics (Latorres et al., 2016; Miles et al., 2004; Ruby et al., 2019).
The conclusions of these studies showed that consumers are not able to understand the problem of food additives, due to their little knowledge about the subject, although they are very concerned about additives and chemicals in their diet. Also, these studies revealed that there is a positive attitude towards using natural origin ingredients in all types of food, including meat products.
The present study focused on consumers' awareness of antioxidants use and their potential health benefits, perceptions of the taste and quality of meat products containing antioxidants, and the affordability of such products.

MATERIALS AND METHODS

A cross-sectional survey based on a Google form questionnaire was conducted among the general population of Romania from September to November 2022. For this study, 605 adults from 5 macro-regions of Romania filled in the questionnaire.

The questionnaire included three parts with a total number of 27 questions regarding: 1. demographic characteristics, 2. assessment of general knowledge related to antioxidants, 3. consumers´ attitude towards the consumption of meat products.
The first part of the questionnaire included questions about demographic characteristics such as gender, age, educational level, civil status, level of education and income. The second part included questions aimed to evaluate the Romanian population's general knowledge related to antioxidants, such as the types of antioxidants, the advantages and disadvantages of using natural antioxidants in meat products. The third part contained questions about consumers´ attitude towards the consumption of meat products, such as the frequency of meat products consumption, the attitude of purchasing meat products based on the price and the ingredients used.
The participants filled in the questionnaire only in Romanian, with a period of about 10 minutes to complete.
Associations of respondents' personal details with the natural antioxidants and consumption of meat products-related traits of focus on this study were evaluated via logistic regression at a significance level threshold of $p < 0.05$. Independent variables included demographic traits: gender (male, female) and level of education (secondary, high school, graduate and postgraduate level).

RESULTS AND DISCUSSIONS

1. Demographic characteristics

All the demographic data are presented in Table 1.
Analyzing the data it can be seen that 69.09% of the participants (n=418) were female and 30.91% were male. It can be observed that the percent of male respondents is substantially lower than the one for the females. The greater number of the participants, 68.09% (n=412) were young, aged between 18 and 24 years, meanwhile only 1.66% of the respondents was older than 64 years old. The tendency to participate in such questionnaires is higher for young people and it decreases progressively with age. Concerning the region, most of the respondents were from Muntenia 74.38% (n=450) and the lowest rate in Banat 0.66% (n=4). Out of the participants, 62.31% (n=377) reported that their level of education is high school and 23.97% (n=145) were college graduates, whereas only 19.17% (n=116) were married and 37.35% earn less than 1500 lei monthly.

Table 1. Demographic parameters of the participants

Variable		N	%
Gender	female	418	69.09
	male	187	30.91
Age (years)	18-24	412	68.09
	25-34	75	12.39
	35-44	57	9.43
	45-54	39	6.45
	55-64	12	1.98
	≥64	10	1.66
Civil status	married	116	19.17
	in a relationship	274	45.29
	single	215	35.54
Region	Muntenia	450	74.38
	Moldova	69	11.40
	Transilvania	34	5.62
	Dobrogea	48	7.94
	Banat	4	0.66
Education level	secondary	3	0.50
	high school	377	62.31
	graduate	145	23.97
	postgraduate	80	13.22
Income	< 1500 lei (300 EUR) *	226	37.35
	1501-3000 lei (301-600EUR)	176	29.10
	3001-4500 lei (601-900 EUR)	101	16.70
	4500 lei (900 EUR)	102	16.85

*Exchange rate: 1 EURO = 5 lei

2. Assessment of general knowledge related to antioxidants

The evaluation of general knowledge related to antioxidants was assessed in 9 questions (Table 2). In this part of the evaluation, the respondents were requested to answer different questions regarding the definition of antioxidants, the use of antioxidants in meat products and the negative aspects of using synthetic antioxidants in the human diet. General knowledge about antioxidants was considered satisfactory if more than 80% of the participants answered correctly for each statement.
More than a half of the respondents (60.33%) answered correctly to the statement "What does antioxidant mean?". A significant percent of the respondents (74.55%) believe that there are natural and synthetic antioxidants.
For the statement "Antioxidant substances are added to extend the shelf life of meat products",

the responses were 45.78% agree, 33.06% unsure and 21.16% disagree, which led us to the conclusion that more than half of the people are not aware of this aspect.

Table 2. Respondents´ general knowledge related to antioxidants

Statement	Responses	%
What does antioxidant mean?	a substance that is added to food products to prevent spoilage	30.57
	compound that inhibits oxidation	60.33
	a substance added to food products to improve their colour, aroma and taste	9.10
Antioxidant substances are added to extend the shelf life of meat products	Agree	45.78
	Unsure	33.06
	Disagree	21.16
Do you think that the processing of meat foods affects the action of antioxidants?	Agree	44.62
	Unsure	37.85
	Disagree	17.53
Do you think that meat products that contain natural antioxidants are?	Bio	10.74
	Organic	20
	Natural	23.64
	Normal	46.62
There are natural antioxidants and synthetic antioxidants	Agree	74.55
	Unsure	21.65
	Disagree	3.80
Do you think that antioxidants also have negative effects?	Agree	37.20
	Unsure	20
	Disagree	8.09
	Some have	37.71
Synthetic antioxidants are harmful if they are consumed for a long time	Agree	58.18
	Unsure	35.86
	Disagree	5.96
Synthetic antioxidants consumed excessively cause allergies	Agree	47.10
	Unsure	49.26
	Disagree	3.64
The latest studies have shown that synthetic antioxidants consumed excessively and for a long time, cause the appearance of cancer. Do you know this aspect?	Agree	33.05
	Unsure	28.93
	Disagree	38.02

A similar pattern (44.62% agree, 37.85% unsure and 17.53% disagree) was observed for the question "Do you think that the processing of meat foods affects the action of antioxidants?".

A quite high percentage of 74.91% responded that all antioxidants (37.20%) or some of them (37.71%) have negative effects. More than 45% of the respondents agreed that synthetic antioxidants are harmful if they are consumed for a long period (58.18%) and also that they can cause allergy (47.10%). For the last question concerning the possibility of appearance of cancer, due to long term exposure to synthetic antioxidants, the responses were evenly distributed, 33.05% agree, 28.93% unsure and 38.02% disagree.

3. **Consumers´ attitude towards the consumption of meat products**

This parameter was evaluated using 12 multiple response questions (Table 3).

Analyzing the data, it can be seen that the most frequently consumed type of meat according to this survey is chicken (56.52%), followed by pork 29.58%, meanwhile the least consumed is venison (0.17%). The most consumed meat products are the fresh ones (44.14%), while cooked smoked products, dried raw products and specialities had a similar percentage 18.67%, 18.35% and 18.84% respectively. Shops and supermarkets were considered the most reliable sources of acquisition for meat products (51.41%). Traditional products (44.63%) and conventional products (39.34%) were the most consumed meat products compared to ecological/ organic/ bio products with only 16.03%. For the question "How often do you eat meat and meat products?", 47.28% of the respondents answered 3-4 times a week, 36.69% daily, 13.06% once a week, 1.82% once a month and 1.15% never. The manufacturer (35.05%) and ingredients (30.09%) were considered to be the most important criteria for choosing a meat product. A high percentage of the respondents (76.53%) agreed with the fact that the price of the food product influences its quality. More than 83.63% of the respondents answered that they consume meat products with natural antioxidants. A percent of 57.36% and 79.67% would not consume a meat product if the natural antioxidant added to extend the shelf-life would change its colour or its taste respectively. A high number of respondents (n=457) would pay more for a meat product that contains only natural antioxidants.

Table 3. Respondents´ attitude towards meat products consumption

Statement	Responses	%
Among the types of meat sold on the Romanian market, the most frequently consumed:	Beef	7.28
	Pork	29.58
	Mutton	1.66
	Chicken	56.52
	Venison	0.17
	Fish	4.79
Among the meat products, which ones do you consume most frequently?	Fresh meat products (fresh sausages, hamburgers)	44.14
	Cooked smoked products (cabanos sausages, salami)	18.67
	Dried raw products (sausages, salami)	18.35
	Specialties (smoked short ribs, smoked tenderloin, smoked ham)	18.84
Among the meat suppliers, those who will inspire you the most confidence are	Individual sellers/producers certified by the manufacturer (home delivery or internet order)	23.31
	Markets/fairs dedicated to traditional products	18.84
	Markets, fairs, in general	6.44
	Shops or supermarkets	51.41
Among the meat products available on the market, which do you consume more frequently?	Conventional products	39.34
	Ecological/ organic/ bio products	16.03
	Traditional products	44.63
How often do you eat meat and meat products?	Daily	36.69
	3-4 times a week	47.28
	Once a week	13.06
	Once a month	1.82
	Never	1.15
Which is the most common criterion for choosing a meat product?	Manufacturer	35.05
	Appearance of the packaging	4.29
	Ingredients	30.09
	Price	13.05
	Lack of preservatives and synthetic colourings	11.57
	Recommendations from relatives	5.95
	TV/radio/internet advertisements	No responses
Do you consider that the price of the product influences the quality of a food product?	Agree	76.53
	Unsure	8.59
	Disagree	14.88
Do you consume meat products that contain natural antioxidants?	Yes	83.63
	No	16.37
Would you still consume a meat product if the natural antioxidants added to extend the shelf life would change its colour?	Yes	42.64
	No	57.36
Would you still consume a meat product if the natural antioxidants added to extend the shelf life would change its taste?	Yes	20.33
	No	79.67
Are you willing to pay more for a meat product that contains only natural antioxidants?	Yes	75.53
	No	24.47
Do you think that this information will influence the way you purchase meat products in the future?	Agree	57.68
	Unsure	33.23
	Disagree	9.09

The correlations between demographic information and different statements in the survey revealed different knowledge, perceptions and attitudes, mostly between gender and level of education.

For the question "Antioxidant substances are added to extend the shelf life of meat products" there were no significant differences between gender ($p=0.940$), but there were significant differences by the level of education ($p=0.001$). Also, for the question "Do you think that the processing of meat products affect the action of antioxidants?" there were no significant differences between gender ($p=0.386$), but there were significant differences by the level of education ($p=0.0051$). For the statement "Among the types of meat sold on the Romanian market, the most frequently consumed" there were significant differences between gender ($p<0.0001$), but there were no significant by the level of education ($p=0.089$). No significant differences were seen between gender ($p=0.577$) and level of education ($p=0.583$) regarding the consumption of distinct types of meat products. Regarding the consumers preferences, if there were sensorial modifications of colour and taste of meat products by adding natural antioxidants, significant differences could be observed, both by gender and level of education ($p<0.05$).

CONCLUSIONS

Our study shows that the knowledge of the general public in Romania regarding the use of natural antioxidants in meat products is quite low. A gratifying aspect is the fact that the majority of respondents consider long-term consumption of synthetic antioxidants has harmful effects on the human body, causing allergies or even cancer.

As a general conclusion, surveys are an important research tool in order to evaluate consumer knowledge, attitudes and perceptions in different fields of food safety.

ACKNOWLEDGEMENTS

This work was supported by a grant of the University of Agronomic Sciences and Veterinary Medicine of Bucharest, project number nr.1065/15.06.2022, code 2022-0016, title *Obtaining an additive with antioxidant potential from indigenous plant sources intended to improve the preservation of meat products*, within IPC 2022.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

SMOKING TEMPERATURE CHARACTERISTICS AND INFLUENCE OF QUALITY INDICATORS ON PHYTOPHAGUS FILLET (*Hypophthalmichthys molitrix)*

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Abstract

The evolution of modern technologies for preserving fish and fish products has led to the eclipse of the preservation properties of many traditional methods, including the smoking method. Nowadays, the main purpose of smoking has been redirected towards highlighting the sensory quality rather than its preserving effect. The main aim of this paper is to highlight the physicochemical, sensory, and color characteristics of smoked phytophagous fillets. To produce the necessary products, nine specimens of phytophagous (Hypophthalmichthys molitrix) were harvested from the fish farm "Piscicola Vlădeni, CC&C PES SRL" Iasi, which were processed and prepared in the Microproduction Workshops of the Iasi University for Life Sciences "Ion Ionescu de la Brad". Three different smoking methods were applied to the fillets resulting from the phytophagous processing; hot smoking, semi-hot smoking, and cold smoking. From a physicochemical point of view, in the case of the batch with hot smoking quantity, a lipid content of 1.76±0.024% was recorded, higher compared to the L2ASC (0.94±0.04%) and L3AR (0.86±0.024%) batches. As regards protein content, the highest value was also found in lot L1AC (22.2±0.083%), followed by lot L2ASC (22.18±0.02%), with the lowest value in lot L3AR (21.96±0.024%). The most appreciated fillets were those processed by heat treatment with semi-warm and warm smoking.

***Key words**: perishability, phytophagus fillet, quality parameters, smoked fish.*

INTRODUCTION

The antimicrobial and antioxidant characteristics of smoke have been extensively studied over the years by various researchers in different countries of the world (Horner, 1997). Substances such as formaldehyde and phenols are released during the burning of wood, which gives the smoke preservative properties. Thanks to these substances, the smoking process inhibits the growth of many microorganisms and limits oxidative reactions (Abou-Taleb et al., 2011). Exposure of fish to smoke leads to a reduction in moisture levels and an enrichment of the fish meat with various substances in its composition. The preservation effect results from the consecutive or simultaneous action of several of the following factors: thermal inactivation of the product microflora, water activity, pH, antibacterial activity of the additives used before smoking, the concentration of antimicrobial and antioxidant components of smoke in the product, barrier properties of the packaging and storage temperature (Arvanitoyannis & Kotsanopoulos, 2012). Therefore, the high-quality shelf life and practical shelf life of different smoked foods vary from a few days at room temperature to several months at refrigeration temperatures, depending on the type, initial freshness, microbial contamination, and shape of the raw material, salting, and ripening parameters, water loss by dripping or drying, temperature, duration, and density of smoke in the smokehouse and packaging and storage conditions of the product.

Depending on the temperature of the smoking chamber, smoking can be cold, semi-hot, or hot. In the case of cold smoking, the temperature in the smoking chamber must not exceed 40°C. Cold-smoked fish is a product of the complex action of the salt (NaCl), smoke components, dehydration, and proteolytic and lipolytic enzymes (Hakimeh et al., 2010). Cold-smoked fish has a delicate smoky aroma and a longer shelf life than hot-smoked fish because it contains significantly less water and more salt. In the case of semi-hot smoking, the temperature ranges from 40 to 80°C.

The proteins in the fish are partially denatured and the enzymes are completely inactivated.

For hot smoking (boiling), the temperature varies between 80 and 170°C. The fish proteins are completely denatured and the enzymes are inactivated. The product has a low salinity and high water content, is lightly smoked, soft, and juicy, has a slightly smoky aroma, and cannot be stored for a long time (Puke et al., 2020).

As such, the main objective of this work was to highlight the applicability of differentiated smoking parameters (hot smoking, semi-hot smoking, and cold smoking) to obtain fish dishes with a pleasant sensory appearance and well-defined shelf-life characteristics (Másílko et al., 2015).

MATERIALS AND METHODS

The current work was carried out in the Food Technologies Department of IULS Iasi, the research activity being carried out in the Meat Processing Workshop, the Meat and Meat Products Technology Laboratory, and the Sensory Analysis Laboratory.

The necessary raw material, phytophagous (*Hypophthalmichthys molitrix*), was purchased from the fish farm "Piscicola Vlădeni, CC&C PES SRL" in Iasi County.

The experiment included the production of three batches of smoked phytophagus by three different smoking methods including different parameters of the applied heat treatment. The smoking methods applied are described in Table 1. The harvested phytophagous was transported under refrigerated conditions to the Meat Processing and Processing Plant, and operations including deboning, gutting, heading, and filleting were carried out. The resulting fillets were divided into three equal batches and salted at a rate of 1.2% of the quantity of raw material, applying the salting method by manual kneading so as not to detach the muscle tissue from the skin and to keep the fillets intact. The salted fillets underwent a dry maturation period under refreezing conditions at 2-4°C for 12 hours.

Table 1. Smoking heat treatment

Lot code	**L1AC**		**L2ASC**			**L3AR**	
Parameter	***Drying***	***Smoking***	***Drying***	***Smoking I***	***Smoking II***	***Drying***	***Smoking***
Time (min/h)	30	30	30	30	120	2	8
Temperature inside the cell (°C)	60	110	16	20	80	16	26
Temperature in the thermal centre (°C)	50	86	12	16	69	12	20
Humidity (%)	40	40	40	40	40	40	40

Raw chemical determinations applied to examine the shelf life of the fillets included quantitative analysis of moisture content, amount of protein remaining after smoking, collagen, fat content, and salt concentration using a versatile near-infrared (NIR) spectroscopic determination method using the Food Check meat analyzer (Bruins Instruments, no. 21F7122065). Physical determinations involved pH analysis using a digital meat pH meter and determination of brightness (L*) and red (a*) and yellow (b*) color indices of the finished product (smoked phytophagous fillets) in the CIELAB system using the Konica Minolta Chroma Meter CR-410 color analyzer.

The sensory analysis was carried out in the Sensory Analysis Laboratory of IULS Iasi in a single tasting session with a panel of 18 tasters. The tasters were represented by second-year students of the same university, of the food engineering profile. The three samples were identified with codes consisting of numbers and sample abbreviations and the fillets were portioned into finger-thick sticks so that the sample contained a part of the anatomical region from the belly and apart from the backbone, for the most accurate evaluation.

A descriptive CATA (Check-All-That-All) method was used to determine the sensory analysis and the samples analyzed with the most appealing appearance, the most uniform appearance, the most uniform color, the most intense color, the most intense smoke smell, the most intense non-specific smell, the most intense fishy taste, the most intense smoke taste, the juiciest sample, and the driest sample were scored.

RESULTS AND DISCUSSIONS

The results obtained from the chemical analysis of smoked phytophagous fillets showed the effectiveness of the heat treatment on the shelf life and the nutritional value of the products resulting from the technological smoking process. From Table 2 it can be seen that the most perishable batch with a low shelf life is represented by the batch with hot smoking (L1AC) which presents the highest percentage of moisture remaining in the product with an average value of 76.76±0.024 %. The high percentage of moisture in this batch is due to the low heat exposure time of the product, which facilitated both the maintenance of a high percentage of protein (22.2±0.083%) and lipids (1.76±0.024%) in the finished product. The semi-warm smoked batch (L2ASC) contains a lower percentage of moisture (76.74±0.050%) than L1AC and high values of protein (22.18±0.02%) and collagen (20.66±0.04%). The batch with the highest shelf life is the cold smoke smoked batch (L3AR) as the moisture value in the product is the lowest (76±0. 044%) compared to the other two batches ($p<0.05$). The low moisture content of the last batch resulted in a concentration of salt in the finished product (3.5±0.044%), the p-value is significant ($p<0.0001$), but also in a decrease of lipid content (0.86±0.024%).

Table 2. Chemical composition of smoked phytophagous fillets

Sample code	Lipid (%)	Protein (%)	Collagen (%)	Humidity (%)	Salt (%)
L1AC	1.76±0.024	22.2±0.083	20.36±0.024	76.76±0.024	2.76±0.067
L2ASC	0.94±0.04	22.18±0.02	20.66±0.04	76.74±0.050	1.2±0.002
L3AR	0.86±0.024	21.96±0.024	20.68±0.02	76.00±0.044	3.5±0.044
p-value	0.000^{**}	0.002^{*}	0.000^{**}	0.010*	$<0.0001^{***}$

ANOVA Tukey test: ns = p > 0.05 ; * = p < 0.05; ** = p < 0.01; *** = p < 0.001

The pH value is closely related to the water activity (wa), and therefore to the moisture content of the finished product. Table 3. reports the averages of the pH values obtained from the 5 pH readings. As the concentration of water in the product decreases, its acidity increases, thus, we can see that the sample with the lowest moisture content, cold smoking, shows the lowest pH value, showing distinctive differences ($p<0.0001$) between the three batches of smoked fillets.

To determine the colour of the smoked phytophagous fillets, the sample brightness (L*), the complementary red-green colour coordinate (a*) and the complementary yellow-blue colour coordinate (b*) were analysed. Table 3 shows that the sample with the highest value of brightness is represented by the sample with warm smoking (L1AC) with a mean value of 54.02±2.38, which also shows the lowest value of red colouration (0.85±0.72), the sample with cold smoking (AR) shows a mean brightness (47.29±2.12) with a red colour value of 3.50±1.22 and the darkest sample is the semi-warm smoked sample (L2ASC) which has a low lightness with an average value of 39.67±1.44 and is the most pigmented sample with an average red colour coefficient of 11.04±1.16.

Table 3. Physical parameters of smoked phytophagous fillets

Sample code	pH-value	L (*) ±SD	a (*)±SD	b (*)±SD
AC	6.516±0.013	$54,02^{A}$ ± 2,38	$0,85^{B}$ ± 0,72	$22,49^{A}$ ± 1,97
ASC	6.2±0.016	$39,67^{B}$± 1,44	$11,04^{A}$ ± 1,16	$20,09^{A}$± 1,58
AR	5.894±0.017	$47,29^{A}$ ± 2,12	$3,50^{B}$± 1,22	$23,22^{A}$ ± 1,05
p-value	<0.0001	0.000^{***}	$<0.0001^{***}$	0.354^{ns}

ANOVA Tukey test: ns = p > 0.05 ; * = p < 0.05; ** = p < 0.01; *** = p < 0.001; A,B - The same superscript letter within the same column means there is no significant difference between any two means (p>0.05).

Since 10 colour readings were taken, 5 on the inner and 5 on the outer side of the fillet, the differences identified for the yellow-blue colour (b*) are insignificant ($p = 0.354$). For the

brightness parameter, the differences recorded between samples were distinctly significant (p=0.000), while the red-green colour coordinates showed highly significant differences (p<0.0001.).
The determination of the quality idea of the finished products was carried out by the CATA sensory analysis method using multiple factor analysis (MFA).
From Figure 1. it can be seen that the semi-warm smoking sample (L2ASC) comprises the most positive indices, presenting the most appealing overall appearance with the most uniform colour.
The overall appearance is closely related to the colour of the sample, which is due to the two smoking stages of the thermal process that helped to form a colour of the most intense red, typical of smoking at high temperatures.
The cold-smoked batch (L3AR), with three indices, was the sample with the lowest moisture content, the most intense smoky taste and the fishiest smell. The intense smoke taste is due to the long exposure time of the phytophagous fillets to the heat treatment which lasted for 10 hours.
The last three quality indexes were positioned towards the sample with hot smoking (L1AC), which due to the short time of heat treatment (1 hour) was perceived as the most intense fish taste and as the most succulent sample. The assessors described this sample as having a "non-specific odour", with no observations of a non-conforming odour recorded by them.

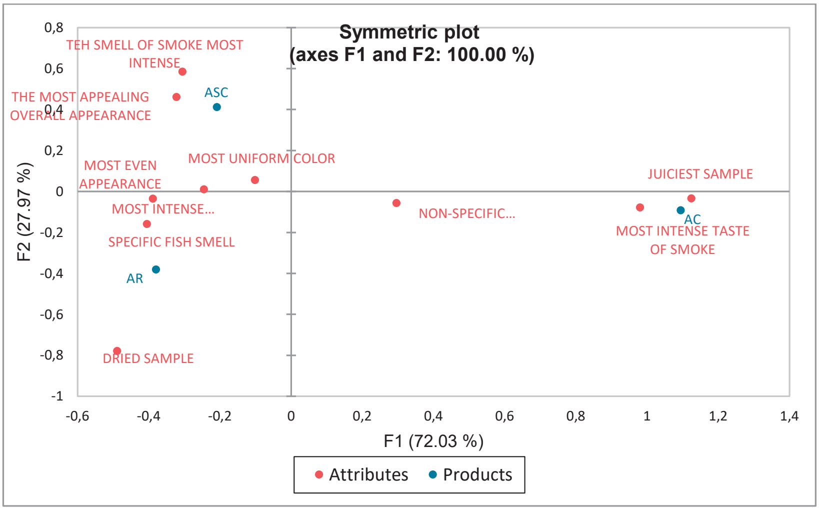

Figure1. Multiple factor analysis (MFA) of smoked phytophagous fillets assessed using the CATA test

CONCLUSIONS

Physico-chemical determinations showed that the hot-smoked batch (L1AC) has a high perishability, with the highest moisture content, but a high nutritional value, with the highest protein content. The chemical composition of semi-warm smoked phytophagous fillets (L2ASC) showed a high percentage of protein content, but had a lower moisture content compared to the hot smoked batch, thus increasing its shelf life. With the lowest moisture percentage and the driest sample, the cold-smoked batch (L3AR) has the highest shelf life. However, due to the long drying time, the protein percentage is low compared to the other two batches.
From a sensory point of view, the hot smoking batch (L2ASC) ranked last, thus the semi-warm smoking batch (L2ASC) ranked first in sensory determination, showing the most uniform appearance and color.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

TECHNOLOGICAL FRAUDS AND MILK ADULTERATIONS: A REVIEW

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Abstract

The milk consumption has increased constantly, with milk being part of the diet of a large proportion of the global population. As a result of this growing demand, the increased competition in the dairy market and the increasing complexity of the supply chain, the producers in the sector of milk and dairy products resort to technological fraud, which is considered to be a predominant problem in countries without a specific legislation. Therefore, this paper aims to review the main adulterants, the counterfeiting techniques and various methods of detecting counterfeiting.

***Key words**: adulteration, detection techniques, fraud, milk.*

INTRODUCTION

Milk, according to the *Codex Alimentarius*, is the normal mammary secretion of milking animals, without any addition or extraction thereof, intended for consumption as liquid milk or for further processing (Morin & Lees, 2018).

Adulteration of milk and dairy products has become a worldwide concern, immediately after the discovery of melamine contamination of infant products in China in 2008, but the history of counterfeiting of milk is very old. In the old German Empire, milk was diluted and then its consistency was restored by adding sugar, flour or calcium carbonate. In adition, this process dates back to 1850, when 8,000 babies died in New York from milk produced by the Swill factories because the milk came from animals fed on by-products from distilleries and was then adulterated by dilution with water, bleached by adding plaster and thickened with starch. Until the early 1900s, the milk was often adulterated with foreign substances, obtained from sick cows or mishandled during milking and storage. As a result, the milk was often the host of tuberculosis, cholera, typhoid fever and other life-threatening diseases. It was not until the end of the 19th century, when scientists began to fully understand theories about microorganisms, that they realized that diseases are transferred through milk and that they can intervene to eliminate this risk (Handford et al., 2015).

Milk is the best source of protein, fat, carbohydrates, vitamins and minerals, but unfortunately, it can be easily counterfeited worldwide. The reasons behind this fraud are mainly the perishable nature of milk, the shortage of supply and demand to meet urban demand and the lack of adequate detection methods (Tanzina & Shoeb, 2016). According to studies, milk is the second most prone to counterfeiting, after olive oil. Thus, it is adulterated with harmful substances that increase its quantity, but considerably reduce its quality (Gawali, 2021).

In addition to its microbiological quality and safety, the quality of milk is usually defined on the basis of the nutrient levels (mainly protein and fat) (Marin et al., 2019). These parameters were used to calculate payment to the supplier. In general, the parameters usually used to assess the milk quality are fat, protein, solids-non-fat and freezing point. The adulterants added to milk improve the value of these parameters, thus increasing milk quality in a dishonest way (Kedjia, 2018).

Since it is equally important to know about common adulterants and their effects on health, appropriate consumer awareness has been taken into account as a solution to prevent counterfeiting (Gawali, 2021).

MATERIALS AND METHODS

The current review was restricted to articles with English full-text availability. MDPI, Google Scholar, MedCrave, Springer Link, Springer Nature, Elsevier, and Juniper Publishers were among the databases used. The most common search terms were: milk quality, milk adulteration, potential milk adulterants, and detection methods. Additionally, searches were conducted using each adulterant in turn. We also looked for additional references in the bibliographies of the included papers. In our review of the literature, we discovered a sizable number of studies that mostly discussed the chemical makeup of milk and how the most crucial elements of milk change when adulterants are added. The results of the thorough search are sorted into categories and listed according to the best techniques discovered. We only included the more than 120 research articles and review papers that were discovered after 2009 because that is when the majority of adulterant detection techniques were created, which was necessary following the significant finding of the falsification of powdered milk from China in 2008.

RESULTS AND DISCUSSIONS

I. The most common milk adulterations

The different types of adulterants found in milk can be categorized into intentionally or accidentally added adulterants. However, only adulterants that have been added intentionally will be presented below. Also, water, vegetable proteins, whey and milk of different species (cow, buffalo, goat, sheep, camel, etc.) form the main constituents of economically motivated adulterants and do not pose a serious health risk. However, adulterants such as urea (to increase the non-protein nitrogen content and make milk white), formalin and boric / benzoic acid (to increase the shelf life of milk), detergents (to emulsify the oil in diluted milk), chlorine (to compensate for the density of diluted milk after adulteration) and ammonium sulphate (to maintain milk density) pose serious health risks (Kumar & Dash, 2021).

1. Adulteration that generally aimed at increasing the volume of milk

This type of adulteration can be done by adding water or skimmed milk to whole milk, as well as double forging using both adding water and reducing the fat content of the milk (Kedjia, 2018).

1.1. ***Addition of liquid whey (a by-product of cheese making)***

This is a well-known practice to increase the volume of milk, especially in areas where a huge amount of cottage cheese is produced. The advantage of this addition is that it does not change the lactose content of the milk. In addition, the whey added to the milk does not change the overall milk density, but reduces the solids and fat content, which decreases in proportion to the whey added. In this particular case, it is the fat that reflects the adulteration. Moreover, the addition of whey cheese to milk is very difficult to detect by formal analytical procedures that make as necessary to implement new experimental procedures/tests (Aquino et al., 2014).

1.2. ***Addition of reconstituted milk or synthetic milk***

Synthetic milk is an excellent imitation of natural milk containing vegetable oil, urea and emulsifiers. It is characterized by the fat, nitrogen, glucose and foam content, and a specific gravity similar to natural milk. When the synthetic milk is mixed with normal milk in different proportions, becomes identical to it including flavour. It is reported that synthetic milk is used to alter milk by 5-10% (Morin & Lees, 2018).

1.3. ***Partial skimming and addition of skimmed milk***

This practice is most often used when milk with a high fat content, milked in the evening, is skimmed and added on top of whole milk. Milk fat is one of the most valuable components and therefore can be subject to fraud, hence removing a quantity of fat leads to changes in the characteristics of the milk: the density increases, and the fat content decreases in proportion to the degree of skimming. The dry matter in the milk may increase, or its value may remain unchanged (Hanganu & Chira, 2021).

1.4. ***Diluting milk with water***

This is the most common, simplest, and oldest method of adulteration, based on the percentage reduction of all milk components and the the nutritional value and other quality control parameters of the milk (such as density, fat, SNF, protein and freezing point) will decrease. Therefore, dilution of milk with water can lead addition of chemicals to bring the density and colour to normal parameters. The milk will increase considerably in volume, hence the producers will make a considerable profit, but to changes in nutritional, hygienic and technological quality in addition to changes in chemical composition (Kedjia, 2018).

Table 1. Variations of milk quality control parameters according to the degree of dilution with water (Vujadinovic et al., 2017)

Models	Density [g/cm^3]	Viscosity [mPas]	Freezing point [°C]	pH	Fat of milk [%]
Undiluted milk	1.0571	33.83	-0.501	6.70	3.20
5% H_2O	1.0531	32.83	-0.484	6.72	3.00
10% H_2O	1.0517	32.63	-0.457	6.73	2.90
15% H_2O	1.0505	31.63	-0.433	6.75	2.80
20% H_2O	1.0478	31.33	-0.405	6.76	2.55
25% H_2O	1.0469	30.90	-0.378	6.77	2.45
30% H_2O	1.0447	29.27	-0.352	6.78	2.25
35% H_2O	1.0436	28.90	-0.327	6.78	2.00
40% H_2O	1.0430	28.73	-0.301	6.80	1.80
45% H_2O	1.0412	27.90	-0.275	6.80	1.73
50% H_2O	1.0397	27.13	-0.250	6.81	1.48

2. Adulterations involving the increased protein content

Nitrogen-rich adulterants are also a well-known issue in recent years precisely because of food safety incidents. This type of adulteration is very common because non-protein nitrogen cannot be distinguished by the Kjeldahl and Dumas methods that are commonly used to determine the total protein content of milk. Melamine, urea and whey are the main adulterants for this purpose due to their high nitrogen content and low cost (Morin & Lees, 2018).

2.1. ***Adulteration of milk with melamine***

According to the World Health Organization, melamine is a nitrogen-rich substance, which is used to increase the apparent protein content and therefore the economic value of milk. Until melamine contamination was reported in China in 2008 the limit of melamine in EU food legislation was not established. Both the European Commission and the United States Food and Drug Administration imposed the maximum allowable limit, which was later followed by the Codex Alimentarius Commission in a new ruling in 2010. According to the Regulation 1881/2006, the maximum level of melamine in food, except infant formula, is 2.5 mg/kg and in powdered infant formula and follow-on formula the maximum level allowed is 1 mg/kg. Melamine is described as harmful if swallowed, inhaled or absorbed through the skin. However, it is not a carcinogenic compound and has low oral toxicity; but it does cause kidney and urinary problems and even infant death when it reacts with cyanuric acid inside the body. The FDA has reported that when melamine and cyanuric acid are absorbed into the bloodstream, they concentrate and form a large number of crystals, which block and destroy kidney cells. The toxic dose of melamine is on par with common table salt with an average lethal dose (LD50) greater than 3 grams per kilogram of body weight (Jalili, 2017).

2.2. ***Adulteration of milk with urea***

Adding water to milk leads to a reduction in whiteness and density, and to maintain these properties, urea is generally used as a adulterating agent. Urea acts as preservative, increases SNF and non-protein nitrogen, but decreases the titratable acidity and suppresses the milk fermentation. At the same time, it makes the milk viscous, giving the impression of thicker milk. Urea, being a natural component of milk, accounts for most of the non-protein nitrogen in milk, i.e. 55%. According to Food Safety and Standards Authority of India (FSSAI), the maximum permissible limit for urea in milk is 70 mg/100 ml. Milk can be adulterated with urea in two ways: by intentionally adding urea and by adding synthetic milk to natural milk (Kumar & Dash, 2021).

2.3. ***Adulteration of milk with proteins resulting from the activity of genetically modified yeasts***

The technology for obtaining these types of protein allows being applied anywhere, even in urbanised areas. This technology is still being studied and the aim is to make these caseins allergen-free. Since these proteins are based on GMO technology, no milk protein produced in this way has so far received regulatory approval in Europe. In the US, the products containing proteins identical to those of natural milk, produced by a controlled fermentation process, are already on the market. In the future, it is likely that these proteins will also be the main ingredient in synthetic milk, as a response to the huge demand for the product, the drawbacks of factory farming and concerns about lactose allergies, hormones and antibiotics (Slane, 2019).

3. Counterfeiting involving the addition of unusual substances

This practice is applied in order to hide another type of falsification and involves the addition of the following compounds:

3.1. ***Detergents or soap***

This type of compounds are added to achieve the natural characteristics of milk, especially to make the milk thicker and to emulsify and dissolve the vegetable oil previously added to replace the extracted fat, forming a solution that resembles the froth of freshly milked milk. Detergent is added mainly to synthetic milk, which is similar to natural milk, being white in colour, and is produced by mixing urea, detergent, vegetable oil, neutralisers, sugar and water. Detergents have been shown to be the essential components of such a milk-like preparation. The anionic detergents are widely used in such practices precisely because of their low cost and easy availability (Barui et al., 2013).

3.2. ***Vegetable oil***

Milk fat is an important component of food and plays a significant role in the economics, nutrition and physical and chemical properties of milk. However, the incorporation of vegetable oils alters the content, the type and distribution of fat droplets in the protein network, causing changes in the microstructure and textural behaviour of dairy products. Olive oil in particular is used to replace milk fat and it is the added element after the fat content is removed. Since milk fatty acids are short-chain fatty acids (caprylic, capric, butyric) and vegetable fats are long-chain fatty acids, only a simple analysis of the fatty acid profile by chromatographic method can show that milk has been adulterated with vegetable oils (Ntakatsane et al., 2013).

3.3. ***Calcium carbonate or calcium chloride***

It is added to correct the density and to mask the dilution of the milk with water. When the calcium value rises above the normal range, the milk can be said to be falsified (Kedjia, 2018).

3.4. ***Maltodextrin***

Maltodextrins are polysaccharides containing dextrose and are obtained either chemically or by enzymatic hydrolysis of starch. Maltodextrins are classified on the basis of the amount of reducing sugar in relation to total carbohydrates, ranging from 3 to 20%. Maltodextrin is highly soluble in water with a solubility of about 1.2 kg/l; it is mainly used in foods and beverages as a thickener, sweetener and/or stabiliser. The maltodextrin imparts important functional properties such as bulking, gelling, binding, prevention of crystallisation, promotion of dispersibility, freezing control. It has been reported that maltodextrin is added as an adulterant to milk mainly to increase the density and also to increase the yield of the product prepared from it (Aparnathi et al., 2020).

3.5. ***Starch, wheat flour or rice flour***

These are added precisely to increase the density of the milk but also to increase the milk solids content. Functional maize starch is also specially designed for introduction into the milk industry. It successfully replaces modified starches with much better stability and is suitable for use in food processes that undergo heating or shearing. It has no impact on product, colour and taste (Kedjia, 2018).

3.6. ***Salt***

It is added mainly to get a correct lactometer reading by increasing the milk density. It is the most commonly used because its properties lend themselves very well to such adulteration. It is relatively difficult to detect, as there is quite a large amount of chlorides in milk, and in some particular situations (milk from cows with mastitis) the chlorides exceed the normal maximum limit. However, the natural content of chlorides in mixed milk (expressed as sodium chloride) varies between 120-170 mg/100 ml of milk, with an average of 140 mg/100 ml, and up to 200 mg/100 ml in colostral milk or milk from cows with mastitis (Aparnathi et al., 2020).

4. Adulterations associated with increasing the shelf life of milk

4.1. ***Neutralizers***

These are added to cover the acidity and sour taste of the milk. The most popular neutralisers are sodium bicarbonate, hydrated lime, sodium hydroxide and sodium carbonate. Although the addition of such alkaline substances is not allowed by law, the producers tend to neutralise milk to avoid rejection in milk collection centres and factories (Kedjia, 2018).

4.2. ***Formaldehyde***

This is an antiseptic, disinfectant and a good preservative. It is used to preserve milk for a long period of time (1 ml per 10 L of milk preserves it for about 10 days), especially during transport and storage to avoid refrigeration costs. It is a toxic and very dangerous substance, considered carcinogenic, and a high dose of formaldehyde can affect the liver and cause kidney damage (Mabood et al., 2017).

4.3. ***Hydrogen peroxide***

It has a similar role to formaldehyde and helps to increase shelf life by acting as a preservative. It decreases the souring of milk when hygiene and low temperature storage rules are not followed by stopping bacterial growth. Hydrogen peroxide (H_2O_2) has a long history of use as a preservative in milk around the world. The use of H_2O_2 to activate the inherent lactoperoxidase enzyme system has improved the quality of milk and dairy products in areas where refrigeration is not widely available. Even though, due to chemical processes within raw milk, it may contain small amounts of hydrogen peroxide (1-2 mg/L), the concentration necessary to inactivate pathogens is 10 times higher. In addition, the presence of H_2O_2 in high concentrations can lead to modifications in the milk chemical composition. At the same time, the addition of hydrogen peroxide for the purpose of presserving milk is prohibited for the following reasons: it masks to some extent negligence in the observance of hygienic conditions; it has a germicidal action which is not selective, acting more strongly on lactic bacteria than on the spoilage microflora; used in larger quantities to ensure preservation for 1-2 days, it can impart undesirable sensory properties to milk (bitter, irritating taste); added even in small quantities, hydrogen peroxide, through the active oxygen released, causes incipient oxidation of milk fat. The US Food and Drug Administration (FDA) only allows hydrogen peroxide in milk that is used for cheese production (Ivanova et al., 2019).

4.4. ***Acids***

These are added to milk as preservatives. The most common are salicylic acid and benzoic acid, which are responsible for increasing shelf life. Salicylic acid added to milk at a rate of 0.04-0.05% preserves the product for several days. Salicylic acid and its salts are prohibited for use in the preservation of milk because of their harmful effects on the body and other negative implications. Benzoic acid is widely used in foodstuffs as a preservative. Although benzoic acid is generally recognised as safe (GRAS) under the food regulations, benzoic acid is not a permitted preservative in milk and milk beverages in EU and China. WHO has assessed and established an acceptable daily intake (ADI) for benzoic acid of 0-5 mg/kg body weight (Qi et al., 2009).

5. Adulterations involving species substitutions

Of the many possible adulterations in milk and dairy products, one of the most common concerns the origin of the species, i.e. replacing milk with higher nutritional value (such as sheep, goat or buffalo) with cheaper cow's milk to reduce production costs and increase profits. This is explained by seasonal fluctuations and lower yields of sheep, goat and buffalo milk (or more exotic species such as camel or donkey), which raises the economic values of these types of milk and derived products. Replacing milk from these species, in addition to having a negative economic impact, is also a problem for many consumer groups for other reasons such as religious, ethical or cultural objections.

In several EU countries, especially those in the Mediterranean and other areas such as the Eastern Europe and the Middle East, a variety of cheeses are prized as traditional products made from goat's milk, sheep's milk, their milk in a mixture or buffalo milk. Traditionally produced cheeses are regarded as specialities and generally fetch higher market prices and are therefore more prone to adulteration of the raw material from which they are made. There is now a growing market for non-cow's milk in some countries, particularly goat's milk, due to its superior nutritional characteristics, but also to other aspects such as its attractive smell and taste and superior digestibility. In addition, according to studies, goat's milk may be a possible alternative to cow's milk as it is considered to be hypoallergenic. In this case, if cow's milk is not listed on the label it could pose a health risk to consumers who are allergic. However, because the proteins are very similar, people allergic to cow's milk proteins can be affected by milk from any species, demonstrating the importance of correct labelling (Morin, 2018).

The composition of milk of different types of farm animals differs significantly in physico-chemical indicators such as the mass fraction of proteins and fats, minerals, vitamins, enzymes, etc. An important identification criterion for the type of milk is also the polymorphism of caseins, the constituent technological components of raw milk that determine its possibility of industrial processing. In order to avoid possible fraudulent substitution of goat and sheep milk by cow's milk, it is necessary to develop analytical procedures capable of detecting such frauds and protecting consumers against misleading mislabelling (Gilmanov et al., 2020).

II. Methods for detecting adulterants in milk

The control of milk quality is very important for the safety reasons. Therefore, the adulteration of milk decreases its quality and can even affects its safety. The methods for detecting adulteration in milk are generally classified into qualitative and quantitative detection methods. Although the quantitative detection methods include complex biotechnological and electrical methods, the qualitative detection methods based on colour chemical reactions (biochemical, physical-chemical) are advantageous because they are simple, fast and very easy to perform, even if not very accurate (Kedjia, 2018).

1. Physical methods (qualitative)

Methods based on the physical properties of milk are density (reading by lactometer), freezing point, refractive index, etc., which are easy to perform but may be inaccurate due to natural variations in milk composition. Physical methods are simple, quick, easy, cheap and convenient. However, the sensitivity of these tests is lower compared to chemical and instrumental methods. The freezing point can be significantly affected by seasonal changes and regional factors. Density (or specific gravity) depends on the composition and temperature history of the milk. Therefore, density measurement may not be a useful tool for detecting adulteration. Thus, physical methods have a number of general limitations due to natural variations, lower sensitivity, poor specificity, susceptibility to manipulation, etc. (Aparnathi et al., 2020)

2. Chemical methods (qualitative)

Chemical methods for detecting milk adulteration are based on observable physico-chemical changes. They can be performed in any biosafety level 1 laboratory with the availability of chemical reagents and the necessary precautions. Chemical changes may occur as a result of chemical reaction between the adulterant in the milk and a specific chemical reagent, resulting in the appropriate colorimetric detection (Rupak et al., 2021). When reviewing and evaluating available qualitative tests for the detection of common adulterants reported in milk, it was observed that there is a wide variation related to the performance of several tests in terms of aspects such as sensitivity, convenience, cost. In view of performance improvement requirements the existing qualitative tests for the determination of common adulterants including detergent, urea, ammonium salts, glucose, sucrose, maltodextrin, starch, hydrogen peroxide, salt, nitrate, sulphate, formaldehyde and neutralisers have undergone a number of changes (Aparnathi et al., 2020).

Table 2. List of qualitative tests for finding common milk adulterants that have been reported (Aparnathi et al., 2020)

Sr. No.	Adulterants	Details of tests selected for optimization	
		Test	**Reference**
1.	Detergent	Methylene blue	Paradkar et al. (2000)
2.	Urea	p-dimethylaminobenzaldehyde test (DMAB)	FSSAI (2016)
3.	Ammonium salts	Nessler	Sharma et al. (2012)
4.	Sucrose	Seliwanoff	Srivastava (2010)
5.	Glucose	Barfoed	Barfoed (1873)
6.	Maltodextrin	Iodine	Sharma et al. (2012)
7.	Starch	Iodine	BIS (1960)
8.	Salt	Silver nitrate test	FSSAI (2016)
9.	Nitrate	Diphenylamine	FAO (1986)
10.	Sulphate	Barium chloride	FSSAI (2016)
11.	Hydrogen peroxide	Iodometric test	FSSAI (2016)
12.	Formaldehyde	Hehner	Draaiyer et al. (2009)
13.	Neutralizers	Rosolic acid	DGHS (2005)

3. Instrumental methods (quantitative)

Modern biotechnology offers a range of rapid, sensitive and accurate methods for the detection and analysis of adulterants in food products. However, recent incidents of adulterated milk contamination have shown that standards in milk quality control are insufficient in identifying poor quality milk. Therefore, different methods have been studied and applied precisely to combat these problems. However, such detection methods require large investments. Another important aspect is that the type of quantitative detection techniques depends on the nature of the adulterants in milk. Although there are fairly well known techniques based on portable equipment, those designed and developed recently require experimental set-up and much more expensive equipment, as well as a number of operational procedures (Kedjia, 2018). A situation often arises when the indicators of the device used, calibrated according to the manufacturer's data, differ from the indicators obtained by reference methods, e.g. the Gerber method for fat determination. The difference can be as significant as 0.3% fat. With the apparent simplicity of the method, overlooking its complexity can lead to systematic errors in the analysis. For example, the temperature regime is not maintained, the isomeric composition of the isoamyl alcohol used is not monitored. This leads to discrepancies between the milk analyser readings and the results obtained from the chemical analysis data precisely because the Gerber method was performed incorrectly. In addition, thanks to new technologies, quality analysis is carried out much faster and is less dependent on the qualifications of employees. Therefore, more and more priority is given to analysers using indirect methods of data collection. The analytical methods by which milk authenticity can be demonstrated are presented below (Smirnova et al., 2020).

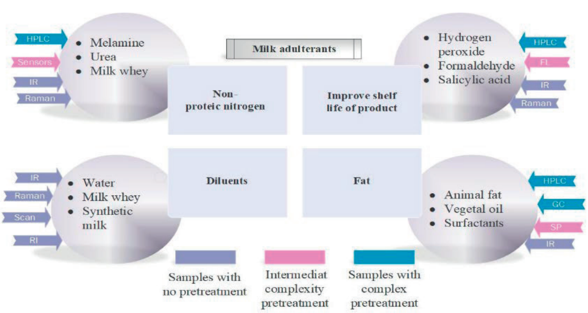

Figure 1. A description of the main milk adulterants, along with information on sample pretreatment complexity and the analytical techniques used for determining the most commonly (Nacimento et al., 2017)

FL: molecular fluorescence; GC: gas chromatography; HPLC: high performance liquid chromatography; IR: infrared spectrometry; RI: measurement of refractive index; Scan: scanometry; SP: UV-vis spectrophotometry

3.1. ***Identification of milk components and quantification of added adulterants by spectroscopy (NIR, Raman)***

Fingerprinting methods are ideal candidates to replace analytical procedures. The term 'fingerprinting' can be defined as a variety of techniques that can measure food composition in a non-selective way. Among these methods, infrared-based vibrational spectroscopy methods and Raman spectroscopic techniques use information from the major compounds present in milk. Organic compounds absorb radiation at specific wavelengths or frequencies, giving rise to spectral signatures that are characteristic of the food composition and can be considered as 'fingerprints' of the product. These signatures also include interference due to variation arising from natural events (e.g. weather, climate, disease, etc.). Vibrational spectroscopy is suitable for implementation in factories and dairy laboratories as it allows on-line control and screening of a large number of samples per unit time. Fingerprinting methods are also of interest to regulatory bodies as they allow for rapid preventive action (Morin, 2018).

3.2. ***Evaluation of proteins by mass spectrometry (MS) or liquid chromatography (LC) coupled with MS***

Evaluation of proteins and/or peptide sequence by mass spectrometry (MS) or liquid chromatography (LC) coupled with MS is increasingly used to prove the authenticity of milk. This has been made possible by several technological advances that allow for accurate protein and peptide analysis using ionization techniques such as electrospray ionization (ESI) and matrix-assisted laser desorption ionization. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) provides informative fingerprints of milk proteins for the authentication of dairy products and is also a simple, fast, sensitive and highly reproducible technique. LC-MS techniques are advantageous in terms of high selectivity and sensitivity, which makes them useful as confirmatory techniques (Morin, 2018).

3.3. ***Different methods of species identification***

In recent years, analytical methods based on DNA analysis have progressed rapidly, going beyond protein analysis and have been successfully applied in testing the authenticity of milk (Morin & Lees, 2018). DNA-based methods have several advantages, in particular the ubiquity of nucleic acids in each cell type and their superior stability compared to proteins. Most DNA tests are based on the polymerase chain reaction (PCR) technique, precisely because of its high specificity, sensitivity, simplicity and speed, allowing the identification of species of origin even in processed foods such as dairy products (Morin, 2018).

According to EU legislation, isoelectric focusing of γ-cazeins after plasmolysis should be used as a reference method to ensure that the products obtained are exactly sheep's milk, goat's milk or buffalo milk or a mixture of sheep's, goat's and buffalo milk. In this method, samples must be analysed together with reference standards containing 0% and 1% cow's milk, and the test is considered positive if both γ2- and γ3-cow's milk exist (obtained by plasmolysis), or the corresponding peak area ratios when densitometry is applied, are equal to or greater than the 1% reference standard level. The method may be used to detect either raw or heat-treated and caseinised cow's milk in fresh or ripened sheep's, goat's and buffalo's milk cheeses or mixtures thereof (Morin, 2018).

Recently, a commercially available kit has been developed that is based on a competitive ELISA using a mouse monoclonal antibody raised against bovine κ-casein that allows screening of both raw milk and heat-treated cow and buffalo milk in milk and cheese from other species and sources (Morin, 2018).

CONCLUSIONS

The milk adulteration represents a main global concerns because milk is consumed as a healthy dairy product everywhere in the world. People are concerned about the quality and purity of milk as a result of the growing fraudulent practice of adulterating milk. As a consequence, the consumers' health may be harmed by milk adulterants like water, vegetable and animal fat, extraneous proteins, and chemical additives like melamine, urea, ammonium sulphate, formalin, acids (e.g. boric acid, benzoic acid, salicylic acid), caustic soda, hydrogen peroxide, detergents, and sugars that are knowingly added. Various techniques have been developed over time to identify adulterants in milk, but the most accurate are instrumental. Therefore, there is a pressingneed for the development of reliable, affordable and non-expensive methods and technologies that could detect and stop the practices of adulterating milk.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

PHYSICO-CHEMICAL AND SENSORY EVALUATION OF THREE TYPES OF PORK MORTADELLA MANUFACTURED IN THE IULS MEAT PROCESSING MICROSECTION

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Abstract

The objective of this study was to develop and characterize three batches of mortadella made at the IULS meat processing microsection from three anatomical regions of the pork carcass: loin, tenderloin, and chop. The proportion of ingredients introduced were: 80% meat, 15% pork fat, and 5% ice flakes. The mortadella samples were characterized physicochemically in terms of moisture, lipid, protein, and collagen content, as well as sensoryly to determine the perception of a group of evaluators on sensory attributes (appearance, color, aroma, taste, texture, general acceptance). The batch of mortadella obtained from tenderloin had the highest moisture and protein content and the lowest lipid percentage compared to the batches obtained from loin and chop. The anatomical region significantly influenced the color parameters of the mortadella, with the L3MC batch showing the most intense lightness, the highest value of the b parameter, and the lowest red intensity compared to the other two samples. Regarding the sensory evaluation, the batches were scored between 6.27-8.03 for the sensory attributes, and the ranking of the overall acceptance of samples was: L1MS, L2MM, L3MC.*

***Key words**: anatomical regions, chop, loin, mortadella, pork, tenderloin.*

INTRODUCTION

Meat is an important source of essential nutrients for the human body. These nutrients include proteins, fats, vitamins, and minerals, which perform various functions in the human body. However, emulsified products are perceived by consumers as unhealthy due to the contribution of saturated fats, additives, and very little or no fibre or calcium in the diet (Feiner, 2006; Horita et al., 2011; Saldaña et al., 2018). In general, emulsified products contain 20-35% fat and 2.2-2.5% salt (Câmara et al., 2020), with fat being a food component that contributes to the sensory perception of the product as it influences the balance, intensity, and release of flavors by affecting the distribution and migration of flavor compounds. Moreover, fat is an element that improves texture and appearance and enhances the feeling of satiety during meals (Cáceres et al., 2004).

Mortadella is an emulsified product known and consumed worldwide, defined as a boiled emulsified product obtained from the meat of various species of animals and introduced into natural or artificial membranes of various shapes and sizes (Muraoka et al., 2019; Biasi et al., 2023). Mortadella is a type of sausage originating from Italy, more precisely from the Emilia-Romagna region. Typically, mortadella is made from finely minced selected pork and beef meat, mixed with small pieces of fat, seasoned with various spices such as pepper, nutmeg, and coriander, and then wrapped in a thin natural membrane. Over time, the recipe and production process for mortadella have evolved, and today there are several varieties of this product, including some that include food additives or other ingredients. However, traditional Italian mortadella is still made today according to the same centuries-old recipe, using simple ingredients and traditional production methods (Olkiewicz & Moch, 2008; Guerra et al., 2011; Doménech-Asensi et al., 2013; Alda et al., 2021). Nowadays, considering the evolution of consumer tastes and the nutritional demands they have from food products, mortadella recipes are becoming more and more oriented towards reducing fat and salt content (Barbieri et al., 2013; do Santos et al., 2020).

Pre-slaughter factors such as species, age, type of feeding, slaughter conditions, and post-slaughter conditions like the rate of occurrence of rigor mortis and method of bleeding all have an impact on final meat quality (Maher et al., 2004).

Moreover, the carcasses of slaughtered animal species comprise several muscle groups whose quality differs in terms of chemical composition, technological quality, and commercial value. In this context, the study investigated the influence of anatomical regions from different quality classes on the physicochemical quality of final products and their influence on consumer preferences.

MATERIALS AND METHODS

The study was carried out at the University of Life Sciences "Ion Ionescu de la Brad" in Iasi; the mortadella was obtained in the Meat Processing Workshop, and the analyses were carried out in the Meat and Meat Products Technology Laboratory.

To achieve the proposed aim, an experimental protocol was developed based on which three batches of mortadella were made from pork meat from three anatomical regions classified in different quality classes: tenderloin, chop, and loin.

The production process of the batches of mortadella involved the introduction of the following raw materials: 80% quality I pork, with loin for batch 1 (L1MS), tenderloin for batch 2 (L2MM), and chop for batch 3 (L3MC); 15% fat; 5% ice flakes. The meat was pre-salted with 2% salt, and black pepper (5 g/kg), garlic powder (10 g/kg) and paprika (2 g/kg) were used for seasoning.

The raw materials were prepared for mincing, for which a grinding machine with a small working capacity (Wolf) was used (with a sieve of 3 mm diameter). The composition obtained was minced until a fine, homogeneous paste (bradt) was obtained, to which ice flakes were added after a few rotations to stop the temperature of the paste from rising during the operation. Throughout the process, the temperature of the paste is checked with a vertical thermometer to prevent it from rising above 13°C. The fat is blanched for 15-20 minutes at a temperature of 80-85°C and then minced through a 10-12 mm diameter sieve.

After primary processing, the raw materials are weighed and then mixed with the fat and spices until evenly distributed in the paste structure. The resulting paste is placed in polyamide filling membranes of 50 mm diameter, which have been previously hydrated to form elasticity.

The heat treatment carried out was the same for the three batches of products; the steps are shown in Table 1.

Table 1. Heat treatment scheme of the mortadella batches

Heat treatment stage	Time	Temperature inside the cell	Temperature in the thermal centre	Humidity
	minute	°C	°C	%
Air drying	30	65	50	60
Boiling	-	78	69	99
Hot air drying	10	78	69	60

The finished products obtained were subjected to physicochemical and sensory evaluations in the Meat and Meat Products Technology Laboratory and the Sensory Analysis Laboratory.

The pH values were determined using a HANNA HI99163 digital pH meter, which uses an amplified pH electrode with a built-in temperature sensor.

The color of mortadella samples was determined using a Chroma Meter CR-410 colorimeter (Konica Minolta Inc., Japan) in the CIELAB scale. The light source of the device was D65, the observation angle was 10°C, and the aperture of the measuring cells was 50 mm. The scale used for the color reading was L*, a*, and b*.

The chemical evaluation involved the determination of proximate composition (moisture, fat, protein, and collagen) using a FoodCheck analyzer (a spectrophotometer that uses infrared light rays). For the sensory evaluation of mortadella samples, 55 potential consumers were selected from students at the University of Life Sciences. The panel ranged in age from 20 to 31, with 62% being female and 38% being male. The sensory evaluation involved the application of a hedonic acceptance test whereby participants were instructed to evaluate the three formulations of pork mortadella through the attributes of appearance, color, aroma, taste, texture, and overall acceptance. A 9-point scale (1 = extremely unpleasant, 5 = neither pleasant nor unpleasant, 9 = extremely pleasant) was used for scoring. The presentation of the samples was in pieces of approximately 3 cm thickness, coded with three-digit codes.

The mean values obtained for the proximate composition, colour parameters and acceptability test were compared using analysis of variance (ANOVA) followed by Tukey's test at 5% significance level ($p < 0.05$), using XLStat software (Addinsoft version, 2022).

RESULTS AND DISCUSSIONS

The chemical composition and mean pH values determined for the batches of mortadella produced are shown in Table 2. Significant differences were observed between the batches made for all five parameters evaluated ($p < 0.05$). The highest moisture content was found in the batch of mortadella made from the tenderloin (L2MM, 66.28%), followed by the batch from the loin (L1MS, 63.46%), and finally by the batch from the chops (L3MC, 63.21%). These findings are comparable to those from Guerra et al. (2011), who described the moisture content of goat mortadella with 10% fat at a level of 65.75%. Similar results were reported by Viuda-Martos et al. (2010), who presented a water content of 65.62% in a control batch of mortadella formulated with 50% lean pork meat and 50% pork backfat.

Regarding the fat content, the L2MM samples had the lowest mean value (12.48%), with the L1MS (16.48%) and L3MC (17.02%) batches coming in second and third, respectively, with similar mean percentages. A study conducted by García et al. (2006) reported a fat content of 12.71% determined in a reduced-fat mortadella product, a percentage similar to the result obtained for batch L1MS. Fat content plays an important role in the technological process of production, as well as in the perception of sensory and instrumental properties of the texture of mortadella-type products (Saldaña et al., 2015; Alda et al., 2021).

The protein content for the three batches of mortadella was in the range of 18.26 ± 0.02 (L3MC) to 19.34 ± 0.02 (L2MM, Table 2). These results could be related to the percentage of fat in the three batches, since the composition of the batch made from tenderloin, which presented the lowest amount of fat (implicitly the highest percentage of water), identified the highest percentages of protein. The other two batches also showed a decrease in protein content and an increase in fat content. The protein content found in the three assortments of mortadella manufactured was higher compared to the values reported by Barbieri et al. (2013), who analyzed 39 samples of mortadella from the Italian markets and found protein contents that varied between 12.25% and 16.85%.

Collagen is a stromal protein of industrial importance because it gelatinizes during cooking, contributing to the texture of the product. The collagen content was expressed as a percentage of protein content. The results obtained for the collagen content of the products revealed percentages directly proportional to the amount of protein content (Table 2); thus, the highest percentage of collagen was identified in batch L2MM.

The salt content of the products did not differ significantly ($p > 0.05$), as all three batches were salted in the technological process of production with the same percentage of salt.

Regarding the pH of mortadella samples, the differences in values were distinctly significant ($p < 0.05$). The highest pH value was recorded for the batch of mortadella made from the tenderloin, while the batches made from loin and chops showed similar pH values, the differences being not significant.

Color is an extremely important quality attribute, as it is the first impact that determines consumers' initial perception of the food product.

Table 2. Analysis of the chemical composition and pH of the mortadellas batches

Parameters	L1MS $\bar{x} \pm s_x$	L2MM $\bar{x} \pm s_x$	L3MC $\bar{x} \pm s_x$	p-value
Moisture (%)	63.46 ± 0.14b	66.28 ± 0.07a	63.21 ± 0.09b	**<0.0001*****
Lipid (%)	16.48 ± 0.16ab	12.48 ± 0.06c	17.02 ± 0.09a	**<0.0001*****
Protein (%)	18.45 ± 0.03b	19.34 ± 0.02a	18.26 ± 0.02b	**<0.0001*****
Collagen (%)	16.76±0.04	17.48 ± 0.02	16.52 ± 0.04	**<0.0001*****
Salt (%)	1.28 ± 0.06	1.26 ± 0.04	1.28 ± 0.04	0.178^{ns}
pH	6.11 ± 0.06b	6.36 ± 0.04a	6.07 ± 0.03b	**0.001****

$\bar{x} \pm s_x$ – Mean value followed by standard deviation. Mean values in the same line with different letters are significantly different by the Tukey test ($p<0.05$).

The results obtained for the color parameters lightness (L*), redness (a*), and yellowness (b*) are presented in Table 3. All three color parameters evaluated showed highly significant differences ($p < 0.05$) between batches of mortadella. The mortadella samples obtained from the loin (L1MS) had the lowest lightness (L*) (59.96 ± 0.65, $p < 0.05$), followed in ascending order by the mortadella batches from the tenderloin (L2MM) and the chop (L3MC). A more intense red hue (a*) was also observed in the samples with the lowest L* lightness, values that decreased with increasing lightness. Regarding the values of the parameter b* (yellowness), these were directly correlated with the lightness, as the yellow coloration was more intense in the samples of lot L3MC with the highest lightness.

Thus, the type of meat used may influence the colour parameters (lightness, redness and yellowness) of each lot of mortadella, mainly due to the concentration of myoglobin present in the respective anatomical region (Alda et al., 2021).

Table 3. Instrumental colour of the mortadellas batches

Parameters	L1MS $\bar{x} \pm s_x$	L2MM $\bar{x} \pm s_x$	L3MC $\bar{x} \pm s_x$	p-value
L*	59.96 ± 0.65	63.23 ± 0.23	68.04 ± 0.24	**<0.0001*****
a*	13.816 ± 0.66	11.23 ± 0.34	7.93 ± 0.25	**<0.0001*****
b*	11.186 ± 0.16	12.49 ± 0.30	13.97 ± 0.17	**<0.0001*****

ANOVA Tukey test: ns = $p > 0.05$; *** = $p < 0.001$

Sensory evaluation of the mortadella batches revealed significant differences ($p<0.05$) in the attributes of appearance, color, aroma, taste, texture, and overall acceptance. Among the three product batches, higher scores were recorded for the L1MS batch, which was perceived by the participating evaluators as the most balanced product in terms of appearance and color. The same sample, L1MS, scored the highest for texture, which correlated with the percentage of fat identified in the product composition. Therefore, the higher amount of fat resulted in a higher appreciation of the texture of the product, the perception of a fine texture, and the composition being better bound and compact. Also, aroma and taste were scored higher for samples with a higher composition of fatty substances. The overall acceptance of the batches scored between 6.67 ± 1.06 (L3MC) and 7.83 ± 0.65 (L1MS), indicating that the products were considered acceptable by the evaluators.

Table 4. Sensory evaluation and acceptability of the mortadellas batches

Sensory parameters	L1MS $\bar{x} \pm s_x$	L2MM $\bar{x} \pm s_x$	L3MC $\bar{x} \pm s_x$	p-value
Appearance	7.53 ± 1.01a	6.33 ± 0.92b	6.43 ± 0.94b	**<0.0001**
Color	7.83 ± 1.26a	6.37 ± 0.85b	6.27 ± 0.91b	**<0.0001**
Aroma	7.87 ± 0.94a	6.90 ± 1.14b	7.12 ± 1.18c	**<0.0001**
Taste	8.03 ± 0.93a	7.47 ± 0.77b	7.73 ± 1.04c	**<0.0001**
Texture	7.70 ± 0.84a	6.87 ± 1.17b	6.57 ± 1.01c	**0.0001**
Overall acceptance	7.83 ± 0.65a	6.97 ± 1.03b	6.67 ± 1.06c	**<0.0001**

$\bar{x} \pm s_x$ – Mean value followed by standard deviation. Mean values in the same line with different letters are significantly different by the Tukey test ($p<0.05$).

CONCLUSIONS

The batches of mortadella produced in the IULS meat processing plant showed positive quality characteristics. The proximate composition of the samples followed the anatomical regions used in the production process. The batch of mortadella made from tenderloin (L2MM) differed significantly from the other two batches by having a higher moisture content and the lowest lipid content. In terms of the instrumental color of the samples, the L1MM batch made from the tenderloin was found to have a lower lightness, and it also showed the most intense color of red. From a sensory perspective, all three batches were positively rated by the evaluators, with the overall sensory acceptance being highest for the L1MS batch.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

CONTENT OF POLYCYCLIC AROMATIC HYDROCARBONS IN FISH AFTER HEAT TREATMENT

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are predominant pollutants in the aquatic environment that can cause a variety of potentially toxic effects, affecting the entire ecosystem. PAHs were evaluated following the application of different heat treatments to fish meat by liquid chromatography (HPLC). The following polycyclic aromatic hydrocarbons were determined: acenaphthene, anthracene, benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(gy)pyrylene, chrysene, dibenzo(a,h)anthracene, phenanthrene, fluorene, fluoranthene, indeno(1,2,3-cd)pyrene, naphthalene and pyrene. The samples analyzed were from raw trout (Salvelinus fontinalis); fried in sunflower oil; fried in olive oil; fried with rapeseed oil; baked in the oven and fried in lard. The brook trout (Salvelinus fontinalis) was harvested from the Mărișel area, Cluj county. The highest values for the hydrocarbons analyzed from raw fish were for naphthalene (5.51±0.21) and benzo(a)anthracene (2.47±0.06). The trout fried in sunflower oil showed the highest values for anthracene (10.34±0.09) and pyrene (10.24±0.30) and Indeno(1,2,3-cd)pyrene was <0.05. Trout fried with rapeseed oil showed the highest values for anthracene (10.34±0.09), followed by pyrene (10.24±0.30) and indeno(1,2,3-cd)pyrene was below <0.05. Trout fried in olive oil showed very high values for pyrene (51.37±0.63) and fluoranthene (20.36±0.89) and dibenzo(a,h)anthracene was <0.05. According to this study, fish meat has the highest percentage of PAHs following the frying process. These compounds play a very important role in the ecosystem, therefore the most affected is the consumer due to the carcinogenic effect they can cause on the body.

Key words: *heat treatments, olive, PAHs, rapeseed, Salvelinus fontinalis, sunflower oil.*

INTRODUCTION

Polycyclic aromatic hydrocarbons are a major class of hydrophobic organic compounds, which are made up of two or more joined aromatic nuclei and are found in both freshwater and aquatic environments (Grimmer et al 1968, 1983; Nielsen et al., 1996; Neff et al., 2005). During processes of incomplete combustion of organic matter or pyrolysis processes, hundreds of PAHs can form and release into the environment (Fetzer, 2000; Llobet et al., 2006). Regarding the natural formation process of polycyclic aromatic hydrocarbons, it is achieved by carbonization of organic matter, and a significant percentage that reach the environment come from incomplete combustion of fossil fuels, used in internal combustion engines, but also from the pyrolysis of organic matter (Adonis, 2003; Grimmer et al., 1979; Nielsen et al., 1996; Wretling et al., 2010). Polycyclic aromatic hydrocarbons, from a biochemical point of view, are lipophilic, with affinity for fatty substances, and their solubility and volatility leads to percentage decrease with increasing molecular weight (Grimmer et al., 1983; Ake, 2012). Therefore, this group of organic compounds, due to their characteristics, are considered pollutants with a large spread in the ecosystem (Grimmer et al., 1983; Boca et al., 1988). PAHs are spread right in the air of the room where fish is prepared through the roasting process, and this process removes most PAHs into the air compared to boiling or other processes, according to a study by Michiko et al. (2001) in Japan. According to the European Communities (2001), following Research has highlighted certain PAHs as substances with the ability to damage DNA in cells, giving them the character of genotoxicity, carcinogenic, and there is no minimum level of safety. The process of contamination of food with PAH can also be done by applying thermal processes: frying, boiling and smoking. Their level (PAH), according to studies, increases significantly in processed foods or foods that have gone through the cooking process (European Communities, 2001; Farhadian et al., 2010; US EPA, 1984; ATSDR, 1999; 2004; 2007; 2012). Fish meat subjected to heat treatments or processes leads to the appearance of carcinogenic, mutagenic and teratogenic lipophilic products, given by the cooking process and increased temperature, according to Ake et al. (2012). In terms of toxicity, PAHs are considered ecosystem contaminants due to their encounters in the food chain, through their accumulation and concentration in air, water or soil. They have action that reflects on the quality of human life (European Communities, 2001; Laslo, 1995). PAHs are also called universal environmental contaminants (air, water, soil), therefore their presence in the food chain is inevitable, the human body being the most affected (Tofană, 2011). As a result of thermal processes for processing and preparing meat, by certain methods either properly applied or abusively performed at high temperature, using traditional procedures (roasting, grilling, smoking) several harmful components are eventually formed, including various mutagens and carcinogens, including PAH in the final product (Jagerstad & Skog, 2005, cited by Aaslyng et al., 2013). Studies conducted on laboratory animals are a confirmation that exemplifies their carcinogenic effect, and from the point of view of chemical analyzes, these substances also result from isolation from certain compounds. To date, more than 500 PAHs have been isolated, only a small percentage has been assessed for toxicity (Banu, 2007; Laslo, 1995). From an epidemiological point of view, there is an increased percentage of cancer risk in various organs (intestines, breast, bladder, prostate and pancreas) after an increased consumption of thermally prepared meat, either through the frying process or through grilling (Kazerouni et al.; Aaslyng et al., 2013). According to Dutta et al. (2022) and other updated studies (Wang et al., 2021), the major PAH exposures of the population are through a diet based on various types of meat, including fish meat subjected to certain heat treatments.

According to the studies carried out by Perelló et al. (2009), the highest concentrations of PAHs were discovered after the frying process of various foods, including fish. The aim of the study is to analyze the content of PAHs in fish according to the thermal treatments applied and the vegetable and animal source applied for cooking.

MATERIALS AND METHODS

PAH analysis was performed by high-performance liquid chromatography (HPLC) with fluorescence detection after solid-liquid extraction.

In this study, the aim was to evaluate 15 PAHs (acenaphthene, anthracene, benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(ghi)pyrylene, chrysene, dibenzo(a,h)anthracene, phenanthrene, fluorene, fluoranthene, indeno(1,2,3-cd)pyrene, naphthalene, pyrene) in brook trout (*Salvelinus fontinalis*).

For analysis, the Perkin Elmer 200 series High Performance Liquid Chromatograph (HPLC) with fluorescence detector and data processing system with FLD detector was used.We used 10 grams of properly ground and homogenized sample, saponified with 100 ml KOH solution in ethanol, then extracted 25 ml Hexane at the ultrasonic bath for about 20 minutes. When evaporation took place, on the rotary evaporator, the samples were returned with 1 ml of acetonitrile and finally injected into the HPLC. The 15 PAHs mentioned above were run on an Inertsil ODS-P 5 pm, 4.6x150mm which was maintained at a temperature of 24°C. Regarding the injection volume, it was 50 pl. Regarding the mobile phase, a water gradient was used, and the substance used was acetonitrile and a time-programmed FLD detector for detection. The samples analyzed for hydrocarbons, were raw Brook trout (*Salvelinus fontinalis*); fried in sunflower oil; fried in olive oil; fried with rapeseed oil; baked in the oven and fried in lard. Brook trout (*Salvelinus fontinalis*) was harvested from the

Mărişel area, Cluj county. Five samples were used for each category analysed. The fish samples were stored in the Faculty of Veterinary Medicine Cluj-Napoca, in optimal conditions, at a temperature of -2ºC in a device equipped with BioFreshPlus technology so that the fish preserves its properties. The weight of the fish was 240-280 grams and a temperature of 180-190ºC was used for cooking, with a duration of 20-25 minutes. The oils and lard used were purchased commercially. Analysis of fish fat and protein was performed by the Soxhlet and Kjeldahl method and minerals by calcination.

RESULTS AND DISCUSSIONS

PAHs are compounds released by various cooking processes. Depending on the cooking process applied and the vegetable and animal source used, PAHs were released in different amounts, much higher compared to the raw fish samples analyzed. Tables 1 to 6 show mean values and variability for polycyclic aromatic hydrocarbons from raw fish compared to thermal baking and roasting processes. Another factor considered is the type of vegetable or animal fat used and the impact on the concentration of polycyclic aromatic hydrocarbons in fish meat.

Table 1. Mean values and variability for polycyclic aromatic hydrocarbons in raw brook trout (*Salvelinus fontinalis*)

Parameter	Raw brook trout (*Salvelinus fontinalis*)	
	X±sx	V%
Naphthalene	5.51±0.21	8.43
Acenaften	<0.05	-
Fluorene	0.67±0.07	22.21
Phenanthrene	1.03±0.02	3.96
Anthracene	2.03±0.12	13.69
Fluoranthene	2.34±0.13	12.68
Pyrene	1.09±0.03	6.01
Benzo(a)anthracene	2.47±0.06	5.36
Crisen	<0.05	-
Benzo(b)fluoranthene	<0.05	-
Benzo(k)fluoranthene	0.87±0.01	2.98
Benzo(a)pyrene	1.04±0.01	3.00
Dibenzo(a,h)anthracene	<0.05	-
Benzo(ghi)perylene	<0.05	-
Indeno(1,2,3-cd) pyrene	<0.05	-

v-variability; x-mean value; sx-standard deviation; n=5

For the raw fish sample, high values were recorded for the following polycyclic aromatic hydrocarbons: naphthalene 5.51±0.21 ng/g, followed by benzo(a)anthracene with a value of 2.47±0.06 ng/g and fluoranthene with a value of 2.34±0.13 ng/g (Table 1). Following the analysis of the sample of fish fried in sunflower oil, high values were observed for PAHs, such as anthracene which has the highest value, which is 10.34±0.09 ng/g. It was followed by pyrene with a value of 10.24±0.30 ng/g. The third type of high PAH is naphthalene with a value of 10.23±0.18 ng/g (Table 2).

Table 2. Average values and variability for hydrocarbons in brook trout (*Salvelinus fontinalis*) fried with sunflower oil

Parameter	Brook trout (*Salvelinus fontinalis*) fried with sunflower oil	
	X±sx	V%
Naphthalene	10.23±0.18	3.93
Acenaften	0.94±0.03	6.50
Fluorene	2.17±0.04	4.55
Phenanthrene	4.18±0.04	2.22
Anthracene	10.34±0.09	1.90
Fluoranthene	9.31±0.13	3.08
Pyrene	10.24±0.30	6.50
Benzo(a)anthracene	6.94±0.04	1.36
Crisen	0.31±0.02	15.26
Benzo(b)fluoranthene	0.16±0.01	14.76
Benzo(k)fluoranthene	2.27±0.04	3.90
Benzo(a)pyrene	4.04±0.19	10.72
Dibenzo(a,h)anthracene	0.51±0.03	13.84
Benzo(ghi)perylene	1.12±0.02	4.90
Indeno(1,2,3-cd) pyrene	<0.05	-

v-variability; x-mean value; sx-standard deviation; n=5

Table 3. Mean values and variability for hydrocarbons in brook trout (*Salvelinus fontinalis*) fried with rapeseed oil

Parameter	Brook trout (*Salvelinus fontinalis*) fried with rapeseed oil	
	X±sx	V%
Naphthalene	16.78±0.16	2.11
Acenaften	1.24±0.08	14.18
Fluorene	5.22±0.06	2.50
Phenanthrene	25.18±0.98	8.63
Anthracene	24.95±0.41	3.64
Fluoranthene	22.36±0.44	4.38
Pyrene	32.17±0.84	5.87
Benzo(a)anthracene	12.57±0.98	17.55
Crisen	0.42±0.08	14.71
Benzo(b)fluoranthene	0.96±0.09	20.87
Benzo(k)fluoranthene	3.04±0.02	1.30
Benzo(a)pyrene	7.76±0.25	7.27
Dibenzo(a,h)anthracene	0.79±0.06	18.12
Benzo(ghi)perylene	3.07±0.39	28.74
Indeno(1,2,3-cd)pyrene	0.87±0.03	7.33

v-variability; x-mean value; sx-standard deviation; n=5

For fish fried in rapeseed oil, four types of polycyclic aromatic hydrocarbons have been observed to have higher values than the others. These were pyrene with the highest concentration of 32.17±0.84 ng/g, followed by phenanthrene with 25.18±0.98 ng/g. Anthracene had a concentration of 24.95±0.41 ng/g and fluoranthene had a concentration of 22.36±0.44

ng/g (Table 3). In the case of fish fried in olive oil, high values for polycyclic aromatic hydrocarbons were observed for pyrene, with a concentration of 51.37±0.63 ng/g, the second high value was for fluoranthene with 20.36±0.89 ng/g, followed by naphthalene with 15.75±12.19 ng/g and anthracene with 15.398±0.27 ng/g (Table 4).

Table 4. Mean values and variability for hydrocarbons in brook trout (*Salvelinus fontinalis*) fried with olive oil

Parameter	Fried brook trout (*Salvelinus fontinalis*) with olive oil	
	X±sx	V%
Naphthalene	15.75±12.19	0.86
Acenaften	0.48±0.01	4.28
Fluorene	4.03±0.18	9.11
Phenanthrene	3.72±0.16	9.91
Anthracene	15.398±0.27	3.87
Fluoranthene	20.36±0.89	9.73
Pyrene	51.37±0.63	2.74
Benzo(a)anthracene	10.56±0.26	5.44
Crisen	0.25±0.07	16.13
Benzo(b)fluoranthene	0.53±0.05	21.69
Benzo(k)fluoranthene	2.128±0.01	1.22
Benzo(a)pyrene	6.72±0.20	6.71
Dibenzo(a,h)anthracene	<0.05	-
Benzo(ghi)perylene	2.15±0.05	4.86
Indeno(1,2,3-cd) pyrene	0.07±0.01	16.62

v-variability; x-mean value; sx-standard deviation; n=5

In the sample of fish fried in pork lard, high values of the following polycyclic aromatic hydrocarbons were observed, namely fluoranthene, pyrene and naphthalene, with values 16.65±0.43 ng/g, 12.42±0.16 ng/g and 12.35 ±0.33 ng/g respectively (Table 5).

Table 5. Average values and variability for hydrocarbons from fried fish with pork lard

Parameter	Fried fish with pork lard (*Salvelinus fontinalis)*	
	X±sx	V%
Naphthalene	12.35 ±0.33	5.97
Acenaften	<0.05	-
Fluorene	3.11±0.22	1.78
Phenanthrene	4.07±0.21	11.56
Anthracene	9.76±0.36	8.28
Fluoranthene	16.65±0.43	5.73
Pyrene	12.42±0.16	2.97
Benzo(a)anthracene	9.02±0.10	2.57
Crisen	<0.05	-
Benzo(b)fluoranthene	<0.05	-
Benzo(k)fluoranthene	1.97±0.12	13.62
Benzo(a)pyrene	1.98±0.24	16.57
Dibenzo(a,h)anthracene	<0.05	-
Benzo(ghi)perylene	<0.05	-
Indeno(1,2,3-cd)pyrene	<0.05	-

v-variability; x-mean value; sx-standard deviation; n=5

In samples of baked fish, high levels were reported for the following PAHs: pyrene, anthracene and naphthalene, which had the following values: 14.75±-0.50 ng/g, 11.58±0.75 ng/g and 9.24±0.09 ng/g respectively (Table 6).

Table 6. Average values and variability for hydrocarbons in baked brook trout (*Salvelinus fontinalis*)

Parameter	Baked brook trout (*Salvelinus fontinalis*) in the oven	
	X±sx	V%
Naphthalene	9.24±0.09	2.23
Acenaften	<0.05	-
Fluorene	1.17±0.04	7.20
Phenanthrene	1.06±0.02	5.08
Anthracene	11.58±0.75	14.51
Fluoranthene	2.47±0.07	6.23
Pyrene	14.75±-0.50	7.53
Benzo(a)anthracene	4.01±0.34	18.98
Crisen	<0.05	-
Benzo(b)fluoranthene	<0.05	-
Benzo(k)fluoranthene	1.974±0.04	4.23
Benzo(a)pyrene	2.67±0.07	6.07
Dibenzo(a,h)anthracene	<0.05	-
Benzo(ghi)perylene	<0.05	-
Indeno(1,2,3-cd)pyrene	<0.05	-

v-variability; x-mean value; sx-standard deviation; n=5

Following the analyzes we notice for samples from raw fish, which has not been subjected to any heat treatment, it still presents polycyclic aromatic hydrocarbons in its composition, but in much smaller quantities compared to those subjected to frying or baking treatment. PAHs with the highest levels in fish meat were observed to be pyrene, fluoranthene, anthracene and naphthalene. In food, PAHs tend to be formed during processing and preparation by different methods: grill use, smoke use, drying, frying, baking or roasting. We can see that rapeseed oil and olive oil used in the thermal roasting process produce the highest amounts of polycyclic aromatic hydrocarbons.The PAHs that were below the value of 0.05 are different according to the thermal process applied and according to the source of vegetable and animal origin applied for cooking. For raw fish, most PAHs (acenaphthene, chrysene, benzo(b)fluoranthene, dibenzo(a,h)anthracene, benzo(gy)perylene, indeno(1,2,3-cd)pyrene) were observed which were below the value of 0.05. In fish fried with sunflower oil, only one hydrocarbon was observed below the value of 0.05, namely indeno(1,2,3-cd)pyrene. Frying fish in rapeseed oil did not identify any hydrocarbons below 0.05, and for fish fried in olive oil, dibenzo(a,h)anthracene was below this value.

For fish cooked in lard and cooked in the oven, the following PAHs (acenaphthene, chrysene, benzo(b)fluoranthene, dibenzo(a,h)anthracene, benzo(ghi)perylene, indeno(1,2,3-cd)pyrene), were below the value of 0.05. The lowest average values are for fluorene, ranging from 3.62 (ng/g) when frying to 1.18 (ng/g) when baking. Benzo(k)fluoranthene has the lowest average values: 2.34 ng/g when frying and 1.98 ng/g when baking. According to the study conducted by Aaslyng et al (2013), on meat of different animal species (pork, beef, chicken), for the determination of PAH and amines, they pointed out that the time and temperature to which meat is subjected is the essential. The study by Farhadian et al (2012) highlights the influence of cooking oil (a component of the meat marinating recipe ingredients) leading to the formation of several PAHs following the thermal process to which the meat is subjected and the introduction. of lemon juice in the basic marinade recipe leads to a significant decrease in PAH concentrations. To confirm the degree of danger to human health, Wang et al. (2021) determined 15 PAHs, from Shandong area, China, and from the obtained results they highlighted that both fried fish and grilled fish represent a danger to the body human, due to concentrations of PAHs and other compounds. Updated studies lead to increased attention for careful monitoring of these potentially toxic compounds. In the process of cooking at high temperature (PAH) is formed. In the study conducted by Sahin et al. (2020), the following samples were analyzed (meat doner, chicken doner, meatballs, grilled chicken and fish), the total PAH contamination was 6.08, 4.42, 4.45, 4.91 and 7.26 μg/kg. Benzo(a)pyrene (BaP) in meatballs and grilled fish samples had a level of 0.70 and 0.73 μg/kg. The physico-chemical parameters from the raw brook trout (*Salvelinus fontinalis*) samples analyzed showed the following average values: water (%) 73.80, fat (%) 4.55, protein (%) 22.66, minerals (%) 1.28. These results are in agreement with the average values reported in the literature for the specific physico-chemical composition for brook trout (*Salvelinus fontinalis*).

CONCLUSIONS

For samples analysed from fish meat, the highest level of PAHs is when roasting in rapeseed oil. The lowest values were recorded in the case of chrysenic, regardless of the type of fat used and regardless of the thermal process applied. Fish meat showed the highest content in PAHs in the roasting process. The lowest values were recorded for the fish sample fried in sunflower oil and in the oven fish sample. Regardless of the thermal process applied, the highest level is represented by: fluoranthene, anthracene, pyrene and naphthalene.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

THE DEVELOPMENT OF *Crocus sativus* L. IN THE AREA OF THE CITY OF SOFIA

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Abstract

Saffron (Crocus sativus L.) is a geophytic plant which is one of the most commonly known medicinal and aromatic plant species in the world. The stigma of saffron is used for dye, food, or beverages additive and in the pharmacology industries. The saffron crocus has been cultivated in our country quite recently, but the areas with this crop are rapidly increasing. Saffron (Crocus sativus L.) is the most expensive spice in the world. This plant species is propagated vegetatively through the formation of daughter corms from the mother one. The field experiment was conducted to determine the most suitable bulb size for saffron cultivation. In the present article, we summarize scientific observations on the influence of air factors on the development of saffron and the size of the bulbs, their mass and also the number of daughter bulbs. One of the most important organs for plant development.

Key words: *corms, Crocus sativus, reproduction, Saffron, vegetatively.*

INTRODUCTION

Globally, saffron is grown primarily in Iran, Pakistan, India, Azerbaijan, Greece, Spain, and Turkey in areas with low labor costs and small family farms under non-irrigated conditions. Saffron has the potential to be used in fields on small family farms, according to Hassan-Beygy (2010). Iran is the largest producer of saffron in the world, with more than 90% of the world's production, and 300 tons of dried product. The saffron crocus is a sterile plant that does not produce seeds and propagates from bulbs. Bulb selection is an important factor in saffron production. According to Basker & Negbi (1983) and Kafi (2002), the plant is the most valuable in the world. The unique characteristics of the saffron crocus, such as its aroma, taste, and yellow dyeing properties, have reserved a special place for this plant in the pharmaceutical, food, and textile industries. Because saffron crocus flowers are completely sterile, they do not produce viable seeds. Therefore, its reproduction depends entirely on human activity: the bulbs are removed by hand, divided, and replanted in the soil.

The mother bulb survives only one season and reproduces by division; from one bulb, about 10 daughter bulbs are formed. The resulting new bulbs subsequently grow as individual plants. The color of the bulbs is brown, the sizes are up to 4.5 cm in diameter, and they are covered with fibers.

According to Hassan-Beygy (2010), the mass and size of bulbs are of great importance when designing and developing equipment for planting, harvesting, sorting, and processing. Because until now, all processes were manual.

The yield and quality of stigmas and flowers are affected by bulb size (Andabjadid et al., 2015). Determining the most appropriate bulb size would contribute to the implementation of cultivation practices and, ultimately, yield and quality.

According to research conducted by Rechinger (1975) and Wendelbo (1977), it is a perennial plant with dimensions of about 20–30 cm. The bulbs are spherical and flat at the base, up to 5 cm in diameter and 50 g in weight, and covered with a sheath that extends upwards about 5 cm above the neck of the plant. Under variable conditions, it is possible to form roots (Negbi, 1999). After blooming, the crocus continues to take nutrients from the soil and accumulate

reserve substances needed for development and flowering the following year.
After flowering, the crocus does not stop feeding on the soil and again accumulates reserve substances, thanks to which it will grow and flower again the next year.
Under favourable conditions, they bloom for only a few weeks. During this very short period, they accumulate nutrients in their root (bulb), tuber bulb, or rhizome. During the long, unfavourable period, the plant hides underground and lives only on the reserves in its bulb.
In traditional cultivation practices, many factors influence saffron propagation. Fungal infections lead to low productivity in daughter bulbs. Conventional propagation methods do not meet the high demand for planting material. In the reproduction of this plant species, the in vitro method is of great importance, as it has many advantages.
According to authors such as Ascough (2009) and Karaoglu (2007), a very good alternative to producing quality planting material is micropropagation, which also reduces the incidence of diseases.
According to Deo (2003), domesticated crocus (*Crocus sativus*) is an autumn flowering perennial plant. According to the author, it is a sterile triploid mutant of the eastern Mediterranean autumn-flowering (*Crocus cartwrightianus*)
Dried stigmas are used medicinally as spices and food colorants. The plant has been considered the most expensive spice for centuries. This plant contains more than 150 volatile and aromatic compounds. In addition, there are many non-volatile active components, such as carotenoids, including zeaxanthin, lycopene, and various α- and β-carotenes.
According to Abdullaev (2002), the yellow-orange color of the stigmas is mainly due to the α-crocin substance. However, saffron's golden yellow-orange color is primarily the result of α-crocin (Abdullaev, 2002).

MATERIALS AND METHODS

The field experiments were carried out in the Experimental Field of the University Forestry, located in the Sofia field, at an altitude of 552 m. The terrain is flat, and the vegetation is grassy. The soil is alluvial-meadow, slightly stony. It is characterized by a modern soil-forming process. Soil-forming materials are represented by clays, sandy loams, sands, and gravels. Their profile is represented by a humus horizon with a thickness of 0.10-0.40m, under which there are alluvial deposits.
The experiment was carried out under scientific project No. B-1217/27.04.2022, "Comparative study of technologies for growing vegetables and spices in urban conditions" In several scientific articles, we followed its development in an urban environment under climatic conditions and on alluvial soils. The bulbs are planted in two parallel furrows 40 m long, with the largest saffron bulbs placed in one furrow and the smaller ones in the other. The inter-row distance is 10 cm, and the inter-row distance is 30 cm. The size and mass of the bulbs before planting were measured. The size of the bulb was evaluated by a calliper and the mass by an electronic scale. No fertilizer was applied.

RESULTS AND DISCUSSIONS

According to Singletary (2020), saffron contains more than 100 biologically active compounds, the most important being crocin, crocetin, picrocrocin, and safranal.
Ghanbari & Khajoei-Nejad (2021) concluded that saffron cultivation is influenced by various factors, including climate, crop density, irrigation, and others. Fertilization is of great importance to obtaining optimal yield and quality of saffron.
According to a number of authors such as Behzad (1992a), Behnia (1999), Kafi (2002), saffron as a perennial crop is usually grown in arid and semi-arid regions in Iran.
According to Renau-Morata (2012), as the growth of the daughter bulbs increases, the mother bulbs are reduced gradually. Each mother bulb produces new daughter bulbs before it dries up. According to Bhagyalakshmi (1999), during the second growing season, each daughter bulb is considered a potential mother At the end of the growing season, the mother bulbs are brown, oval, and flat discs, with daughter bulbs attached to them (Kumar, 2009).
According to a number of authors, such as Gresta (2008), Koocheki & Seyyedi (2019),

and Renau-Morata (2012), the yield of saffron is highly dependent on the growth of daughter bulbs during the previous growing season. At the beginning of the growing season, reproduction is strongly influenced by the concentration of food reserves in the bulbs.

According to Kafi et al. (2022), plant density increases during each growing season. Accordingly, the yield of saffron in the first year is usually low, and the highest yield is recorded in the fourth to fifth year of its cultivation. In relation to the data obtained by Kumar (2009), the large number of bulbs formed in the soil, the increased density due to soil compaction, and the reduced fertility can be observed as a gradual decrease in the yield of flowers.

The field experiment was conducted in 2022 on alluvial-meadow soils. They are characterized by a gray yellow to dark gray color, with this variety depending on the amount of humus. The soils have fewer humus horizons, with certain differences among themselves in the content of coarse particle materials found in large sizes. The mechanical composition depends entirely on the composition of the river sediments on which they are formed.

Analysis of the soil

The mechanical composition of the soil (alluvial meadow) is characterized by a predominant sandy fraction of the soil substrate, both in the horizontal direction and in the depth of the soil profile up to 100-125 cm. The sandy fraction (0.25 mm) increases with depth, varying widely and having a low content of physical clay (0.01 mm) and of or (0.001 mm). These indicators are typical for the surface horizons up to 40-60 cm and cause high water permeability in the soil.

The humus content in the surface horizon of arable land varies from 1-2%. Along the depth of the profile, it either decreases gradually, or horizons or layers with higher or lower humus content alternate. These soils have a medium acid to slightly alkaline volume (pH in H_2O 5.0–6.0). The amount of total nitrogen also varies from 0.040% to 0.30%. The conditions for the mobilization of organic nitrogen are good. As a result, the phosphorus stock is highly subject to plant extraction, and phosphorus deficiency is possible. Groundwater plays an important role in the lowlands, where during the winter-spring season it is high and causes swamping. The volumetric weight of the soil for the layer 0- 100 cm is 1.46 g/cm^3, and the field capacity (FP) in percent relative to the absolute dry weight of the soil is 21.1%. The reaction of the soil in an aqueous solution is neutral to slightly alkaline, and the content of humus in the surface genetic layer defines the soil as medium humus.

Climatic characteristic

The climate in the Sofia valley, where the experiment was carried out, is moderately continental, with an average annual temperature of 9-10.5°C. Winters are cold and snowy, except for the last one. In winter, temperatures drop to -15°C or lower on the coldest winter days. The coldest month is January. At the beginning of the winter season, fog is a very characteristic phenomenon. Snow cover in winter in Sofia averages 58 days. In the last few years, snow cover has been a rare phenomenon. Summer is hot and sunny. Due to the higher altitude, it is slightly cooler in the summer than the rest of the country.

However, on the hottest summer days, most often in July and August, temperatures can exceed 35°C. Changeable and dynamic weather is characteristic of spring and autumn. The recorded average annual rainfall is within 581.8 mm, reaching its maximum in late spring and early summer. Thunderstorms are also observed during this period, which are not rare.

Figure 1 presents the data from the measured temperatures and precipitation rate after planting the bulbs.

The recorded rainfall since the beginning of the growing season for saffron is about 3.86 mm. Two of the highest rainfall amounts were recorded in early and mid-August, at 12.5 and 15.4 mm, respectively. Despite the relatively small amount of rainfall, it is very evenly distributed and is sufficient to meet the water needs of saffron in the initial stages of development. In the month of September, twice as much precipitation was reported 26 and 24.3 mm.

The rainfall is less compared to the previous month but in larger amounts, which is sufficient for the development of saffron. Total rainfall for October was 37.1 mm, which combined with the drop in average daily

temperatures provided sufficient soil moisture at the end of the saffron flowering period.
The average daily temperature during the growing season is between 24.6 and 8.9ºC. At the end of September, there is a sharp drop in temperatures. For the month of October, the temperatures are between 8.7 and 15.9ºC.

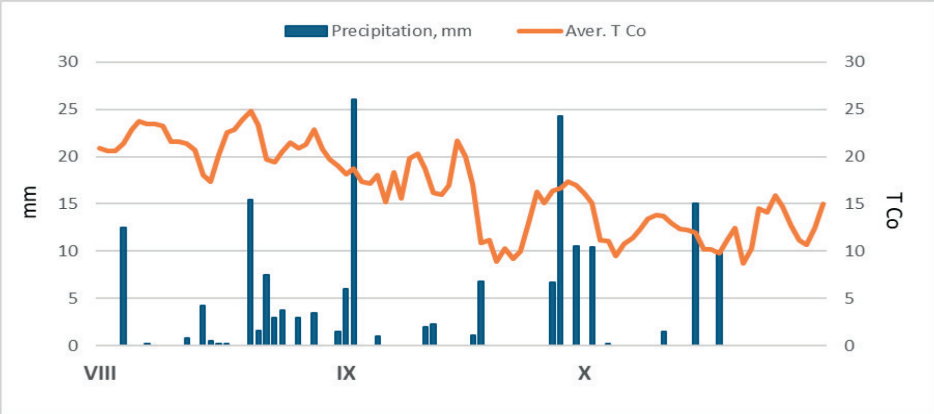

Figure 1. Precipitation and average air temperatures for 2022

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For the production of saffron, the size of the bulb is essential. Reported sizes vary from 1 cm/small fraction/to 3 cm/large fraction/. To get the maximum yield from the production of flowering saffron, it is advisable to start with a larger bulb size, as shown in Figure 2.

Figure 2. Size of the bulbs

Figure 3 shows the bulb sizes of the two fractions. Values range from 10-10.5 mm for small bulbs to 30 mm for larger bulbs. To produce daughter bulbs, a larger size may be recommended.
The results in Figure 4 show that the mean bulb mass values ranged from 2.89 to 3.57 g.
Larger bulbs develop better and accumulate more nutrients.

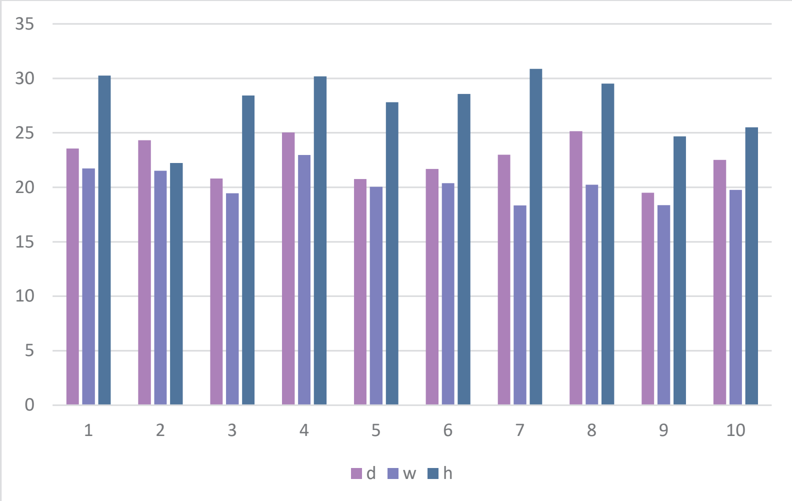

Fig. 3. Size of the bulbs depending on the diameter (mm)

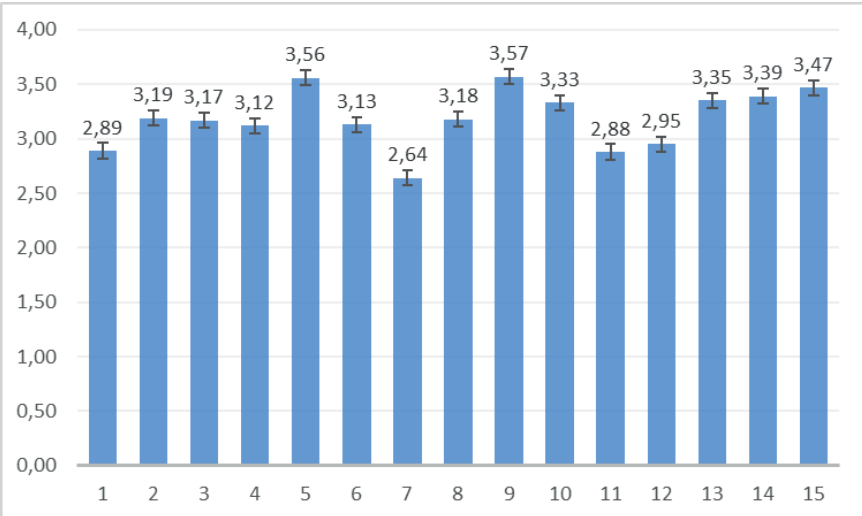

Figure 4. Mass of the bulbs

CONCLUSIONS

The climatic characterization made during the field experience, the type of soil and the growing conditions are favourable for growing saffron in the Sofia basin. Temperature and humidity during the period have a positive effect on its development. Larger bulb size results in a higher number of flowers per m^2 and a higher total yield of daughter bulbs.

ACKNOWLEDGEMENTS

Project NIS- No. B-1217: 27.04.2022 "Comparative study of technologies for growing vegetables and spices in urban conditions".

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

IMPACT OF CLIMATE FACTORS ON THE HONEY-BEARING QUALITY OF SAFFRON CROCUS (*Crocus sativus*)

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Abstract

The saffron crocus (Crocus sativus) is a flowering plant of the Iridaceae family A spice called saffron is obtained from the dried red stigmas of the flower and is used in the pharmaceutical, cosmetic, perfumery and textile industries. It has been grown in our country for a short time, but the areas with this culture are rapidly increasing. To date, no scientific research has been conducted on this type of plant in our country. In the present article, we summarize scientific observations on the influence of climatic factors, temperature, and air humidity on the honey qualities of the saffron crocus. We determined the amount of nectar and its sugar content, the amount of individual pollen from one flower, and the size and shape of the pollen grains in the pollen. We tracked the flowering period and the number of flowers against the age of the planted bulbs.

Key words: *climatic factors, Crocus sativus, flowers, honey qualities, nectar, pollen.*

INTRODUCTION

The crocus is a perennial plant from the Iridaceae family, distributed from the Eastern Mediterranean to Central Asia.

About 40 species exist, most of which are cultivated. There are nine of them in Bulgaria. Crocus has sharp, grass-like dark green leaves that are slightly hairy, usually 4 to 8 in number. It reaches up to 10 cm in height. They are most often pollinated by bees, but also by other insects. If the stamen pollen lands on the pistil after the plant has finished flowering, the fruit also appears. Because they are sterile, saffron crocus flowers do not produce viable seeds. (McGee, 2004)

Saffron crocus (*Crocus sativus* L.) is an autumn-flowering geophyte that reproduces exclusively vegetatively (Mzabri et al., 2017). It is cultivated for its red stigmas, which after drying are the most expensive and valuable spice in the world (Zhang et al., 2019).

More recently, the demand for stigmas has increased dramatically, amid the discovery of new-to-science pharmacological applications, especially those based on cytotoxic and antitumor properties (Cavusoglu, 2017; Mzabri et al., 2019). Saffron adapts extremely well to different environmental conditions, it grows well in arid and semi-arid regions (Gresta et al., 2008), but it can also adapt to temperate and subtropical climates.

The saffron crocus (*Crocus sativus* L.) is a sterile geophyte with autumnal flowering, which reproduces exclusively by vegetative means (Mzabri et al., 2017). It is cultivated for its red stigmas which, after drying, are the most expensive and valuable spice in the world (Zhang et al., 2019). More recently, the demand for stigmas has increased dramatically, especially with the discovery of new pharmacological applications, especially those based on cytotoxic and antitumor properties (Cavusoglu, 2017; Mzabri et al., 2019). Saffron is adapted to various environmental conditions, it grows well in arid and semi-arid areas (Gresta et al., 2008) but it can also adapt to temperate and subtropical climates.

Among all environmental factors, the temperature is considered one of the key elements that control the growth and development of saffron.

The plant tolerates cold winters, withstands frosts down to −10 °C and snow cover. Despite the long period during which it has been cultivated, the scientific literature on flowering in this species is far from complete (Plessner et al., 1989).

The beginning of flowering begins in mid-autumn when the average air temperature is below 15-17◦C. These temperatures appear to be close to optimal for flower emergence (Plessner et al., 1989; Molina et al., 2005).

Saffron flowers are set 3-4 months earlier, from early spring to mid-summer depending on location (Koul, 1984; Azizbekova, 1999; Negbi, 1999; Molina et al, 2005), soon after bud release in rest. The optimum temperature for initiation of flowering is in the range of 23–27◦C, but long exposure to these temperatures results in delayed flowering (Molina et al., 2005).

Their colour can vary from light to dark purple with or without stripes. When flowering, the saffron crocus reaches an average height of 30 cm. Each flower is triple-branched, ending in three dark orange-red licks. They can be between 25 and 30 mm in length.

Their colour can vary from light to dark purple with or without stripes. When flowering, the saffron crocus reaches an average height of 30 cm. Each flower is triple-branched, ending in three dark orange-red licks. They can be between 25 and 30 mm in length.

Pollen from flowers is necessary for germination, fertilization, and seed formation. Because the crocus flowers are brightly coloured, pollination is done by insects (entomophilia).

The flowers are hermaphroditic, releasing a lot of pollen and nectar to attract insects. Pollination begins when the flowers open. In most spring flowering crocuses, the flowers open when the temperature is around 15°C, but in highland species the flowers open earlier when the temperature is between 5°C and 10°C.

Flower opening in sunny, dry weather results in rapid maturation of the pollen sac and microspores (Grilli & Canini, 2010). Pollen sacs open longitudinally by an adhesion mechanism and release the pollen.

Crocus flowers are allogamous. This is the normal case of pollination and seed development. C. sativus is triploid and sterile, therefore triploid saffron results in abnormal chromosome pairing, chromosome misdistribution and sterile gametes (Chichiricco & Grilli, 1987, Rudall, 1984).

However, we rarely observe sexual reproduction and seed formation. In his experiments, Molina (2005) tracked the environmental temperature and phenological stages of crocus (*Crocus sativus* L.) The optimum temperature for flower formation was in the range of 23 to 27°C, with a temperature of 23°C slightly better. To ensure the formation of the maximum number of flowers, incubation at these temperatures should exceed 50 days.

The appearance of flowers requires moving the bulbs to a lower temperature (17°C). Various factors influence saffron cultivation, including climate, crop density, irrigation, and other agricultural methods (Madini et al., 2019; Rezvani-Moghaddam, 2020), but balanced and timely fertilization is a prerequisite for obtaining optimal yield and potential for quality of saffron (Ghanbari & Khajoei-Nejad, 2021). Despite the low fertilization requirements of saffron, studies confirm that most changes in saffron flower yield depend on fertilizers and soil amendments (Kamili et al., 2007).

MATERIALS AND METHODS

The study was conducted in 2022 at the Educational Experimental Field of the Forestry University in Sofia. According to the scientific project No. B -1217/27.04.2022 "Comparative study of technologies for growing vegetables and spices in urban conditions", we set up a Polish experience with saffron crocus. In several consecutive scientific articles, we followed its development in the conditions of the urban environment under the climatic conditions and on alluvial soils. The bulbs were planted in two parallel furrows 40 m long. The intra-row distance was 10 cm, and the inter-row distance was 30 cm.

RESULTS AND DISCUSSIONS

Although saffron grows well in temperate and dry climates, its vegetative growth coincides with cold weather and freezing conditions in our country. In October 2022, the temperatures are close to the norm and with precipitation below the average for the month.

Temperatures are slightly higher than usual for the season, maximum temperatures between 16 and 24 degrees. Mostly sunny with temporary overcast and high clouds.

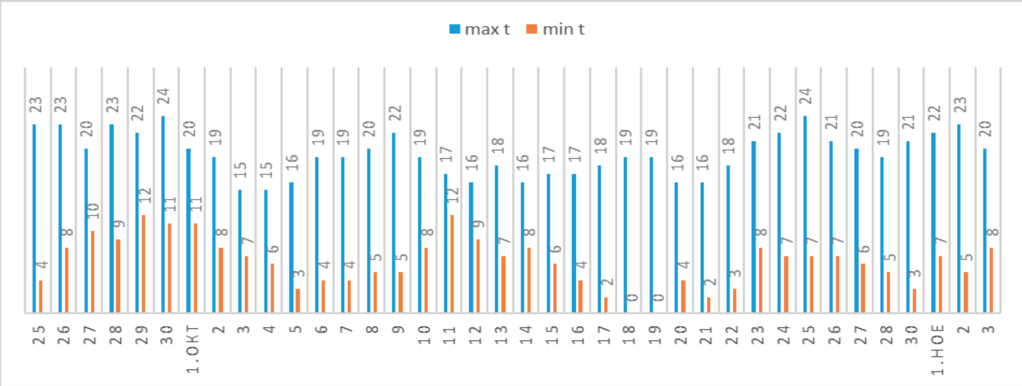

Figure 1. Air temperature, °C

Figure 1 shows the results of the temperature during the flowering period of the crocus. The temperature is favourable for normal plant development and pollinator visitation due to the warm autumn. Of all environmental factors, temperature is considered one of the key elements that control saffron growth and development and flower production.

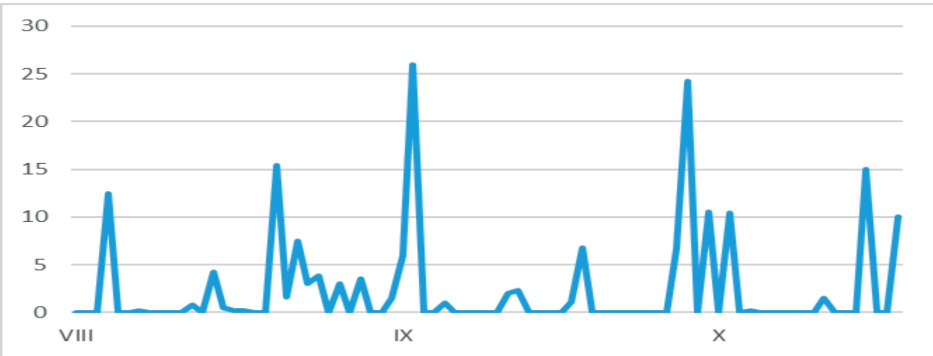

Figure 2. Average amount of precipitation during the flowering period

Figure 2 shows average rainfall data. Despite the relatively small amount of precipitation, in the period after planting the bulbs, it is evenly distributed throughout the period.

Rainfall in the initial stages of development is sufficient to meet the water needs of saffron, even in non-irrigated growing conditions.

The results in Figure 3 show that a larger number of bulbs on the plant produced an average of 2-3 and less often four flowers.

Figure 4 shows the average flowering duration of one flower in days. The duration of flowering of each flower varies within 4 to 6 days in the large bulbs of the plants. The flowering period is strongly dependent on the temperature during the period.

The onset of flowering in the large bulbs of Crocus sativus began on October 10 and lasted a total of 25 days. This period is strongly influenced by weather conditions during the period. High temperatures in summer have a positive effect on flowering in autumn. The bulbs receive enough heat in the soil during the summer period.

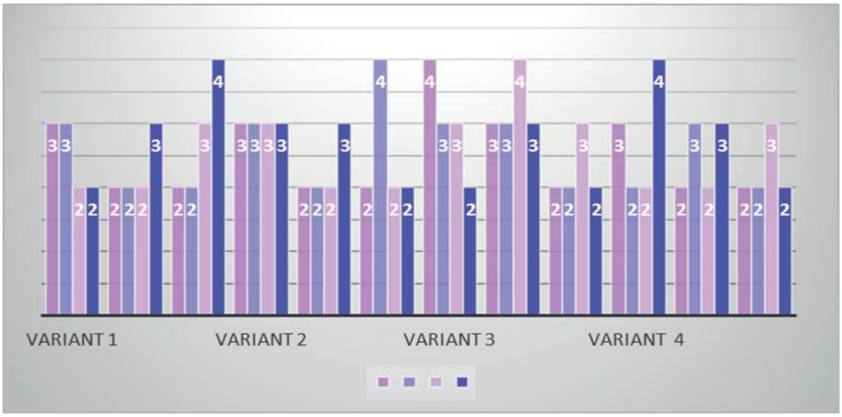

Figure 3. Number of flowers from one tuber

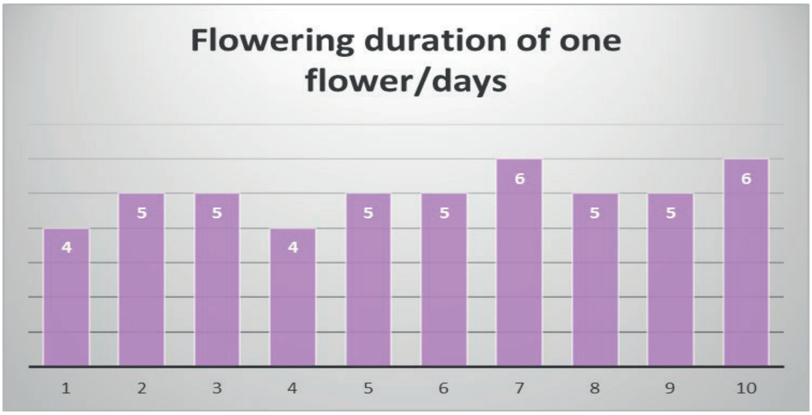

Figure 4. Flowering duration of one flower/days

Figure 5. Saffron crocus flower, stamens, and stigmas

Table 1. Flowering period

Beginning of flowering	Mass flowering	End of flowering	Flowering period in days
10.10. 2022	15. 10.2022	03.11.2022	25

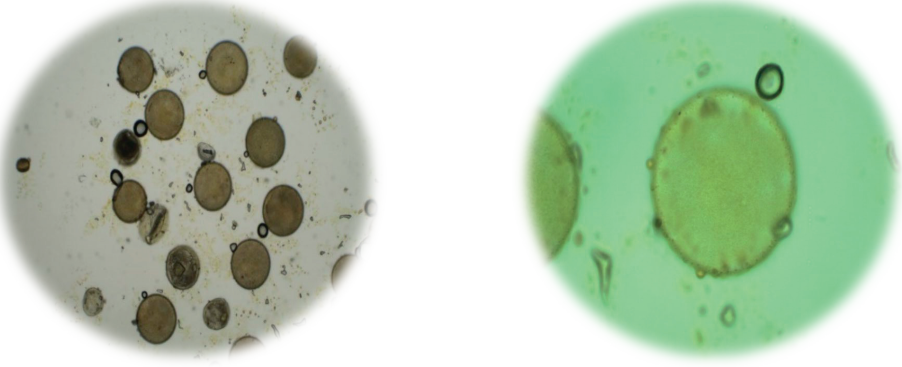

Figure 6. Pollen grains of *Crocus sativus*

Figure 6 shows the results of microscopic observation of *Crocus sativus* pollen grains. Pollen grains are of the correct shape for the species, they are viable and have no deviations. Saffron crocus pollen is heavy and sticky, but it can be collected by insect pollinators, such as honeybees and bumblebees and other insects, in areas where climatic conditions permit. Saffron produces a good amount of pollen and nectar with a nectar sugar content of 2% Brix.

CONCLUSIONS

Larger bulb sizes result in a larger number of flowers per m^2. Climatic conditions in the Sofia valley and alluvial soils have a favourable effect on the development of saffron.

The plants develop well and produce many flowers, therefore also stigmas, for this the recorded temperature and humidity during the vegetation period of the plants are favourable.

In areas where the climate allows bees to fly, the saffron crocus is a good plant as a source of food and honey.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

BAKERY PRODUCTS WITH VALUE-ADDED PREMIX BASED ON LUPIN (*LUPINUS ANGUSTIFOLIUS*) SPROUTS

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Abstract

The paper aims to study the nutritional and sensorial properties of bakery products fortified with lupin sprouts. The lupin seeds were germinated, and the sprouts collected after 14 days of germination were dried and ground. Wheat flour mixed with different percentages of sprout flour (10-30%) was used to obtain bun-type bakery products obtained from leavened dough. The proximate composition (proteins, lipids, mineral substances, carbohydrates), macro and microelements composition and the content of total polyphenols, as well as the antioxidant activity of fortified products was determined. The obtained results showed that the maximum mineral content was recorded in the case of buns with 30% (2.225%). The content of total polyphenols varied between 8.0-28.9 µM GAE/g.d.m.) and antioxidant activity (2.52-9.44 µM Fe^{2+}/g d.m.). Elemental composition highlighted an increase of Cu (0.873-1.382 ppm), Ni (0.647-1.348 ppm), Mn (0.622-283.409 ppm), Fe (2.182-12.197 ppm), Zn (3.440-14.133 ppm), Na (607.973-851.325 ppm), Mg (165.781-389.073 ppm), Ca (451.163-502.318 ppm), K (202.683-420.596 ppm) with the percentage of lupin sprouts addition. Sensorial analysis showed that the addition of 10% lupin sprouts was appreciated by consumers regarding all the sensory parameters analyzed.

Key words: *antioxidant activity, legume sprouts, polyphenols, macro and microelements.*

INTRODUCTION

As a result of consumer demand for healthy and naturally processed foods, interest in germinated products has increased over the past decade. The proof lies in the growing number of products contained germinated cereals launched each year (www.mintel.com).

Sprouts are living foods, very rich in vitamins, minerals, antioxidants and other nutrients obtained by germination. During germination an increased volume of fiber is obtained inside the seed, with real benefits for accelerating digestion, the proper assimilation of nutrients and the elimination of fats. The sprouts contain approximately 20-30 times more nutrients compared to the mature plant having a positive impact on the health of the human body (Galanty et al., 2022). Also, during the germination process, a series of vitamins, minerals, enzymes, flavonoids and volatile oils are developed which are known as vital elements for the growth and development of the respective plant, but also for human health. Germination enhances the content of phytochemicals such as polyphenols and flavonoids (Dueñas et al., 2009). The health benefits of phenolic compounds from legume sprouts include anticarcinogenic, anti-thrombotic, anti-ulcer, anti-allergenic, anti-inflammatory, anti-artherogenic, antioxidant, anti-microbial, cardioprotective and analgesic effects (Singh et al., 2017).

Legumes are one of the most important categories of plant products with the potential for germination and use of sprouts obtained in food safety (Goncearov et al., 2004; Gulewicz et al., 2008). Previous studies highlighted the role of germination to enhances the nutritional value and the macro and microelements content of legumes (Gulewicz et al., 2008; Swieca et al., 2019; Farag et al., 2023).

Lupin (*Lupinus angustifolius*) is an herbaceous plant belonging to the Fabaceae family, which also includes peas, lentil, bean and soy. Lupin is a plant cultivated in the Mediterranean basin as food or as an ornamental flower. Lupin seeds – sometimes called Lupini beans because of their pods – are used in mediterranean diet (Lemus-Conejo et al., 2023).

In addition to protein (40%), lupin seeds contain fiber (32%) and probiotics, which strengthen the immune system and support the health of the digestive tract (Msaddak et al., 2023). They also contain B vitamins, magnesium, manganese, copper and zinc (Petterson, 2016).

Lupin seeds do not contain cholesterol, gluten or gastric irritants, unlike soy, which has a large amount of saponins. The fat content is characterised by high percentage of mono and poly-unsaturated fats, omega-3, 6 and 9 fatty acids (Abreu et al., 2023). They are probiotics, helping the development of good bacteria in the body (van de Noort, 2017).

Regarding the impact on health, lupin seeds have a diuretic, hypoglycemic, cicatrizing, and carminative effect (Devkota et al., 2023). The lupin sprouts shown cytotoxic effects on some breast and prostate cancer cells (Galanty et al., 2022).

The use of germinated legumes in bakery has been a real success in recent years, studying, in addition to the nutritional effects, the influence of germination on the rheological properties of the dough (Atudorei et al., 2022; Atudorei, et al., 2023).

The objective of this paper was to study the nutritional, phytochemical and sensory characteristics of bread obtained with added-value premix based on lupin sprouts.

MATERIALS AND METHODS

Obtaining of lupin sprouts

The lupin seeds (450 g) were immersed in 1000 mL could water for 12 hours. After hydration, the lupin seeds were placed in an even layer in a pan (55x34 cm) with moistened cotton wool in a bright room with constant temperature (20-24^{0}C). The seeds germination begins after two days and after 14 days majority of sprouts was obtained. The constant humidity during germination was maintained by daily sprinkling with water. After 14 days the lupin sprouts were harvested by cutting green shoots and then dried in the incubator (INB, 500 Memmert, Schwabach, Germany) at a temperature of 60^{0}C for 4 days. The sprouts were grinded in the mill (GRINDOMIX RETSCH, GM 200, Haan, Germany) and the obtained flour was used to prepare the composite sprouts flours.

Preparation of composite sprout flours

Wheat flour type 000 purchased from Baneasa Company, Romania, was used as control sample. Three types of added value premix were obtained by adding 10%, 20% and 30% milled lupin sprouts flour in wheat flour.

Obtaining of composite sprout buns

The composite premix was used in different percentage regarding wheat and lupin sprouts flours: 100 g/90 g/80 g/70 g superior white wheat flour 000 from Baneasa, Romania company and 10 g/20 g/30 g flour obtained from lupin sprouts obtaining four samples: C (control), LSB1 (buns with 10% lupin sprouts flour), LSB2 (buns with 20% lupin sprouts flour), LSB3 (Buns with 30% lupin sprouts flour).

The buns were prepared using the indirect bakery method according to (Plustea et al., 2022). The method included following steps: preparing the ingredients and their dosage, kneading the sourdough, fermenting, kneading the dough, fermenting the dough, dividing, shaping, leavening, baking the dough and cooling the final product.

The sourdough was obtained by dissolving 3 g fresh yeast in 10 mL water at 30^{0}C, 1g crystal white sugar and 1/3 from the flour mixture for each sample. After 30 minutes the sourdough was added to the rest of flour mixed initially with 2 g iodized salt.

The dough obtained was kneaded manually until obtaining a non-sticky dough, well bound, with a porous aspect in structure. During kneading, an additional 3 mL of sunflower oil with emulsifying role was added. The obtained dough was divided into 2 equal parts and left to ferment for 30 minutes in the leaven equipment at 35^{0}C.

After leavening the dough was baked in the electric oven at the temperature of 180^0C for 20 minutes. The buns obtained are presented in Figure 1.

The proximate composition of lupin sprouts buns

The proximate composition of composite sprout buns and control was analyzed using standardised methods: moisture SR 91/2007 pct.10, protein SR EN ISO 8968-1:2014; total lipid SR 91:2007 pct.14.4; minerals SR ISO 2171/2010, starch 2009R0152-RO-004.001-61. The carbohydrate content (%) was calculated as the difference between 100 and the sum of the following fractions: proteins, lipids, proteins, mineral substances and moisture.

Taking in account the contribution of lipids (1 g lipid = 9 kcal), proteins (1 g protein = 4 kcal) and carbohydrates (1 g carbohydrate = 4 kcal) it was calculated the energy value. All determinations were done in triplicate. Also, it was determined the contribution of intake consumption of 100 g samples calculated by reporting the values obtained to the reference intake established for an average adult according to the Regulation CE 1169/2011, namely: lipids 70 g; proteins 50 g; carbohydrates 260 g and energy value 2000 Kcal (https://eur-lex.europa.eu/legal-content/).

Determination of macro and microelements

The macro and microelements content were determined after calcination of samples at 650^0C using a calcination oven (Naberthem GmbH, Lilienthai/Bremen, Germany) until a white color of ash.

The quantification of macro and microelements was performed by atomic absorption spectroscopy (AAS) using the Varian 220 FAA equipment according to the method described by (Plustea et al., 2022).

The determinations were performed in triplicate and the results were expressed in ppm.

The contribution of intake consumption of 100g samples calculated by reporting the values obtained to the daily reference intake (DRI) established for an average adult according to the Regulation CE 1169/2011 (potassium: 2000 mg, magnesium: 375 mg, calcium: 800 mg, zinc: 10 g, iron: 14 mg, copper: 1 mg, manganese: 2 mg).

Evaluation of the total phenolic content (TPC)

The TPC was determined using Folin-Ciocâlteu method adapted according to (Obistioiu et al., 2021). The extract was prepared in ethanol 70% at the ratio 1:10 (sample: ethanol). After colour reaction the absorbance of the samples was read at 750 nm using the Specord 205 spectrophotometer (Analytik Jena AG, Jena, Germany). Results were expressed as mg gallic acid equivalent (GAE) per 100 g sample. The calibration curve was performed using GAE solutions in the concentration range between 2.5-250 μg/mL. All the determinations were performed in triplicate.

Evaluation of antioxidant activity (AA)

The AA was determined according FRAP method (Horablaga et al., 2023). The results are expressed as mM Fe^{2+}/100 g sample and the calibration curve ranged between 0.05-0.4 μM/mL. All the determinations were performed in triplicate.

Sensory analysis

The consumer acceptability of lupin sprouts buns was evaluated using sensory analysis performed by 16 untrained consumers, with ages between 19 and 26. The samples were presented in cardboard plates with four-digit characters, once at a time to each panelist. The panelists were asked to evaluate the bun samples in relation to the control sample. Appearance, odor, texture, taste/chewiness, and overall acceptability was evaluated using a 5-point hedonic scale (5 for "like extremely" and 1 for "dislike extremely") (Alomari, 2013). Evaluators were asked to rinse their mouths with still water and eat unsalted crackers between samples evaluation (Molnar et al. 2020). All 16 panellists were trained according to ISO 6658:2017.

Statistical analysis

Al determinations were made in triplicate and the results are reported as mean values ± standard deviation (SD). Differences between means were analyzed by t-test (two-sample assuming equal variances) using Microsoft Excel 365. Differences were considered significant when p-values < 0.05.

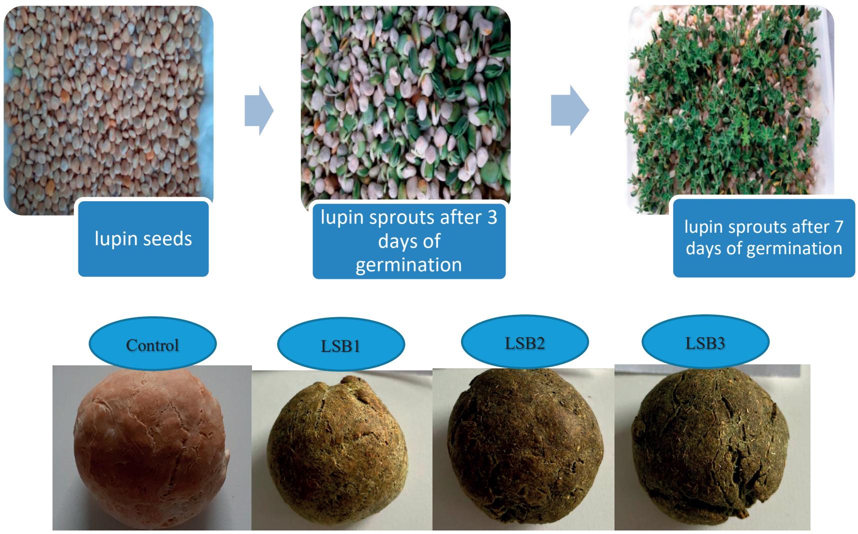

Figure 1. Lupin sprouts buns. Control - wheat bun, LSB1 - Bun with 10% lupin sprouts, LSB2 - Bun with 20% lupin sprouts, LSB3 - Bun with 30% lupin sprouts

RESULTS AND DISCUSSIONS

Proximate composition

The results presented in Table 1 show the nutritional characteristics of buns with lupin added sprouts. The lipid content varied between 1.234% in control sample and 7.836% in the sample with addition of 30% lupin sprouts. The increasing of lipids content is due by the legume matrix and sprouts. Similar results regarding the lipids content in bread supplemented with lupin flour were reported in the literature (Alomari, 2013; Plustea et al., 2022). Protein content was enhanced when different percentage of lupin sprouts was added in the dough composition. The maximum level was recorded in the sample LSB3 (20.97%) that mean a 100.66% increase compared to the control (buns with wheat flour). Sprouting process caused significant increases in moisture, protein, ash, crude fiber, free amino acids, total, reducing and nonreducing sugars (Fouad and Rehab, 2015).

The effect of germination on lentil protein content was reported by Santos S et al. (2020) and highlighted the increase in all lentil varieties with a mean of 29% protein in sprouts. Significant differences were reported between protein levels in seeds and sprouts (Santos et al., 2020). The use of lupin both in the form of flour from un-germinated seeds and from sprouts in the bakery industry represents a viable method of increasing the protein content without increasing the fraction of gluten proteins in the dough.

The addition of lupin sprouts leads to an increase in the content of mineral substances in the bakery products obtained. The maximum increasing percentage was observed in the case of LSB3 sample (69.33%).

Germination involves biochemical and physiological processes in the plant that favour the accumulation of active principles and mineral substances. Similar results regarding the mineral substances in lupin flour processed by several methods were reported by (Devkota et al., 2023).

However, the starch and carbohydrates content decreases with the addition of sprouts in bakery products. The maximum starch content was observed in the control sample (61.30%) and decreased until 32.84% when 30% lupin sprouts were added in the buns composition. In the same manner the carbohydrates were reduced at 43.11% in the sample LSB3 with 28.8% compared with the white wheat sample.

Significant differences ($p<0.05$) were recorded according to t-test, between samples in term of protein content, mineral substances and starch.

Other study highlighted the decreasing of carbohydrates from 67.2% in control bread to

36.85% when 20% lupin seeds flour was added (Alomari, 2013).
Previous studies reported the antidiabetic role of legumes germinated seeds and explained the mechanisms that mediated the delaying of gastric process and inhibition of carbohydrate digestive enzymes (Tefera, 2020) and (Farag et al., 2023).
As a major bioactive component responsible for the hypoglycemic action in legume sprouts was detected the trigonelline (Farag et al., 2023). The use of legumes and especially their sprouts as lupin represents a possibility to replace the floury matrix in bakery products and to obtain hypoglycemic foods intended for people with diabetes.
Regarding the contribution of 100 g bakery product with lupin sprout to the daily reference intake (DRI) according CE Regulation 1169/2011, it can be observed that 30% sprouts added in the buns dough provided 41.94% from the daily protein requirement of an adult, 11.19% lipids, 16.58% carbohydrates and 16.34% from energy value.

Macro and microelements
Tables 2-3 show the macro and micronutrient content of the buns with lupin sprouts. It was observed the increasing of macro and microelements content with the percentage addition of lupin sprouts, the maximum values for all analysed parameters being recorded in the LSB3 sample. The most abundant macroelement was sodium (Na) with concentrations between 607.941-851.312 ppm higher in the case of lupin sprouts addition.
In the LSB1 sample, calcium (Ca) and potassium (K) recorded a lower level as in control, but the concentration increased with the sprouts percentage added. The higher concentration of Ca was 502.319 ppm, respectively 420.602 ppm the level of K recorded in LSB3 sample.
The supplementation of bakery products with lupin sprouts lead to increasing of microelements content. Statistically significant differences ($p<0.05$) between all samples were recorded in terms of all microelements content, but also in Ca and Na content.
The influence of lentil germination on macro and micronutrients content was previously reported by (Santos et al., 2020). This study highlighted that Mg and Ca were not affected by germination, while K concentration has been identified as a possible marker for germination capacity. In terms of K concentrations, other study reported that soybean seed sprouts present a concentration five-times higher when compared to the seeds (Plaza et al., 2003).
The highest increase was observed in the case of Mn where the content varied from 0.645 ppm in the sample obtained from wheat flour to 283.418 ppm in the sample with 30% lupin sprouts. Lupin seeds from Romania are characterised by a high content of manganese. Previous study reported 136.74 ppm Mn in lupin seeds (Plustea et al., 2022). Taking in account the improvement of Mn level during germination process as a phenomenon noted by other authors (Santos et al. 2020), the consumption of lupin sprouts is an important dietary source for people with Mn deficiency.
Cu content increased with 64.07% in the LSB3 sample compared with the control when 30% lupin sprouts was added. Similar pattern was observed in the case of Ni, Zn and Fe. Ni values varied between 0.638 ppm in the control and 1.322 ppm in the sample with highest content of lupin sprouts.
Regarding microelements composition, of the 12 varieties analysed by Santos et al. (2020), only five did not register a significant increase in Zn concentration after sprouting.
The content of Fe and Mn increased by germination in most of the lentil varieties analysed. Legume germination induces the reduction of phytate content, bonded with minerals in seeds and forming insoluble complexes (Santos et al., 2020).

Table 1. Proximate composition of buns with lupin added sprouts

Samples	Lipids %	% from DRI*	Proteins (%)	% from DRI*	Mineral substances %	Humidity (%)	Starch (%)	Carbo-hydrates (%)	% from DRI*	Energy value [kcal/ 100 g]	% from DRI*
Control	1.234±0.119 ^{a}	1.76	10.45±0.254 ^{a}	20.90	1.314±0.084 ^{a}	26.402±0.503 ^{a}	61.30±1.64 ^{a}	60.60	23.31	295.30	14.76
LSB1	5.320±0.147 ^{b}	7.60	13.87±0.266 ^{b}	27.74	1.555±0.095 ^{b}	22.425±0.470 ^{b}	54.73±1.36 ^{b}	56.83	21.86	330.68	16.53
LSB2	5.826±0.154 ^{b}	8.32	17.64±0.305 ^{c}	35.28	1.872±0.117 ^{c}	24.668±0.399 ^{c}	43.79±1.25 ^{c}	49.99	19.23	322.97	16.15
LSB3	7.836±0.155 ^{c}	11.19	20.97±0.384 ^{d}	41.94	2.225±0.112 ^{d}	25.855±0.476 ^{a}	32.84±0.88 ^{d}	43.11	16.58	326.86	16.34

*$^{a-d}$ data within the same column sharing different superscripts are significantly different ($p < 0.05$) according to t-test; *DRI (daily reference intake according CE Regulation 1169/2011*

Table 2. Macroelements content of buns with lupin added sprouts

	K (ppm)	% from DRI*	Ca (ppm)	% from DRI*	Mg (ppm)	% from DRI*	Na (ppm)
Control	202.649±0.182 ^{a}	1.01	451.176±0.175 ^{a}	5.64	165.761±0.206 ^{a}	4.42	607.941±0.195 ^{a}
LSB1	68.929±0.196 ^{b}	0.34	257.631±0.189 ^{b}	3.22	214.181±0.209 ^{b}	5.71	762.839±0.180 ^{b}
LSB2	256.188±0.206 ^{a}	1.28	357.845±0.191 ^{c}	4.47	229.152±0.19 ^{b}	6.11	893.093±0.194 ^{c}
LSB3	420.602±0.212 ^{c}	2.10	502.319±0.197 ^{d}	6.28	389.122±0.193 ^{c}	10.38	851.312±0.201 ^{c}

$^{a-d}$data within the same column sharing different superscripts are significantly different ($p < 0.05$) according to t-test; *DRI (daily reference intake according CE Regulation 1169/2011

Table 3. Microelements content of content of buns with lupin added sprouts

	Cu (ppm)	% from DRI*	Ni (ppm)	Zn (ppm)	% from DRI*	Fe (ppm)	% from DRI*	Mn (ppm)	% from DRI*
Control	0.835±0.181^{a}	8.35	0.638±0.172^{a}	3.447±0.208^{a}	3.44	2.196±0.182^{a}	1.57	0.645±0.172^{a}	3.22
LSB1	1.268±0.158^{b}	12.68	1.046±0.179^{b}	9.187±0.204^{b}	9.18	7.110±0.181^{b}	5.08	66.874±0.169^{b}	334.37
LSB2	1.370±0.178^{c}	13.70	1.233±0.168^{c}	10.523±0.20^{c}	10.52	8.844±0.175^{c}	6.32	162.136±0.185^{c}	810.68
LSB3	1.480±0.159^{d}	14.80	1.322±0.198^{d}	14.183±0.20^{d}	14.18	12.202±0.19^{d}	8.72	283.418±0.199^{d}	1417.09

$^{a-d}$data within the same column sharing different superscripts are significantly different ($p < 0.05$) according to t-test, *DRI (daily reference intake according

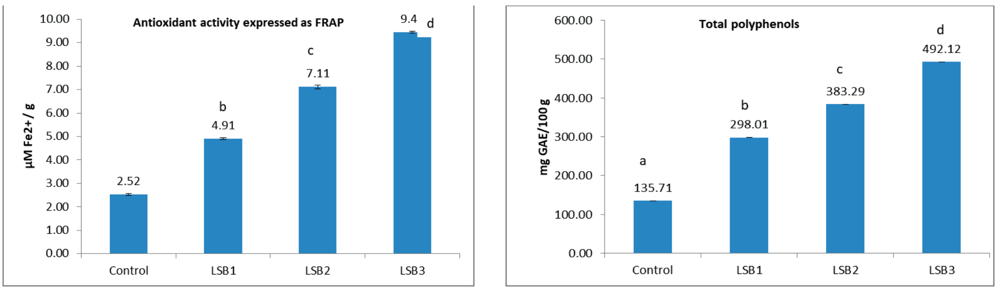

Figure 2. AA and TPC of buns with lupin added sprouts
Control - wheat bun, LSB1 - bun with 10% lupin sprouts, LSB2 - bun with 20% lupin sprouts, LSB3 - bun with 30% lupin sprouts

A significant increasing of iron content was recorded with addition of lupin sprouts in buns. The iron content was 2.196 ppm in control and increased until 12.202 ppm in the case of LSB3, representing an iron supplementation of 455.64%. The natural supplementation of iron in bakery products represents a special issue that comes to meet the increased requirements of recent years regarding the fortification of bakery products with iron in Romania. Currently, in Romania there is no obligation to fortify wheat flour with iron, even if the incidence of anemia among the population is high, almost 60% of children aged between one and two years having iron deficiency anemia. In the USA, since 1998, bread and other cereal products have been fortified with folic acid, by order of the Food and Drug Administration. All US cereals, rice and corn are fortified with 140 µg folic acid/100 g grain, providing consumers with 100 µg/day of

this vitamin (Hagiu, 2021). In addition, fortification with folic acid causes a reduced bioavailability of iron, which is facilitated when iron is found naturally in food. As a result, the use of sprouts of lupin in bakery products can solve problems related to the high level of anemia among the Romanian population.
The contribution of 100 g product consumption to daily reference intakes (DRI) for minerals (adults) according to the Regulation CE 1169/2011 (Tables 2-3) highlighted that 100 g products LSB1-LSB3 provides a daily intake of macro-elements corresponding to: 0.34-2.1% K, 3.22-6.28% Ca, 5.71-10.38% Mg from DRI for an adult person.
In term of micronutrients, 100 g products provide a daily intake corresponding to: 12.68-14.8% Cu, 9.18-14.18% Zn, 5.08-8.72% Fe, 334.37-1417.09% Mn from DRI for an adult person. The important increase of Mn content through lupin seeds germination, as well as the high content of Mn in lupin seeds from Romania, recommends these products to people with a deficiency of this microelement.

Phytochemical proprieties

The Figure 2 shows the total polyphenol content and antioxidant activity of bakery product with lupin sprouts addition. The results shown a significant increasing of TPC with the percentage of lupin sprouts added in the dough. The highest value was recorded in the LSB3 sample (492.12 mg GAE/100 g), comparatively with the control obtained from wheat flour (135.71 mg GAE/100 g). The increasing of TPC was 119.59% by addition of 10% lupin sprouts, 182.43% by addition of 20% lupin sprouts, respectively 262.62% for the highest level of substitution. Taking in account the importance of TPC on the development of metabolic processes and maintaining the health of the body, the consumption of bread supplemented with lupin sprouts has a positive impact on the quality of life.
The addition of lupin sprouts is positively reflected also in the antioxidant activity values. The values increased with the addition of lupin sprouts; the maximum level was recorded in the LSB3 sample (9.4 μM Fe^{2+}/g). Similar pattern was observed when lupin seeds flour was added to wheat flour in order to obtain bread (Plustea et al., 2022). The reported increasing of TPC was between 5.58-58.99%, and between 32.88-55.47% for AA, respectively. The values recorded in our study, when lupin sprouts are used in the bread composition, are higher and highlights the importance of germination in order to enhance the phytochemical profile of foods. Values between 46.4-63.9 ppm for TPC were reported in legume sprouts, the level being significantly reduced with increase of sprout age (Chon, 2013). Phenolics content increased from 1341.13 mg/100 g in raw lentil seeds to 1630.20 mg/100g, the maximum value being recorded after 5 days of germination (Fouad & Rehab, 2015).

Sensory analysis

Sensory evaluation of the buns with lupin sprout flour was carried out in order to assess the consumer acceptance and preferences.
Figure 3 indicates the mean scores for the sensory attributes (appearance, odor, texture/porosity, taste/chewiness and overall acceptability) of the studied buns with lupin sprout flour in different proportions (10%, 20% and 30%). The highest scores were recorded in LSB1 samples (buns with 10% lupin sprouts flour) concerning the attributes: appearance (4.813), texture/porosity (4.688), taste/chewiness (4.813) and overall acceptability (4.75). In terms of taste/chewiness attribute, the scores increased in the following order LSB1> Control > LSB2 > LSB3 and in terms of appearance the panellists ranked the bun samples as follows: LSB1> C > LSB2 > LSB3. Regarding the overall acceptability attribute, the score of the studied samples increased in the following order LSB1 >Control>LSB2> LSB3 (Figure 3).

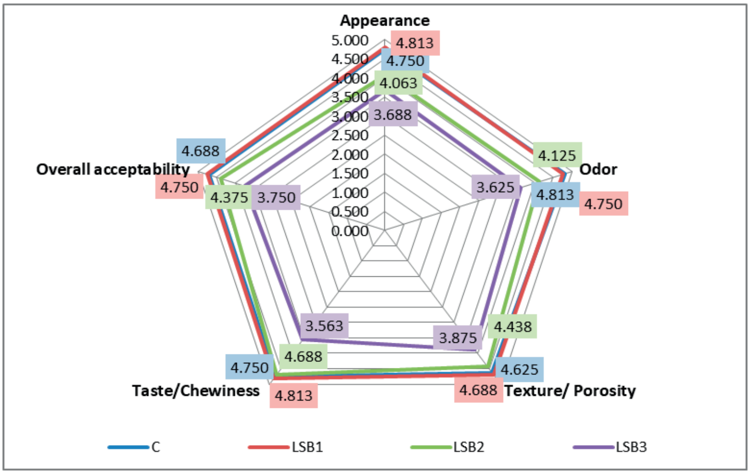

Figure 3. Global values of the sensory evaluation (consumer acceptance) of bread with Moringa (MWB1, MWB2, MWB3 and MWB4; WB (control bread) by using 5-point hedonic scale (n = 36)

Following the sensory evaluation, which highlights the consumers' acceptance, can be pointed out that the addition of lupin sprouts flour in buns significantly influenced the appearance, taste, texture and overall acceptability being highly appreciated by panelists.

Similar studies were carried out by Alomari & Abdul-Hussain in 2013 who pointed out that by substituting wheat flour with lupin flour up to 20% level it is possible to produce healthy and nutritionally bread, rich in proteins, mineral substances, lipids and fiber, without affecting sensory propertie.

The extensive study performed by Abreu et al. (2023), regarding the consumer perception and acceptability of lupin-derived products, indicated as optimal percentage 15% of lupin addition in novel food products, but some studies suggest less than 10% of lupin addition (Yaver & Bilgiçli, 2021; Atudorei et al., 2022). Higher as 20% lupin addition resulted in some sensory losses on breads (Abreu et al., 2023).

CONCLUSIONS

The results obtained in this study highlight the nutritional role of sprouted lupin and the added value obtained for bakery products.

The use of lupin sprouts in a proportion of 10-30% increases the supply of proteins, lipids, macro and microelements in bakery products.

Important improvement regarding the compounds with the biologically active value reflected by the antioxidant activity and polyphenolic compounds is also observed.

However, the sensory analysis points to the fact that the addition of more than 10% lupin sprouts in bakery products is reflected in a worsening of the sensory properties in term of: appearance, texture/porosity, taste/chewiness and overall acceptability.

ACKNOWLEDGEMENTS

This study was performed during the doctoral studies of the PhD Loredana Plustea (Paven), University of Life Sciences “King Mihai I” from Timisoara, PhD coordinator: Prof. Ersilia Alexa.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

BIODEGRADABLE ACTIVE PACKAGING APPLICATION ON FRESH MINCED BEEF

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Abstract

Meat quality and safety is of great importance, and it frequently depends on the packaging technology. Recently, active packaging gained more and more attention in food industry, due to its ability to carry antimicrobial and antioxidant ingredients, which could lead to enhanced properties of the packed food. The aim of this study was to determine the quality parameters of fresh minced beef during storage in the presence of an active packaging material based on PLA, PHBV and nano emulsion of nisin and dill essential oil. Physical-chemical and microbiological analysis were performed for fresh minced beef quality determination. Furthermore, a challenge test was performed using Escherichia coli ATCC 8739 as test microorganism to determine the developed materials antimicrobial efficacy. The results showed that the application of active packaging decreased the microbial load during storage at 4°C.

***Key words**: active packaging, essential oil, food safety, minced beef, nisin.*

INTRODUCTION

Food safety represents a very important factor during storage, especially the microbiological safety of the products. Fresh meat is generally categorized as a highly perishable food product, acting as a great environment for microorganisms' growth, therefore being susceptible to microbial spoilage as the main cause of deterioration (Cercel et al., 2017; Şimşek et al., 2017). Due to the negative effects that spoilage can have on consumers (health issues) but also economical loss for producers there is an increasing need to develop new techniques to maintain fresh meat quality. One of these techniques is represented by active packaging development with antimicrobial properties. As a general requirement from both food manufacturers and consumers for natural, safe and minimally processed foods a group of antimicrobial agents has been often used, represented by antimicrobial peptides which present various properties such as antifungal, antibacterial and antiviral properties (Bahrami et al., 2019). These peptides can be incorporated into films, coatings and food matrices, having a wide possibility of use within food industry. One of the most used peptides, especially in meat industry is nisin, which is a bacteriocin belonging to the lantibiotic class, produced by *Lactococcus lactis* subsp. *lactis*. It is composed of 34 amino acids and has a molecular mass of 3.5 kDa (Leelaphiwat et al., 2022). It has been recognised as safe (GRAS) by both Food and drug Administration (FDA) (Santos et al., 2018; Jia et al., 2021) and also approved by the Food Standards Australia New Zealand and the European Food Safety Authority (Gedarawatte et al., 2021). Furthermore, nisin possess great antimicrobial activity against a great variety of Gram-positive bacteria (Cui et al., 2016; Martillanes et al., 2021; Martinez-Rios et al., 2021). Bacteriocins incorporation in polymeric food packaging has been intensively studied in the past years due to their great ability to interact with the external surface of the microorganisms, not being necessary the internalization of the bacteriocin in order to fulfil its role (Santos et al., 2018). Therefore, application of nisin in packaging materials was studied using as base polymers like chitosan

(Divsalar et al., 2018; Remedio et al., 2019; Cao et al., 2019), cellulose (Wu et al., 2018), sugarcane bagasse (Yang et al., 2020), polyvinyl alcohol (Wang et al., 2015; Settier-Ramírez et al., 2021), pullulan (Hassan & Cutter, 2020) or polylactic acid (PLA) (Gulzar et al., 2022; Arias et al., 2022).

The aim of the present study was to assess the quality parameters of fresh minced beef which was stored at refrigeration temperatures (4±0.5°C) in the presence of a newly developed active packaging material based on PLA, poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) and nano emulsion of nisin and dill essential oil. The antimicrobial efficiency of the developed packaging materials was also tested using a challenge test with *Escherichia coli* ATCC 8739 as test microorganism.

MATERIALS AND METHODS

Materials

In this experiment (Figure 1), beef samples kept in the presence of films obtained by electrospinning (ES), after different periods of storage at refrigeration temperatures (4±0.5 °C), were analysed from a physical-chemical and microbiological point of view.

The tested materials were obtained by deposing on the surface of PLA and PHBV films (using electrospinning technique) of a nanoemulsion formed of dill essential oil (Dill EO) and nisin as antimicrobial agents.

The fresh beef purchased from a local butchery in Bucharest, Romania, was processed by cutting and mincing and packaged as follows:

I. 100 g beef packed in a PET tray (L=8 cm, l=8 cm, h=4 cm), in contact with the PLA film obtained by ES (circular form) which was placed at the base of the tray, in direct contact with the sample.

II. 100 g beef packed in a PET tray (L=8 cm, l=8 cm, h=4 cm), in contact with the PHBV film obtained by ES (circular form) which was placed at the base of the tray, in direct contact with the sample.

III. 100 g beef packed in a PET tray (L=8 cm, l=8 cm, h=4 cm), considered control sample.

After packaging, the samples were stored at 4±0.5°C, to determine the shelf life of minced fresh beef. In Table 1 the samples and their coding are presented. As the first moment of analysis (Day 0), fresh minced beef sample was analysed (before packaging).

Table 1. Coding and sample description

Sample code	Description
Beef control	Minced beef sample packed in a PET casserole, considered the control sample and stored at 4°C
Beef PLA/Nisin/Dill EO	The minced beef sample packed in the PET casserole in contact with the PLA film obtained by ES and stored at 4°C
Beef PHBV/Nisin/Dill EO	The minced beef sample packed in the PET casserole in contact with the PHBV film obtained by ES and stored at 4°C

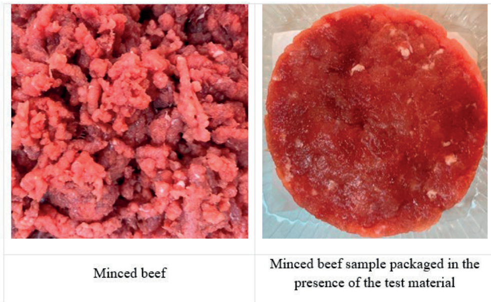

Figure 1. Experimental design

Methods

Further, during the refrigeration period, the following physical-chemical analyses were performed: pH determination; determination of free acidity; determination of dry matter; determination of the a_w index; determination of free ammonia (freshness analysis), determination of colour and also microbiological analysis such as determination of total viable count (TVC), *Enterobacteriaceae* and *E. Coli*/Coliforms.

Briefly, the pH determination was performed using a pH meter WTW INOLAB 720 series type with automatic temperature compensator. Free acidity was determined by titration with NaOH 0.1 N in the presence of phenolphthalein as indicator. Dry matter content (DM) was performed by weighing 5 g of sample, which was further subjected to drying at 105°C using a RADWAG MAC 50 thermobalance. The results were expressed as a percentage (%). Water activity index (a_w) of the tested samples was determined using a NOVASINA equipment by introducing the sample into specific recipients of the equipment and the value of a_w was read when stable at 25°C. Free ammonia was determined using the Nessler reagent. Colour determination was performed at room temperature using a HunterLab colorimeter, Miniscan XE Plus.

RESULTS AND DISCUSSIONS

Regarding the pH values (Table 2) of the minced beef, it can be observed that they did not vary significantly compared to the sample analysed on the day of packaging (Day 0 of the analysis). The acidity of the minced beef samples decreased during the storage period, indicating the beginning of degradation of the samples.

However, the Beef PHBV/Nisin sample presented the highest acidity value after five days of refrigeration, compared to the other two studied samples. In Table 3, the values of dry matter and humidity of samples are presented. No significant changes were observed in the values obtained for moisture and dry matter for the analysed samples.

Table 2. Evolution of pH and free acidity during the refrigeration storage

Sample / Moment of analysis	pH			Acidity (oleic acid/100 g)		
	Day 0	Day 3	Day 5	Day 0	Day 3	Day 5
Beef control	5.77 ± 0.014	5.75 ± 0.183	5.68 ± 0.035	8.315 ± 1.378	5.345 ± 0.318	5.065 ± 0.261
Beef PLA/Nisin/Dill EO	5.77 ± 0.014	5.71 ± 0.134	5.69 ± 0.021	8.315 ± 1.378	5.725 ± 0.233	5.255 ± 0.063
Beef PHBV/Nisin/Dill EO	5.77 ± 0.014	5.68 ± 0.028	5.75 ± 0.028	8.315 ± 1.378	5.645 ± 0.615	5.400 ± 0.056

Table 3. The values of dry matter and moisture determined during the refrigeration storage

Sample / Moment of analysis	Day 0		Day 3		Day 5	
	Humidity%	DM%	Humidity%	DM%	Humidity%	DM%
Beef control	76.518 ± 0.449	23.482 ± 0.449	76.841 ± 0.424	22.159 ± 0.424	76.597 ± 0.347	23.403 ± 0.347
Beef PLA/Nisin/Dill EO	76.518 ± 0.449	23.482 ± 0.449	76.700 ± 0.283	23.299 ± 0.283	76.682 ± 0.101	23.318 ± 0.101
Beef PHBV/Nisin/Dill EO	76.518 ± 0.449	23.482 ± 0.449	73.373 ± 4.927	26.626 ± 4.927	76.738 ± 0.376	23.261 ± 0.376

Table 4. The a_w index values determined during the refrigeration storage

Sample / Moment of analysis	Day 0	Day 3	Day 5
Beef control	0.980 ± 0.002	0.980 ± 0.000	0.985 ± 0.001
Beef PLA/Nisin/Dill EO	0.980 ± 0.002	0.971 ± 0.002	0.979 ± 0.000
Beef PHBV/Nisin/Dill EO	0.980 ± 0.002	0.975 ± 0.002	0.979 ± 0.000

Following the analysis of the data in Table 4, a decrease tendency of the a_w index can be observed for the meat samples packed in the presence of PLA/Nisin and PHBV/Nisin films and stored in a refrigerated state at a temperature of 4±0.5°C, compared to the Control sample. This leads to the conclusion that the water available for the development of microorganisms has decreased, thus preventing the alteration of the product from a microbiological point of view.

Table 5. The NH_3 content of minced beef during refrigeration storage

Sample / Moment of analysis	Day 0	Day 3	Day 5
	NH_3		
Beef control	Negative	Negative	Weakly positive
Beef PLA/Nisin/Dill EO	Negative	Negative	Negative
Beef PHBV/Nisin/Dill EO	Negative	Negative	Negative

The content of NH_3 is directly related to the freshness assessment of the meat. The results of the analyses regarding free ammonia (Table 5) show that the minced beef sample packed in the presence of the studied films and stored at 4±0.5°C did not show signs of the beginning of product degradation on day 5 of storage, compared to the control sample.

Regarding the colour of the minced beef samples, a decrease of the values of L*, a* and b* parameters was observed for all the studied samples during the refrigerated storage period, compared to the initially analysed sample (Table 6), the colour of the samples being modified on the last day of the analysis (Figure 2).

Table 6. The values of the L*, a* and b* parameters for the tested samples during refrigeration storage

Moment of analysis / Sample	Beef control			Beef PLA/Nisin/Dill EO			Beef PHBV/Nisin/Dill EO		
	L*	**a***	**b***	**L***	**a***	**b***	**L***	**a***	**b***
Day 0	39.84 ± 0.65	22.89 ± 0.79	21.00 ± 0.37	39.84 ± 0.65	22.89 ± 0.79	21.00 ± 0.37	39.84 ± 0.65	22.89 ± 0.79	21.00 ± 0.37
Day 3	35.77 ± 0.48	14.3 ± 0.80	16.08 ± 0.22	39.25 ± 1.16	11.78 ± 0.76	15.53 ± 0.32	35.04 ± 0.65	11.20 ± 0.72	15.41 ± 0.22
Day 5	40.28 ± 1.46	17.28 ± 0.46	18.39 ± 0.25	39.04 ± 0.67	11.22 ± 1.21	16.22 ± 0.32	35.14 ± 0.23	14.29 ± 1.15	16.94 ± 0.52

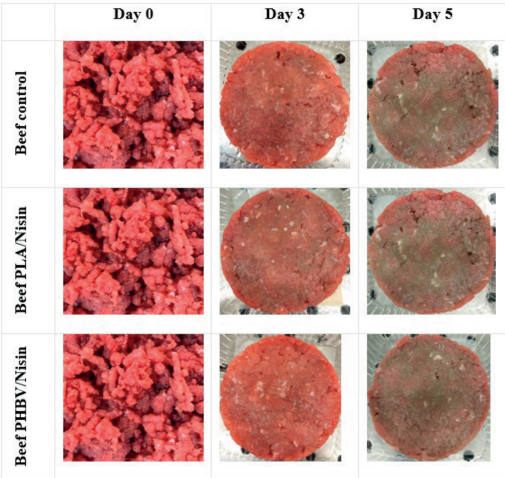

Figure 2. Appearance of minced beef samples during refrigerated storage

Regarding the microbiological analysis, the evolution of TVC values, as presented in Table 7, is of significant increase for the Beef control and Beef PHBV/Nisin samples, namely by approximately 2 logarithmic cycles, while for the Beef PLA/Nisin sample there was an increase of approximately 1 logarithmic cycle, during the refrigeration storage.

Further, in Table 8 it can be observed the presence of both *Enterobacteriaceae* and *E. coli*/Coliforms in all analysed samples. After 3 days of refrigeration storage of the packed minced beef in the presence of the two tested films, no more *Enterobacteriaceae* colony forming units (CFU) were identified in the samples compared to the control sample, proving the antimicrobial efficiency of the two tested films.

Table 7. TVC values of the tested beef samples

Sample / Moment of analysis	**Day 0**	**Day 3**	**Day 5**
	Total viable count (lgCFU)		
Beef control	2.26	3.69	4.42
Beef PLA/Nisin/Dill EO	2.26	3.03	3.82
Beef PHBV/Nisin/Dill EO	2.26	3.27	4.26

Table 8. The values obtained following the determination of *E. Coli* / Coliforms and *Enterobacteriaceae*

Sample / Moment of analysis	Day 0	Day 3	Day 5	Day 0	Day 3	Day 5
	*Enterobacteriaceae**			*E. Coli* / Coliforms*		
Beef control	+	+	+	-/+	-/-	-/+
Beef PLA/Nisin/Dill EO	+	-	+	-/+	-/-	-/-
Beef PHBV/Nisin/Dill EO	+	-	+	-/+	-/-	-/+

* - no CFU were identified + under 50 CFU ++ over 50 CFU +++ over 100 CFU

Challenge test

A "challenge test" was performed on the developed packaging materials using a bacterial strain of interest, namely *Escherichia coli* ATCC 8739. The test performed was of the "single challenge" type, namely a suspension of *Escherichia coli* ATCC 8739 (10^6 CFU) was added to the product in a proportion of 0.5 ml per 100 g of minced beef (Russel, 2003) and homogenized very well. There were prepared five samples for testing (Figure 3), as follows: - Control Sample – minced beef inoculated with the bacterial strain and stored at 4±0.5°C; - PLA/Nisin sample – minced beef inoculated with the bacterial strain and packed between 2 PLA/Nisin films, then stored at 4±0.5°C; - PHBV/Nisin sample – minced beef inoculated with the bacterial strain and packed between 2 PHBV/Nisin films, then stored at 4±0.5°C; - Nisin 1% sample - minced beef sample to which 1% nisin was added, inoculated with the bacterial strain and stored at 4±0.5°C; - Nisin 5% sample - minced beef sample to which 5% nisin was added, inoculated with the bacterial strain and stored at 4±0.5°C.

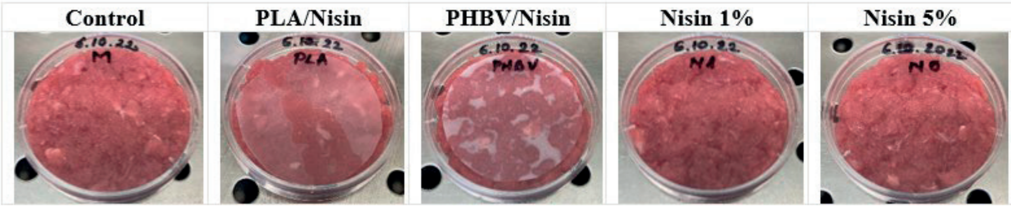

Figure 3. The appearance of the samples subjected to analysis

The samples were analysed after 24 and 48 hours to determine the growth rate of the applied bacterial strain. The results are further presented in Table 9. A decrease in the development of *E. coli* ATCC 8739 can be observed both after 24h and after 48h for all the samples in the presence of the tested films or the nisin, compared to the control sample, by approximately 1 logarithmic cycle. Applying nisin directly to minced beef was more effective, at the end of the storage period for these samples the lowest values were obtained. These results prove the antibacterial efficiency of nisin against *E. coli* ATCC 8739, on the studied product.

Table 9. Growth rate of *Escherichia coli* ATCC 8739 bacteria in the tested samples

Sample / Moment of analysis	0 h (lgCFU)	24 h (lgCFU)	48 h (lgCFU)
Control	5	4.30	4.95
PLA/Nisin/Dill EO	5	4.69	4.04
PHBV/Nisin/Dill EO	5	4.84	4.38
Nisin 1%	5	4.38	4.02
Nisin 5%	5	4.09	4.02

CONCLUSIONS

According to the obtained results, the minced beef samples packed in the presence of the two studied films (PLA/Nisin and PHBV/Nisin) and stored at 4°C demonstrated a good behaviour for 5 days, while the control sample started the process of degradation after only 3 days from packaging. The microbiological analyses showed that the microbial load of the tested samples had a continuous decrease during the refrigeration period for all the analysed samples. However, the samples packaged in the presence of PLA/Nisin and PHBV/Nisin films presented lower values of the microbial load, compared to the control sample, during the storage period, demonstrating that these materials have the potential to slow down the development of microorganisms in the tested minced beef samples.

Furthermore, nisin proved to be efficient against *E. coli* ATCC 8739, reducing the bacterial load in the tested samples with about 1 logarithmic cycle in 48 hours.

ACKNOWLEDGEMENTS

This work was supported by a grant of the Romanian Ministry of Education and Research, CCCDI - UEFISCDI, project number PN-III-P2-2.1-PED-2019-2878, within PNCDI III, contract no. 379PED/2020, acronym BIOMATFOOD.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

EVALUATION OF DATA CONCERNING THE PRODUCT OPTIMISATION OF THE ACID DAIRY PRODUCTS

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Abstract

Acidic dairy products are produced using a specific formula that relies on a multitude of variables. The process of optimizing this formula involves systematically adjusting one or more of these variables to achieve the desired outcome. Diary acid products formulation undergo rigorous testing to identify one that resonates with consumers and meets their acceptance criteria. In the quest to create a novel product with specific predefined attributes, an experimental design is employed based on the outcomes of sensory analyses conducted on representative products slated for optimization. The mathematical modelling of consumer acceptance responses provides a valuable tool for researchers to pinpoint the ingredients and/or processes that yield the greatest product acceptability while minimizing costs. This approach ultimately aids in the formulation of precise manufacturing specifications tailored to meet consumer expectations. In this study, seven traditional acidic dairy products available in the market were carefully evaluated to determine the most desirable attributes. Subsequently, two unique product formulations were developed, which exhibited exceptional sensory characteristics based on the findings. To assess the significance of these results, a statistical analysis was conducted using SPSS® version 16. The outcomes of this analysis, following the sensory evaluation, confirmed that the optimized products successfully met the specific qualities originally intended in the design.

***Key words**: acidity, consistency, consumers acceptability, odour, sensorial analysis.*

INTRODUCTION

Innovations in milk processing have been a significant area of research and development within the food industry. Scientists and food technologists continuously work on improving the quality and variety of dairy products, including acid dairy products like yogurt and buttermilk. A key factor in improving milk processing is to enhance the quality of dairy products (Adesogan & Dahl, 2020). This can involve improving taste, texture, nutritional content, and shelf-life. For example, optimizing the fermentation process for yogurt production can result in a creamier and more flavourful product (Deshwal et al., 2021). In the same time, sustainable practices are becoming increasingly important in dairy processing. Innovations may involve reducing water and energy usage, finding eco-friendly packaging solutions, or optimizing production processes to minimize environmental impact (Peerzada et al., 2023). However, it is important to note that the dairy industry is highly regulated to ensure food safety and quality (Garcia et al., 2019). Any innovation in milk processing must adhere to these regulations and often undergo rigorous testing and certification processes. Additionally, consumer demand and market trends play a significant role in driving innovation in dairy product development

The acid dairy products are the result of the development of specific lactic bacteria in the milk and some microorganisms which we can call associated (Dash et al., 2022). The lactic acid produced in the fermentation has the role to determine the curdling ore the increasing of the viscosity of the milk and producing a sour taste and sometimes of a specific flavour (Deshwal et al., 2021). All around the world, the yoghurt is the main fermented dairy product, the other products could be considered specific to some geographical areas (Arfini & Bellassen, 2019; Dusabe et al., 2022; Moga et al., 2020).

Yogurt, a type of acidic dairy product, undergoes a relatively short fermentation period lasting approximately 3 to 5 hours (Achaw & Danso-Boateng, 2021). This transformation is achieved with the assistance of thermophilic bacteria, specifically *Lactobacillus delbrueckii subspecies bulgaricus* and *Streptococcus salivarius subspecies thermophiles* (Dan et al., 2023).

Buttermilk, on the other hand, shares similarities with yogurt but distinguishes itself through its fermentation cultures, which include *Lactococcus lactis* subspcies. *lactis, Lactococcus lactis cremoris, Lactococcus lactis lactic biovar. diacetylactis*, and *Leuconostoc meszenteroides* (O'Toole & Lee, 2006). The buttermilk fermentation process operates within a temperature range of 28°C to 32°C and extends for a longer duration, typically spanning 8 to 12 hours (Bezie, 2019). These variations in bacteria and fermentation parameters contribute to the dairy acid products qualities.

In this paper we are tackling an original approach of the acid dairy products quality optimizing, presenting the stages to follow for raising the quality of same dairy products taking into account chemical and physical analyses and microbiological analyses, sensorial analyses, food engineering and biotechnology.

MATERIALS AND METHODS

Selection of Products

Seven acid dairy products were selected from the market. These products were likely different types or variations of acid dairy products, such as yogurt and buttermilk, from four different manufacturers or processors. Almost 8 litters of each dairy product were purchased (local producers), and microbiological, physical-chemical and biochemical analysed (unpublished data).

Production Date Calculation

The production date of these products was determined based on the expiration date, with a consideration of a 21-day shelf life. This calculation is important for ensuring that the products were tested at an appropriate stage of freshness.

Product Codification. Each of the selected products was assigned a unique code for anonymity. The codes consisted of two letters and were not known by the testing team conducting sensorial analyses or by the analyst's performing chemical, physical, and microbiological tests. The given codes were: yoghurts: CA01, CL02, CP03, CB04, and buttermilk: BA05, BL06 and BB08. Aiming the CR09 and BR10 optimisation. The codification was double, the product could be recognized only by the first two letters, the first representing the type of product thermophile or mesophyll and the second letter was a letter of the name of the producer. In addition to the previous products were introducer other two optimized acid lactic products CR09 and BR10.

Sensorial Analysis

The sensorial analysis was conducted in according with the European standards SR ISO 4121:2008, and national standard SR 6345:1995 for specific for lactic acid products procedure, by using the standardised sampling standard SR ISO 5497:2006, the hedonic scale method STAS 1265-88, and establishing the sensorial profile with the dairy acid products profile SR EN ISO 13299: 2016.

Panellists group and conditions

In order to select and train the naive assessor group, the European standard SR ISO 8586:2014, SR ISO 8586:2023, and supplemented by ISO 6658 were employed. Twenty persons were employed (n=10 ♂, and n=10 ♀, with the ages between 21 up to 60 years of age, having different education levels). Before testing, sensorial panellists were tested for lactose intolerance, followed by voluntary participation to the study.

The panellist activity took place in an special destined environment (SR EN ISO 8589:2010), with the mandatory testing conditions STAS 12655:88.

Statistical analyses

Statistical analyses were performed using SPSS program, version 16 and LSD process. The data were registered, organized and analyzed. Each sample was analyzed in triplicate (n=3), and results were expressed as the mean values, the standard differences and then compared the averages of the scores given by the members of the group for the level of acceptability, acidity, consistency, surface radiance and the odor. There also were compared the averages of the scores with the t- student test for pair observation. There were registered the values of this test for each of the pairs compared, also the level of signification. The results were registered, and their acknowledgement was performed for each pair in turn, for all the aspects taken into account.

RESULTS AND DISCUSSIONS

In the following Table 1 and Figure 1 are shown the mean values of the total scores of acidic dairy products analysed. The highest average value for traditional products existing on the market, was the product BR10, when compared with all experimental data, followed by the BA05 samples. The sample CR09 obtained the best mean value when compared with all the yoghurt samples, followed by CP03 yoghurt samples. Regarding the high levels of acidity of the experimental groups, might be a direct influence of the product pH negative trend, thus increasing the product specific attribute (Körzendörfer & Hinrichs, 2019; Salehi et al., 2021).

Table 1. Average total score given by panellists included in the study and standard deviation for traditional products existing on the market

Product	Mean ± Standard deviation
CA01	16.76±3.2052
CL02	15.92±2.7677
CP03	16.96±3.3352
CB04	13.04±2.4576
BA05	18.28±2.3544
BL06	14.92±2.8711
BB08	12,84±1.8184
CR09	18.16±1.7954
BR10	18.44±2.3108

In Figure 2 could be notice that in what concerns the level of acceptability, of the studied products the highest score was recorded for sample BA05, when compared with all experimental data. The CR09 optimised product sample had the acceptability score similar to the BA05 and BR10 samples scores, indicating similarities concerning the consumers decision (Torrico et al., 2020), being higher than the mean averages of the studied products.

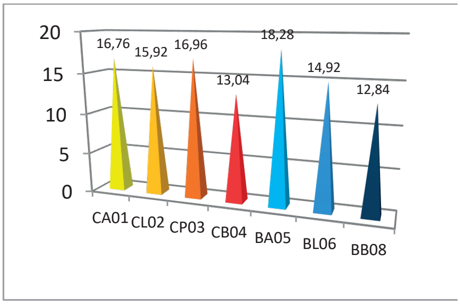

Figure 1. Average total score given by panellists included in the study and standard deviation for traditional products existing on the market

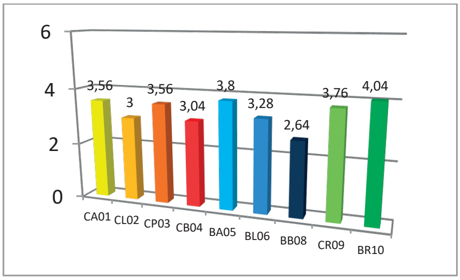

Figure 2. The average score awarded by the panellist in the study for acceptability

The acidity score is shown in Figure 3 for all experimental samples. It is noticeable that the highest average value of acidity had products CP03 and BA05, and the optimized products CR09 and BR10, had an average score equal or higher than the products studied. Similar to our findings Camacho Flinois et al. (2019), had high values for the acidity attribute score.

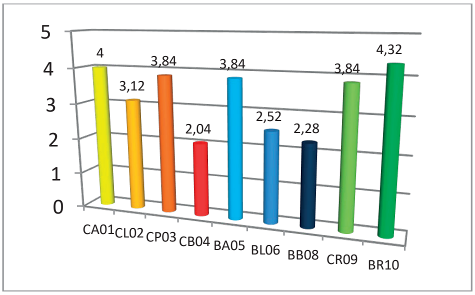

Figure 3. Average score awarded by the panellist included in the test for acidity

In Figure 4 are presented the average values concerning the consistency of the dairy acid products analysed. The highest average values belonged to the products CL02, CP03 and BA05. For the optimized products the average values were for: CR09-4.12 and for BR10-3.24, so the best in what concerns the consistency is CR09.

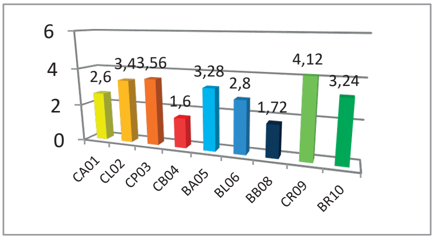

Figure 4. Average score awarded by the members of the group included in the study for consistency

Figure 5 presents the average scores concerning the surface shine for acid dairy products in analyses. The highest average values were for the CL02 product. For the optimized products average scores were: for CR09-2.80 and for BR10-2.76, so in what concerns the surface shine of the optimized products they had close values to the classical acid dairy products.

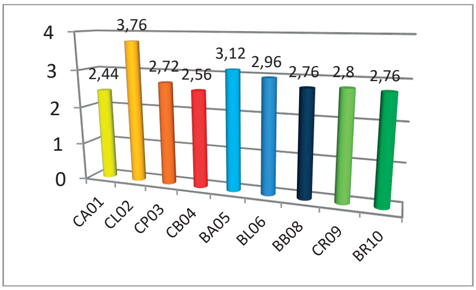

Figure 5. The average score awarded by the panellist in the study for surface

The average values concerning the odor of the acid dairy products analysed are presented in Figure 6. For the classical the acid dairy products the average values were between 2.64 and 4.24. The highest values were for products CL02, CP03 and BA05. For the optimized products, the average values were for CR09-3.64 and for BR10-4.56, so the best in what smell is concerned was CR09.

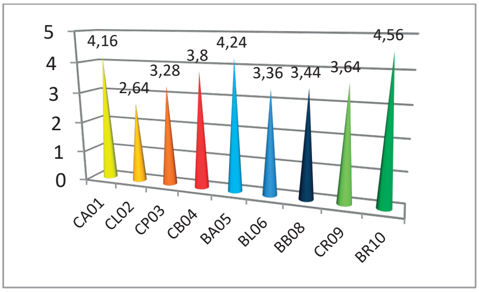

Figure 6. The average score awarded by the panellists included in the study for odor

Figure 7 shows the mean values of the overall scores of the acid dairy products analysed. The highest average value for the classical products was the one of BA05 product, and for the optimized product the highest average value was for BR10-18.44 so the best total score was awarded to BR10.

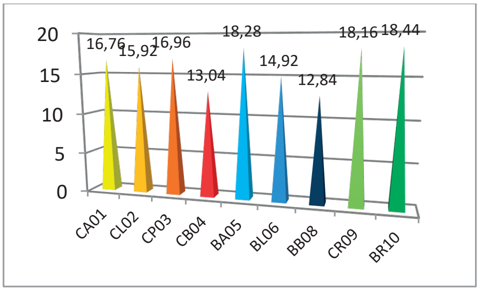

Figure 7. The overall score awarded by the panellist in the study for surface

Current results of statistical analysis using test t - student test for the degree of pleasure, acidity and consistency are given in the following tables (Table 2 and 3). The fact that there are statistically significant differences means that rejecting the hypothesis of equal averages two products significant compared to a level of p <0.05. The fact that there are no statistically significant differences means that we cannot reject the hypothesis of equal averages two products significant compared to a level of p <0.05.

Table 2. Results of statistical analysis using test t - student test for the degree of pleasure, acidity and consistency

Degree of pleasure		Acidity		Consistency	
SD	NSD	SD	NSD	SD	NSD
CA01 – CL02	CA01 – CP03	CA01 – CL02	CA01 – CP03	CA01 – CL02	CA01 – BL06
CA01 – CB04	CA01 – BA05	CA01 – CB04	CA01 – BA05	CA01 – CP03	CL02 – CP03
CA01 – BB08	CA01 – BL06	CA01 – BL06	CA01 – CR09	CA01 – CB04	CL02 – BA05
Ca01 – BR10	CA01 – CR09	CA01 – BB08	Ca01 – BR10	CA01 – BA05	CL02 – BR10
CL02 – BA05	CL02 – CP03	CL02 – CP03	CP03 – BA05	CA01 – BB08	CP03 – BA05
CL02 – CR09	CL02 – CB04	CL02 – CB04	CP03 – CR09	CA01 – CR09	CP03 – BR10
CL02 – BR10	CL02 – BL06	CL02 – BA05	CB04 – BB08	Ca01 – BR10	CB04 – BB08
CP03 – CB04	CL02 – BB08	CL02 – BL06	BA05 – CR09	CL02 – CB04	BA05 – BR10
CP03 – BB08	CP03 – BA05	CL02 – BB08	BL06 – BB08	CL02 – BL06	
CP03 – BR10	CP03 – BL06	CL02 – CR09		CL02 – BB08	
CB04 – BA05	CP03 – CR09	CL02 – BR10		CL02 – CR09	
CB04 – BB08	CB04 – BL06	CP03 – CB04		CP03 – CB04	
CB04 – CR09	BA05 – CR09	CP03 – BL06		CP03 – BL06	
CB04 – BR10	BA05 – BR10	CP03 – BB08		CP03 – BB08	
BA05 – BL06	CR09 – BR10	CP03 – BR10		CP03 – CR09	
BA05 – BB08		CB04 – BA05		CB04 – BA05	
BL06 – BB08		CB04 – BL06		CB04 – BL06	
BL06 – CR09		CB04 – CR09		CB04 – CR09	
BL06 – BR10		CB04 – BR10		CB04 – BR10	
BB08 – CR09		BA05 – BL06		BA05 – BL06	
BB08 – BR10		BA05 – BB08		BA05 – BB08	
		BA05 – BR10		BA05 – CR09	
		BL06 – CR09		BL06 – BB08	
		BL06 – BR10		BL06 – CR09	
		BB08 – CR09		BL06 – BR10	
		BB08 – BR10		BB08 – CR09	
		CR09 – BR10		BB08 – BR10	
				CR09 – BR10	

SD = statistically significant differences; NSD = no statistically significant differences.

Table 3. Results of statistical analysis using t - student test for surface shine, odor and total score

Degree of pleasure		Acidity		Consistency	
SD	NSD	SD	NSD	SD	NSD
CA01 – CL02	CA01 – CP03	CA01 – CL02	CA01 – CB04	CA01 – CB04	CA01 – CL02
CA01 – BA05	CA01 – CB04	CA01 – CP03	CA01 – BA05	CA01 – BA05	CA01 – CP03
CA01 – BL06	CA01 – BB08	CA01 – BL06	Ca01 – BR10	CA01 – BB08	CA01 – BL06
CL02 – CP03	CA01 – CR09	CA01 – BB08	CL02 – CP03	CA01 – CR09	Ca01 – BR10
CL02 – CB04	Ca01 – BR10	CA01 – CR09	CP03 – CB04	CL02 – CB04	CL02 – CP03
CL02 – BA05	CP03 – CB04	CL02 – CB04	CP03 – BL06	CL02 – BA05	CL02 – BL06
CL02 – BL06	CP03 – BA05	CL02 – BA05	CP03 – BB08	CL02 – BB08	CP03 – BA05
CL02 – BB08	CP03 – BL06	CL02 – BL06	CP03 – CR09	CL02 – CR09	CP03 – CR09
CL02 – CR09	CP03 – BB08	CL02 – BB08	CB04 – BA05	CL02 – BR10	CB04 – BB08
CL02 – BR10	CP03 – CR09	CL02 – CR09	CB04 – BL06	CP03 – CB04	BA05 – CR09
CB04 – BA05	CP03 – BR10	CL02 – BR10	CB04 – BB08	CP03 – BL06	BA05 – BR10
CB04 – BL06	CB04 – BB08	CP03 – BA05	CB04 – CR09	CP03 – BB08	CR09 – BR10
BA05 – BR10	CB04 – CR09	CP03 – BR10	BL06 – BB08	CP03 – BR10	
	CB04 – BR10	CB04 – BR10	BL06 – CR09	CB04 – BA05	

	BA05 – BL06	BA05 – BL06	BB08 – CR09	CB04 – BL06
	BA05 – BB08	BA05 – BB08		CB04 – CR09
	BA05 – CR09	BA05 – CR09		CB04 – BR10
	BL06 – BB08	BA05 – BR10		BA05 – BL06
	BL06 – CR09	BL06 – BR10		BA05 – BB08
	BL06 – BR10	BB08 – BR10		BL06 – BB08
	BB08 – CR09	CR09 – BR10		BL06 – CR09
	BB08 – BR10			BL06 – BR10
	CR09 – BR10			BB08 – CR09
				BB08 – BR10

SD = statistically significant differences; NSD = no statistically significant differences.

CONCLUSIONS

Sensorial analysis is often used in scientific research to assess the sensory attributes of various products, such as taste, smell, texture, and appearance. Typically, sensory analysis is used as a final step to evaluate a limited number of products with a small panel of trained tasters. However, in our study, the sensorial analysis employed directly contributed to comprehensive product development and effective process evaluation.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

PORK JERKY USING SUGAR ANTS NIRA AND NaCl SALT DURING STORAGE

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Abstract

This study was conducted to determine the effect of palm sugar and NaCl salt on pork jerky stored at room temperature. In this study, the Split Plot design was used in a time design that was arranged as follows: Factor A was the concentration of palm ant sugar + NaCl salt (divided into 3 combinations), namely A1 = Palm ant sugar 15% + NaCl 5%, A2 = Palm sugar 10 % + 10% NaCl salt, A3 = 5% palm sugar + 15% NaCl salt and factor B is storage duration at room temperature (20-25°C) (divided by 3 different time durations) as follows B1 = 10 days, B2 = 20 days, B3 + 30 days, with three replications. The variables observed were water content, pH and microbial count and peroxide value. The results showed that the use of palm sugar + NaCl gave a very different effect (P <0.01) on the water content and the number of microbes in pork jerky and peroxide value but not (P>0.05) on the pH of pork jerky. Storage time at room temperature had a significantly different effect (P<0.01) on water content, pH, and the number of microbes and peroxide numbers. In short, the use of palm sugar 5% + NaCl 15% can extend the shelf life of pork jerky up to 30 days.

***Key words**: palm sugar sap, pork jerky, salt.*

INTRODUCTION

In Indonesia, especially the province of North Sulawesi, the availability of pork is quite a lot and the price is relatively cheaper than beef. In meeting the needs of animal protein, pork has a high nutritional content with a chemical composition of 60-70% water content, 6-10% fat and 20-28% protein (USDA, 2009). Meat preservation is a way of storing meat for a long period of time so that the quality and cleanliness is maintained. One way of preservation is to make beef jerky which is a traditional processed product from meat which is the result of a combination curing and drying processes. Pork jerky is a plate-shaped food product made from sliced fresh pork from healthy pork that has been seasoned and dried. Jerky is a semi-wet food (intermediate moisture food) with a moisture content of 20 to 40% (Said & Ahmad, 2007). Curing seasoning is table salt, saltpeter (nitrate salt and/or nitrite), sugar is the main ingredient, while pepper, laos, coriander, and garlic are additional spices that can increase the palatability of beef jerky (Suharyanto et al., 2008). The problem faced in making pork jerky is the use of saltpeter as a coloring agent which can be detrimental to health, so palm ant sugar is used. The maximum limit for the use of nitrites according to Permenkes No. 722/MENKES/Per/IX/1988 is 50 ppm for corned beef (single/mixed with KNO_2), if it exceeds the standard it will cause poison to the human body (Ministry of Health RI, 1998). Humectants that are often used are from the sugar, polyol and salt groups which can be used singly or can be combined to produce semi-wet food. Palm sap is a liquid produced from palm types such as palm sugar and is used as an ingredient for making palm sugar and can then be made into powdered ant sugar. Ant sugar is produced with a yield of 11.48-11.50% and a water content of 3.57-4.05% (Inayatul et al., 2019), ash content 0.19%-1.24%, and sucrose content 90.77%-97.24% with favorable organoleptic properties (Nurjanna et al., 2020). Palm sugar has a distinctive taste so that its use cannot be replaced by other types of sugar, besides having a function as a natural sweetener and giving the impression of a brown color to food (Said & Ahmad, 2007). NaCl salt is one of the complementary needs for food and a source of electrolytes for the human body (Purbani, 2000). In the processing industry, salt is generally used to improve the taste, appearance, and functional properties of the resulting product. serves as a preservative, as well as to improve the appearance of the texture (Assadad and Utomo, 2011). Most of the beef jerky is stored at room temperature, which is 26-27 °C with a relative humidity of 70%. Another opinion states that beef jerky is a semi-wet food ingredient with a moisture content of 15 to 50% (Purnomo, 1995) As an area with a large pig farming industry, processing pork into beef jerky is not well known and pork jerky products are not widely circulated in Manado city. And the problem is how far the use of palm sugar + NaCl salt in pork jerky during its storage period at room temperature.

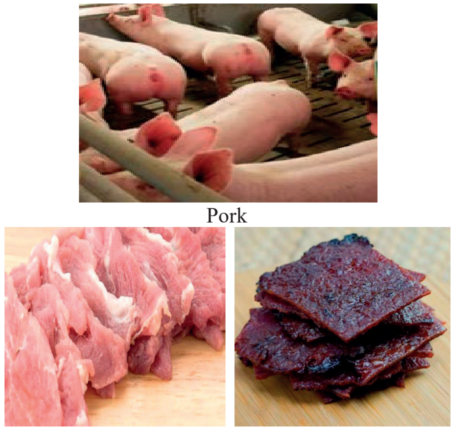
MATERIALS AND METHODS

This research was conducted at the Laboratory of Livestock Technology, Faculty of Animal Husbandry, Sam Ratulangi University, Manado for 40 days. Materials and tools this research uses materials such as ground pork, NaCl, palm sugar, PCA, Physiological NaCl, distilled water, 70% alcohol, and tools such as pH meter, oven, analytical scale, blender, weighing bottle, desiccator, autoclave, incubator, coloni counters and glassware. Design and Variables The research design was split plot in time on the basis of RAL with three replications. Factor A combination of palm sugar + NaCl consists of: A1 = Pork + (15% palm sugar-NaCl 5%), A2 = pork + (10% palm sugar-NaCl 10%), A3 = pork + (palm sugar 5%-NaCl 15%) and Factor B storage time at room temperature (26-27^{0}C) consisted of: B1 = 10 days, B2 = 20 days, B3 = 30 days.
The composition of the treatment as listed in Table 1.

Table 1. Treatment Composition

Material	Treatment		
	1	2	3
Palm ant sugar	15	10	5
Salt NaCl	5	10	15
Garlic	0.5	0.5	0.5
Galangal	0.2	0.2	0.2

How to make pork jerky:
1. The pork is washed and drained, then Ground;
2. Mixed with materials according to treatment;
3. Shaped flat round/rectangle;
4. Dry at 70 °C for 6 hours;
5. Stored at room temperature according to treatment and analyzed (Figure 1).



Pork

Fresh Pork Pork Jerky

Figure 1. Experimental design

Research Variables
1. Moisture content (AOAC, 2005)
Measurements were carried out using the heating method. The material is heated at a temperature (100-10°C) for 6 hours, then weighed until a constant weight of.
2. pH is obtained (AOAC, 2005). Measurement using a pH meter where the material is blended with distilled water is then measured and read when the pointer shows a constant scale.
3. Number of microbes (BSN, 2000) Measurement of Total Plate Count (TPC) with PCA tools and media was autoclaved and diluted with physiological NaCl, then the material was blended with distilled water and incubated at 37°C for 48 hours. The calculation of the number of microbes was carried out using a handtally counter.
4. Peroxide Number (Ketaren, 2005)
The sample is put into a funnel which is given ammonium chloride, shaken until the fat is clumped and separated with hexane. Then evaporated until the fat is left behind. Then put it in the erlemeyer, add acetic acid and chloroform, then heat it and add indicator and titrate with 0.1 N thiosulfate until the yellow color disappears.

Data Analysis
The data obtained from the test (moisture content, pH, number of microbes) were analyzed for variance in Split plot in time and if the results were significantly different, then continued with the BNJ test (Steel and Torrie, 1991).

RESULTS AND DISCUSSIONS

Water Content of Pork Jerky

The average water content of pork jerky using palm sugar (GSA) + NaCl during storage at room temperature can be seen in Table 2. The average value is in the range of 14.47-38.86%, not much different from the study of Saleh et al (2012) the water content of chicken jerky soaked in betel leaf juice ranged from 49.83% and the soaking time factor ranged from 55.23%. and is in the range of 15-50% semi-wet food moisture content (Purnomo, 1995). Based on the Indonesian National Standard on the quality of beef jerky, the maximum water content is 12% (w/w). Thus, the water content obtained in this study is still high (>12%).
The results of the analysis of diversity showed that the combination of GSA+NaCl had a highly significant ($P<0.01$) effect on the water content of pork jerky, as well as the length of storage at room temperature. The BNJ follow-up test showed that pork jerky using the combination of GSA+NaCl A1 and A2 had the same water content but significantly different ($P<0.01$) higher than A3 (Table 2). It can be concluded that the best one is the one using the GSA+NaCl combination A3, this is because NaCl salt can extract meat protein and can bind more water because of its hygroscopic nature which is stronger than palm sugar. Salting can remove water from the surface of the meat. The higher salt concentration can remove more water from the meat (Desniar et al., 2009). Furthermore Syarief and Halid (1993) added that dissolved sugar causes a lower vapor pressure so that water evaporates more easily from the dried material. Inayatul et al (2019) stated that the more salt and sugar concentration in beef jerky, the water content tends to decrease. This reduction in water content can be achieved by using high processing temperatures and/or using preservatives (Saleh et al., 2012). BNJ follow-up tests showed that the storage time at room temperature B1 was significantly different ($P<0.05$) with lower water content with B2 and B3, then B2 had a significantly ($P<0.05$) lower water content than B3 (Table 2). It can be concluded that B1 is the best because of the lowest water content. The low initial water content is very decisive and increases the absorption of moisture from the environment in pork jerky. In line with Dewi and Ratna (2008), Ikhsan et al, 2016 stated that the water content of beef jerky had increased during storage at room temperature by ± 2% from its initial water content. Delviani et al. (2021) stated that jerky with vacuum packaging has a significant effect on water content and storage time of 0 days to 9 days can affect quality and shelf life. The results of the research by Istihastuti et al (1998) showed that eel fish jerky with different packaging (vacuum and non-vacuum) each contained 20.33% and 20.54% moisture after being stored for 8 weeks, increasing to 22.61% and 20.79% (Istihastuti et al., 1998). Dewi and Ratna (2008), stated that jerky packed in plastic bags after 30 days of storage had a higher water content than jerky packaged in a vacuum manner.

Table 2. Average effect (palm sugar + NaCl salt) and storage time at room temperature on the moisture content (%) of pork jerky

Palm ant sugar concentration + NaCl salt	Storage Time			Average
	B1(10 days)	B2(20 days)	B3(30 days)	
A1 (GSA 15% and NaCl 5%)	27.59±0.44	27.35±1.69	38.29±1.27	31.08[a]
A2 (GSA 10% and NaCl 10%)	20.96±1.80	26.86±1.34	38.86±1.83	28.89[a]
A3 (GSA 5% and NaCl 15%	14.47±2.06	24.72±1.96	24.72±2.01	21.30[b]
Average	36.93[a]	26.31[b]	26.31[b]	27.09

Note: Different superscripts (abc) in the same row and column show differences ($P<0.01$)

The pH of Pork Jerky

The average pH of pork jerky can be seen in Table 3 ranging from 4.43 to 5.26. Almost the same as the results of research from Miwada et al (2015), that the average pH of pork jerky with drying and seasoning concentrations ranged from 5.29-6.64 and using liquid smoke 5.94-6.4. Analysis of variance showed that the use of the glycerol-NaCl combination had no significant effect (p>0.05) on the pH of pork jerky while storage time at room temperature had a significantly different effect (P<0.01) on the pH of pork jerky (Table 3).

Table 3. The average effect of palm sugar + NaCl salt and storage time at room temperature on the pH of pork jerky

Concentration palm ant sugar + NaCl salt	Storage Time			Average
	B1(10 days)	B2(20 days)	B2(20 days)	
A1 (GSA 15% and NaCl 5%)	4.94±0.22	5.13±0.28	5.26±0.18	5.11
A2 (GSA 10% and NaCl 10%)	4.76±0.18	4.97±0.18	5.16±0.16	4.96
A3 (GSA 5% and NaCl 15%)	4.43±0.11	4.87±0.11	5.03±0.11	4.78
Average	4.71^{a}	4.99^{a}	5.15^{b}	4.95

Note: Different superscripts (abc) on the same line indicate differences (P<0.01)

Furthermore, the BNJ test showed that B1 and B2 were the same but significantly different (P<0.01). B3 had a higher pH of pork jerky (Table 3). This is because during storage protein decomposition occurs resulting in changes in hydroxyl groups and hydrogen groups which causes an increase in the pH of pork jerky. An increase in meat pH can also occur due to changes in meat chemical proportions (Rotinsulu et al., 2019). The magnitude of the pH is related to the formation of alkaline compounds during storage and will affect microbial growth (Hadiwiyoto, 1993) and low H+ concentrations indicate higher pH and vice versa low pH indicates high H+ concentrations.

In addition, microbes can grow in the pH range of 6.0-8.0, usually they are destructive and yeast and lactic acid bacteria grow well in the pH range of 3.0-6.0 (Sutrisna et al*.,* 2015).

Pork Jerky Microbes

The average number of pork jerky microbes can be seen in Table 4, which is in the range of 4.18-4.93 log cfu/g, much lower than Rotinsulu et al (2019) study on beef jerky using glycerol-Nacl salt, namely 6.04-6, 60 log cfu/g. Meat starts to spoil if the microbial count exceeds log 6.69 cfu/g (Wilson et al, 1981).

The BNJ test showed the number of pork jerky microbes using palm sugar (GSA) –NaCl salt showed that A1 was different from A2 and A3, then A2 was different from A3 (Table 4). It can be concluded that the best was the A3 treatment with the smallest microbial count, namely 4.34 log cfu/g. This is because NaCl salt has a stronger ability to inhibit microbial growth than sugar palm ants. Salt can cause plasmolysis and Ci_ ions are toxic to microbes and can cause microbial dehydration due to osmotic forces (Madigan et al, 2011).

Table 4. The average effect of palm sugar + NaCl salt and storage time at room temperature on pork jerky microorganisms (log cfu/g)

Palm ant sugar concentration + NaCl salt	Storage time			Average
	B1 (10 days)	B2 (20 days)	B3 (30 days)	
A1 (GSA 15% and NaCl 5%)	4.57± 0.02	4.87±0.02	4.93±0.02	4.80^{a}
A2 (GSA 10% and NaCl 10%)	4.36±0.03	4.54±0.02	4.61±0.00	4.52^{b}
A3 (GSA 5% and NaCl 15%)	4.18±0.06	4.42±0.03	4.45±0.01	4.34^{c}
Average	4.37^{a}	4.66^{b}	4.74^{c}	4.55

Note: Different superscripts (abc) in the same row and column show a difference (P<0.01)

Sugar has a high solubility which can reduce the relative humidity (RH). In addition, salt and sugar which are humectants can reduce water activity and water content thereby inhibiting microbial growth, because metabolic activities require water (Purnomo, 1995). The BNJ test showed that the storage time at room temperature B1, B2 and B3 was highly significant (P<0.01) on the number of microbes (Table 4). The longer the storage, the increased the number of microbes. This is due to the availability of nutrients and water for microbial growth. In addition, there is degradation of nutrients in pork jerky by microbes which can cause spoilage over time. Microbes need nutrients such as carbon, nitrogen, non-metal elements such as sulfur and phosphorus, metal elements such as Ca, Zn, Na, K, Cu, Mn, Mg and Fe, vitamins, water and energy (Cappucino, 2014). Microbial growth is relatively fast if nutrition is good and enzymes are formed to break down the substrate and then adapt to the environment (Madigan et al, 2011).

Pork Jerky Peroxide Numbers

The average peroxide value of pork jerky can be seen in Table 5, which is in the range of 2.56-12.13 milliequivalence/kg of material.
Analysis of variance showed that the use of the glycerol-NaCl combination and storage time at room temperature as well as the interaction between the two factors had a significantly different effect (p<0.01) on the peroxide value of pork jerky. The BNJ test showed that the peroxide value of pork jerky using palm ant sugar (GSA) – NaCl salt showed that A1 was different from A2 and A3, then A2 was different from A3.

Table 5. The average effect of palm sugar + NaCl salt and storage time at room temperature on the peroxide number (milli equivalent / kg of ingredient) of pork jerky

Combination of ant sugar-Nacl	Storage time			Average
	B1	B2	B3	
A1	2.86^{g}±0.04	5.03^{e}±0.11	8.26^{c}±0.18	5.38^{a}
A2	3.66^{f}±0.04	6.23^{d}±0.11	9.13^{b}±0.11	6.34^{b}
A3	6.40^{d}±0.07	9.03^{b}±0.16	12.13^{a}±0.18	9.18^{c}
Average	4.30^{a}	6.76^{b}	9.84^{c}	

Note: Different superscripts (abc) on the same line indicate differences (P<0.01)

It can be concluded that the best was treatment A1 with the smallest peroxide value, namely 5.38 milliequivalence/kg beef jerky. This is because the ant sugar salt has a stronger ability to prevent oxidation than NaCl. Sugar has a high solubility can dissolve in beef jerky. Antioxidants from palm sugar added to the product are able to inhibit or prevent auto-oxidation reactions and act as donors of hydrogen atoms for lipid radicals. The stability of derivatives of antioxidant radicals is better than free radicals (Kosim et al., 2015). In addition, salt and sugar which are humectants also have the ability as antioxidants to reduce peroxide numbers (Purnomo, 1995). The BNJ test shows the storage time at room temperature B1, B2 and B3 were highly significant (P<0.01) on the jerky peroxide value (Table 4). With longer storage there is an increase in peroxide value. This is due to the longer storage at room temperature the more fat jerky oxidized by oxygen.
Under these conditions, the fat content affects the rate of oxidation (Purnamasari et al., 2012).

CONCLUSIONS

The combination of GSA+NaCl had a highly significant (P<0.01) effect on the water content of pork jerky, as well as storage time at room temperature. And the combination of GSA+NaCl on pork jerky pH gave no significant effect (P>0.05) while storage time at room temperature had a significantly different effect (P<0.01) on pork jerky pH. The number of pork jerky microbes using palm sugar (GSA + NaCl salt) showed a very significant difference (P<0.01) and The BNJ test shows the storage time at room temperature B1, B2 and B3 were highly significant (P<0.01) on the jerky peroxide value. It was clear that using 5% palm sugar + 15% NaCl could extend the shelf life of pork jerky up to 30 days.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

CONTENT AND SOURCES OF CONTAMINATION OF DONKEY MILK BY HEAVY METALS - REVIEW

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Abstract

Agricultural practice, the methods used in increasing and feeding of animals, may be a main factor in the appearance of harmful elements in the products obtained from them. Due to the high consumption of milk worldwide, the question has arisen of determining the compounds that can be harmful to consumers, such as the presence of heavy metals. Heavy metals are toxic to the human body, consumed even in small proportions, and their sources in milk, its products or by-products, can be both natural and anthropogenic, as the main sources being water used in irrigation, agricultural practices, air pollution and contaminated feed used in the ration of animals. The most common heavy metals determined in fresh donkey milk are iron, copper, magnesium, lead, zinc, cadmium, arsenic and chromium, their determination being possible both by means of standard methods and by means of the mass spectrometry method. The determination of these toxic compounds which may be present in milk is of importance, in particular as regards babies and children, on which they may have a carcinogenic effect.

Key words*: donkey, factor, heavy metals, lead, milk, zinc.*

INTRODUCTION

In the human diet, milk and milk products are almost always found, because of this, the determinations of the potentially harmful and chemical compounds that may appear in these products are of increasing interest (Meshref et al., 2014).

Milk is an important source of nutrients, being indispensable for the growth and development of children, and donkey milk being rich in elements such as lactose and whey proteins, its usefulness in making milk substitutes has been demonstrated, thus attracting the attention of researchers (Brumini et al., 2016).

Due to the medical and nutritional benefits of donkey milk, obtained from domestic donkeys, the question was raised of determining the possible contamination of this product and determining the benefits it presents for the body (Li et al., 2021).

Also once, the level of protein it contains, donkey milk is recognized as having a high biological value, being similar to human milk (Proikakis et al., 2021).

Also, donkey milk has aroused great interest, due to its antiallergenic properties, being otherwise named as a pharmaceutical product (Perna et al., 2015) having anti-inflammatory action and acting on oxidative stress (Li et al., 2022).

Being a product that is becoming increasingly consumed internationally, with a high comercial value at the moment, the importance of determining possible risks is increasing (Conte et al., 2019).

As uses, donkey milk has been frequently used to obtain cosmetics and also to obtain supplements with antioxidative, anti-inflammatory and immunomodulatory roles (Li et al., 2021).

In order to maintain a high level of consumer health and to avoid the most significant dangers that may arise in donkey milk, it is important to consider risk analysis also in the case of this species, the main consumers of which are children and the elderly (Conte et al., 2019).

The main heavy metals found in both milk and dairy products were mercury, chromium, arsenic, lead and cadmium, their level being dependent on both contamination from the external environment and feed and materials used in product packaging (Yan et al., 2022), all of which were determined in milk by means of standard methods for determining heavy metals (Hasan et al, 2022).

MATERIALS AND METHODS

We systematically reviewed the latest and pertinent research papers accessible through prominent databases such as PubMed, MDPI, Research Gate. This review aims to present a comprehensive synthesis of the scientific findings related to the specified topics regarding the heavy metals in donkey milk.

RESULTS AND DISCUSSIONS

Heavy metals in donkey milk

In milk, various chemical hazards may occur, many of which are introduced during production or various processes for the production and packaging of milk products, also other factors may be veterinary medicinal products, radionuclides, mycotoxins, pesticides and, last but not least, heavy metals, which consumed by animals, leave their residues in their productions (Conte & Panebianco, 2019).

According to the legislation of our country, the maximum limits of heavy metals in milk should not exceed values of 0.1mg /1 l in the case of lead and arsenic and 0.01 mg / 1 l in terms of mercury and cadmium (Hygienic-sanitary norm for food from 16.12.1998).

The translocation of heavy metals and their storage in milk is possible due to non-biodegradation and their ability to keep themselves under the action of temperatures being extremely persistent in the environment (Abdel-Rahman, 2022).

The main consumers of milk are represented by vulnerable people, children and the elderly, who are the most exposed to heavy metal contamination (Ismail et al., 2017).

Heavy metal concentrations in milk and dairy products are one of the main risk factors that have been determined lately, with a harmful action on consumers (Meshref et al., 2014).

Not being metabolized, their retention occurs in all tissues of animals and in its intestine, being transported to the product or products we obtain from animal species (Eskandari & Pakfetrat, 2014).

Due to the accumulation in different organs, heavy metals can cause various liver, nervous and cardiovascular diseases, therefore solutions have been sought over time to eliminate their concentrations in the environment and food (Masoud et al., 2022).

As a result of studies carried out in Italy, residues of toxic metals such as arsenic, lead, cadmium, nickel, mercury and antimuonium were determined in donkey milk and feed used to grow and maintain them (Conte & Panebianco, 2019).

As factors that influence the presence of heavy metals in donkey milk, the lactation period and the geographical area from which the animals come are listed (Longodor et al., 2018), the highest concentrations being highlighted in the 4th lactation, compared to the first lactation, when the values of heavy metals are much lower (Longodor et al., 2019).

Compared to cow's milk in terms of the amount of vanadium and titanium, concentrations were similar or lower, and molybdenum, caesium and strontium showed higher values (Conte et al., 2019).

Also, in other studies conducted this time on donkey breeds in Turkey, the level of Ni, Cd, V and barium concentrations, were 1 mg/L, being lower than the permissible limit (Conte, 2019).

The level of heavy metals found in milk are dependent on their level in the environment, this being associated primarily with the risks to which consumers are exposed (Yan et al., 2022).

Both fresh and processed milk and spilled products, such as butter, cheeses or yogurts, showed high concentrations of heavy metals such as cadmium, zinc, copper and lead (Meshref et al., 2014).

As for copper, it does not have a carcinogenic effect for humans and animals, but in large quantities, it can cause liver and kidney damage (ATSDR, 2004)

Studies have determined, however, that due to the biological filter that the udder of dairy animals presents, the transport of heavy metals is still small being reported as 1:5000, but even so contamination with them is harmful (Ismail et al., 2017).

According to the studies carried out by other authors in Romania, variations in heavy metals depends on the number of lactation were found, thus identifying lead concentrations of 29.7 µg/L, while the identified cadmium concentrations were 5.66 µg/L (Longodor et al., 2018).

However, in the studies carried out on the concentration of heavy metals in donkey milk in donkey breeds in Turkey, they have not been detected, however, with concentrations of essential elements such as magnesium, calcium, potassium and copper being found (Paksoy et al., 2018).
Within our country, the level of heavy metal concentrations in different areas, respectively in Cluj and Sălaj, were determined, being determined differences in the concentrations detected between the two regions (Longodor et al., 2018).
The level of lead concentrations, being lower in the case of Cluj County, respectively 19.59 µg/L, resulting in a weaker contamination with heavy metals of pastoral soils in this county (Longodor et al., 2018).

Table 1. Standard for milk

Heavy metals	Standard for milk and milk product	Reference
Mercury	500 mg/l	Aggarwal et al., 2022
	0.002 mg/kg	Masoud et al., 2022
Arsenic	20 mg/l	Aggarwal et al., 2022
Lead	140 mg/l	Aggarwal et al., 2022
	0.2 mg/kg	Masoud et al., 2022
	0.40 ppm	Meshref & Moselhy, 2014
Cadmium	200 mg/l	Aggarwal et al., 2022
	0.02 mg/kg	Masoud et al., 2022
	0.104 ppm	Meshref & Moselhy, 2014

Sources of heavy metal contamination

The source of contamination by toxic compounds is dependent for both humans and animals on the period of exposure and the level of contaminants found in food or feed (Hasan et al., 2022).
Described as a group of very heterogeneous elements that vary widely in terms of chemical properties and biological functions, heavy metals, are very harmful to the body, and their appearance is determined mainly due to industrialization and high urbanization (Sharma & Agrawal, 2005).
Following the recent industrialization of all processes, and also due to agricultural practices, the emission of heavy metals is favored, both in the air and in water, soil and plants (Meshref et al., 2014).
Heavy metals found in milk and dairy products can be grouped according to their toxicity, into essential elements such as zinc, iron and copper, and into toxic and non-essential elements of the body, such as cadmium and lead (Meshref et al., 2014).
Studies conducted on heavy metal contamination have shown that, on a daily basis, this contamination occurs on animals and humans, and their sources can be multiple, from water to food (Pandey & Madhuri, 2014).
Milk-producing animals are increasingly exposed to these toxic compounds, so ingesting heavy metals from pastures or after feeding with contaminated feed, and their transfer to the body to milk is very variable (Meshref et al., 2014).
As a result of studies carried out in Italy, residues of toxic metals such as arsenic, lead, cadmium, nickel, mercury and antimuonium were determined in donkey milk and feed used to grow and maintain them (Conte et al., 2019).
The food chain and feed are the main gateway to toxic substances for animals and higher organisms (Caggiano et al., 2005).
The source of heavy metals in the body is usually an anthropogenic one, as they come from the external environment as a result of massive industrialization and urbanization (Caggiano et al., 2005).
Through the alloying of animals with contaminated feed (Dai et al., 2016) or due to emissions from atmospheric air, they are exposed to heavy metals, being bioaccumulated by the body, lead to the appearance of toxicity, depending on the degree of exposure (Pandey et al., 2014).
The consumption of food contaminated with heavy metals poses an increased risk to human health, many of which are considered the main factors of different types of cancer (Meshref et al., 2014).
Some toxic substances may be found in additive feed or even in medicinal products administered to animals which are generally metabolized into non-clinical constituents, but if this feeding stuff or medicines are contaminated with heavy metals and dioxins, they will remain in the tissues of the consuming animals and then in their products, having a negative effect (Eskandari et al., 2014).
In both the United States and Europe, the presence of heavy metals in feed used in the ration of animals has been reported (Dai et al., 2016).

In concentrated feed for farm animals, it is known that arsenic, zinc and copper can be introduced as additive minerals that prevent the growth and growth of bacteria, which are consumed by animals, the spread of metals on land is favored (Zhang et al., 2012), also contamination of plants on pastures is inevitable (Eskandari et al., 2014).

Animals raised on pasture are prone to contamination by heavy metals, so, as a result of fertilization processes through the use of inorganic fertilizers, such as phosphate, a variable quantity of heavy metals will accumulate in plants (Sharma et al., 2005.).

Lead reaches the animal organism through plants, in which it is bioaccumulated, this happens especially in pastures close to rivers contaminated with this metal (Puschner et al., 2010).

As the main source of arsenic contamination of pasture plants and forages, it is polluted water used in irrigation, which is absorbed by plants (Eskandari et al., 2014), its action leads to the slowing down of the activities of enzymes such as peroxidases in the leaves and changes the hydrolytic activity of chlorophyll, thus affecting plants (Sharma et al., 2005).

It is known that manure used to fertilize the soil has beneficial properties on it (Zhang et al., 2012), but studies have determined that overuse of it can have a negative and corrosive effect on the soil, leading to its bioaccumulation with heavy metals, then affecting the plants used in animal feed and then the animals themselves, but manure obtained from donkeys shows a low level in these compounds (Adesoye et al., 2014).

The accumulation in the leaves in very large quantities of cadmium is responsible for the use of these fertilizers, and the long repetition of fertilizers such as phosphate creates in the agricultural soil an excessive accumulation of these elements (Sharma et al., 2005), especially favoring the contamination with cadmium (Pandey et al., 2014).

Other extremely important sources are the chemical amendments, the pesticides used in the grain crops, also sewage sludges are the most important sources of heavy metals, affecting the soil, the plants and then the animals on the pasture (Sharma et al., 2005).

The transport to arable land of different types of agricultural machinery, the incineration or keeping of waste on them, has an important source of heavy metals, with a negative effect on future feed (Sharma, 2005).

In addition to feed that can be contaminated with heavy metals, another important source can be drinking water, if it is contaminated as a result of human activity due to soil erosion processes (Pandey & Madhuri, 2014).

Lead can have as a source of contamination in addition to feed, and drinking water, having a toxic effect on the synthesis of haemoglobin, the gastrointestinal tract and the reproductive system (Pandey et al., 2014).

Studies conducted on equines have shown that heavy metals such as arsenic, cadmium, iron, molybdenum, zinc, copper and lead were accumulated in various organs other to the body, among which, the largest reserves were in the liver and kidneys, and their concentrations were higher in animals over 2 years old (Plumlee et al., 1996).

Areas where industrially developed metallurgic or overpopulated ones, which have heavy traffic of motor vehicles, have been determined to be extremely contaminated with heavy metals and other chemical contaminants that can end up in animal productions, having hepatotoxic and even neurotoxic effect on consumers (Porova et al., 2014).

In donkeys, studies have been carried out on the level of heavy metals in the blood, with an emphasis on toxic metals such as lead, which causes cardiovascular, renal, gastrointestinal and nervous system diseases, but also infertility, obesity and seizures (Yipel et al., 2014).

Lead and cadmium are mainly occurring as a result of the irrigation of the soil, of the contaminated waters used in this agricultural practice, also as a result of thermal processes carried out in different factories, where materials containing heavy metals are used, cause their emission of snub vapor form, which in combination with water leads to the formation of atmospheric aerosols, which affect both plants and animals directly (Sharma et al., 2005).

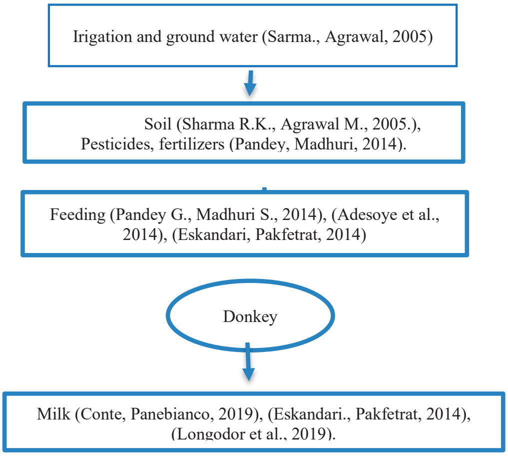

Figure 1. The main sources of heavy metals in donkey milk

The action of heavy metals on the animal organism

For living organisms, heavy metals are highly toxic, having bioaccumulation properties, are persistent, causing serious health problems, morbidities or even death (Pandey & Madhuri, 2014).

The danger that heavies metals cause on the animal and human body is extremely high, toxic elements such as lead and mercury can cause neurodegenerative conditions such as Alzheimer's disease and multiple sclerosis (Giacoppo et al., 2014).

Heavy metals, have a very high toxicity, being very quickly accumulated by extra and intra-cellular proteins, affecting all the time the functions of cells and the body (Buckler et al., 1986).

As regards elements such as zinc, iron and copper, which usually have benefits and have an important role in the various biological functions of organisms, their high content with these elements and their consumption in too large quantities can have negative effects on the body, producing toxicity (Meshref et al., 2014).

Of all heavy metals, not all of them are necessary for the functions of the body, they have toxic action, but even those necessary for functioning processes, such as iron, in large quantities can have a negative effect (Pandey & Madhuri, 2014).

In some circumstances, beryllium can be toxic to the body, also the essential elements can undergo changes caused by these compounds, causing toxicity (Pandey & Madhuri, 2014).

Some heavy metals can mimic the action of organoleptic compounds, for example radium, can be strained in bones, imitating the action of calcium (Pandey & Madhuri, 2014).

Another heavy metal, determined by studies from Iran, in animal feed, is cadmium, which accumulates in the body, especially in the liver and kidneys, contamination with it is favored in highly industrialized areas (Eskandari & Pakfetrat, 2014).

Antibiotics and medicines used in animals can also cause contamination with residues in their milk (Rezaei et al., 2014).

Studies by Porova et al., in 2014, demonstrated that the use of methods such as that of low-frequency ultra-sound, on the production lines of dairy products, would solve the problem of heavy metals in them, without affecting all at once the chemical and physical properties of milk.

CONCLUSIONS

Studies show the problem that the accumulation of heavy metals represents for animal production and ultimately for the human body.

Thus, their accumulation in the case of donkey milk is, as in the case of other species, a problem.

The main problem that causes this contamination is represented by grazing on surfaces on which chemical treatments have been applied or due to the wastewater used in irrigation.

But studies have shown that in terms of this species, heavy metal accumulations are not so high compared to other species.

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WILD LIFE MANAGEMENT, FISHERY AND AQUACULTURE

Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

EVOLUTION OF FISH PRODUCTION ACHIEVED FROM COMMERCIAL FISHING IN THE DANUBE RIVER IN THE PERIOD 2015-2021

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Abstract

The paper presents the evolution of fish production from commercial fishing in the Danube River during 2015-2021 and is based on the analysis of statistical data collected by the National Agency for Fisheries and Aquaculture as part of the data collection program. Production data were collected annually by authorized commercial fishermen, quantitatively and by species. During the analysed period, there is a quantitative decrease in the production of wild carp and raptors, and significant increases in catches of Asian carp species. Catches of Danube record annual fluctuations with decreasing trends in the analysed period. At the end, the causes of the catches decrease of commercial fishing are analysed and the fisheries management measures that must be taken, based on ecosystem principles, to protect and restore the declining fish populations in the Danube River.

Key words*: biodiversity conservation, catches, fisheries management, sustainability.*

INTRODUCTION

According to Fish Base, 119 species of fish livein the Danube. As endemic species we mention the brook lamprey (*Eudontomyzonvladykovi,* Oliva & Zanandrea, 1959) and the European mudminnow (*Umbra krameri,* Walbaum, 1792). An emblematic specie in the Danube are the sturgeons, most of them almost extinct, excepting the sterlet (*Acipenser ruthenus*, Linnaeus, 1758). Also, sturgeon population depend, mostly, on repopulations.

Among the newly introduced speciescan be mentioned: the grasscarp, cosas (*Ctenopharyngodon idella*, Valenciennes 1848), silver carp, sanger (*Hypophthalmichythys molitrix,* Valenciennes, 1848) end big had carp, novac (*Aristichtys nobilis*, Richardson,1844).

The Danube supports both commercial and recreational fishing, depending on the country. In Germany, Austria and Slovakia an important role in the fishing catches is played by the recreational fishing. In contrast, Hungary, Romania, Bulgaria, Serbia and Ukraine, commercial fishing is more important, even though in this countries, large flood plains support both commercial and recreational fisheries (Schiemer et al., 2004). The largest fisheries in the Danube River area are in Serbia and Romania, but the construction of the Iron Gates Dams determined the decrease in the quotas of this type of fishing.

There are about 40 species are important for commercial and recreational fishing, among which we mention: the bream (*Abramis brama*), the pike-perch *(Sander lucioperca*), the barbel (*Barbus barbus*), the sterlet (*Acipenser ruthenus*), the crusian carp (*Carassius auratus gibelio*), the carp (*Cyprinus carpio*), the pike (*Esox lucius*), the asp (*Aspius aspius aspius*) and the tench (*Tinca tinca*) (Schiemer et al., 2004; Smederevac-Lalić et al., 2012). In Serbia and Hungary, 40% to 70% of commercial catches consist of bream, barbel and carp, while in Germany the recreational catches consist of whitefish (*Coregonus* spp.), perch, northern pike, and common carp (Smederevac-Lalić et al., 2012).

Since the 1970s, sturgeon fishing has decresed drastically, and the quality of catches has shown a continuous decline due to the poor connectivity between the river and its

floodplain and the blockade of the migration corridors (Schiemer et al., 2004).
A major importance for commercial fishing is hold by the Pontic shad (*Alosa pontica*), in Romania, being reported (according to ANPA statistical data) between 200-600 tons, annually.
The most complex and complete evaluation of the value of all the fishery components, from the point of view of stock diversity and quality and of the bioresources regeneration rate, is based on scientifically well-founded data. This represents the basis of an efficient and responsible management.
Depending on this, in fisheries management can take relevant decisions only through a good cooperation with all responsible factors; thus, the exploitation methods will be improved, the overexploitation risk will be reduced, the measures of protection and conservation of biodiversity will be observed, and a sustainable exploitation of all resources will be ensured (Călin et al., 2013, Maximov, 2006).
The purpose of the present work is to analyze the data of the reported catches but also of the data of the Total Allowable Catch (TAC), as well as the share of species of interest for commercial fishing in the Danube River.

MATERIALS AND METHODS

Fish data

The analysis of thefishcatches' dynamicsin 2015-2021 was made usingtheofficialrecords of the National Agency for Fisheriesand Aquaculture. It has also been compared to the annual ordersissued establishing the quotas and fishing effort (Common Order no. 368/391/2015, Common Order no. 284/613/2016, Common Order no. 13/142/2017, Common Order no. 546/352/2018, Comon Order no. 243/354/2019, Common Order no. 124/1159/2020, Common Order no. 99/814/2021).
Data processing was carried out using Microsoft Excel for Windows.

Study area

The studied fishing area includes the Romanian sector of the Danube from the entrance in the country at Baziaș to the border with the Danube Delta Biosphere Reserve. The Romanian sector of the Danube unfolds from Baziaş to the Black Sea is 1075 km long, representing 78% of the Danube (Figure 1).

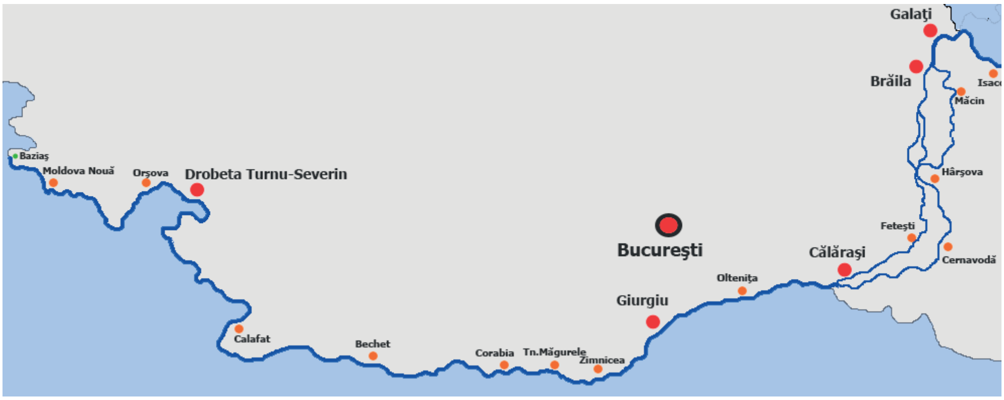

Figure 1. Danube river in Romania - study area

The ecological structure of the Danube is determined by two major factors, conditioning the composition of the aquatic biocenosis: bottom of theriver, stony or silty, and the speed of the river current.
Based of the previous elements, for the Danube River were identified and established the following exploitation sectors:

Sector 1 - lenght 14 km - *Delimitation*: Mm 76 (Cotu Pisicii - up stream the RBDD limit) - km 155 (confluence with Siret river);
Sector 2 - lenght 75 km - *Delimitation*: km 155 (confluence with Siret river) - km 227 (Călmățui river), including The Valciu Branch;
Sector 3 - length 98 km - *Delimitation:* km 0 - km 98 Macin Branch (Vadu Oii);

Sector 4 - length 128 km - *Delimitation:* km 248 (Vadu Oii) - km 366 (lower from the Chiciu - Island);
Sector 5 - length 71 km - *Delimitation*: km 227 (Gura Călmăţui) - km 50 Borcea Branch;
Sector 6 - length 137 km -*Delimitation:* km 50 Borcea Branch - km 452 (Greaca);
Sector 7 - length 74 km - *Delimitation*: km 452 (Greaca) - km 526 (Vedea river);
Sector 8 - lenght 89 km - *Delimitation:* km 526 (Vedea river) - km 615 (Gârcov);
Sector 9 - lenght 50 km - *Delimitation:* km 615 (Gârcov) - km 665;
Sector 10 - length 152 km - *Delimitation:* km 665 - km 817 (upstream of the Citadel);
Sector 11 - lenght 46 km - *Delimitation:* km 817 (upstream of the Citadel) - km 863 (lower from the Iron Gates II Dam), including Gogoşu Branch;
Sector 12 - length 80 km - *Delimitation:* km 863 (Iron Gates II Dam) - km 943 (Iron Gates I Dam);
Sector 13 - lenght 69 km - *Delimitation:* km 943 (Iron Gates I Dam) - km 1012 (Cozla);
Sector 14 - lenght 63 km - *Delimitation:* km 1012 (Cozla) - km 1075 (Baziaş).

RESULTS AND DISCUSSIONS

The total annual catches (in tonnes) for most important fish species (cyprinids, clupeids, predatory fish, others species) from reported by commercial fishermen to the National Agency for Fisheries and Aquaculture in the period 2015-2021 are shown in Table 1. Also, in the table are shown the catch quotas which have been established by annual orders (TACs) and the fishing effort quantified by number of boats.

Also, in the table are shown the reported catch (tons), the catch quotas which have been established by annual orders (TACs) and the fishing effort quantified by number of boats.

The data in Table 1 indicate an increasing trend from year to year in both the fishing effort, the TAC, and the reported catches. But the reported catch quantities do not even reach 50% of the annual TAC.

Total allowable catches covered by annual orders varied between 764,80 tons in 2015 and 1122,50 tons in 2021, which indicates an allocated fishing quota/boat that varies between 1.18 tons/boat in 2015 and 1.37 tons/ boat in 2021.

Tabel 1. Evolution of reported catch and annual TACs values during 2015-2021

Year	The reported catch (t)	The quotas from annual TAC's	Fishing effort (Number of boats)
2015	179.28	764.80	646
2016	249.50	1086.50	696
2017	242.54	1087.50	696
2018	318.45	1087.50	696
2019	429.97	1084.60	800
2020	350.01	1105.80	810
2021	528.72	1122.50	814

The fishing effort (in number of boats) does not have large variations between 2015 and 2021, with a minimum of 646 boats (in 2015) and a maximum of 814 boats (in 2021).

The species structure in the Danube River (Figure 2) of the reported catches is dominated by cyprinids (carp, silver carp, grass carp, bighead carp, crucian carp, fresh water bream, barbel, etc.) 68%, then predatory fish (European catfish, pikeperch, pike) 20%, Pontic shad (8%), another species with low economic value (roach etc.). This structure of the reported catches was similar with Ibănescu, 2019, 2020, which analyzes the structure of fish species that are the object of commercial fishing in inland waters in Romania.

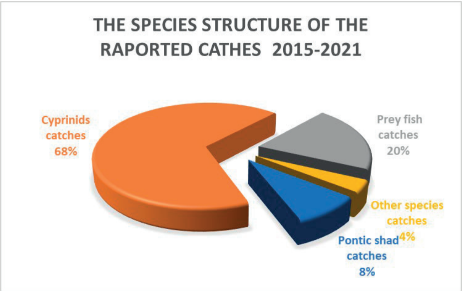

Figure 2. The species structure of the reported catches (2015-2021)

According to the Ibănescu (2020), the species structure of the catches only partially reflects the composition of the Danube rivers ichthyofauna because the type of gear conditions the report between the different species of fish caught.

If we analysed the evolution of cyprinid catches in the Danube (Figure 3), we can see that the maximum value reported was recorded in 2021 - 371.36 tons and the minimum value reported was 114.54 tons in 2015. It can also be observed that only in 2021 the value of the reported catch fishing.

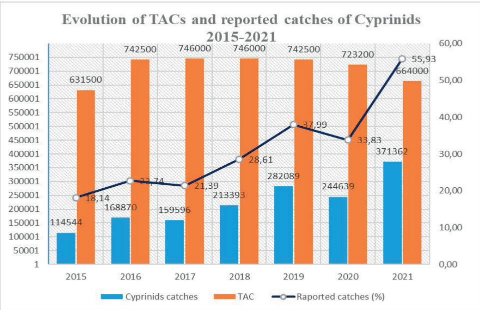

Figure 3. Evolution of TACs and reported quantities of Cyprinids (2015-2021)

The analysis of the evolution of predatory fish species in the Danube (Figure 4) indicates a maximum value of reported catches of 93.17 tons recorded in 2021 and a minimum value of 47.91 tons in 2015.

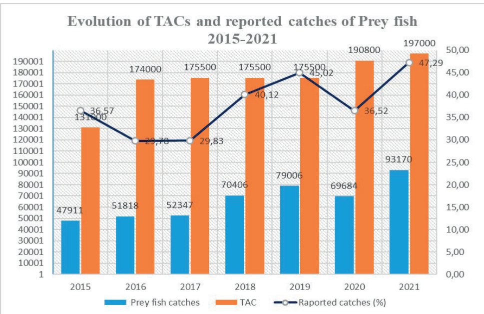

Figure 4. Evolution of TACs and reported quantities of predatory fish (2015-2021)

Radu (2012) and Chioveanu (2019) observed that in years or seasons with higher water levels and flow, peaceful species are advantaged, with more chances to escape the attack of predators, and years or seasons with low water levels favoring predatory fish species. According to the data reported by fishermen, it can be considered that the ratio between peaceful and predatory fish species indicates an ecosystem balance of the Danube River during the analyzed period.

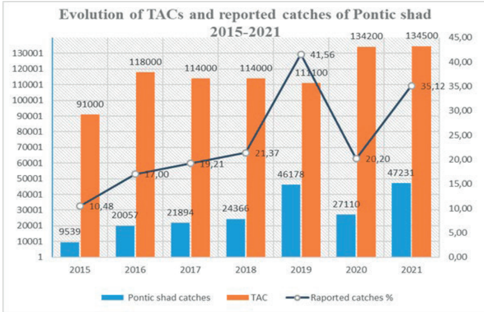

Figure 5. Evolution of TACs and reported quantities of Pontic shad (2015-2021)

Regarding the Pontic shad it can be said that despite the seasonality of fishing in the Danube (during the migration period), considerable quantities are reported, so the maximum value reported was 47.23 tons in 2021 and the minimum is 9.53 tons in 2015 according to Figure 5.

Other fish species with low economic value (roach, small silver bream etc) appear in accidental catches, but nevertheless their values varied between 7.28 tons in 2015 and 16,9 tons in 2021 (Figure 6).

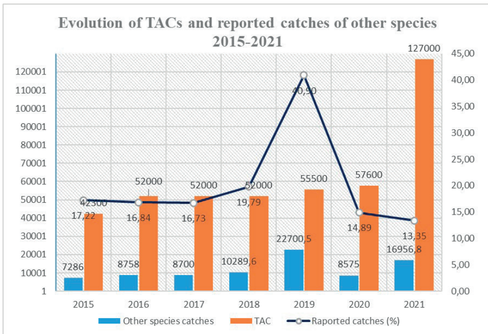

Figure 6. Evolution of TACs and reported quantities of other species (2015-2021)

Not even in the case of these species of fish with low economic value, the value of the total allowable catch is not even reached at 50%.

CONCLUSIONS

The species structure in the Danube River of the reported catches is dominated by cyprinids (grass carp, silver carp, bighead carp, common carp, crucian carp, fresh water bream, vimba bream, common barbel) 68%, then predatory fish (European catfish, pikeperch, pike) 20%,

Pontic shad (8%), and other species with low economic value (roach, small silver bream etc).
Between 2015 and 2021, the total quantity of fish allocated by annual TACs was 7.470,20 tonnes and that reported by fishermen to the National Agency for Fisheries and Aquaculture was 2.298,47 tonnes.
Due to the fact that each year there are significant differences between allocated and reported quotas, it indicates that there are under reported in the case of all analyzed fish species.
Catches reported in the period 2015-2021 represent on average 31% of the total allowable catch, which leads us to believe that fishermen reported only a small percentage of the actual catch.

ACKNOWLEDGEMENTS

This research work is a part of the PhD thesis elaboration, and was carried out with the support of Faculty of Engineering and Management of Animal Production, University of Agronomic Sciences and Veterinary Medicine of Bucharest.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

NEW DATA FOR HELMINTH FAUNA OF *Esox lucius* (Linnaeus, 1758) FROM MARITSA RIVER, BULGARIA

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Abstract

Twelve specimens of northern pike (Esox lucius (Linnaeus, 1758)) were caught from the Maritsa River during 2020-2021 and examined for parasites. Helminth parasites were found in 50% of the examined northern pikes (6 specimens) from the Maritsa River. Two species of parasites were fixed: one trematode species (Bunodera luciopercae (Müller, 1776)) and one nematode species (Raphidascaris acus (Bloch, 1779)). The dominant structure of the established species is represented at the level of component communities and infracommunities. R. acus is a core species for the component community of Esox lucius from the Maritsa River. B. luciopercae is an accidental parasite species for northern pike helminth communities. The data for the infracommunities were used to determine the basic biotic indices. The bioindicator significance of the established parasite species was discussed for an ecological assessment of the studied river ecosystem. As a result of the conducted research, new data on the parasite fauna of E. lucius from the Maritsa River have been presented.

Key words: *bioindication, Esox Lucius, helminths, Maritsa River.*

INTRODUCTION

The longest river on the Balkan Peninsula is the Maritsa River (427 km), the upper and middle reaches of which are located on the territory of Bulgaria (321.6 km). The Maritsa River springs from Rila Mountain. East of Svilengrad, the river flows eastward, forming the border between Bulgaria on the northern coast and Greece on the southern coast and then between Turkey and Greece. The two main tributaries, Tunja and Arda, flow into Edirne (Turkey), and the river pass through Turkish territory on both banks. The Maritsa then turns south and forms the border between Greece on the west coast and Turkey on the east coast reaching the Aegean Sea, which enters near the Enez.

Parasites are considered an essential and necessary component of biodiversity (Dobson et al., 2008). They are also valuable indicators for food web structure and ecosystem state (Marcogliese, 2004; Marcogliese, 2005; Hudson et al., 2006). The complex life cycles of endoparasites reflect relationships with a range of invertebrate and vertebrate hosts. The assemblage of these different parasites in a host organism reflects not only the trophic position of that host in the food web but also the presence in the ecosystem of any other organisms that participate in the different life cycles of parasites.

This study aims to reveal parasite species biodiversity and structure of parasite communities of *Esox lucius* from the Maritsa River, Bulgaria.

MATERIALS AND METHODS

Twelve specimens of northern pike (*Esox lucius* (Linnaeus, 1758)) were caught from the Maritsa River (Plovdiv region) in the two years 2020-2021 and examined for parasites. The object of the present study is a section of the Maritsa River near the village of Orizari (Rhodopi municipality, Plovdiv region). After the mouth of the Luda Yana River to the state border with the Republic of Greece and the Republic of Turkey, the Maritsa River belongs to river type R12: Large lowland rivers in Ecoregion 7 (Eastern Balkans).

A total of twelve *Esox lucius* (Pisces: Esocidae) are collected from the Maritsa River in the vicinity of the village Orizari and examined for parasites. Fish are caught with a fishing line during the two years of 2020-2021. The studied fish specimens were measured (mean total length 41.08±2.40 cm; range 39-44 cm) and weighed (mean total weight 1.105±0.38 kg; range 0.950-1.4 kg). The fish specimens were examined with standard techniques immediately after capture. Trematodes were stained with acetic carmine, differentiated in 70% acid ethanol, dehydrated in ascending ethanol series, cleared in eugenol and mounted in Canada balm as permanent slides (Bykhovskaya-Pavlovskaya, 1985; Georgiev et al., 1986). The identification of parasite samples was made using the keys of Bauer et al. (1981), Bauer (1987), and Bykhovskaya-Pavlovskaya (1985). Nematodes were examined in glycerine as temporary microscopic preparations (Moravec, 2013).

The common and taxonomic name of fish is used in accordance with Fröse and Pauly (2022). The mean intensity (MI), mean abundance (MA) and prevalence (P%) were used according to Bush et al. (1997).

The helminth community structure was studied at levels of component community and infracommunity. Based on the prevalence (P%) as suggested by Kennedy (1993), the parasites are grouped as core (P%>20), component (P%<20) and accidental (P%<10).

RESULTS AND DISCUSSIONS

During 2021-2022, twelve specimens of *Esox lucius* (Linnaeus, 1758) were collected from the Maritsa River and examined for helminths. The northern pike has Holarctic distribution. In Bulgaria, the species is widespread in the Danube River and the lower reaches of its tributaries, in the Kamchia River, in the rivers of the Aegean Basin, in dams and other stagnant and slow-flowing waters (Karapetkova & Zivkov, 2010). *Esox lucius* is not listed in the Bulgarian Red Data Book (Golemanski (Ed.), 2011) and is rated as LC=Least Concern species (IUCN Red List Status). The northern pike is a freshwater, predatory brackish, potamodromous species (Fröse & Pauly, 2022). *Esox lucius* is typical predatory species that feed on fish and other vertebrates, feeding on zooplankton only in its earliest stages (Karapetkova & Zivkov, 2010). The northern pike is highly territorial and usually solitary (Fröse & Pauly, 2022). Habitat alternations can impact *Esox lucius* locally (Kottelat & Freyhof, 2007).

Parasites were found in 50.00% (Table 1) of the examined northern pikes from the Maritsa River (6 fish). Two parasite species were identified, which were represented by 13 specimens (p), one species belonging to the class Trematoda (*Bunodera luciopercae* (Müller, 1776)) and one to the class Nematoda (*Raphidascaris acus* (Bloch, 1779)). All established species are adults.

In the component community of the northern pike from the Maritsa River, nematodes are represented by the largest number of specimens - 11 belonging to one species. One species with only two specimens represent trematodes. *Raphidascaris acus* is a core species (P%=50.00). The prevalence of *Bunodera luciopercae* (P%=8.30) determines it as an accidental parasite species for the communities of northern pike (Table 1).

Table 1. Parasite diversity of *Esox lucius* from Maritsa River (N - Number of studied fish, n - Number of infected fish)

Helminth species	N = 12					
	n	p	P%	MA±SD	MI±SD	Range
Bunodera luciopercae (Müller, 1776)	1	2	8.3	0.17±0.55	2.0±0.0	0-2
Raphidascaris acus (Bloch, 1779)	6	11	50	0.92±1.19	1.83±1.07	1-4

Species richness in infracommunity of northern pike ranged from 1 to 2 species. Five fish (41.66%) were infected with one helminth species and only one (8.33%) with two helminth species. The largest number of helminths found in one host is 4. The infracommunity of northern pike is presented by 0.58±0.64 species per host and 1.08±1.32 helminths per host (Table 2). The infracommunities of *E. lucius* from the Maritsa River showed low Brillouin's diversity index, HB=0.278.

Table 2. Parameters of the infracommunities of *Esox lucius*

	Number of endohelminth species				
	0	1	2	Mean±SD	Range
Esox lucius	6	5	1	0.58±0.64	0-2

	Number of endohelminth specimens			
	Total number	Mean±SD	Range	Brillouin's index HB
Esox lucius	13	1.08±1.32	1-4	0.278

Bunodera luciopercae is an intestinal parasite of fish, representatives of families Percidae, Gadidae, Siluridae, etc. The development of *Bunodera luciopercae* is accomplished with the precipitation of two intermediate hosts. The first is mollusc - *Sphaerium corneum* (Linnaeus, 1758) and *Sphaerium rivicola* (Lamarck, 1818), and the second are crustaceans - *Thermocyclops oithonoides* (Sars, 1863), *Daphnia pulex* (Leydig, 1860), *Mesocyclops crassus* (Fischer, 1853), *Acanthocyclops vernalis* (Fischer, 1853) (Kakacheva-Avramova, 1983).

For Bulgaria, there is data for seven fish species belonging to 4 families as hosts for *Bunodera luciopercae* (see Table 3). River Maritsa is a new locality for *Bunodera luciopercae* in Bulgaria.

Table 3. Overview of registered in Bulgaria hosts of *Bunodera luciopercae* and their locality

Fish host	Locality	References
Family Esocidae		
Esox lucius	Danube River	Kakacheva-Avramova (1977)
Family Salmonidae		
Salmo trutta fario	Arda River	Kirin (2002)
Family Percidae		
Perca fluviatilis	Danube River	Kakacheva-Avramova (1977)
	Arda River	Kirin (2005)
	Srebarna Lake	Shukerova et al. (2010)
Sander lucioperca	Danube River	Margaritov (1959)
		Margaritov (1966)
		Atanasov (2012)
Zingel zingel	Danube River	Kakacheva-Avramova et al. (1978)
Zingel streber	Danube River	Kakacheva-Avramova et al. (1978)
Family Cyprinidae		
Alburnus alburnus	Danube River	Atanasov (2012)

For the conditions of Europe, the typical definitive host of *R. acus* is the northern pike (*E. lucius*) and quite often also the *Salmo trutta fario* (Moravec, 2013).

Raphidascaris acus has a very complex life cycle that may include: exogenous development, development in invertebrates, and development in intermediate host vertebrates and also paratenic hosts (both for second-stage larva and for third-stage larva) and postcyclic hosts (see Moravec, 2013). This nematode species was reported from Bulgaria from 10 fish hosts (Table 4) from different localities.

Table 4. Overview of fish hosts of *Raphidascaris acus*

Fish host	Locality	References
Esox lucius	Maritsa River	Kirin (2013)
*Abramis brama**	Danube River	Chunchukova et al. (2017)
*Neogobius kessleri**	Danube River	Ondračková et al. (2006)
Neogobius melanostomus *	Danube River	Francová et al. (2011)
*Cyprinus carpio**	Srebarna Lake	Shukerova (2006)
*Perca fluviatilis**	Srebarna Lake	Shukerova et al. (2010)
*Barbus barbus**	Danube River	Chunchukova & Kirin (2018)
*Carassius gibelio**	Srebarna Lake	Shukerova (2005)
*Chondrostoma nasus**	Danube River	Zaharieva & Kirin (2020).
Salmo trutta fario	Tamrashka River	Kirin et al. (2020)

*The fish species reported as host of the larval stage of *Raphidascaris acus*

The parasite fauna of *Esox lucius* in Bulgaria was subject to ecogoparasitological investigation in previous studies only from two river ecosystems – Danube River (Margaritov, 1959; Margaritov, 1964; Kakacheva-Avramova, 1977; Atanasov, 2012), and Maritsa River (Kirin, 2006; Kirin, 2013). The spectrum of northern pike parasites differed depending on the number of examined fish, the study site, and various ecological factors. The number of parasite species reported by the above papers ranged between one (Margaritov, 1964) and seven taxa (Kakacheva-Avramova, 1977) (see Table 5). In the earliest study of the parasite fauna of *Esox lucius* from the same river ecosystem, the fish host was free of parasites (Margaritov, 1965). The number and diversity of parasite species reflect the study site, its ecological factors, and the behaviour of the examined specimens. It must be taken into consideration that the northern pike is a selective predator that can influence the fish communities and, at the same time, is influenced by other predators and the available prey (see Craig, 2008). For example, some studies reveal the effects of prey size and gape-size limitation on *Esox lucius* behaviour (Nilsson & Brönmark, 1999; Nilsson & Brönmark, 2000).

For Bulgaria, there is also data for myxosporean parasites of *Esox lucius* from the specific ecosystem of the marshes of Belene Island.

Kakacheva-Avramova et al. (1978) reported *Myxidium lieberkuhni* Bütschli, 1882, *Henneguya lobosa* (Cohn, 1895), *Henneguya psorospermica* Thelohan, 1895 with host *Esox lucius* from the marshes of Belene Island.

Table 5. List of parasite species of *Esox lucius* and their locality registered in river ecosystems in Bulgaria

References / Parasite species	Margaritov (1959)	Margaritov (1964)	Kakacheva-Avramova (1977)	Atanasov (2012)	Kirin (2006)	Kirin (2013)	This study
Diplostomum pseudospathaceum				*			
Azygia lucii	*		*				
Bunodera luciopercae			*				*
Tetraonchus monenteron	*		*		*	*	
Triaenophorus nodulosus		*	*				
Triaenophorus meridionalis			*	*			
Acanthocephalus tenuirostris					*		
Pomphorhynchus laevis			*	*	*		
Raphidascaris acus						*	*
Contracaecum bidentatum			*				
Caryophyllaeus laticeps				*			
Locality	Danube	Danube	Danube	Danube	Maritsa	Maritsa	Maritsa

CONCLUSIONS

As a result of the examination for parasites of twelve northern pikes from the Maritsa River, thirteen specimens belonging to two classes were found. One from class Trematoda (*Bunodera luciopercae* (accidental species)) and one from class Nematoda (*Raphidascaris acus* (core species). *Bunodera luciopercae* is reported for the first time in the river ecosystem of Maritsa. Thereby River Maritsa is a new locality for *Bunodera luciopercae* in Bulgaria.

ACKNOWLEDGEMENTS

This research was carried out with the support of Agricultural University - Plovdiv, providing the laboratory and technical equipment.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

Anurans (*Amphibia*) - VECTORS OF THE PARASITIC AGENTS TO WILD AND DOMESTIC ANIMALS IN MOLDOVA

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Abstract

In this scientific work are exposed data with reference to the description of the diversity of the helminthic fauna of ecaudata amphibians from Ranidae and Bufonidae families, and the determination of its role as vectors for various groups of helminths to wild and domestic animals. As result of helminthological investigations during 2020-2022 years, 17 helminths species (secernentea, trematoda, palaeacanthocephala) was established: H. variegatus Rudolphi, 1819; G. varsoviensis Sinitzin, 1905;C.urniger Rudolphi, 1819; P. robusta Szidat, 1928; P. brumpti Buttner, 1951; P. confusus Looss, 1894; T. excavata Rudolphi, 1803; D. subclavatus Pallas, 1760; O. ranae Froelich, 1791 S. falconis Szidat, 1928; H. cylindracea Zeder, 1800; P. medians Olsson, 1876, C. ornata Dujardin, 1845; O. filiformis Goeze, 1782; I. neglecta Diesing, 1851; S. lupi Rudolphi, 1809; A. ranae Schrank, 1788). Of the 17 helminth species detected in amphibians, a special importance is attributed to the presence of 4 species of medical-veterinary importance helminths (Spirocerca lupi, Codonocephalus urniger, Parastrigea robusta, Strigea falconis), which can cause sterility, parastrigeosis and strigeosis in birds, as well as spirocercosis in dogs, foxes, wolves and occasionally in goat, horse, cattle and other.

Key words*: anurans, Moldova, parasitic agents, vectors, wild and domestic animals.*

INTRODUCTION

In recent decades, the effect of anthropogenic factors on natural ecosystems has been significantly highlighted. One of the consequences of this process was certain changes in the parasitic cenosis of amphibians. During the anthropogenic transformations of the environment, the biological diversity of communities is reduced, their structure is simplified, and a number of ecological niches are released or destroyed. At the same time, invasive species appear, carrying with them parasitic complexes unusual for these ecosystems. This creates a potential risks of zoonotic invasions, such as cercariosis, alaryosis, spirocercosis, etc.

Thus, the helminhstudy of the parasitic fauna and the structure of invasive species is very relevant, because the amphibians can be serve as intermediate hosts (Bolt et al., 1993; Gonz´alez & Hamann 2007; Jackson & Tinsley, 1998; King et al., 2007; Moravec & Kaiser, 1994) or as paratenic hosts (Iacob, 2019; Eberhard & Brandt, 1995; Gonz´alez & Hamann, 2007; Jackson & Tinsley, 1998; Moravec & Skorıkova, 1998; Nickol, 1995), for a wide variety of helminths specific to the vertebrates.

The helminthological and ecological study of amphibians from various types of ecosystems, depending on the degree of synanthropization and contact with other species of invertebrate and vertebrate animals allow the establishment of the parasitological condition, some characteristics in the pathogenesis of the formation of outbreaks of parasitic agents and the elaboration of biological measures to control and prevent the parasitic diseases in productive animals, because in the structure of the helminth fauna of amphibians are some helminths species that are antagonistic to other helminths species specific to the animals (Gherasim, 2016).

The importance of such research is very high, developing a conceptual framework about particularities in helminthology populations of anurans throughout the annual life cycle that will lead to the possibility to identification of new helminths species in the fauna of Moldova, but also, elaboration of new effective and harmless methods of biological control of some helminths specific to productive animals.

MATERIALS AND METHODS

The study of anurans from Ranidae (*Rana dalmatina* Fotzinger in Bonaparte, 1839; *Pelophylax lessonae* Camerano, 1882; *Pelophylax ridibundus* Pallas, 1771; *Pelophylax esculentus* Linnaeus, 1758) and Bufonidae (*Bufo bufo* Linnaeus, 1758; *Bufo viridis* Laurenti, 1768) families was performed in aquatic and terestrial ecosystems from Moldova.

Helminthological research in amphibians was carried out according to the specific research methodology, which involves the examination of all the internal organs of the amphibians (Moravec & Skorıkova, 1998). The obtaining, collection, processing, determination and fixing of the helminthological material was carried after the methods proposed by various authors (Koprivnikar et al., 2006; Koprivnikar & Poulin, 2009; Krone & Streich, 2000; May & Anderson, 1983; Moravec & Kaiser, 1994; Nickol, 1995; Okulewicz, 2008).

After collection, fixing and processing, the assembly of the helminthological material is carried out using paraffin rings according to the method proposed by Seinhorst (1959) and described by Erhan & Gherasim (2022).

In order to assess the risk of vectorization of dangerous helminth species for other groups of animals, the main parasitological indices (II, specimens; EI, %) of helminth species were evaluated depending on the host species.

RESULTS AND DISCUSSIONS

Amphibians as definitive, intermediate, complimentary and reservoir hosts, for various species of parasites, are "vector" organisms which, being obligatory in the development of parasites, constitute the favorable environment for the penetration, development and conservation of evolutionary forms of parasitic agents.

Amphibians are a category of biological vectors that, moving from the aquatic environment to the terrestrial one, ensure development and multiplication, at least for one biological stage, which gives them the role of source of a pathogen and, at the same time, transmits a variety of parasitic forms. They have a special role in the contamination of areas favorable to certain parasites and participate directly in the formation of parasitic zoonoses.

According to the investigations of amphibians from Ranidae (*Pelophylax ridibundus, P. lessonae* , *P. esculentus, Rana dalmatina*) and *Bufonidae* (*Bufo bufo*, *Bufo viridis*) families in Moldova, 17 species of helminths were identified: *Haematoloechus variegatus, Codonocephalus urniger*, *Opisthioglyphe ranae, Paralepoderma brumpti, Prosotocus confuses, Tylodelphys excavate, Diplodiscus subclavatus, Parastrigea robusta, Strigea falconis, Gorgodera varsoviensis*, *Haplometra cylindracea, Pleurogenoides medians, Cosmocerca ornate, Oswaldocruzia filiformis, Icosiella neglecta, Spirocerca lupi,* and *Acanthocephalus ranae*, which from a taxonomic point of view fall into 3 classes, 7 orders, 16 families and 17 genera.

4 of the 17 species of helminths determined in amphibians species previously mentioned, cause various parasitosis common to wild, domestic and pet animals.

In the context of the development of science and the increase of the diversity of animals helminthologically investigated in our country, it is certain that the number of species that are registered in wild, domestic animals and to humans has increased in the last 10 years.

The parasitic diseases of animals (domestic, wild) include spirocercosis, which is caused by the *Spirocerca lupi* Rudolphi, 1809 nematode. This disease is spread all over the world, but in Moldova this nematode, which causes the disease, was detected for the first in 2019 in the southern area.

This species of nematode forms spirocercosis in carnivores (dog, fox, wolf), and accidentally in goats, horses, cattle, pigs, etc., it is located in the esophagus, clinically characterized by digestive, cardiovascular and general disorders (Iacob, 2019).

Another species of helminths with veterinary medical importance found in the investigated amphibians is *Codonocephalus urniger* Rudolphi, 1819 - a trematode with trixene life cycle.

The species of aquatic snails *Lymnaea stagnalis* and *L. palustris* are the intermediate hosts, but the amphibians for this trematode are the complementary hosts. In their body parasitize the larval forms, in the stage of metacercariae of the trematode *Codonocephalus urniger*. The species of birds as *Botaurus slellaris*, *Ixobrychus minutus*, *Ardea purpurea*, *Egretta garzetta* et al, are the definitive hosts. The infection of amphibians occurs from the tadpole stage and ends with adult specimens. The metacercariae has a pathogenic action on the hosts in the sense that in the strong infestations of the genital glands of amphibians with this parasite, total castration is found.

Amphibians stop laying eggs and does not show a reproductive instinct any more. The infested ovary has a dirty yellowish colour and is very small in volume.

Parastrigea robusta Szidat, 1928, from the Strigeidae family is another species of trematode with veterinary medical importance that was detected in amphibians in the muscles and less often on the mesentery. The larval form of this species (metacercarie) is also found in fish (*Abramis brama*, *Atherina mochon pontica*, *Alburnus alburnus* and other). The adult forms are parasitic in the intestines of herons and of day predators, especially of the Ardeiformes order - *Ardea cinerea, A. purpurea*. The infection of birds with trematode of *Parastrigea robusta* species, this is causes parastrigeosis.

The *Strigea falconis* Szidat, 1928 is a trematode species similar to family Strigeidae that was found in amphibians under the muscular fascias around the neck, chest, legs, under the serosa of the esophagus and goiter, in the connective tissue between the trachea and esophagus, under the skin of the neck, chest and legs.

Adult forms parasitize in the bird intestines of different orders: Falconiformes, less often Strigiformes and accidentally Passeriformes (*Oriolus melanocephalus*), Galliformes (*Meleagris gallopavo*), Gharadriiformes (*Charadrius dubius*) and Columbiformes (*Streptopelia chinensis*) causing Strigeosis. The larval forms of meso- and metacercariae are found in birds of the Ardeiformes, Columbiformes, Ralliformes, Steganopodes, Anseriformes, Charadriiformes, Lariformes, Falconiformes, Coraciiformes, Galliformes, Strigiformes, Piciformes, Passeriformes orders, as well in amphibians *P. lessonae* species.

Tylodelphys excavata is another species, wich is characterized by the trixenic life cycle, with the obligatory participation of 3 hosts: 1intermediate host, 2 intermediate host and the definitive host. Marita *Tylodelphys excavata* parasitizes the intestines of storks of the genus *Cocinia*, which are also their obligate definitive hosts. *Planorbarius corneus* mollusk species, in the life cycle of this trematode, are 1 intermediate hosts, in whose body cavities the stage of cercar develops (Erhan & Gherasim, 2022).

The metacercar of *Tylodelphys excavata* is a specific parasite freshwater fish (2intermediate host) and parasitizes in the vitreous body of the eyes.

Of all the investigated amphibian specimens from the Ranidae family, the *P. ridibundus* species (n = 45) was established to be the most common, and assessing the diversity of their helminth fauna, the helminth infestation in 75.0% of cases was established, among which in the aspect of mono invasion in 25.0% of cases, and in aspect of poly invasion in 50.0% of cases (Figure 1).

In the investigated *P. lessonae* specimens (n = 19), it was found that 69.3% of cases were infested, all of which were in the aspect of polyinvasion, while in their hybrid - *P. esculentus* (n = 16), the infestation in 68.0% of cases was established, of which in the aspect of monoinvasion - 18.7% of cases, and in the aspect of polyinvasions the infestation in 49.3% of cases was established, and in the specimens *R. dalmatina* (n = 12), the infestation was registered in 52.0% of cases, of which monoinvasions in 15.8% of cases and respectively 36.2% of cases in terms of polyinvasions (Figure 1).

At the investigated amphibians of the Bufonidae family (*Bufo bufo* n = 30, *B. viridis* n = 11) was established that the infestation predominates in the same way in aspect of polyinvasions, thus in the *Bufo bufo* species the infestation in 88.0% of cases was established, of which in aspect of monoinvasions in 24.0% of cases and polyinvasions in 64.0% of cases, and in *B. viridis* the species the infestation in

85.0% of cases was established, of which in aspect of monoinvasions in 35.0% of cases and respectively 50.0% of cases in terms of polyinvasions (Figure 1).

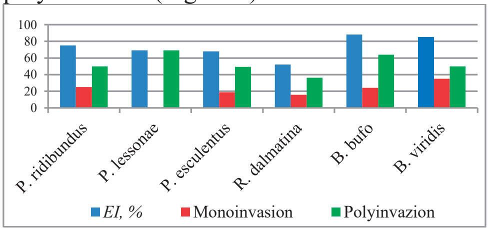

Figure 1. Parasitological indices of amphibians in mono - and polyinvasion aspect

The analysis of the helminthological data obtained during the spring-summer-autumn seasonal period allowed us to establish the nature of the distribution of helminths depending on the host and the research area, with a particularly important value, which contributes to the determination of the seasonal dynamics of the formation of parasitic zoonoses. Of all species of helminths detected in amphibians, 4 species of trematodes (*Tylodelphys excavata, Codonocephalus urniger, Parastrigea robusta, Strigea falconis*) and one species of nematode (*Spirocerca lupi*) are of medical-veterinary importance, causing tylodelphyosis (blindness) in fish, codonocephalosis, parastrigeosis, strigeosis in birds and spirocercosis of insectivorous mammals and canids, respectively.

When assessing the degree of helminth infestation of amphibians, taking into account the area and time of detection of helminths, the age of the host and the parasite, it is possible to assess the period, stations and places of infection of the definitive hosts (fish, reptiles, birds, mammals), which is particularly important for determining the role of amphibians as vectors in the formation and maintenance of outbreaks of parasitic zoonoses. Thus, for the trematode species *Tylodelphys excavata*, *Parastrigea robusta* and *Strigea falconis*, which were determined only in the southern part of the country, the most favorable vectorization period is autumn, for the nematode species *Spirocerca lupi* - summer, but for the trematode species *Codonocephalus urniger* - spring and summer, respectively, which were determined in the center and southern part of the country. Although these data are remarkable for the high abundance of the invasion, only in the spring period were all 5 species of helminths of medical-veterinary importance established (Table 1).

The causes of differences in the degree of infestation of amphibians with helminthes in different biotopes are complex. Thus, the faunal diversity in biotopes populated by amphibians depends of the high abundance of definitive, intermediate, paratenic hosts (mollusks and insects), which determine the degree of infestation with helminthes transmitted through the food chain. The high density of amphibians themselves, as a result, batrachophage predators, leads to their intensive infestation with helminthes in the larval stage.

Table 1. The degree of infestation with helminthes of medical veterinary importance depending on the seasonal period

No.	Invasion	Seasonal period		
		Spring	Summer	Autumn
1.	*Tylodelphis excavata*	40.00%	-	59.50%
2.	*Parastrigea robusta*	5.00%	-	33.30%
3.	*Strigea falconis*	10.00%	42.40%	88.10%
4.	*Codonocephalus urniger*	23.80%	73.90%	-
5.	*Spirocerca lupi*	25.00%	87.90%	-

In addition to the fact that amphibians vector various species of helminths to vertebrate animals (domestic, pet, wild), amphibians also vector species of helminths to invertebrates. In this study, we established that in the case of vectorization of helminthic elements to invertebrate by amphibians, we found that the presence of the trematode species *H. cylindracea* is antagonistic to the Fasciola hepatica species, which causes fasciolosis in farm animals.

In the Republic of Moldova, this disease is quite widespread, and veterinary specialists apply medicinal methods to combat this species of trematodes that cause considerable damage.

It should be noted that these methods have a series of shortcoming: drug treatment involves expenses and consists of the toxic and immunosuppressive action of the antiparasitic preparations on the animal organism treated. At the same time, the way of using these preparations orally presents a difficulty for administration to a large number of animals. As a result of deworming through the administration of anthelmintics, after a short period of time, the parasitic forms (eggs) are eliminated again in the external environment (water, vegetable mass), which then contribute to the infestation and re-infestation of the animals.

The role of amphibians in the biological control of ruminant fasciolosis is explained by the fact that they are definitive hosts of the trematode species *Haplometra cylindracea* which in the cercariae stage parasitizes in the snail species *Lymnaea truncatula* - intermediate hosts for the *species Fasciola hepatica*.

These antagonistic relationships between trematodes *Fasciola hepatica* and *Haplometra cylindracea* trematodes we have realized both in field conditions and in the laboratory conditions. Initially, *Fasciola hepatica* and *Haplometra cylindracea* miracidia were obtained. To achieve the proposed goal, adult fascioles were collected from animals slaughtered at the slaughterhouse, from which eggs were obtained. In the thermostat at a temperature of 24-26^{0}C, over 10 days, from the eggs *Fasciola hepatica* miracidia were obtained.

The miracidia of *Haplometra cylindracea* were obtained by examining all the internal organs of amphibians (*Rana ridibunda, Rana lessonae, Rana temporaria, Bufo viridis*) applying the standard method proposed by the academician Ryzhikov et al. (1928). *Haplometra cylindracea* specimens were kept alive in a thermostat, at a constant temperature of 37^{0}C, in physiological solution.

In order to achieve the method of combating fasciolosis in the laboratory conditions, the experiments were carried out in 3 glass vessels of identical sizes and according to the same scheme.

In the first glass vessel, with a volume of 0.5 liters of water, 50 miracidia of *Fasciola hepatica* and 50 miracidia of *Haplometra cylindracea* were introduced, in a ratio of 1:1.

In the second glass vessel, with a volume of 0.5 liters of water, 50 miracidia of *Fasciola hepatica* and 25 miracidia of *Haplometra cylindracea* were introduced, in a ratio of 2:1.

In the third glass vessel, with a volume of 0.5 liters of water, only miracidia of *Fasciola hepatica* (control batch) were introduced.

During 14 days, at regular time intervals (T = 24 h), the number of *Fasciola hepatica* and *Haplometra cylindracea* miracidia was calculated.

During the experiments, in vessels no. 1 and no. 2 significant results were obtained starting from the second day of contact between *Fasciola hepatica* and *Haplometra cylindracea* species.

As a result, in vessel no. 1, in which the species were introduced in a 1:1 ratio, *Fasciola hepatica* miracidia were absent at the 8th day of research. In vessel no. 2, in which the species were introduced in a ratio of 2:1, *Fasciola hepatica* miracidia were absent at the 9th day of research. In vessel no. 3 (control), there was practically no numerical change in the *Fasciola hepatica* miracidia, a fact that demonstrates its presence in the absence of the antagonistic species *Haplometra cylindracea*.

The results of the experiments are presented in Table 2.

Table 2. The result of the antagonistic action between *Haplometra cylindracea* on *Fasciola hepatica* miracidia

No. day	Vessel no. 1		Vessel no. 2		Vessel no. 3	
	No. of miracidia of *F.hepatica*	No. of miracidia of *H.cylindracea*	No. of miracidia of *F.hepatica*	No. of miracidia of *H.cylindracea*	No. of miracidia of *F.hepatica*	No. of miracidia of *H.cylindracea*
1	50	50	50	25	50	-
2	49	50	48	25	50	-
3	32	50	40	25	50	-
4	24	50	35	25	50	-
5	16	50	22	25	50	-
6	7	50	14	25	50	-
7	3	50	8	25	50	-
8	0	50	2	25	50	-
9	0	50	0	25	50	-
10	0	49	0	25	50	-
11	0	49	0	25	50	-
12	0	49	0	25	49	-
13	0	49	0	24	49	-
14	0	49	0	24	49	-

The experiences in the field were carried in the private cattle breeding farm of SRL "STRAPID", Călăraşi district.

In order to achieve the proposed goal, 100 heads of cattle, aged 3-5 years, were helminthologically investigated by the coprological method - Darling. As a result of these investigations, it was established that cattle were infected with *Fasciola hepatica* in 35.0% of cases.

In the spring, in the household favorable to fasciolosis, the land where the cattle were to graze, parasitological investigations were carried out and the presence of snails of *Lymnaea* genus was established. This land was divided into two lots.

The first lot - experimental, in which 1000 specimens of miracidia of the *Haplometra cylindracea* species were introduced into the environment. The second lot - control, in which *Haplometra cylindracea* miracidia were not introduced.

In the same time, the cattle were divided into two lots of 50 heads each. The research was carried out on cattle over the course of the entire year 2016: in the spring - until the start of the grazing period and in the autumn - before their transition to the stable. Coprological samples were taken, in order to establish the level of fasciola infestation. The results of the experiments are presented in Table 3.

Table 3. The result of using *Haplometra cylindracea* miracidia in combating fasciolosis in ruminants

The experimental lot	**Number of cattle, n**	***Haplometra cylindracea* introduced, n**	**Level of infestation by *Fasciola hepatica* (EI - %)**	
			The fifth month	**The tenth month**
The first experimental lot	50	1000	17.0%	19.0%
The second control lot	50	-	18.0%	84.0%

During the research period of the level of infestation with Fasciola hepatica in the cattle that grazed on first Lot and second Lot, different values were established.

Therefore, during the 5th month, the cattle that grazed on Lot no. 1 and Lot no. 2 with *Fasciola hepatica* in 17.0% and 18.0% of the cases, respectively were infected. Contrary to the research carried out during the 5th month, in the 10th month, in the cattle that grazed on Lot no. 1, in which of the *Haplometra cylindracea* miracidia were introduced, a maintenance of the level of infestation with *Fasciola hepatica* was established (19.0 % of cases), while, in the cattle that grazed on Lot no. 2 (the control lot), in which *Haplometra cylindracea* miracidia were not introduced, the level of infestation of the cattle with *Fasciola hepatica* increased considerably and their infestation was established in 84.0% of cases.

Thus, in order to reduce the risk of contamination of domestic and wild animals with fasciola, it is effective to implement this previously described method, which consists in the biological elimination of fascioliasis in ruminants by introducing amphibians (Amphibia: Anura) which are infested with the trematode *Haplometra cylindracea* in a biotope that is favorable to the development of the trematode *Fasciola hepatica*. This is a low difficulty method, without the toxicity and immunosuppressive action of chemicals, and with minimal expenses.

The advantages of the biological strategies for the dehelmintization of ruminants attributes a new qualitative effect, which allows to increase the productivity and viability of cattle. At the same time, the method can be applied for combating fascioliosis in all affected biotopes.

CONCLUSIONS

It has been studied the helminth fauna of amphibians in the Ranidae and Bufonidae families and has been established by 17 species of helminths: *Haematoloechus variegatus*; *Codonocephalus urniger*; *Opisthioglyphe ranae*; *Paralepoderma brumpti*; *Prosotocus confusus*; *Tylodelphys excavata*; *Diplodiscus subclavatus*; *Parastrigea robusta*, *Strigea falconis*; *Gorgodera varsoviensis*; *Haplometra cylindracea*; *Pleurogenoides medians*, *Cosmocerca ornata*; *Oswaldocruzia filiformis*; *Icosiella neglecta*; *Spirocerca lupi*; *Acanthocephalus ranae* from 3 classes, 7 orders, 16 families and 17 genera.

The presence of 5 species of medical-veterinary importance helminths, (*Spirocerca lupi, Codonocephalus urniger, Parastrigea robusta, Strigea falconis, Tylodelphis clavata*) has been established, which can cause sterility, parastrigeosis, strigeosis in birds, as well as spirocercosis in dogs, foxes, wolves and occasionally in goat, horse, cattle and other and tylodephiosis in fish.

The degree of helminth infestation in amphibians in the aspect of mono- and polyinvasions was evaluated and it was found that in all investigated species the polyinvasion infestation predominates as a result of the coexistence of several helminth species in the same host.

ACKNOWLEDGEMENTS

This research work was carried out with the support of framework of the state projects "Diversity of hematophagous arthropods, zoo- and phyto-helminths, their vulnerability and tolerance strategies to climatic factors and elaboration of innovative procedures for integrated control of species with socio-economic value" no. 20.80009.7007.02 and "Helminthic fauna of amphibians (Amphibia), their importance as vectors in the formation and maintenance of parasitic zoonoses" no. 23.00208.7007.05/PDI

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

OVERVIEW OF ECOSYSTEM SERVICES PROVIDED BY LESSER KESTREL IN ITS MAIN-BREEDING HABITAT IN BULGARIA

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Abstract

The Lesser Kestrel (Falco naumanni, Fleischer, 1818) strongly attached to agro-environmental landscapes, showing high preferences towards extensively managed wheat crops and extensively grazed or otherwise maintained pastures. The colonies of that species are often nesting in urban areas usually surrounded by agricultural fields or open uncultivated grasslands, securing food resources. This defines the species as a typical representative of farmland birds, whose main foraging and breeding habitats in Bulgaria fall into two main types of ecosystems - agroecosystems and grassland ecosystems, and its breeding habitats cover urban ecosystems. The aim of the present study is to assess the potential ecosystem services provide by Lesser Kestrel after recovering the species as a breeder in Bulgaria by Green Balkans NGO. MAES Ecosystem classification and data from the largest colony of the species in the country, located within SPA Sakar, part of the ecological network NATURA 2000 used. As a result, two major ecosystem services provided by the species: the provision of regulating ecosystem services by suppressing arthropods, reptiles and rodents populations and cultural ecosystem services through opportunities of ecotourism, environmental education, birdwatching were identify. Because of the critically endangered status of the Lesser Kestrel in Bulgaria, the species further contributes to the protection of habitats and thus, to the ecosystem services they provide.

***Key words**: agroecosystems, Falco naumanni, farmland birds, grassland, NATURA 2000.*

INTRODUCTION

The Lesser Kestrel (*Falco naumanni*, Fleischer, 1818) is strongly associates with agro-environmental landscapes, showing high preferences towards extensively managed wheat crops and extensively grazed or otherwise maintained pastures (Barov, 2002; Donazar et al., 1993; Franco et al., 2004; Garcia et al., 2006; Kmetova et al., 2012; Parr et al., 1997). The colonies of the species are often located in urban areas, perhaps since at least 2000-2500 years ago (Negro et al., 2000), as they provide nesting sites and reduced nest predation and are usually surrounded by agricultural fields or open uncultivated grasslands, securing food resources (Bustamante, 1997; Hiraldo et al., 1996). This defines the species as a typical representative of the farmland birds, whose main foraging habitats are grasslands, semi-natural grasslands, and cultivated non-irrigated croplands (Morganti et al., 2021; Christakis & Sfougaris, 2021; Assandri et al., 2022). These species are considered as a threatened farmland bird (de Frutos et al., 2010; Tella et al., 2020). As a strategy for the conservation of the Lesser Kestrel, the preservation of traditional cereal cultures with a number of field margins, and low treatment on the fields with biocides has been emphasized (Tella et al., 1998).

While, farmland landscapes provide key ecosystem services, the intensification of agroecological practices in the last century the capacity of these sources has significantly decreased these areas (Tscharntke et al., 2005, Emmerson et al., 2016). The last confirmed records of the Lesser Kestrel breeding in Bulgaria date back to the late 20th century (Iñigo & Barov, 2010). The primary aims of this study were to make an overview of ecosystem services provided by Lesser Kestrel after the reintroduction as a breeding species in Bulgaria by Green Balkans NGO (Gradev et al., 2016a) in its main-breeding habitat in Bulgaria.

MATERIALS AND METHODS

The study was carried out in the area of Levka village, Sakar SPA (BG0002021), part of the ecological network NATURA 2000 (MOEW 2013), where the species has been recovered as breeder in Bulgaria by Green Balkans NGO (Gradev et al., 2016a). For this purpose, a Lesser Kestrel Release and Adaptation Module (LKRAM) has been established. The building where LKRAM is located also houses the Environmental Centre of Levka village which has a demonstration and information hall. In the Environmental Centre the hosts offer the following options for tourists and guests: observation of Lesser Kestrels and other rare birds from the region; presenting various decorative nest box models, models of Lesser Kestrels and their eggs; diorama with the hunting and nesting habitats of the Lesser Kestrels, as well as other species typical for the region like Eastern imperial eagle, European ground squirrel, etc.; thematical lectures about the biodiversity of SPA Sakar; video surveillance of the Lesser Kestrels' colony; videos and films about environmental protection; information materials and souvenirs; educational activities for kids and teenagers; work with volunteers and trainees (www.lesserkestrellife.greenbalkans.org). This is Bulgaria's largest colony of the species, with the birds nesting primarily in nest boxes specially designed and placed to support the individuals of the restored colony. In addition to this one, there are two more colonies in our country at the moment (Gradev et al., 2021). In biogeographical terms, the area falls into the Southern biogeographical region and, more specifically, according to the biotic basis, it refers to the "Dolnomarishko - Dolnotundzhansky" subregion (Gruev & Kuzmanov, 1999), as Mediterranean influence penetrates the sub-region along the Maritsa and Tundzha rivers' valleys. The Lesser Kestrel is included in the subject and conservation objectives of the Sakar SPA (State Gazette 2010), it is subject of conservation under Annex 2 and Annex 3 of the Biological Diversity Act. According to Red Data Book of Republic of Bulgaria, conservation status of the species in Bulgaria is Critically endangered (CR) (Barov et al., 2015). At the international level, under the IUCN Red List of Threatened Species, Lesser Kestrel listed as Least Concern (LC) (BirdLife International, 2021) and protected species by the EU Birds Directive 2009/147/EC, listed in Annex 1.

In order to determine the habitats used by the falcons in the target area, combined data from radio-telemetry of Lesser Kestrel (Zhelev et al., 2016), satellite tracking of birds originating from the recovered colony in Levka (Gradev et al., 2016b), and direct visual observations of birds of prey in the area were used. The established home ranges from these surveys cover areas ranging in size from 29.70 to 46.80 km2 which are significantly overlapping in the field.

In order to determine the type of ecosystems and the ecosystems services potentially provided by them, data from project "Improving Bulgarian Biodiversity Information system", Activity №2 "Module for collecting, mapping and analysis of the status of the ecosystems and their services" and "Guidelines for Monitoring the Status and Development of Ecosystems and Ecosystem Services" (Chipev et al., 2017) was used. Also, the approach to evaluate ecosystem services related to the Lesser Kestrel and their habitats based on the MAES Ecosystem type is used (Maes et al., 2018).

RESULTS AND DISCUSSIONS

Our data on the foraging behaviours support the classification of the Lesser Kestrel as a species associated with agroecosystems and grassland ecosystems (Chipev et al., 2017). Based on Mapping and Assessment of Ecosystems and their Services - MAES (Maes et al., 2018) the habitats of Lesser Kestrel fall into 4 basic ecosystem pilots - Agroecosystem pilot, Urban pilot, Soil pilot and Nature pilot, and out of 12 described Ecosystem types, the habitats of the species are covered by a total of 3 types (25%) - Cropland, Grassland and Urban.

We identified two main categories of ecosystem services provided by the Lesser Kestrel - Regulating ecosystem services (suppressing arthropods, reptiles and rodents) and cultural ecosystem services (ecotourism, environmental education, birdwatching, conservation of natural resources, etc.) (Figure 1). In general, there are four categories of Ecosystem services described

- provisioning, regulating, supporting, and cultural services which also are provided by birds (Michel et al., 2020).

Figure 1. Lesser Kestrel with captured prey *Tettigonia* sp.

Lesser Kestrel in its main habitat in Bulgaria provide 50% of categories described for birds. (Figure 2).

Figure 2. Grassland ecosystems in Sakar SPA Lesser Kestrel's foraging habitats

Regulating ecosystem services provided by the Lesser Kestrel

These birds feed mainly on large insects (Orthoptera, Coleoptera) (Kok et al. 2000), lizards and some small mammals (Parr et al., 1997). The species is described as primarily insectivorous, as in Europe its prey consists mainly of grasshoppers, beetles and Myriapoda (Rodriguez et al., 2010). In Thessaly, Greece previous work has found that 98.9% of prey are arthropods (insects and centipedes), while mammals comprise only 0.9% (Makri et al., 2018). In Albania the diet of the species consists of mainly invertebrates and more specifically orthopteran insects (Krištín et al., 2020). Data from Turkey suggest food preferences is mainly invertebrates, especially Orthoptera and Coleoptera; however, Rodentia and Sauria fragments (lizards), are frequently encountered in pellets (Avci, 2018). For the colony in Levka village, Sakar SPA that we study, the species diet comprises Orthoptera, *Scolopendra sp.*, Cicadidae, and Coleoptera: 94% of observed food items used by the Lesser Kestrel are insects, 4% are rodents Muridae and Arvicolinae, and 2% reptiles, mainly green lizard (*Lacerta viridis*) (Mihtieva, 2015). Similar data are obtained from the analysis of a total of 54 pellets collected during May 2022 (Figure 3).

Figure 3. Collected pellets from Lesser Kestrel

Their analysis led to the identification of 74 prey items.

During the breeding season Lesser Kestrels, were feeding mainly on Coleoptera (60.5%), Hymenoptera (17.65%) Orthoptera (5.88%), as well as Scolopendromorpha (3.36%) and Rodentia (8.4%) (probably voles). *Bradyporus dasypus* recorded by us on 29.06.2014 and reported by Mihtieva, 2015, is not so frequent prey items for the birds from the colony in Levka village. We have also observed several cases when Lesser Kestrels' prey included *Passer sp*. These were mainly pulls whose nests situated in close proximity to the nest boxes where Lesser Kestrels were breeding. In the same colony, we have not detected even a single case of European Mole Cricket (*Gryllotalpa gryllotalpa*) among prey items unlike other colonies in neighbouring countries – Greece and Türkiye – where that is one of the most common preys of the Lesser Kestrel. Our field studies have revealed that prey items vary according to the life cycle of the prey and its abundance during different period of the Lesser Kestrel's breeding season. On 02.07.2020 in one of the checked Lesser Kestrel nest boxes were found 11 Common Voles (*Microtus arvalis*) (Figure 4).

Only one pair raising two chicks captured the prey in the morning hours no later than noon. This high intensity of foraging and oversupply with food is most probably caused by higher density and accessibility of prey used during that period.

Potential reasons for that are typical agricultural activities like ploughing of fields, hay mowing or other agricultural activity that expose voles making them easier prey for the Lesser Kestrel, as they prefer foraging habitat with low vegetation (Cioccarelli et al., 2022). The capture of House Mouse (*Mus musculus*) by Lesser Kestrel, is also registered by us, but in much rarer cases.

Figure 4. *Microtus* sp. caught by a Lesser Kestrel just a half day

Relative calculations of mass and quantity of consumed prey show that the studied colony uses as food hundreds of kilograms of insects, small mammals and reptiles. Petrov et al. (2022) reports that the minimum amount of food for raising a single Lesser Kestrel in ex-situ conditions during the breeding season is approx. 80 g of cut rats or 40 g chicken hearts, or 2 pcs mice, 2 pcs day-old chicks, as total mass of mice and day-old chicks is approx. 60-80 g/day.

Based on this data, we can estimate the minimum amount of biomass that is required for the survival of a Lesser Kestrel in the wild. Probably for the free-living birds, which expend much more energy, it is higher than 80 g/day, but still this value can be conditionally accepted for the purposes of the present study. Considering that in 2022 in Lesser Kestrel colony, in Levka village, Sakar SPA (BG0002021) about adult 50 birds were observed, of which 17 pairs successfully raised young with Fledging success (FS), which is 3.58, hypothetically the following values can be calculated:

Table 1. Amounts of biomass per 2022 required for the survival of a Lesser Kestrel in the wild

Biomass used from adult birds during 01.04. - 31.07				
Total number of days in colony	Total number of birds in colony (individuals)	Food per day for one bird (kg)	Fledging success (individuals)	Used biomass (kg)
122	50	0.08	n/a	**488**
Biomass used from juveniles 01.06 - 31.07				
Total number of days in colony	Total number of successful breeding pairs	Food per day for one bird (kg.)	Fledging success (individuals)	Used biomass (kg)
62	17	0.08	3.58	**301.87**
Total used biomass from colony for one breeding season				
				789.87

Considering the amount of total biomass (over 789 kg) potentially exploited by the colony, as well as the individual mass of some of the taxa most commonly used for food by the Lesser Kestrel, the number of exploited individuals that are agriculture pests (Table 2).

Of course, the number of individuals of the individual species could be in these values only if the given taxon would be the only prey for the birds of the colony during the entire breeding season. Given that Lesser Kestrel always use a variety of prey and catch the most common or the most abundant prey, the number of individuals is most likely a combination of the above-described species in different proportions.

The data from the both Tables (1, 2) indisputably confirm regulating ecosystem services which Lesser Kestrel provided agroecosystems and prove the positive effect that this species has in maintaining biological control in agriculture.

Table 2. The calculated number of used prey individuals of Lesser Kestrel in SPA Sakar

Prey taxon	**Source**	**Average ind. mass (kg.)**	**number of used individuals (thousands)**
Microtus arvalis	Popov & Sedefchev, 2003	0.04	19,747
Mus musculus	Popov & Sedefchev, 2003	0.02	39,493
Chilopoda	Rodriguez et al. 2010	0.0023	343,420
Gryllotalpidae	Rodriguez et al., 2010	0.0035	225,676
Gryllidae	Rodriguez et al., 2010	0.00067	1,178,904

Cultural ecosystem services provided by the Lesser Kestrel

All activities provided in the demonstration and information hall of the Environmental Centre in the LKRAM building are directly related with this type of ecosystem services - ecotourism, environmental education, birdwatching, conservation etc. In the yard of the Centre created the only photo hide providing opportunity for photographing Lesser Kestrels in the country. Approx. 300 people annually visit the Centre and learn about the Lesser Kestrel and its habitats. Given that the permanent number of people in the village of Levka is ~300, the centre provides an important boost to the local economy.

Every year over 50 children from three different schools in Levka and the municipal centre town of Svilengrad visit the Centre. In addition to participating in lectures about the kestrel and biodiversity at Sakar SPA, children are involved in various training, painting, quizzes and other educational activities. The centre is also a place for conducting practical trainings and developing Bachelor, Master and PhD theses, as well as scientific papers. Together with professors and students from the leading universities in Bulgaria in the field of ecology, biology, veterinary medicine, zoo engineering and other Earth Sciences, including Agricultural University - Plovdiv, Trakia University - Stara Zagora, University of Plovdiv - Paisii Hilendarski, Sofia University, University "Prof. Dr. Asen Zlatarov" - Burgas and others. The Green Balkans team works to improve the qualifications of young people.

Green Balkans' Environmental Centre in the LKRAM is in partnership networking with the Historical Museum in Svilengrad, BSPB's Nature Conservation Centre Eastern Rhodopes in Madjarovo, Green Balkans' Wildlife Rescue Centre in Stara Zagora, and many others from which both organized and individual tourists and groups interested in nature in the region. In the yard and Information Hall of the LKRAM, exhibitions, events and Plain-Air dedicated to the International European Green Belt Initiative (organized in partnership with European Green Belt Association and EuroNatur), European Natura Day 2000 organized by the European Commission are held every year.

In addition, some initiatives of other European programs held here - Erasmus, INTERREG-IPA CBC Bulgaria - Türkiye Programme, etc. as well as many other informational, educational and public events.

CONCLUSIONS

As a result, two categories of ecosystem services provided by the Lesser Kestrel: the provision of regulating ecosystem services by suppressing arthropods, reptiles and rodents populations and cultural ecosystem services through opportunities of ecotourism, environmental education, birdwatching were identify. Because of the critically endangered status of the Lesser Kestrel in Bulgaria, the species further contributes to the protection of habitats and thus, to the ecosystem services they provide. Strong correlation between biodiversity and cultural ecosystem services was found. Considering that birds are helpers of man in the fight against harmful species, further efforts have done for conserving Lesser Kesrtrel and birds in general. This will contribute to the preservation of biodiversity and sustainable development at the regional level. Cultural ecosystem services (ecotourism, birdwatching and education), will increase public interest in biodiversity conservation and this will lead to improved human well-being.

ACKNOWLEDGEMENTS

These results achieved through "LIFE for Lesser Kestrel" LIFE19 NAT/BG/001017 project funded by the LIFE program of the European Union.

This work was partly supported by the Bulgarian Ministry of Education and Science under the National Research Programme "Healthy Foods for a Strong Bio-Economy and Quality of Life" approved by DCM # 577/17.08.2018

We are indebted to Dr. Nedko Nedyalkov, theriologist at National Natural History Museum - Bulgarian Academy of Sciences for the identification of Common Voles and Associate professor Dr. Daniela Miteva for the critical review on the manuscript.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

THE REDISCOVERY OF *Lycaena helle* (Lepidoptera: Lycaenidae) IN DORNA DEPRESSION (ROMANIA), 125 YEARS AFTER ITS FIRST MENTION

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Abstract

At present, the known populations of Lycaena helle from Romania are found at a lower altitude than those from Western and Central Europe. Besides land-use changes, climate warming severely threatens this specie that prefer humid and cool habitats. Withdrawal to higher altitudes is also restricted by the species' low dispersal ability. Therefore, the future of the Romanian population is uncertain. While implementing a peatlands restoration project in the northeastern part of the country, we investigated the local invertebrate fauna around a bog woodland from Dornelor Depression (Suceava County). Here we found a mosaic of wet habitats sheltering a violet copper population. This population was mentioned long ago, representing the first recorded instance of this species in present-day Romania. The violet copper butterfly has not been spotted in the area since the initial record was published in 1897, and current literature only refers to it as historical data from the Dorna Depression. We describe the habitat occupied by this population, bring up to date the specie's distribution, and prompt for the designation of a special area of conservation for this European protected species.

Key words: *Bistorta officinalis, Lycaena helle, protected species, Romania.*

INTRODUCTION

Lycaena helle (Denis & Schiffermüller, 1775) is one of the smallest endangered coppers with a wing span smaller than 27 mm. Males have a distinctive, broadly distributed, purplish-blue iridescence on the upper side of the wings; this iridescence is more restricted to the marginal area of the wings in females. The males of the second generation are less violet-iridescent, and therefore they seem to be darker. The orange colour on the females' dorsal side of the wings varies within each generation (Lafranchis, 2004; Tshikolovets, 2011; Craioveanu et al., 2014; Settele et al., 2015; Leraut, 2016, Gergely et al., 2017). Due to the populations' isolation, many other subspecies were described in Europe, and their validity needed to be genetically confirmed (Rakosy, 2013).

The butterfly is bivoltine in Eastern Europe and has a lifespan of 7.6 days for the first generation and 3.3 days for the second generation (Craioveanu et al., 2014). The larvae are feeding on the lower side of the leaves of *Bistorta officinalis*, causing characteristic feeding scars, and the adults use the same plant species as primary nectar sources (Székely, 2008). This butterfly inhabits wetlands, sometimes (like in Romania) loosely wooded, with patches of shrubs, trees, and host plants (Székely, 2008; Rákosy, 2013).

At a European scale, following the Red Data Data Book of European Butterflies, this species is endangered (EN) and it is protected by the Council Directive 92/43/EEC of 21 May, 1992, (Annexes 2 and 4) on the conservation of natural habitats and of wild fauna and flora (Van Swaay & Warren, 1999; Van Swaay et al., 2000). In Romania, this species is critically endangered (CR)/endangered (EN), being protected through the Emergency Ordinance no. 57/2007 regarding the regime of natural protected areas, conservation of natural habitats, flora and fauna (Annexes 3 and 4 A) (Rákosy, 2021; Rákosy et al., 2021).

Due to climate warming and land-use changes, including improper forest management and abandonment of grassland, the populations all over Europe are threatened with extinction (Rákosy L., 2013; Craioveanu et al., 2014; Modin & Öckinger, 2020) and a loss of connection within a metapopulation is often considered when favorable habitats are still available (Mutanen & Välimäki, 2014).

In Romania, the populations of this butterfly are still present in three Sites of Community Importance (SCI) within the Continental region: ROSCI0205 Poienile cu narcise de la Dumbrava Vadului, ROSCI0214 Râul Tur and ROSCI0421 Pădurea celor Două Veverițe, but there are no recent data on the existence of viable population in Alpine region (Craioveanu et al., 2014).

Our primary goal was to conduct an invertebrate inventory in the areas where peatland restoration activities are being implemented. In the context of the rediscovery of this population of rare butterfly species, we present here a literature analysis regarding this species in Romania, clarify the taxonomic identification of some specimens, and discuss the importance of setting protection measures for this population of *Lycaena helle*, and its mosaic habitats that support this species.

MATERIALS AND METHODS

The Institute of Biology Bucharest implements two projects, aiming to restore 19 peatlands covering 802 ha in Suceava, Maramureș, Brașov, and Sibiu counties, Romania. The wetland where *Lycaena helle* was found is near Coșna village, Suceava County, in the Teșna river basin. Teșna left tributaries Tocila, and Bancu brooks define the studied area. After the junction with Bancu, Teșna flows into the Dorna River. Morphologically, the whole area belongs to the intramontane depression of Dorna.

In May 2022, we conducted an initial invertebrate fauna survey through direct visual observation with no capture, aiming for several invertebrate groups. On-site, observed butterfly species were noted and photographed, and the GPS coordinates of each observation point were recorded. Adult butterfly individuals were identified to species level using identification keys (Lafranchis, 2004; Tshikolovets, 2011; Settele et al., 2015; Leraut, 2016, Gergely et al, 2017).

In a follow-up visit to the site in June 2022, we used a DJI Mini 2 drone to evaluate the specific habitat distribution and the extent of the host plant distribution. The aerial imagery was integrated with a botanical survey conducted on the ground to delineate the critical area for the survival of the *Lycaena helle*. Plant nomenclature used for the listed taxa is the one agreed by Flora Europaea through Euro+Med PlantBase, with up-to-date information on the taxa present in the European and Mediterranean region (https://europlusmed.org/). The phytosociological nomenclature follows the synthesis works on the vegetation of Romania (Coldea et al., 1997; Sanda et al., 2008; Doniță et al., 2009).

To update the distribution of the species in Romania, we reviewed the literature to clarify older records, especially indirect citations. Specimens from Oltenia Museum - Department of Natural Sciences, Craiova (catalogue numbers 33534, 33535, 33536, 33537) were revised by one of the authors. The updated distribution map of the violet copper in Romania, based on the map from The Red Book of Romanian Invertebrates (Rákosy, 2021), was generated in RoBioAtlas WebApp. The habitat map for *Lycaena helle* population in Coșna was produced using the collected data in ArcGIS 10.7.1.

RESULTS AND DISCUSSIONS

Field data

At least 30 specimens of *Lycaena helle*, both males and females, were observed and photographed within a half an hour at a single observation point in an area covered with *Bistorta officinalis*, close to the border of the forest situated N-V of Coșna primary school, on 17 May 2022. After examining photographs taken in the field, we also identified several specimens in the area right behind the school, 250 m from the first point. The temperature was 19ºC, the sky was clear, and the wind speed was five kph. Fresh adults were on wing, feeding on species of *Viola sp., Cardamine pratensis, Ranunculus acris,* and *Ranunculus repens*, basking on *Bistorta officinalis* (not yet in with flowers), or even mating (Figure 1). On 12 June 2022, at the second visit, the temperature was 22ºC, the sky was partially cloudy, and the wind was 20-30 kph. While the flowers of *Bistorta officinalis* were now open (Figure 2), the butterflies were not active, hiding in the lower part of the vegetation, mainly because of the unfavorable weather.

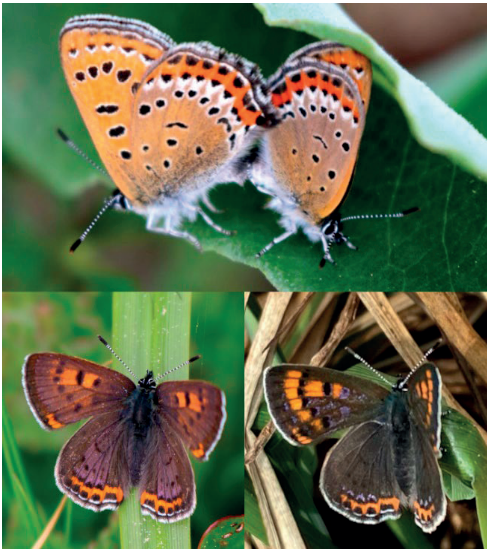

Figure 1. *Lycaena helle* specimens from Coșna, Romania

The area covered by the favorable habitat for *Lycaena helle*, delimited based on the vegetation study and aerial images, was no more than 22.2 ha (Figures 3 and 4) and in close proximity to human settlements.

We found a complex landscape within the area characterized by patches of wet meadows with eutrophic ecology and under different land uses that alternate with the bog woodland. These meadows, developed on a 1.2-4 m thick peaty soil (Pop, 1960), originate from the denaturation of some parts of the bog by draining and cutting trees and shrubs. They grow on flat or slightly sloping land with a high humidity regime, ensuring the soil has excess water throughout the year.

Three types of meadows were identified, creating a mosaic beneficial for butterfly species in general and for *Lycaena helle* in particular.

Fenced and ungrazed wet meadows groups, a series of hygrophilous phytocenoses that belong to *Scheuchzerio - Caricetea fuscae* R. Tx. 1937 and *Molinio - Arrhenatheretea* R. Tx. 1937 (Figure 2). With a coverage between 80-90%, the herbaceous layer has a very special compact physiognomy, especially during the flowering period of *Bistorta officinalis*, the dominant species. In the floristic composition, *Rumex acetosa*, *Cirsium rivulare*, *Ranunculus repens*, *R. acris*, *Succisa pratensis* can be found, and isolated bushes of *Salix repens subsp. rosmarinifolia* and *S. caprea* were also reported.

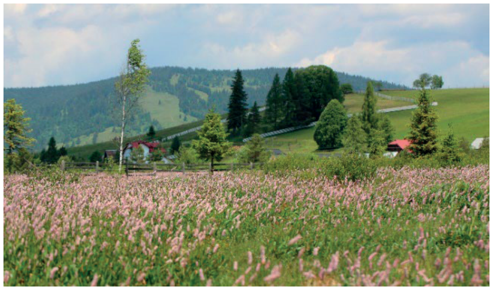

Figure 2. Fenced and ungrazed wet meadows with *Bistorta officinalis*

Figure 3. Aerial photo of the mosaic of habitats inhabited by *Lycaena helle*, Coșna, Suceava, 12 June, 2022

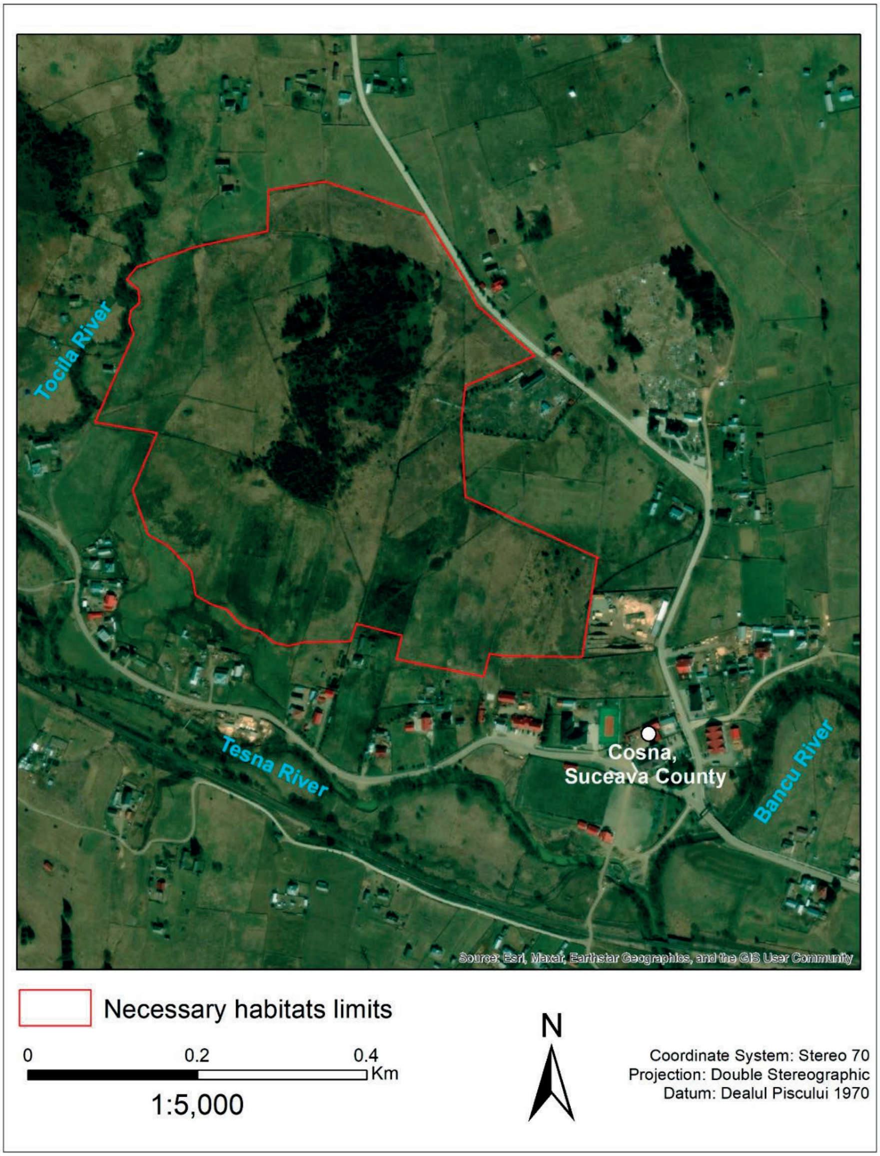

Figure 4. Boundaries of the critical habitats for the survival of the *Lycaena helle* population from Coșna area, Romania

Interleaved with the previous, low-intensity grazed meadows with poor floristic composition characterizes the *Agrostio stoloniferae - Deschampsietum caespitosae Ujvarosi* 1947 and *Juncetum tenuis* (Diemont, Siss. et Westhoff 1940) Schwik. 1944 phytocenoses; among the dominant species: *Deschampsia caespitosa*, *Agrostis capillaris*, *A. canina*, *Ranunculus repens*, *Trifolium repens, T. pratense*, *Caltha palustris* s.a. (Figure 5).

The marshy land behind the Coșna elementary school is an open habitat with scattered trees species of *Picea abies*, *Betula pendula*, *Frangula alnus*, *Salix capraea*, next to numerous *Betula humilis* and *Salix repens* subsp *rosmarinifolia* shrubs. In the upper herbaceous layer we recognised *Filipendula ulmaria*, *Succisa pratensis*, *Comarum palustre*, *Cirsium rivulare*, *Dryopteris cristata*, *Epilobium palustre*, *Valeriana officinalis*, *Equisetum* *palustre*, *Crepis paludosa*, *Lysimachia vulgaris*, *Rumex acetosa*; among other small species that make up the lower herbaceous layer: *Galium aparine*, *Galium palustre*, *Galium uliginosum*, *Caltha palustris*, *Plantago lanceolata*, *Potentilla erecta*, *Viola declinata*, *Galeopsis speciosa*, *Cardamine pratensis*, *Agrostis canina*, *Campanula abietina*, *Carex diandra*, *Vaccinium oxycoccos* (Figure 6).

Figure 5. Low-intensity grazed meadows

A series of drainage channels cross these meadows, hosting hygrophilous species such as *Typha angustifolia, Carex rostrata*, *Carex nigra*, *Mentha aquatica*, *Menyantes trifoliata*, *Potamogeton natans* and *Callitriche palustris* where the water is more than 40-50 cm deep.
The violet copper specimens were spotted near the edge of the bog woodland in a wet meadow with large stands of *Filipendula ulmaria* along with *Thelypteris palustris*, *Ligularia sibirica*, *Parnassia palustris*, *Potentilla anserina*, on *Sphagnum* substrate (Figure 7). Here, the host plant *Bistorta officinalis* is distributed in uneven size patches and ensures variable coverage that can go up to 80%.

Figure 6. Marshy land with *Betula humilis*

The bog woodland vegetation is assigned to *Vaccinio-Pinetum sylvestris* Kleist 1929 em. Matuszk. 1962.

Figure 7. Wet meadow at the bog woodland edge

The floristic composition of the bog is relatively poor due to the restrictive conditions offered by the oligotrophic swamp. In the structure of the plant community, four layers can be defined, respectively: the arborescent layer, which has as its dominant species *Pinus sylvestris* with rare individuals of *Betula pendula*, *Sorbus aucuparia* and *Rhamnus frangula*, and together achieve a coverage of 50-70%; the shrub layer includes the juveniles of *Pinus sylvestris* with *Betula pendula* and isolated bushes of *Betula humilis*; the layer of grasses and small shrubs is dominated by *Vaccinium vitis-idaea*, alongside whith *Vaccinium myrtillus*, *V. oxycoccos* and *Eriophorum vaginatum* and achieve large coverages, up to 70-80%, where the tree layer is less developed. The moss layer is well-developed and dominated by *Sphagnum* species (Figure 8).

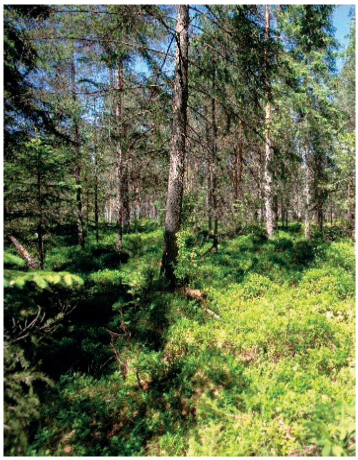

Figure 8. The bog woodland

Literature and museum data

Older papers on *L. helle* in Romania have almost exclusively faunistic data, but recently semi-

quantitative data on population size and structure, along with the dispersal capacity of individuals, were published based on studies of existing populations in Maramures county (Craioveanu et al., 2014).
Based on the literature published, we compiled a timeline of data regarding *L. helle* presence and extinctions in Romania (Figure 9).

Most of the older discovered populations are considered extinct: Sighișoara (Czekelius, 1899; Rákosy & Weber, 1986), Chitila near Bucharest (Salay, 1910; Szabó, 1982), Valea Cernei at Crucea Ghizelei (Rebel, 1911, Craioveanu et al., 2014), Vlădeni Brașov (Ciochia & Barbu, 1980) Livada 1 Satu-Mare (Bálint & Szabó, 1981; Craioveanu et al., 2014).

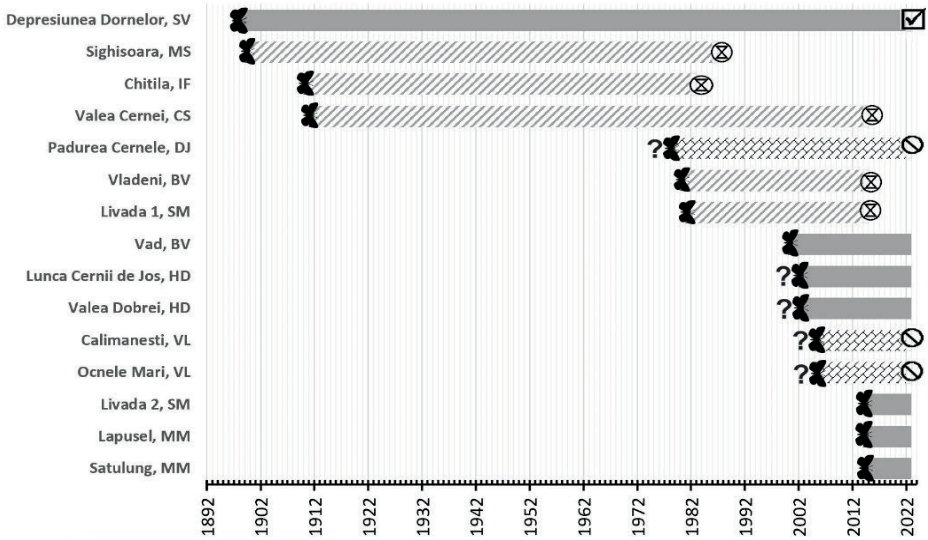

Figure 9. Timeline of literature data regarding *Lycaena helle* presence in Romania
(The butterfly symbol indicates the year of the first mention in the area. A question mark beside the first mention indicates a doubtful record. The grey horizontal bar marks the populations still viable or unproven to be extinct. Diagonally hatched bars symbolize the populations declared extinct, and the hourglass symbol points to the year of the published extinction. Brick-hatched bars mark the populations proven here to be wrong species identification. Check-mark symbol points to the year of confirmation in the field of the population in Dornelor Depression)

The revision of the specimens on which the doubtful report from Cernele Forest, Dolj county (Stănoiu et al., 1978), Călimănești and Ocnele Mari, Vâlcea county (Chimişliu & Goga, 2005) were based, proved that in fact, these were misidentified specimens of the congeneric large copper - *Lycaena dispar rutila* (Werneburg, 1864) (Figures 10, 11).
The exact location of several other populations, e.g. those from Hunedoara county, Lunca Cernii de Jos, and Bătrăna-Bunila (Dobrei valley) (Burnaz, 2002), is unknown. These locations have never been checked out or evaluated following the publication of these records. Hence, up to now, the only known viable populations that still survive are those from Vad (Brașov) (Székely et al., 2000), Livada 2 (Satu-Mare) (Craioveanu et al., 2014), Lăpușel and Satulung (Maramureș) (Craioveanu et al., 2014) all within the Continental region and situated at low altitudes, up to 500 m.

Figure 10. *Lycaena dispar rutila,* specimen no. 33.535 from Oltenia Museum, Craiova

Figure 11. *Lycaena dispar rutila,* specimen no. 33.537 from Oltenia Museum, Craiova

Hormuzaki's initial report (1897) is the first one for *Lycaena helle* on the present day territory of Romania. In the years to come, this record has been repeated, without being supported by new data, by several authors (e.g., Fleck, 1900; Pax, 1906). Following some misinterpretations, several recent authors (Dincă & Goia, 2005) have erroneously attributed to Pax (1906) a record of the violet copper from Rodnei Mountains. However, in the text, the author only mentions other nearby areas with Alpine elements and mentions the Dorna region with a list of butterfly species quoting Hormuzaki (1897). In his paper on the butterfly fauna of Bucovina, Hormuzaki (1897) listed *L. helle* under the name *Polyommatus amphidamas*: "in our country only in the higher mountains; on the peat bogs in the upper Dorna valley at the end of May 1894 we captured a large, vivid violet iridescent specimen". Hence the population we found near Coșna, between tributaries of Dorna river, might be a part of, or a remnant of, the long-ago reported population found by Hormuzaki, more than 125 years ago.
Found in an area at about 959 m altitude, the population from Coșna it is also the highest reported altitude for a purple copper population in Romania and the only one found at an altitude comparable to that at which similar populations are found in the Pyrenees or in Central Europe, thus distinguishing itself from the other three viable populations of this butterfly from Transylvania. It is also the only population of this butterfly found on the territory of Romania within the Alpine bioregion.
In the updated distribution map, superimposed with the bioregion map of Romania, the rediscovered population is marked with a red triangle (Figure 12). Old records with blue dots, and viable present-day populations with red dots. Light green represents the Continental region, while the Alpine region is in a darker green shade.

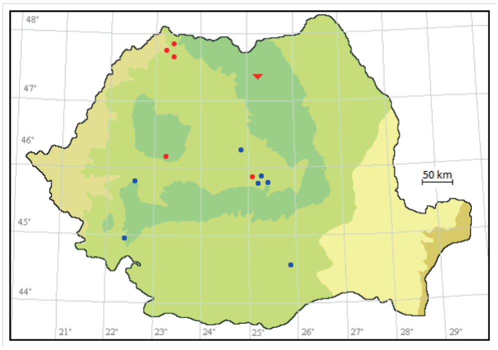

Figure 12. Distribution map of *Lycaena helle* in Romania

Future studies on this population will have to focus on a better estimation of the population size and the threats that confront it since the cover in *Bistorta officinalis* is considerable, and the opened, mixed habitat needed to support a large population of violet copper is found over a relatively large area (Székely, 2008; Rákosy, 2013; Craioveanu et al., 2013). Also, as the species is known to form metapopulation (Modin & Öckinger, 2020), and there are several peatland and wetlands areas around, we will further look for the host plant and individuals of this species while implementing the reconstruction projects.
Another aspect to consider is whether the population here is uni or bivoltine due to the high altitude and lower temperatures. While in mid-September 2022, there were still some flowers of *Bistorta officinalis*, it was too late in the year, even in a bivoltine situation as is the case of the other populations in Romania (Székely, 2008; Craioveanu et al., 20144).
The two points where the violet copper was spotted at Coșna, are within the average flight distance measured for the population in Maramureș county (Craioveanu et al., 2014). This small distance can ensure an exchange of

individuals necessary for the population's survival. However, the terrain at the back of the school was considered for urban development by the local administration.

The main threats of *L. helle* are climate warming, land-use changes (drainage, peat extraction, afforestation, forest grazing, transformations into arable land, urbanization), burning, and chemical treatment (Mutanen & Välimäki, 2014). Lack of appropriate forest management (clear cuts of the whole forest body or large area cuts at the edge of the forest body) and the abandonment of grassland are the leading causes of the species decline (Rákosy, 2013; Craioveanu et al., 2013).

Being situated in the Alpine area, unlike the other viable populations in Romania, the population from Coșna could remain the only refuge of the species in Romania, in case of climate warming. This aspect emphasizes the importance of our discovery, the need for further research, and immediate measures to protect the area, at least within the boundaries delimited in Figure 4. It is important to note that rare and protected plant species exist in the area. The terrain at the back of the school hosts numerous plants of *Betula humilis* an important glacial relict included in the red plant list of Romania (Oltean et al., 1994) and considered critically endangered (CR) at the national level (Dihoru & Negrean, 2009). Close to the forest edge, we also found *Ligularia sibirica.* This plant is also a rare glacial relict plant of great conservative value, protected at the European level (Annex 2 and 4 Habitat Directive) and threatened by the drainage of marshlands (Mânzu & Cîșlariu, 2019)

CONCLUSIONS

Careful examination of old literature, now easier to access through digitalized libraries, can reveal important information on rare species distribution.

When designed with care for flora and fauna, habitat restoration projects can enhance knowledge and help species protection.

Protection and careful management measures must be implemented to protect this rare butterfly population, rediscovered after 125 years of its first mention, and the other rare plant species found in the area.

ACKNOWLEDGEMENTS

This research work was funded by project no. RO1567-IBB03/2023 through the Institute of Biology Bucharest of Romanian Academy and the EEA Grants: PeatRo2 and PeatRo3 Projects.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

STUDIES ON THE HELMINTH FAUNA OF TWO FISH SPECIES OF THE GENUS *Ballerus* Heckel, 1843 FROM THE BULGARIAN SECTION OF THE DANUBE RIVER

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Abstract

During the period 2019-2021, the helminth fauna of two species of freshwater fish of the family Cyprinidae, genus Ballerus Heckel, 1843 - white-eye bream (Ballerus sapa (Pallas, 1814)) and zope (Ballerus ballerus (Linnaeus, 1758)) were examined. Six specimens of white-eye bream and one specimen of zope were collected from a total of three biotopes located in the Bulgarian section of the Danube River between 845 and 807 river km. Three species of helminths were found - 2 species of the class Trematoda (Asymphylodora imitans (Mühling, 1898) Looss, 1899; Nicolla skrjabini (Iwanitzky, 1928) Dollfus, 1960)) and 1 species of the class Nematoda (Contracaecum sp. (larvae)). The present study aims to provide new data on the species composition and helminth ecological indices of the two examined fish species. B. sapa is reported as a new host record for the three helminth species in Bulgaria. Koshava biotope is a new habitat for the established helminths in the white-eye bream.

Key words*: Ballerus ballerus, Ballerus sapa, ecological indices, helminths, Vidin Province.*

INTRODUCTION

The Danube River, with a length of 2,850 km, ranks among the longest rivers in Europe. The length of the Danube River in Bulgarian territory is 470 km. The Bulgarian section of the river starts from the mouth of the Timok River at the Danube River (at 845 river km) and reaches the town of Silistra (at 375 river km). The river is distinguished by exceptional biological diversity, with 68 fish species reported for the Bulgarian section (Zarev et al., 2013). Species from the families Cyprinidae, Percidae, Gobiidae, Cobitidae, and others predominate (Keckeis & Schiemer, 2002). Different authors study the species composition of parasites of white-eye bream from the Danube River in Bulgaria (Kakacheva-Avramova et al., 1978; Kirin et al., 2013); in Serbia (Đikanović et al., 2013). There are also studies from the river basin in Romania (Cojocaru, 2003); Slovakia (Oros & Hanzelová, 2009; Hanzelová et al., 2011), and Serbia (Djikanovic et al., 2011). Studies on the parasite fauna of zope have been conducted from the Bulgarian section (Kakacheva-Avramova et al., 1978) and Serbian section (Đikanović et al., 2013) of the Danube River, as well as for the river basin in Romania (Cojocaru, 2003) and Serbia (Djikanovic et al., 2011).

The present study aims to study the species composition of the helminths of two fish species of genus *Ballerus* Heckel, 1843, inhabiting the freshwater ecosystem of the Danube River in Bulgaria; to provide new data on the mean intensity, mean abundance, and prevalence of endohelminths of the studied fish species.

MATERIALS AND METHODS

The objects of study are white-eye bream (*Ballerus sapa* (Pallas, 1814)) (syn. *Abramis sapa* (Pallas, 1814)) and zope (*Ballerus ballerus* (Linnaeus, 1758) (syn. *Abramis ballerus* (Linnaeus, 1758)). The fish were caught from the Danube River, on the vicinities of three villages - Kudelin, Novo selo, and Koshava (designated as biotopes), located in the border zone of Bulgaria, Vidin Province. The biotopes are located at 844, 833 and 807 km along the Danube River, respectively (Figure 1).

Fish were caught according to BS EN 14757:2015 Water quality - Sampling of fish with multi-mesh gillnets. Net fishing devices were used under permits issued by the Executive Agency for Fisheries and Aquaculture. Scientific species names were given according to Fröse & Pauly (2022). Immediately after capture, all fish specimens were measured and weighed. Mean values for total body length (TL), maximum body height (MH), and body weight (BW) of *B. sapa* specimens were calculated: 16.12 ± 6.39 cm, 4.33 ± 1.79 cm and 49.17 ± 39.71 g, respectively.

Figure 1. Location of the studied section of the Danube River (844-807 river km), Vidin Province, Bulgaria (https://www.google.bg/maps/place/Видин)

The captured specimens white-eye bream and zope were subjected to helminthological examination by methods indicated by Zashev & Margaritov (1966); Kakacheva-Avramova (1983); Bauer (Ed.) (1987); Moravec (2013); others. For all types of helminths ecological indices were calculated: mean intensity (MI); mean abundance (MA) and prevalence (P%) according to Bush et al. (1997). The helminthological studies were carried out in the laboratory of the Department of Agroecology and Environmental Protection, Agricultural University - Plovdiv. To determine the taxonomic affiliation of the isolated parasites, a microscope "XS-213", China, was used.

RESULTS AND DISCUSSIONS

Fish species

Two fish species of the genus *Ballerus* Heckel, 1843; family Cyprinidae were selected as model fish species. *B. sapa* is a freshwater, brackish and benthopelagic fish. Inhabits fast-flowing waters. The diet of the species consists of a variety of crustaceans, molluscs, and aquatic vegetation. *B. ballerus* is a freshwater, benthopelagic fish. It occurs in slow-flowing waters. Uses zooplankton for food. Both species grow at a slow rate. The body length of white-eye bream and zope is up to 30 cm and 45 cm, respectively, and the weight is up to 800 g and up to 1.5 kg, respectively. Visually, the two species are similar, as zope is distinguished from white-eye bream by the position of the mouth, which is upturned; by the snout, which is pointed; by the smaller scales; and by the size of the eyes, which are smaller (Karapetkova & Zhivkov, 2006; Kottelat & Freyhof, 2007). They are included in the IUCN Red List with the category "Least Concern" and Annex III of the Bern Convention (Convention on the conservation of European wildlife and natural habitats, 1982; Freyhof & Brooks, 2011).

Ecologohelminthological examinations

A total of six specimens *B. sapa* (5 specimens from Koshava biotope and 1 specimen from Novo selo biotope) and one specimen *B. ballerus* from the Kudelin biotope were subjected to helminthological examination. Infection was found only in white-eye bream from Koshava biotope. Two out of five examined specimens of *B. sapa* (40%) were infected. Three types of endohelminths - two species of class Trematoda (*Asymphylodora imitans* (Mühling, 1898) Looss, 1899; *Nicolla skrjabini* (Iwanitzky, 1928) Dollfus, 1960) and one species of class Nematoda (*Contracaecum* sp. (larvae)) were found (Table 1).

Table 1. Taxonomic position, synonyms, localization, biotopes, season of detection of *Asymphylodora imitans*, *Nicolla skrjabini*, and *Contracaecum* sp.

Helminth species	***Asymphylodora imitans*[1]**	***Nicolla skrjabini*[2,3]**	***Contracaecum* sp.**
Taxonomic position	CLASS TREMATODA RUDOLPHI, 1808 Family Monorchidae Odhner, 1911 Genus *Asymphylodora* Looss, 1899	CLASS TREMATODA RUDOLPHI, 1808 Family Opecoelidae Ozaki, 1925 Genus *Nicolla* Wišniewski, 1933	CLASS NEMATODA RUDOLPHI, 1808 Family Anisakidae Skrjabin et Karokhin, 1945 Genus *Contracaecum* Railliet & Henry, 1912
Synonyms[1,2]	*Asymphylodora dneproviana* Iwanitzky, 1928; *Distoma imitans* Mühling, 1898	*Coitocaecum macrostomum* Pigulewsky, 1931; *Coitocaecum ovatum* Pigulewsky, 1931; *Coitocaecum skrjabini* Iwanitzky, 1928; *Coitocaecum macrostomum* Pigulewski, 1931; *Coitocaecum ovata* Pigulewski, 1931; *Crowcrocaecum skrjabini* (Iwanitzky, 1928) Skrjabin & Koval, 1956; *Excoitocaecum skrjabini* (Ivanitzkii, 1928) Slusarski, 1958; *Nicolla macrostoma* (Pigulewsky, 1931) Wisniewski, 1934; *Nicolla ovata* (Pigulewsky, 1931) Wisniewski, 1934; *Nicollia macrostoma* (Pigulewsky, 1931) Wisniewski, 1933; *Nicollia ovata* (Pigulewsky, 1931) Wisniewski, 1933	-
Localization	Intestine	intestine	in capsules on the serous membrane of the organs in the abdominal cavity of the fish
Biotope	Koshava	Koshava	Koshava
Season	Spring	spring	spring

[1]WoRMS (2022a); [2]WoRMS (2022b)

In the present study, a total of 711 endohelminth specimens were isolated. The trematode *As. imitans* had the highest values for MI and MA (MI = 700.00 and MA = 140.00), and the nematode *Contracaecum* sp. had the lowest (MI = 3.00 and MA = 0.60). All three isolated species of helminths had equal prevalence (P% = 20.00) (Table 2).

Table 2. Species diversity and ecological indices in the helminth community of *Ballerus sapa* from the Danube River

***Ballerus sapa* (N = 5 / Koshava) Parasite species**	**n**	**p**	**MI**	**MA**	**P%**	**R**
Asymphylodora imitans (Mühling, 1898) Looss, 1899	1	700	700.00	140.00	20.00	700
Nicolla skrjabini (Iwanitzky, 1928) Dollfus, 1960	1	8	8.00	1.60	20.00	8
Contracaecum sp. (larvae)	1	3	3.00	0.60	20.00	3

N - number of investigated fish; n - number of infected fish; p - number of fish parasites; MI - mean intensity; MA - mean abundance; P% - prevalence; R - range

Definitive hosts of *As. imitans* are freshwater fish species, such as *Blicca bjoerkna* (Linnaeus, 1758); *Abramis brama* (Linnaeus, 1758); *B. ballerus*; *B. sapa*; *Scardinius erythrophthalmus* (Linnaeus, 1758). The development cycle of this helminth species is not sufficiently studied. Definitive hosts of *N. skrjabini* are fish species of families Cyprinidae, Percidae, Gobiidae, Cobitidae, Siluridae, Gadidae, Esocidae, Acipenseridae, Salmonidae. The specific hosts are *Gymnocephalus acerina* (Gmelin, 1789) and *Silurus glanis* Linnaeus, 1758. *N. skrjabini* has a one-year development cycle involving two intermediate hosts. The first intermediate host is the snail *Lithoglyphus naticoides* (Pfeiffer, 1828). In it, sporocysts develop, localized in the liver, gonads, and gills. The second intermediate hosts are the crustaceans *Gammarus balcanicus* Schäferna, 1923; *Pontogammarus crassus* (Sars, 1894); *Dikerogammarus haemobaphes* (Eichwald, 1841). The metacercariae encyst in the dorsal musculature and fins. They are also found in the body cavity of crustaceans (Bykhovskaya-Pavlovskaya et al., 1962; Gaevskaya et al., 1975; Kakacheva-Avramova, 1983; Bauer (Ed.), 1987). *Contracaecum* sp. has definitive hosts of waterfowl (*Ardea*, *Egretta*, *Podiceps*, *Phalacrocorax*). Intermediate hosts are copepods (*Cyclops*, *Acanthocyclops*, *Macrocyclops*, *Mesocyclops*, *Eucyclops*, *Arctodiaptomus*, *Diaptomus*) (Bauer (Ed.), 1987; Moravec, 2013).

During a parasitological examination of *B. sapa* from the Bulgarian section of the Danube River *Apophallus muehlingi* (Jägerskiöld, 1899) Lühe, 1909; *Caryophyllaeus laticeps* (Pallas, 1781) Lühe, 1910; *Caryophyllaeides fennica* (Schneider, 1902) Nybelin, 1922; *Contracaecum bidentatum* (Linstow, 1899); *Acanthocephalus lucii* (Müller, 1776) Lühe, 1911 and *Pomphorhynchus laevis* (Zoega in Müller, 1776) Porta, 1908 (Kakacheva-Avramova et al., 1978) were reported in the area of the cities of Ruse, Svishtov, Silistra, Lom, Tutrakan; and *Pomphorhynchus tereticollis* (Rudolphi, 1809) Meyer, 1932 (Kirin et al., 2013) in the area of the village of Vetren. For the parasite fauna of *B. sapa* from the Serbian section of the Danube River near Zemun (1,173 river km) and Visnjica (1,162 river km) *C. fennica*; *Caryophyllaeus* sp.; *C. laticeps*; *Triaenophorus nodulosus* (Pallas, 1781) Rudolphi, 1793; *Proteocephalus* sp.; *Proteocephalus torulosus* (Batsch, 1786) Nufer, 1905; *Ligula intestinalis* (Linnaeus, 1758) Gmelin, 1790; cestode cysts were reported (Đikanović et al., 2013). White-eye bream from the Danube River basin in Serbia (Djikanovic et al., 2011), in Slovakia – Tisa and Latorica rivers (Oros & Hanzelová, 2009; Hanzelová et al., 2011), and Romania – Timiş and Bega rivers (Cojocaru, 2003) was examined for the presence of parasites. Djikanovic et al. (2011) found *Rhipidocotyle campanula* (Dujardin, 1845); *Ap. muehlingi*; *C. fennica*; *Tr. nodulosus*; *Pr. torulosus*; *L. intestinalis*; *Acanthocephalus tenuirostris* (Achmerov & Dombrovskaja-Achmerova, 1941) Yamaguti, 1963. Oros & Hanzelová (2009) and Hanzelová et al. (2011) reported the helminths *C. fennica*; *Caryophyllaeus brachycollis* Janiszewska, 1953; *C. laticeps* (for Tisa River) and *Aspidogaster limacoides* Diesing, 1834; *As. imitans*; *Palaeorchis incognitus* Szidat, 1943; *Nicolla skrjabini* (Iwanitzky, 1928) Dollfus, 1960 (syn. *Crowcracoecum skrjabini*); *Nicolla testiobliqua* (Wisniewski, 1933) Dollfus, 1958; *C. fennica*; *C. laticeps* (for Latorica River). Cojocaru (2003) found *As. imitans* (Table 3).

Table 3. Distribution of the found helminths (in the present study) of *Ballerus sapa* from the Danube River and its basin

Biotopes / Helminth species	Koshava biotope	Novo selo biotope	Danube River in other countries	Danube River Basin in other countries	Danube River in Bulgaria	Danube River Basin in Bulgaria
Asymphylodora imitans (Mühling, 1898) Looss, 1899	+	-	-	+	-	-
Nicolla skrjabini (Iwanitzky, 1928) Dollfus, 1960	+	-	-	+	-	-
Contracaecum sp.	+	-	-	-	-	-

During a study of the parasite fauna of *B. ballerus* from the Bulgarian section of the Danube River in the area of Vidin, Silistra, Svishtov, Lom, Ruse, Tutrakan, the helminths *N. skrjabini*; *C. fennica* and *P. laevis* were found (Kakacheva-Avramova et al., 1978). For zope from the Serbian section of the river near Zemun (1,173 river km) and Visnjica (1,162 river km) the cestodes *C. fennica*; *C. laticeps*; *Proteocephalus* sp.; *Pr. torulosus*; cestode cysts were reported (Đikanović et al., 2013). Cojocaru (2003) studied *B. ballerus* from the Danube River basin in Romania (Timiş and Bega rivers) and reported the trematode *As.*

imitans. Djikanovic et al. (2011) reported *Pr. torulosus*; *Philometra ovata* (Zeder, 1803), and others of zope from the Danube River basin in Serbia.

CONCLUSIONS

During the period 2019-2021, five specimens of *B. sapa* from Koshava biotope; one specimen *B. sapa* from Novo selo biotope, and one specimen *B. ballerus* from Kudelin biotope were examined for helminths. Infection was found only in white-eye bream from Koshava biotope. Three taxa of helminths - *As. imitans* and *N. skrjabini* (class Trematoda), and *Contracaecum* sp. (class Nematoda), were found. *As. imitans* was distinguished by the highest mean intensity (MI = 700.00) and mean abundance (MA = 140.00). All three parasite species had an equal prevalence (P% = 20.00). Koshava biotope is a new habitat for the found helminths of white-eye bream. *B. sapa* is a new host for *As. imitans*, *N. skrjabini* and *Contracaecum* sp. in Bulgaria.

ACKNOWLEDGEMENTS

We thank the leadership of the Centre of Research, Technology Transfer and Protection of Intellectual Property Rights at the Agricultural University - Plovdiv for funding this research.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

BIODIVERSITY AND HELMINTH COMMUNITIES OF *Barbus cyclolepis* Heckel, 1837 FROM CHERNA RIVER, BULGARIA

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Abstract

The study presents for the first time the Cherna River, southern Bulgaria, Aegean water basin, the results of research on the biological diversity and helminth communities of the Round-scaled barbell Barbus cyclolepis Heckel, 1837. 30 specimens of B. cyclolepis are studied. Infection by 5 species of helminths are found (Allocreadium isoporum (Loos, 1894); Bathybothrium rectangulum (Bloch, 1782); Caryophyllaeus laticeps (Pallas, 1781); Capillaria petruschewskii Zeder, 1800; Neoechinorhynchus rutili (Müller, 1780)). The infection indices and the dominant structure of helminth communities are presented. Basic biotic indices are determined. Helminth communities are analyzed at two levels: infracommunity and component community. All established parasite species are autogenous for the helminth communities of the Round-scaled barbell from the freshwater ecosystem of the Cherna River. New data for helminths and helminth communities of B. cyclolepis are presented. The bioindicator significance of helminths and helminth communities are discussed.

Key words: *bioindication, Barbus cyclolepis, Cherna River, helminth communities.*

INTRODUCTION

Cherna River is distinguished by its exceptional biological diversity. It springs 5 km southeast of Mugla village, Smolyan municipality (1770 m above sea level). The river flows entirely on the territory of the Smolyan region. It flows into the village of Leshtak (Madan municipality) as a left tributary of the Arda River (624 m above sea level). Along its entire length, it flows in an eastern direction in a narrow canyon-like valley with a single valley extension in the area of the town of Smolyan. The river ecosystem is not subjected to intense negative anthropogenic impacts. Cherna River falls into the protected area BG0001030 Rhodope-Western, declared under the Habitats Directive (Directive 92/43/EEC) and protected area BG0002113 Trigrad - Mursalitsa, declared under the Birds Directive (Directive 79/409/EEC). Helminths are characterized by complex development, often involving more than one type of host. Therefore, the reduction of helminth infections and helminth diversity species is often reduced in the species diversity and population size of a number of species of free-living organisms (Thompson et al., 2016; Kevin & Lafferty, 2012). According to some scientific studies, parasites influence the behavior of the hosts and their health status (Preston & Johnson, 2010). Parasites and parasite communities are of fundamental ecological importance because they influence trophic relationships, food chains and webs, and biodiversity, especially for keystone species. The increase in parasite populations, especially in some species, has also been linked to impacts on host health, including and the human (Preston & Johnson, 2010; Zaharieva & Zaharieva, 2020a, b; Zaharieva & Zaharieva, 2020c,d; Zaharieva & Zaharieva, 2021a; Zaharieva & Zaharieva, 2021b). Parasites are an essential part of the elements of biological diversity, but at the same time, both their diversity and their communities are not well studied (Selbach et al., 2020). Parasites are also biological elements for bioindication and assessment of the state of the environment (degradation, pollution, loss of biodiversity, etc.) (Nachev & Sures, 2016; Vidal-Martinez & Wunderlich, 2017; Zaharieva & Zaharieva, 2021a; Zaharieva & Zaharieva, 2021b). Parasites and parasite communities of *B. cyclolepis* were studied by a few authors in Bulgaria (Kirin, 2002, 2003; Kakacheva-Avramova, 1965, 1972; Margaritov, 1965; Chunchukova, 2020; Chunchukova et al., 2020; Kirin et al., 2020, etc.). There are also few studies from other countries about parasites and parasite communities of pound-scaled barbel from the Aegean Water basin (Bazsalovicsová et al., 2014, etc.). They refer mainly to representatives of the class Monogenea (Simkova et al., 2007; Benovics et al., 2018; Řehulková et al., 2020, etc.). Cherna River has not been the subject of systematic ecological, ecologoparasitological, and biomonitoring studies with the biological element endoparasites. The present research aims to present the biological diversity of the parasites of *B. cyclolepis* from the Cherna River, the structure of the component, and the infracommunities formed by them.

MATERIALS AND METHODS

In 2019, a total of 30 specimens *Barbus cyclolepis* Heckel, 1837 are examined for helminths. The scientific and common names of the fish are presented according to the FishBase database (Fröse & Pauly, 2022). Helminthological examinations are conducted following research methods described by Petrochenko, 1956; Zashev & Margaritov, 1966; Bauer, 1987; Moravec, 2013. Helminth specimens were fixed and preserved in Eppendorf tubes with 70% ethylalcohol. The specimens of classes Trematoda and Cestoda are studied by methods of Georgiev et al., 1986; Scholz & Hanzelová, 1998 and of Acanthocephala and Nematoda - by methods of Zashev & Margaritov, 1966; Moravec, 2013. Analyses of the helminth community structure have been implemented in both levels: infracommunity (total and mean number of species; total and mean number of specimens; Brillouin's index of diversity (HB)) and component community (prevalence (P%) and mean intensity (MI) for each species) (Bush et al., 1997; Kennedy, 1993, 1997; Magurran, 1988). The species are divided into core species (P%>20), component species (P%>10), and accidental species (P%<10) (Kennedy, 1993). The diversity measures are calculated by software products Statistica 10 (StatSoft Inc., 2011) and MS Excel (Microsoft 2010).

RESULTS AND DISCUSSIONS

Model fish species Round-scaled barbell (*Barbus cyclolepis* Heckel,1837; Cyprinidae) is a fresh water, benthopelagic and subtropical fish, distributed in Europe and Asia - Aegean Water Basin (Bulgaria, Turkey, and Greece), Black Sea Basin, etc. (Kottelat & Freyhof, 2007; Karapetkova & Zhivkov, 2009). In Bulgaria, the species is widespread in Maritsa, Mesta, and Struma rivers as well as its tributaries (Karapetkova & Zhivkov, 2009). The fish inhabits streams, lakes, and upper and middle streams of the fast-flowing rivers, but prefers areas with clear water and sandy-gravel bottom (Bianco, 1998; Kottelat & Freyhof 2007; Karapetkova & Zhivkov, 2009). It reaches a maximum length of up to 30 cm and a weight of up to 1000 g (Karapetkova & Zhivkov, 2009), as mean body length in decreasing age groups, sex varies in different rivers and habitats (Marinov, 1986; Dikov et al., 1994; Vasiliou & Economidis, 2005; Rozdina, 2009; Raikova & Kolev, 2015; Kolev, 2019; Çelik & Özuluğ, 2021).

The development of *B. cyclolepis* from rivers in Bulgaria was studied by Mihaylova, 1965; Marinov, 1986; Vasiliou & Economidis, 2005; Rozdina, 2009; Raikova-Petrova & Rozdina, 2012; Kolev & Raikova, 2019, etc.

In the food spectrum, from 14 food components at trophic systems of Round-scaled barbel from the middle part of the Maritsa River, the highest prevalence and index of dominance were determined for chironomid larvae as well as for plant detritus but mainly during the summer season (Rozdina et al., 2008). In Istranca Stream (Istanbul, Turkey), from 11 food components, the dominant Insects and fish was determined as selective to Diptera, reported by many other authors (Saç et al., 2021). *B. cyclolepis* is included in IUCN Red List as a Least Concern (LC) species (Bianco, 1998; Crivelli, 2006; Rozdina et al., 2008). For Bulgaria and Balkan Peninsula *B.cyclolepis* is an endemic fish species (Rozdina et al., 2008; Raikova & Kolev, 2015).

Helminths and helminth community structure

As a result of the ecologoparasitological studies carried out on 30 specimens of the Round-scaled barbel (*B. cyclolepis*), infection with 5 species of parasites was found: *Allocreadium isoporum* (Loos, 1894); *Bathybothrium rectangulum* (Bloch, 1782); *Caryophyllaeus laticeps* (Pallas, 1781); *Schulmanela petruschewskii* (Schulman, 1948) Ivashkin, 1964; *Neoechinorhynchus rutili* (Müller, 1780). The identified parasite species belong to 4 orders: Trematoda, Cestoda, Nematoda, and Acanthocephala (Table 1). *All. isoporum* parasitizes the intestines of family Cyprinidae fishes. The life cycle includes a first (*Sphaerium* Scopoli, 1777) and a second intermediate host (*Ephemera* Linnaeus, 1758, *Anabolia* Stephens, 1837, *Chaetopterix* Cuvier, 1827, larvae). *B. rectangulum* is a specific helminth species of *Barbus barbus* (Linnaeus, 1758) and *B. petenyi* Heckel, 1852. *C. laticeps* is an intestinal cyprinid helminth with intermediate hosts *Tubifex tubifex* (Müller, 1774), *T. barbatus* Grube, 1891, *Limnodrilus claparedeanus* Ratzel, 1868. *Sch. petruschewskii* infected the liver of freshwater fish (*Gymnocephalus cernua* (Linnaeus, 1758), *Cobitis taenia* Linnaeus, 1758, *Lepomis gibbosus* (Linnaeus, 1758), *Sander lucioperca* (Linnaeus, 1758), *Perca fluviatilis* Linnaeus, 1758, etc.). Intermediate host is *Eiseniella tetraedra* (Savigny, 1826). *N. rutili*is an intestinal parasite of freshwater fish species from the families Cyprinidae, Salmonidae, Esocidae, Percidae, Gobiidae, Cottidae, etc. Intermediate hosts are the species of insects *Sialis fulinginosa* Pictet, 1836 and *Apogonniger* Döderlein, 1883, as well as the species of crustaceans, *Cyclocyris laevis* (Müller, 1776) and *Cypria turneri* Hoff, 1942 (Petrochenko, 1956; Kakacheva-Avramova, 1983; Bauer (Ed.), 1987; Moravec, 2013). The established species of helminths are characterized by complex development cycles. *N. rutili* (Acanthocephala) and *Sch. petruschewskii* (Nematoda) are core species for the parasite communities of the barbel.

Component community

N. *rutile* (Acanthocephala) and *Sch. petruschewskii* (Nematoda) were distinguished with the highest prevalences (70% and 40%, respectively), followed by those of *C. laticeps* (27%) (Cestoda) and *All. isoporum* (Trematoda) (24%). The species *C. laticeps* and *All. isoporum* are component species of the host's parasite communities (Table 1).

B. rectangulum is an accidental species to the parasite communities of the barbel. The highest mean intensity is *N. rutili* (3.14), and the lowest is *B. rectangulum* (1.0) (Table 1). Ecological intensity and prevalence for *N. rutili* have the highest values and for *B. rectangulum* they are the lowest. Populations of *Sch. petruschewskii* in the barbel have a lower ecological intensity but a higher prevalence. In the remaining two populations, of *All. isoporum* and *C. laticeps*, almost the same ecological intensity and prevalence were observed. All identified parasite species are autogenous to the parasite communities of the barbel. The number and mean intensity of parasite species are closely related to the distribution, number, and intensity of intermediate and final hosts in the river ecosystem of the Cherna River.

Table1. Biodiversity, mean intensity (MI), and prevalence (P%) of parasite species of *Barbus cyclolepis* Heckel, 1837

Parasite species	Intermediate hosts	Definitive host *B. cyclolepis* (N^1= 30)	
		P%[2]	MI[3]
Trematoda			
1. *Allocreadium isoporum* (Loos, 1894)	Mollusca, I; Insecta, larvae, II	24%	1.4
Cestoda			
2.*Bathybothrium rectangulum* (Bloch, 1782)	Crustacea	7.0%	1.0
3.*Caryophyllaeus laticeps* (Pallas, 1781)	Oligochaeta	27%	1.6
Nematoda			
4.*Schulmanela petruschewskii* (Schulman, 1948) Ivashkin, 1964	Oligochaeta	40%	1.5
Acanthocephala			
5.*Neoechinorhynchus rutili*(Müller, 1780)	Insecta Crustacea	74%	3.14

Legend: [1]N = total number of examined fish specimens.
[2]P% = prevalence.
[3]MI = mean intensity.

B. rectangulum is an accidental species to the parasite communities of the barbel. The highest mean intensity is *N. rutili* (3.14), and the lowest is *B. rectangulum* (1.0) (Table 1). Ecological intensity and prevalence for *N. rutili* have the highest values and for *B. rectangulum* they are the lowest. Populations of *Sch. petruschewskii* in the barbel have a lower ecological intensity but a higher prevalence. In the remaining two populations, of *All. isoporum* and *C. laticeps*, almost the same ecological intensity and prevalence were observed. All identified parasite species are autogenous to the parasite communities of the barbel. The number and mean intensity of parasite species are closely related to the distribution, number, and intensity of intermediate and final hosts in the river ecosystem of the Cherna River.

Infracommunity

Out of the 30 specimens of barbel examined, no parasites were found in only two specimens of fish (6.67%). The largest share of barbels infested with two types of helminths (50%), followed by those infested with one helminth (30%) and three types of helminths (13.34%). Brillouin's index of diversity is HB = 0.64 (Table 2).

Table 2. Infracommunity data

Number of helminth species				
Total number	5			
Number of infected fish	0	1	2	3
Number of helminth species	2	9	15	4
Number of helminth specimens				
Total number	51			
Mean ± SD	10.2±7.49			
Range	1-5			
Mean HB ± SD	0.64±0.21			

DISCUSSIONS

The helminth species of *B. cyclolepis* found in this study have also been reported for other localities in Bulgaria (Table 3).

Table 3. Endohelminth species of *Barbus cyclolepis* reportedfrom other studies in Bulgaria

Parasite species	Authors	Locality - rivers
Trematoda		
Allocreadium isoporum (Loos, 1894)	Kakacheva-Avramova, 1965	Syuyutlijka, Asenitsa
	Margaritov, 1965	Vycha
Parasite species	Authors	Locality - rivers
	Kirin, 2002	Luda Yana
	Kirin et al., 2020	Tamrashka
Cestoda		
Caryophyllaeus brachycollis Janiszewska, 1951	Kakacheva-Avramova, 1965	Asenitsa, Sushitsa
	Margaritov, 1965	Maritsa, Vycha, Topolnitsa
Caryophyllaeides fennica (Schneider, 1902) Nybelin, 1922	Kakacheva-Avramova, 1965	Asenitsa, Harmanlijska, Topolnitsa, Syuyutlijka, Sushitsa, Bedechka
	Margaritov, 1965	Topolnitsa
	Kirin et al., 2020	Tamrashka
Caryophyllaeides sp.	Kakacheva-Avramova, 1965	
Bathybothrium rectangulum (Bloch, 1782)	Kakacheva-Avramova, 1965	Asenitsa, Syuyutlijka
	Margaritov, 1965	Maritsa, Vacha, Chepinska
	Kirin, 2002	Luda Yana
	Kirin, 2003	Arda
Cestoidea g. sp.	Margaritov, 1965	Vycha
Acanthocephala		
Acanthocephalus anguillae (Müller, 1780)	Margaritov, 1965	Chepinska
Neoechinorhynchus rutili (Müller, 1780)	Kakacheva-Avramova, 1965	Syuyutlijka
	Kakacheva-Avramova, 1972	Tundzha
	Chunchukova et al., 2020	Topolnitsa
Pomphorhynchus laevis (Zoega in Müller, 1776)	Chunchukova, 2020	Chepelarska
	Kirin et al., 2020	Tamrashka
Nematoda		
Rhabdochona denudata (Dujardin, 1845) Raillet, 1916	Kakacheva-Avramova, 1965	inТракия
	Margaritov, 1965	Maritsa, Vycha, Chepinska, Topolnitsa
Rhabdochona hellichi (Šramek, 1901) Chitwood, 1933	Chunchukova, 2020	Chepelarska
	Kirin et al., 2020	Tamrashka
Rhabdochona gnedini Skrjabin, 1948 (syn., *Rhabdochona sulaki* Saidov, 1953)	Matgaritov, 1964	Tundzha, Vycha
	Margaritov, 1965	Maritsa, Vycha
	Kirin et al., 2020	Tamrashka
Capillaria sp.	Margaritov, 1965	Maritsa
*Schulmanella*sp.	Kakacheva-Avramova, 1972	Tundzha
Rhabdochona sp. juv.	Kakacheva-Avramova, 1965	Maritsa, Asenitsa
Nematoda d. sp.	Margaritov, 1965	Topolnitsa

Sixteen taxa have been reported from previous studies of *B. cyclolepis*. Two species are reported for the first time for the barbel helminth communities, *C. laticeps* and *Sch. petruschewskii* in this study. From the total of 18 taxa, 5 species were identified for barbel from the Cherna River (27.78%).

Schulmanella sp. was reported as a helminth species of *round-scaled barbel* from the Tundzha River, Bulgaria (Kakacheva-Avramova, 1972). Helminth species found in *B. cyclolepis* have also been reported for other fish species in Bulgaria (Kakacheva-Avramova, 1983; Chunchukova et al., 2020a, b; Zaharieva & Zaharieva, 2020a, b; Zaharieva & Zaharieva, 2020c, d; Zaharieva & Zaharieva, 2021a; Zaharieva & Zaharieva, 2021b).

CONCLUSIONS

As a result of the examination of 30 specimens of *B. cyclolepis* from the Cherna River, infection with 5 types of endohelminths was found. Of these, two species are reported for the first time for the barbel fauna in Bulgaria, *C. laticeps* and *Sch. petruschewskii*. *C. laticeps* is a component species (P%=27) and *Sch. petruschewskii* (P%=40) is core species for the helminth communities of the barbell from the studied freshwater ecosystem. The five parasite species are autogenous to the parasite communities of the barbel.

ACKNOWLEDGEMENTS

This research work was published with the support of Agricultural University – Plovdiv, Centre of research, technology transfer, and protection of intellectual property rights, and was financed from Project 17-12.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

EVALUATION OF THE PROBIOTIC SUPPLEMENT *Saccharomyces cerevisiae* BB06 AS A BENEFICIAL GROWTH PROMOTER FOR CARP (*Cyprinus carpio*) IN RECIRCULATING AQUACULTURE

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Abstract

The current study investigates the effect of the probiotic Saccharomyces cerevisiae BB06 on the growth performance of carp (Cyprinus carpio). The experiments were conducted in a laboratory-scale re-circulating system over an eight-week trial period. Ninety healthy carp were randomly divided into three groups and fed with fish feed containing 0% yeast biomass (control), 3% yeast biomass (YBV3%), and 5% yeast biomass (YBV5%). The fish were fed twice daily with a quantity of feed corresponding to 2.5% of their body weight. Growth performance increased significantly (p<0.05) with increasing levels of probiotic S. cerevisiae BB06. The YBV5% group performed the best in terms of the final body weight, weight gain rate of Cyprinus carpio. The mortality rate of the fish fed with the probiotic S. cerevisiae BB06 was 0%. An increase in the yeast content of the feed had a tendency to decrease in the value of the Fulton condition factor. In addition, fish fed diets enriched with S. cerevisiae BB06 showed improvements in feed conversion ratio and protein efficiency ratio. Water quality parameters were maintained at acceptable levels in all tanks during the eight-week trial period, allowing high growth rates and production of the carp. In conclusion, dietary supplementation with S. cerevisiae BB06 can improve the growth performance of carp (Cyprinus carpio).

***Key words**: carp, growth performance, Saccharomyces cerevisiae, feed, water quality.*

INTRODUCTION

Considering that per capita fish consumption has increased by 1.5% over the last half-century, expanding aquaculture production is seen as a viable solution to fulfill the world's increasing food demands, as well as an excellent source of income and employment opportunities (Béné et al., 2016; FAO., 2020; Saha et al., 2022). Feed quality and efficacy are critical factors in aquaculture that have an impact on fish growth, survival, and production, as well as water quality (Kong et al., 2020). Therefore, probiotics are considered suitable additives to improve feed production and utilization (Jamal et al., 2020; Tuan et al., 2013; Chauhan and Singh, 2019). Probiotics are viable microorganisms which, when administered in adequate quantities, can provide health benefits to the host (FAO/WHO., 2002; Hill et al., 2014). Lactic bacteria (such as *Bifidobacterium, Lactobacillus,* and *Pediococcus*) and *Bacillus* spp. have been extensively investigated for use as probiotics in aquaculture to improve fish performance and welfare (Coulibaly et al., 2023; Chizhayeva et al., 2022; Kawser et al., 2022). Nonetheless, there is a possible threat of the transfer of antimicrobial resistance genes to pathogenic bacteria via bacterial probiotics

(Daniali et al., 2020; Li et al., 2020). Instead, yeasts are non-pathogenic and due to their resistance to the gastrointestinal environment (including pH, body temperature, the presence of digestive enzymes, and bile salts), and their ability to resist antibiotics, may be a valuable probiotic adjunct to antibiotic therapy (Agbola et al., 2021; Jack et al., 2015; Alkalbani et al., 2022; Fernández-Pacheco et al., 2021; Mahdy et al., 2022; Navarrete & Tovar-Ramírez, 2014). *Saccharomyces cerevisiae,* isolated from various sources, is the most extensively studied probiotic yeast for aquafeeds (Dumitrache et al., 2022; Mogmenga et al., 2023; Del Valle et al., 2023; Diguță et al., 2023; Abass et al., 2018). Increased scientific research has been conducted to identify and characterize novel non-*Saccharomyces* species, such as *Debaryomyces* sp., *Kluyveromyces* sp., *Hanseniaspora* sp., *Rhodotorula* sp., *Wickerhamomyces* sp., and *Yarrowia* sp., which reportedly enhance fish production (El-Feky et al., 2017; Adel et al., 2017; Rekha et al., 2022; Vidakovic et al., 2019; Corbu et al., 2020; Reyes-Becerril & Alamillo, 2021). As part of the current circular economy, new technologies are being promoted to produce large quantities of yeast from cheap inorganic compounds and low-value agricultural and industrial wastes, particularly for use as aquaculture feed (Ma et al., 2013; Bărbulescu et al., 2018).

Furthermore, with rapidly expanding aquaculture and limited fishmeal availability, it is critical to identify sustainable protein replacements. In aquafeed production, the substitution of fishmeal for plant protein sources (mainly soybean meal or concentrate, and grains glutens) has become an important standard practice (Øverland & Skrede, 2016). However, certain fish species are unable to efficiently assimilate plant-based feed due to nutritional deficiencies, an imbalanced amino acid composition, and the presence of antinutritional compounds (Hardy, 2010; Zhou et al., 2018). Single-cell ingredients derived from bacteria, yeast, and microalgae, are used as supplements in food or feed Bratosin et al., 2021; Jach et al., 2022; Ferreira et al., 2010; Shah et al., 2017; Glencross et al., 2020; Banerjee end Ray, 2017; Balcazar et al., 2006). In particular, due to its high content of protein, carbohydrates (alpha-glucan, beta-glucan, alpha-mannan), nucleic acids, vitamins, antioxidants, and minerals, the yeast *S. cerevisiae* has been widely used as a valuable source of functional ingredients in the production of feed and food (Agboola et al., 2021; Navarrete & Tovar-Ramírez, 2014; Jach et al., 2022; Ferreira et al., 2010).

Due to their probiotic abilities and high protein content, yeast supplementation improves growth performance (Abass et al., 2018; El-Fely et al., 2017; Adel et al., 2017; Vidakovic et al., 2019; Korkmaz et al., 2011; Zhanga et al., 2020; Banu et al., 2020), feed digestibility (Rekka et al., 2022; Vidakovic et al., 2019; Korkmaz & Cakirogullari, 2011), intestinal microbiota (Adel et al., 2017; Vidakovic et al., 2019; Islam et al., 2021), stress tolerance (Abass et al., 2018), immune system (Adel et al., 2017; Ma et al., 2013; Li et al., 2003; Øverland et al., 2013; Tewary and Patra, 2011; Saini et al., 2014) and disease control (Abass et al., 2018; Reyes-Becerril and Alamillo, 2021; Ma et al., 2013; Chiu et al., 2010; Zhanga et al., 2020) in aquaculture species.

Therefore, the aim of this study was to investigate whether the partial replacement of fishmeal with probiotic *Saccharomyces cerevisiae* BB06 has a beneficial effect on growth performance, survival of carp (*Cyprinus carpio*), as well as water quality.

MATERIALS AND METHODS

Yeast strains and feed preparation

S. cerevisiae strain BB06 was isolated from grapes and identified by 5.8S rRNA gene sequencing (NCBI GenBank accession no. OL757483). According to a previous study, the strain was found to possess valuable probiotic properties (Diguță et al., 2023). The strain was preserved at -20°C in the Microbial Collection of the Faculty of Land Reclamation and Environmental Engineering (USAMV Bucharest, Romania), as suspensions in 25% (v/v) glycerol. The yeast strain was cultivated in a yeast-malt medium consisting of yeast extract 0.5%, malt extract 2%, sucrose 2%, peptone Hy-Soy 0.5%, and agar 2% (abbreviated as YMSP) and kept at 30°C for 18h (Frîncu et al., 2022). Cells were harvested by centrifugation at 3500 - 4000 rpm for 5 min at 4°C, as described in (Bărbulescu et al., 2018). The yeast biomass was stored by freeze-drying as described in (Diguță et al., 2023). Cellular viability was confirmed by plate counting prior to the use of yeast biomass as a functional supplement.

Diet formulation

Three experimental diets were formulated according to nutrient requirements. A yeast-free product (YBV0%) was used as the control diet and two other diets were supplemented with 3% (YBV3%) and 5% (YBV5%) freeze-dried *S. cerevisiae* BB06, respectively. The composition of the ingredients is given in Table 1. To prepare the desired diets (YBV3% and YBV5%) (Table 1), a dough was prepared with 30% water and mixed for 15 min. The dough was then pelleted to a thickness of 6 mm using a feed mill, dried overnight at 30°C, crushed and stored at 4°C until use. All experimental diets, including the control, were prepared according to the manufacturer's instructions for Carp Aller Classic (https://www.aller-aqua.com/species/warm-freshwater-species/carp) using the same size and pellet extrusion parameters and 6 mm pellet size.

Table 1. Composition of experimental feeds (% freeze-dried weight)

Ingredients (%)	Diet 1	Diet 2	Diet 3
Formulation			
Fish meal	20.30	17.30	15.30
Cereals	20.60	20.60	20.60
Wheat bran	15.10	15.10	15.10
Vegetable proteins	14.00	14.00	14.00
Protein concentrate from fish	18.10	18.10	18.10
Algae	7.40	7.40	7.40
Oils and fats	1.40	1.40	1.40
freeze-dried *S. cerevisiae* BB06	0	3	5
Proximate chemical composition of feeds			
Ash	8.6	7.1	7.5
Crude protein	40.00	42.49	45.07
Crude lipid	4.32	4.2	4.3
Carbohydrates	6.74	6.93	6.81
Moisture	8.1	8.6	9.2

Trial and experimental design

The present study was carried out under laboratory conditions in a recirculating aquaculture system (RAS), during an experimental period of eight weeks. The aquaculture system consists of 9 rearing units of water (120 L) where the fishes were distributed randomly. Aquaculture system include several components such as a fish tank, a biofilter, water pumps, and air pumps to ensure proper circulation. Maintained under standardized illumination, all tanks experienced identical natural photoperiods with 12 to 13 hours of light. To support the growth of bacteria involved in the nitrification process, expanded clay aggregates were used for biofiltration (Figure 1), resulting in a reduction of ammonia and nitrite concentrations (Estim et al., 2019). A total number of 90 fish Cyprinus carpio, provided by the aquaculture farm of SCDP Nucet (Dambovita, Romania) were used. Ten carp fish stocked in each of the aquariums containing 120 L of treated water fitted with air stones to provide an adequate mixture of oxygen and water in the aquarium.

Figure 1. Recirculation aquaculture system (RAS) used in the study and compartments with expanded clay aggregates as biofiltration substrates. The water circulated through an overflow in the biofiltration compartment and returned through a submersible pump

The mean initial weight of the fish was 81.88 ± 0.2 g. The fish were acclimated for 2 weeks with a commercial fish pellet feed containing

40% crude protein and 8% crude fat (JBL GranoMix, JBL GmbH & Co. KG, Germany). Subsequently, the fish were fed on experimental diets twice daily for eight weeks, which were equal to 2.5% of their body weight to diminish feed wastage and prevent the fish from becoming overfed and vulnerable to diseases (https://www.fao.org/fishery/docs/CDrom/FAO_Training/FAO_Training/General/x6709e/x6709e10.htm). The schedule of biometric measurements was as follows: one at the beginning of the experiment, one at the end of it, and every 7 days interval, between.

Growth performance

The length and body weight of each fish were recorded in both the control and experimental aquariums at the start and end of the experiment, as well as after 7-day intervals. The number of fish was also noted to calculate the survival rate. The average values of the measured parameters of all fish in each aquarium were used for the interpretation of the data.

Various parameters have been approximated using different formulas, such as:

- Survival Rate (SR%) = (Initial fish number-Final fish number/Initial fish num-ber) × 100);
- Weight gain (WG%) = [(Final weight (Wf), g–Initial weight (Wi), g)/Initial weight (Wi), g] × 100;
- Specific growth rate (SGR%) = [(Final weight, g–Initial weight, g)/No. of day] × 100;
- Relative growth rate (RGR%) = WG/days of experiment/Wi(g/g day−1) x 100;
- Feed Conversion Ratio (FCR%) = Feed consumed (g)/(Wf-Wi) (g);
- Fulton`s condition factor (K) = (Tw/TL3) x 100; Tw-total weight, TL-total length;
- Protein efficiency ratio (PER) = weight gain of fish (g) / total protein(g).

Chemical Analysis

The study followed the procedures for the handling and use of the aquatic animals, in line with the Romanian legislation (Low 43 of 11 April 2014) and approved by the Ethical Commission of UASMV Bucharest (Decision No. 26 of 23 June 2022). The fish were sacrificed with a lethal dose of 2-phenoxyethanol 2 ml/L^{-1} (Sigma, Germany) in a bucket for 3 minutes to obtain data on moisture, ash, protein, lipid, and carbohydrate content. Samples of fish meat were refrigerated at 8°C until chemical analysis was performed. The head, the viscera, the skin, and the bones were removed manually to obtain the meat and then freeze-dried. For the freeze-dried process, the fish meat was frozen at -80°C for 24 hours, and under the vacuum applied by the freeze-dried machine the water goes directly into the gaseous state and condenses on the cooled coil of the device. At the end of the process, the samples were bagged in polyethylene bags and kept at 4°C until the analyses.

Moisture and Ash

The moisture content of fish was determined by calcination for 24 h. After the samples had been burned in a muffle oven at a temperature of 600°C for 2 hours, the total ash content was determined.

Crude Lipid, Carbohydrates, and Crude Protein Analysis

The total protein content was determined by CHNS elemental analysis using the Dumas method with the EA 3100 elemental Analyzer (manual operating v1.3/2019). Cystine (B2131-1, elemental Microanalysis) has been used as a calibration standard according to SR EN ISO 16634-1:2009. The measured total nitrogen was converted to equivalent crude protein (%) using a conversion factor of 6.25. CHNS elemental analysis is the most widely used method of determining nitrogen and protein content in food and feed due to its high level of accuracy and reproducibility. The total fat content was determined with the Soxhlet automatic extractor (model R254, Behr Labor-Technik) and the extraction was carried out using a well-determined volume of solvent (petroleum ether). The total soluble carbohydrates was determined after the method described by Scott and Melvin, 1953.

Water Quality Parameters

Key physicochemical parameters were monitored to check water quality. Dissolved oxygen, electrical conductivity, water pH, and temperature were measured using a HI-9811-5 pH/EC/TDS/°C portable meter (Hanna Company, Cluj-Napoca, Romania). The nitrate concentration was measured using the phenol disulphonic acid method. Nitrate is typically converted to nitrite, which is then quantified by the standard Griess reaction (the sample was treated with sulphanilic acid and naphthyl-1-amine in an acidic medium) (Stavrescu-Bedivan et al, 2015). Ammonia nitrogen concentration was determined using the Nessler reagent spectrophotometric method at 420 nm wavelength. Phosphate concentration was determined using the molybdenum blue method at 700 nm wavelength. The measurements were performed using an SP-830 Plus spectrophotometer (Metertech Inc, Taipei, Taiwan). All determinations were performed according to ASRO Standard Methods (SR EN ISO 10523:2012, 5814:2013, 7150:2001). All water parameters were measured weekly.

Statistical analysis

The experiment was performed in triplicate and results were expressed as arithmetic mean ± standard deviation (SD). Quantitative data were statistically analyzed using repeated measures ANOVA followed by a post hoc test ($p < 0.05$). The analysis was performed using Jasp version 0.16.3.0 (an open-source statistical analysis program supported by the University of Amsterdam).

RESULTS AND DISCUSSIONS

Growth performance

In our study, we used freeze-dried *S. cerevisiae* BB06 with a cell viability of 10^9 CFU/g. Growth performance and feed utilization of *Cyprinus carpio* treated with and without different *S. cerevisiae* BB06 levels (YBV0%, YBV3%, and YBV5%) are shown in Table 2.

Table 2. Growth performance of common carp for each of the experimental diets

Indicators	Control (YBV0%)	YBV3%	YBV5%
Initial weight (IW-g)	81.63±0.50	82.50±0.70	81.88±0.62
Final weight (FW-g)	130.58±1.22	161.28±0.94	181.29±0.85
Weight gain (WG %)	59.96±2.29	96.41±2.13	108.04±2.17
Initial length (cm)	17.71±0.10	17.70±0.26	17.97±010
Final length (cm)	23.50±0.10	28.08±0.36	29.72±0.10
Feed conversion ratio (FCR %)	5.07±0.10	3.39±0.05	3.11±0.08
Condition factor (K)	1.41±0.01	1.02±0.01	1.16±0.01
Specific growth rate (SGR %/day)	0.84±0.02	1.20±0.02	1.31±0.02
Relative growth rate (RGR %/day)	0.01±0.00	0.02±0.00	0.03±0.00
Survival rate (SR %)	90.00±0.00	100±0.00	100±0.00
Protein efficiency ratio (PER g/g)	1.42±0.05	2.38±0.02	2.54±0.05

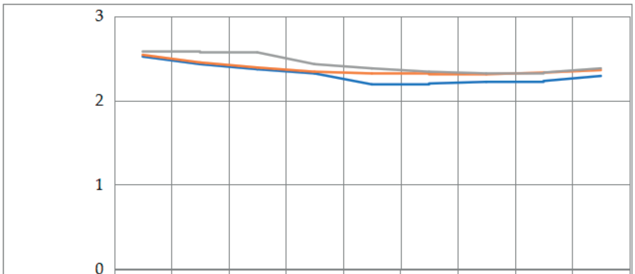
Experimental diet: YBV0% - Control, commercial feed without yeast, YBV3% - diet supplemented with 3% yeast biomass, YBV5% - diet supplemented with 5% yeast biomass. Values are means ± SD (n=10)

Parameters like weight gain, final fish weight, and specific growth rate increased significantly ($p <0.05$) with the increase in yeast biomass addition. The group that received no yeast supplement (YBV 0%) showed the lowest growth. The group that received a 5% yeast biomass supplement had a significantly higher final weight compared to the group that received a 3% yeast biomass supplement.

The YBV5% group had a higher percentage of weight gain (108.04%) compared to YBV3% (96.41%) and almost double that of the control group (59.96%). By statistical analysis, it was found that the length and final weight of *Cyprinus carpio* fish in the three aquariums were significantly different between the control (YBV0%) versus YBV3% and YBV5% ($p < 0.05$). Among the treatments, the SGR % was highest in YBV5%. Nevertheless, the condition factor (K) for the control group (YBV0%) was significantly higher than for the two groups receiving probiotic *S. cerevisiae* BB06. The survival rate of *Cyprinus carpio* was 100% for the groups receiving probiotic *S. cerevisiae* BB06. The feed conversion ratio (FCR) provides an objective metric for quantifying the amount of feed necessary for producing 1 kg of fish. However, practical applications of FCR are subject to variations resulting from diverse conditions such as temperature, water quality, etc. (Barbacariu et al., 2021; Mocanu et al., 2021).The feed conversion ratio (FCR) of

Cyprinus carpio treated with *S. cerevisiae* BB06 was significantly improved compared to the control. The protein efficiency ratio (PER) is the amount of body protein obtained per unit of dietary protein consumed and is used to assess the quality of different dietary proteins. In addition, the protein efficiency ratio was significantly higher ($p < 0.05$) in fish receiving yeast-treated diets than in the receiving control diets. In the current study, diets containing *S. cerevisiae* BB06 significantly increased the protein efficiency ratio (PER) compared to the yeast-free diet (control). An increase in the yeast content of the feed had a slight tendency to decrease the value of the Fulton condition factor.

At the same time, were determined the allometry coefficient to observe if the fish had grown more in mass or length. The allometric coefficient during the entire experiment for each experimental diet is presented in Figure 2.



	week 0	week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8
YBV0%	2.52	2.43	2.37	2.32	2.19	2.20	2.22	2.23	2.29
YBV3%	2.54	2.45	2.39	2.34	2.32	2.31	2.31	2.33	2.36
YBV5%	2.58	2.57	2.57	2.43	2.38	2.34	2.32	2.33	2.38

Figure 2. Allometric coefficient of fish weight *versus* length during the experimental period

In our case, the allometry coefficient obtained had values between 2.29 and 2.58. Weight increases faster than length (about 2.5 times faster).

Meat composition in carp

Meat composition in carp is presented in Table 3. Using the Jasp version 0.16.3.0 Pearson correlations (Pearson's R) were made between crude lipid and crude protein content in fish meat for each experimental diet Figure 3. It is well known that in the case of carp, the fat content is an important marketable parameter and depends very much on the growth conditions. Content over 15% can adversely affect the taste of the meat and, in some countries, allow on the market only carp with a fat content below 10% (Tewary and Patra, 2011; Bauer end Schlott, 2009).

All parameters, except crude protein, were significantly influenced ($p<0.05$) by supplemented yeast biomass. The analysis of meat composition indicates a significant decrease in lipid content alongside increased protein content. YBV5% showed significantly higher carbohydrate and ash content than the control (YBV0%), however, the lipid content was found to be the lowest.

Since the values obtained for Pearson's R correlation are negative, R = -0.92 for the YBV3% diet, with a significant difference ($p< 0.05$) and R = -0.85 for YBV5% ($p< 0.05$), indicate that large values of one variable involve small values at the other variable. Thus, the higher the percentage of fat the lower the percentage of protein, and vice versa, the lower the percentage of fat the higher the percentage of protein. There was a significant correlation between crude fat content and crude protein in diets supplemented with probiotic *S. cerevisiae* BB06 and the yeast-free diet control group ($p>0.05$).

Table 3. Meat composition of fish fed with different experimental diets (% freeze-dried weight)

Indices	Control (YBV0%)	YBV3%	YBV5%
Ash (%)	3.45±0.12	3.08±0.42	4.24±0.62
Crude protein (%)	79.20±1.44	80.27±1.85	81.35±1.07
Crude lipid (%)	8.11±0.23	8.06±0.77	7.26±0.17
Carbohydrates (%)	4.13±0.03	4.19±0.15	4.22±0.10
Moisture (%)	3.15±0.23	4.41±0.25	2.80±0.48

Experimental diet: YBV0% - Control, commercial feed without yeast, YBV3% - diet supplemented with 3% yeast biomass, YBV5% - diet supplemented with 5% yeast biomass. Values are means ± SD (n=10)

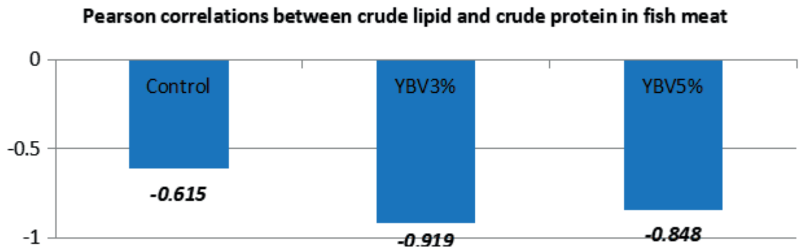


Figure 3. Pearson correlations of meat composition with different experimental diets

The analysis of the meat composition showed a considerable decrease in fat content, from 8.11% at YBV0% to 7.26% at YBV5%. In our study, the fat content was below the maximum recommended on the market, so the fish can be accepted from a qualitative point of view. Also, the addition of probiotic *S. cerevisiae* led to a significantly higher content of carbohydrates and ash content in the fish diet, and this may help the optimal growth of common carp. However, should be emphasized that in some studies was proved that the yeast addition led to a protein and ash content increase, while in others no changes were noticed; in this regard, it is most probable that the results depend on the employed yeast and the fish studied specie (Ljubojević et al., 2013). Crude protein is significantly higher in diet YBV5% compared to other diets, but a high protein content can lead to diseases caused by excessive growth (Guo et al., 2019). Fish require diets containing 30% to 55% crude protein and a supply of amino acids focused on specific requirements for maximum growth (Aragão et al., 2022). If the diet includes easily digestible ingredients, the high demand for protein in fish food can be met.

Water quality parameters

In our study, water quality parameters were regularly monitored in recirculating aquaculture systems (RAS), which conducted in them being maintained at acceptable levels in all tanks over eight weeks. The mean values (±SD) of the water quality parameters during the experimental variants are detailed in Table 4. Statistical analyses showed that the studied parameters: ammonium, nitrite, nitrate, phosphate, pH, electrical conductivity (EC), and dissolved oxygen (DO) were not significantly different ($p>0.05$) between the three experimental sets. Water pollution, caused by uneaten feed, high levels of ammonium and nitrite discharges, and toxic metabolites in fecal waste, is a major factor affecting fish growth and increasing the incidence of disease (Balcazar et al., 2006). In our study, we chose to use an ecological recirculating aquaculture system, which allows fish to be reared in controlled indoor conditions, limiting the likelihood of adverse environmental effects on carp production. The data showed no significant differences ($p> 0.05$) in any of the three aquariums in terms of water pH, temperature, and OD. Ammonia and nitrate concentrations were lowest ($p< 0.05$) in the aquarium with YBV3%, followed by YBV5% and control. Although N-NO_3 is not very toxic to fish, its accumulation in high concentrations for long periods is not suitable for fish growth (Estim et al., 2019). Toxic compounds, including ammonium and nitrogen compounds, are regulated by naturally occurring microorganisms, particularly denitrifying bacteria. However, various studies have demonstrated the effectiveness of certain

probiotics, particularly Gram-positive bacteria, in reducing ammonia nitrogen levels, indicating their potential as a sustainable additive to enhance water quality (Mohapatra et al., 2013; Zorriehzahra et al., 2016; Camargo et al., 2005; Verschuere et al., 2000; Jóźwiakowski et al., 2009; Cha et al., 2013). Nonetheless, certain differences may arise depending on the yeast and fish species (Essa et al., 2011).
However, further research is needed to understand how probiotics work to improve water quality.

Table 4. Average values of the water parameters in recirculation aquaculture system

Water quality parameter	GROUPS			*p*-Value
	CONTROL[1]	***YBV3%***[1]	***YBV5%***[1]	
$N\text{-}NH_4$ (mg/L)	0.079±0.029	0.048±0.004	0.048±0.004	0.06
$N\text{-}NO_2$ (mg/L)	0.34±0.02	0.01±0.03	0.14±0.01	0.29
$N\text{-}NO_3$ (mg/L)	29.67±3.24	23.57±5.28	25.13±7.85	0.74
PO_4 (mg/L)	1.47±0.51	0.73±0.10	0.43±0.19	0.50
pH	6.70±0.46	7.28±0.19	6.64±0.35	0.14
EC (µS/cm)	524.44±168.53	671.11±276.33	620.0±210.53	0.28
DO (mg/L)	4.77±0.09	4.91±0.36	5.26±0.44	0.94
Temperature (ºC)	24.94±0.58	25.33±0.64	24.86±0.66	0.92

[1] Mean values ± standard deviation of the water parameters during the experiments (56 days) (temperature, pH, salinity, and oxygen: n = 56; ammonium, nitrite, and nitrate: n = 28).

CONCLUSIONS

The current study showed that the addition of freeze-dried probiotic *S. cerevisiae* BB06 at 3% and 5% in the diet significantly improved the body weight, weight gain, specific growth rate, and feed conversion ratio compared to the control group. The results show that diets yeast supplemented represent alternatives worthy of consideration for ensuring sustainable aquaculture. The high content of additionally administered yeasts contributes to the increase in the protein content of the carp meat. Previous research has shown that the *S. cerevisiae* BB06 strain has functional properties such as high resistance to the gastrointestinal environment, hydrophobicity, self-aggregation, antioxidant, and antibacterial activities, as well as safety properties such as γ-hemolysis and resistance to bacterial antibiotics, making it a suitable candidate (Diguță, 2023). It also has high freeze-drying survival, making it optimal for storage purposes. (Diguță, 2023).
The results of the study confirm that the partial replacement of fish meal with 3% or 5% probiotic *S. cerevisiae* BB06 has a beneficial effect on growth parameters, in agreement with previously published literature (Aragão et al., 2022; Essa et al., 2011; Ma et al., 2019; Oliva-Teles and Gonçalves, 2011; Tovar et al., 2002). In our study, including probiotic *S. cerevisiae* BB06 in the fish diet may have contributed to maintaining the water quality by improving feed utilization and reducing the food conversion ratio (FCR), leading to high growth rates and carp production. Substituting fishmeal partially with probiotic *S. cerevisiae* BB06 was shown to promote beneficial growth of carp (*Cyprinus carpio*), while maintaining meat fat content and improving protein retention. In addition, the feed conversion ratio was reduced. Overall, the results indicate that yeast-supplemented diets represent viable alternatives to ensure sustainable aquaculture. The recommendation for future carp feed recipes is to completely replace fishmeal with yeast to ensure better growth performance, viability and maximize sustainability in aquaculture.
Further research is required to explore the role of the probiotic *S. cerevisiae* BB06 in disease resistance, as well as on the host fish's immune system.

ACKNOWLEDGEMENTS

The present work was funded by the University of Agronomic Sciences and Veterinary Medicine of Bucharest – Romania, Research Project 1262/30.07.2021, acronym FEED4FISH in the Competition IPC 2021.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

SIGNS OF THE PRESENCE OF THE EURASIAN BEAVER (*Castor fiber*, Linnaeus, 1758) IN THE PRE-DELTAIC AREA OF ROMANIA

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Abstract

The beaver has a historical presence on Romania's territory. The European beaver is nicknamed the "engineer of ecosystems" for the ingenuity through which it builds a mosaic of habitats. In their habitats it stores water and, in the same time, does something more like expanding wetlands which are helpful for adapting to the climate change. Moreover, this herbivorous species has a long-term impact on the environment in which it lives, enriching the biodiversity of these habitats. The purpose of this manuscript is to update the information about the beaver populations in the pre-deltaic area of Romania. The information was collected during period 2019-2022, when we effectuated researches in weekly in the dig-mal flood zone of the Danube River, between Grindu locality and Tulcea city and in the area of the Somova-Parches aquatic complex. All the researches were done using the method of signs of presence, a methodology also approved by the IUCN working group. Finding signs of presence on other territories apart from the well known us, led us to the conclusion that the number of beavers in Danube's avandelta has expanded since it was reported in 2010 by occupying favourable habitats.

Key words*: Danube's avandelta, Eurasian beaver, presence.*

INTRODUCTION

The Anthropocene is the historical period we are currently living in, characterised by a phase of severe decline of biodiversity worldwide, mostly due to habitat loss and fragmentation, and biological invasions (Dirzo et al., 2014; Bellard et al., 2016, Mori et al., 2021). As a young region, in continuous formation and consolidation, the Danube Delta is the most suitable place for the development of a unique fauna in Europe.

Inside the Delta, several main ecosystems can be distinguished: flowing waters, stagnant waters, marshy and floodable surfaces, fluvial and maritime dykes. The areas of contact between fluvial and maritime waters constitute a special ecosystem called Avandelta.

For millions of years, beaver has been an integral part of the Danube basin fauna. This rodent mammal loves to carry his life out in habitats like: the ones of freshwater, with woods all around, different types of canals maybe agriculture one or both areas suburban and urban. The Romanians known the beaver as the "breb", but more than that has also is nicknamed as "engineer of ecosystems". An important quality: ingenuity of building mosaic of natural surface, having the capability of retaining water and expanded a wetland, conducted to this nicknamed. This capability is very useful in the action condition like enlonged droughts and the other changes because of the climate (https://www.carpathia.org/ro/castorul-revine-in-sud-estul-muntilor-fagaras/).

Beaver's introduction in a certain area must be preceded by a feasibility study because it's presence there can be helpful or unwanted by humans. The advantages can be: the shifts of

river dynamics, nutrient cycling, biodiversity, and human cultural experience while the disadvantages are associated damages caused to humans (Blewett et al., 2021).
After the disappears of this specie in the first part of 19th century from all the Romania and the most of the Europe, it became a strictly protected species. He disappeared for the same reasons: excessive hunting for fur and for the used castoreum in the perfume industry and habitat modification. Then, through some projects, the beaver was reintroduced almost in all countries.
Thus, in 2020, the majority of the beaver population in Europe was located in the central and eastern parts of the continent (Wrobel, 2020). According Pasca et all, 2018 the estimation is 2145-2250 exemplares.
In Romania, in the Somova-Parches complex, the presence of beavers was reported in 2010 on Parches Lake. During the last years, the beavers have been noticed along the Danube, downstream from the confluence with the Ialomiţa until the Danube Delta.
Via the trap's camera, the teams of WWF-Romania and Rewilding Europe have revealed to media images with beavers taken from Somova - Parches Complex.
Even in the Small Inland of Brăila was seen a family of beavers, this being the most recent reports. Thus, nowadays the beaver can be seen in Romania in places like rivers Olt, Mures, Ialomița and the Danube basin downstream of the confluence with the Ialomița and the Somova-Parcheș complex area of the Danube Delta.

MATERIALS AND METHODS

Research zone

The research zone contains: mainly of Isaccea-Tulcea floodplain, Somova - Parcheș aquatic complex and Danube River between Cotul Pisicii and Isaccea. Isaccea-Tulcea floodplain is located upstream of Tulcea.
Depending on the intensity and duration of the floods in the meadow, different lands and water categories are distinguished, floodable or non-floodable lands and water basins, some permanently with water, others temporary, which, with the decrease of the waters, significantly reduce their surface and depth or sometimes it dries up completely.
The aquatic complex Somova-Parches is considered a mini delta of Romania, being located in a field on the right side of Danube, upstream to entrance in the Danube Delta. Its surface is 9170 ha and it is a proper habitat for a large number of species of animals. This complex consists of fishing resources of fresh water of the Danube Delta Biosphere Reserve.
All the channels and brooks have a length of 35.5 km. The most important lakes of the complex are: Rotundu (228 ha), Gorgonel (141 ha), Telincea (188 ha), Parches (196 ha), Somova (123 ha), Caslita (153 ha) and Somova (149 ha) (Burada, 2016; Popescu, 2022).

Methodology

Our investigations regarding European castor of aquatic complex Somova-Parches, Isaccea-Tulcea floodplain and Danube River between Cotul Pisicii and Isaccea were made between years 2019-2022.
We used the indirect monitoring method, based on the analysis of the traces left by the beaver in its living environment. Indirect methods allow not only the detection of the presence of species (in the case of rare ones), but also estimates of their abundance as well as obtaining data on various aspects of their biology or ecology (Ionescu et al., 2013). To respect the methodology, we effectuated transects of 1200 m, keeping the distance of 2 meters between then. For those too small habitats, we investigated the entire aquatic body.

RESULTS AND DISCUSSIONS

The monitoring of beavers in the Danube Delta, carried out by the WWF-Romania team and Rewilding Europe, in partnership with the Forestry Research and Development Institute (ICAS) Brașov, began in April 2014 at the recommendation of the Danube Delta Biosphere Reserve Administration (ARBDD). The reintroductions of the species, which were carried out in different parts of Europe, including Romania, as well as the rate of natural growth led to an increase in the number of individuals.

According to Ionescu et al. (2010), the exemplars of beavers from the repopulated Romania come from Germany and more precise from Bavaria. We remark a growth from 64 individuals to 217 individuals in Ialomita (Carpathian Foundation, 2014). Kiss et al. (2011) made a report to let us known that the population extended these territories: along the Danube, reaching the Danube Delta downstream.
Using the transect the team fossed on finding: shelters, footprints, bites, beaver paths, territorial marking, or others. We starting the investigations in July 2019. We only presented the successfully transects.
The first observation of signs of beaver (inactive shelter) was identified on August 02, 2019.
As signs of inactivity, we highlight the covering of entrances with spontaneous vegetation, a sign that they are not used.
Almost one year after the first observation, in order to monitoring the shelter, on May 20, 2020, at the same coordinates, the shelter was damaged and the surrounding vegetation burned (Figure 3 A, B). The channel was transited by fishermen with motor boats, towards Telincea Lake. 2019, on the hill of Parches Lake (45°22′56″ N 28°56′66″ E) (Figure 1).

Figure 1. Inactive shelter of beaver (photo: Ibanescu)

Vegetation is crucial for the beaver' s habitat (Bouroș et al., 2022).
The shelter was observed on a 1200 m transect with mixed vegetation: forest, shrubs and grassy vegetation (Figure 2).

Figure 2. Hill of canal shelter (photo: Popescu)

Figure 3A. Burned vegetation (photo: Cristescu)

Figure 3B. Burned vegetation (photo: Nica)

On November 11, 2020, on a transect carried out on the channel bed (beginning: 45 º 22'87" N, 28 56 º 73'75''E; ending: 45 º 23'89'' N, 28 º 56'89''E), a large entrance to the shore was identified, at the coordinates: 45 º 22'92''N/ 28 º 56'65''E (Figure 4).

Figure 4. Large entrance to the shore (photo: Ibanescu)

The next observations were done in July, 16, 2021, on a transect carried out by boat on Parches Lake.
Following the visual investigation of the banks and the monitoring of the above-ground beaver shelter, fresh cracks were observed around the shelter (Figure 5), willow branches with a diameter of 2-3 cm and 5-6 cm (Figure 6A and 6B).

Figure 5. Active shelter of beaver (photo: Cristescu)

Figure 6A. Fresh rosacea (photo: Popescu)

Figure 6B. Beaver rosacea (photo: Nica)

On 17.12.2021 we carried out a new transect at the beaver shelter, on the canal connecting Parcheș and Telincea, coordinates; transect start: 45°23'05" N/ 28°57' 58" E; end of transect: 45°23'25"/28°56'60". Its condition was good, the shelter was rebuilt, height approximately 1.5 m, diameter 4 m (branches, plant debris were added); you can see willow branches with recent traces of frostbite.
Willow twigs and stumps with old rots, willow twigs (diameter = 10-20 cm) and willow stumps with very fresh rots, willow trunks (3 willows) with very fresh rots were found (Figure 7).

Figure 7. Willow trunk with rodents (photo: Nica)

Figure 8. Beaver rosacea (Photo: Cristescu)

Figure 9. Investigated habitat (Photo: Popescu)

Figure 10. Branches of different diameters (photo: Ibanescu)

All branches, stumps and gnawed trunks were found at the following coordinates: 45º23’04” N / 28º57’57’’; 45º23’04’’ N / 28º57’55” E ; 45º23’05”/ 28º57’54” E; 45º23’01” N /28º57’49” E; 45º23’01” N /28º57’53” E; 45º23’00” N / 28º57’49” E; 45º23’01”N / 28º57’50” E; 45º22’99” / 28º57’47” E.

On 22.02.2022, new signs of the presence of the beaver were found at the coordinates: 45 º 44'86" N / 28 º 20'36" E (Figure 8).

The habitat investigated is presented in Figure 9.

Observations: the beam was full of branches with different fresh spines, branches of different diameters, a red willow trunk, with a diameter of approximately 30 cm (Figure 10).

Also, branches with rotten bark were found (Figure 11).
So, the beaver family passed the winter well.
Coordinates**:**
- 45 º 23’03” N / 28 º 57’53”;
- 45 º 23’03” N / 28 º 57’55” E;
- 45 º 23’02” N /28 º 57’53”E;
- 45 º 23’00” N / 28 º 57’49” E;
- 45 º 22’95” N / 28 º 57’37” E.

Figure 11. Branches with rotten bark (photo: Cristescu)

On 28.04.2022, a transect was carried out on the gravel between the Ivanova channel and Corciovata Mică lake, where the habitat is optimal for the *Castor fiber* species, with mixed vegetation, predominating shrubs.

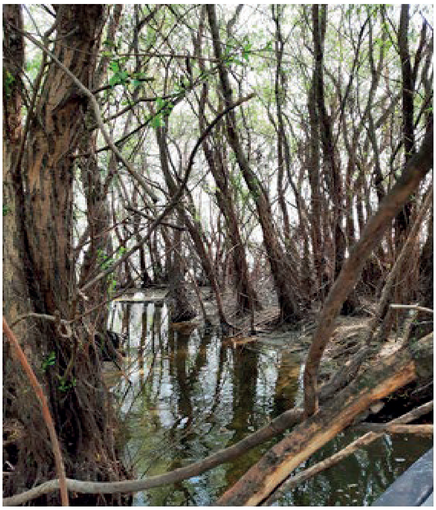

Figure 12. A ditch built by beavers

Figure 13 highlights the places where we found indisputable signs of the presence of the Eurasian beaver.

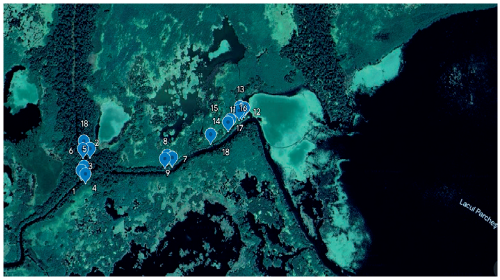

Figure 13. The coordinates of signs of beaver presence

CONCLUSIONS

The reintroductions played an important role in restoring the beaver population in Romania.
Beaver gradually returns to the area, from which he was thoroughly eliminated away by people's selfishness.
The discovery of new territories occupied by the Eurasian beaver demonstrates that the beaver population in Danube's avandelta has expanded since it was reported in 2010 by occupying favorable habitats.

ACKNOWLEDGEMENTS

Acknowledgments to the project “Revision of the management plan and the DDBRA regulation”, contract no. 253/2019, POIM / 178 / 4.1 / 123322.
The present research was supported by the project An Integrated System for the Complex Environmental Research and Monitoring in the Danube River Area, REXDAN, SMIS code 127065, co-financed by the European Regional Development Fund through the Competitiveness Operational Programme 2014–2020, contract no. 309/10.07.2021.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

THE IMPACT OF ENVIRONMENTAL FACTORS ON THE METABOLIC RATE IN FISH: INTEGRATION OF EXISTING DATA

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Abstract

As far as we known there are many scientists doing a lot of researches about animal physiologic regarding the influence and the result of different factors on fish metabolic rate. We can mention as an example: mass, temperature or chemical pollutants on metabolic rate. Regarding intraspecific variation, the reasons why it isn't very well known less about factors causing. It is well known that feeding control influences the metabolism, they interlinked each other. During the last years we have been seeing a lot of climate changes which affect also the fish. Many areas like ecological niches, metabolic performance is definitively influenced by two very important factors like oxygen or temperature. Sometimes, when we have most important environmental variables, the answer for these variables is the temperature of the water and as an effect of this environmental variables we could see the metabolic rates of ectotherms. For this reason, wise minds studied besides water temperature the also the effects of this factor on fish metabolic rate. The also concluded that other environmental factors like oxygen and salinity play very important role as potential determinations of the fish metabolic rate. This review aims to integrate the currently existing data regarding the influence of environmental factors on metabolic rate in fish.

***Key words**: environmental factors, impact, fish, metabolic rate.*

INTRODUCTION

Fishes have evolved physiologically to live within a specific range of environmental variation and existence outside of that range can be stressful or fatal (Jahan, 2018).
Metabolic rate is a very important point for keeping fish alive that determines survival in the field because behavior such as providing their food escape from other strong animals and rivals, and also other important physiological process, that require oxygen (Eliason & Farrell, 2016). Metabolic rate, the amount of energy consumed per unit of time, for an organism to sustain life, is influenced by both intrinsic and extrinsic factors (Tanaka et al., 2023). There are a lot of factors in our environmental, like: temperature, oxygen concentration, and pH levels (Campos et al., 2018) with important influences on each metabolize. In conclusion, all these factors affect also the fish metabolism. To some up, we conclude that the issue of this overview is to gather the literature study about those factors which affect the metabolic rate in fish.

MATERIALS AND METHODS

For realising this overview were consulted a number of speciality articles from this domain which approaches the mentioned subject. The most studies are recent, but there are a few older ones that are representative in this domain. The scientific papers we spoke about

are indexed in GS data based, a percentage of 58.06% of all these papers are, as well, indexed in the Web of Science (WOS) database and another percentage of 48,38 % are indexed in Scopus. We have red zone, yellow zone and white zone in the WOS database. According to our scientifical studies, our percentages are: 35,48% in the red area, 16,12% in yellow one and 48,4 % in the white area.
After Simionov et al. (2019), the best results can be obtained when multiple research methods electronic and manual compete at the wishing result. To be in the position to given a complete response, we taken to considers o large a period between 1965 and 2023. They were prior the published papers those three zone (red, yellow and white) having a good visibility. The searching of the articles was done using keywords for the approached subject.

RESULTS AND DISCUSSIONS

1. The influence of temperature on the metabolic rate in fish

Under a global warming system, extreme variation in environmental temperature could cause significant stress to aquatic species. The most important effect on fish metabolism, on all its levels, those levels being between the standard metabolic rate and maximus, is temperature (Brett, 1965; Norin & Clark, 2016).
Fish have the capacity of acclimating to temperature changes when are exposed for longer periods. This factor es a key one with an important impact on the metabolic processes. The clownfish was exposed to a rapid increase in temperature combined with limited food availability (one meal after fasting) by Pham et al. (2022) in a study regarding metabolic rate food intake and intestinal transit. The conclusion shows up that less forage combined with more degrees led to assessed he stimulation of anorexigenic metabolically ways for clownfish. The meaning of this action is that: a bit of food steel goes to a higher metabolic rate steel having an induced temperature.
These results show up those small quantities availability and elevated temperature excites anorexigenic steps in, resulting in significantly lower feed intake despite the temperature-induced increase in metabolic rate.
The thermal curve includes the metabolism effects.
This shows up an increasing of temperatures followed by a value's constancy and severe drop (Rezende & Bozinovic, 2019).
Another study made by Liang et al. (2022) aimed to determine whether water temperature played a role in regulating the growth and nutritional metabolism of juvenile grass carp. He is mentioning that *Grass carp* reared at different temperatures seem to show different metabolic patterns.
Kuhn et al. (2023) were exanimating the effects of temperature on feeding actions, food eating and the expression of this in three characid fish (black tetra, neon tetra and cavefish) by submitting them to four different temperatures and showed that temperature influences feeding in *Characidae* fish and induces species-specific changes in the expression of appetite regulators. It is well known that the fish are ectotherms and because of that they have fluctuations of their body's temperature in the same time with the environmental temperature (Haesemeyer, 2020). The increasing temperature is direct proportional with the increasing of biochemical reaction rates, with the metabolic rate, energy balance subsequently increasing energetic demands (Johansen et al., 2021).
Another study observed that when repeatedly exposed to warm water temperatures daily for 53 days, juvenile lake trout had higher maximum metabolic rates compared to those that stayed at cool temperatures, offering the capacity of adapting to thermal exposure (Guzzo et al., 2019).
Moore et al. (2023) investigated how temperatures associated with future warming and present-day marine heatwaves (+3°C) impact the growth, metabolic rate, and more aspects regarding developmental steps of clownfish larvae (*Amphitryon ocellaris*). There results indicate that clownfish development could be altered under future warming, with developmental rate, metabolic rate, and gene expression all affected.
The importance of temperature results also comes out of the researches Grimmelpont et al. (2023). They focused on golden grey mullet

Chelon auratus which they expose to marine heatwaves getting the conclusion that increasing temperature and increasing metabolism are direct proportional.

When Beuvard et al. (2022) concentrated on examining on the effect of different temperatures regarding survival, the growth rate metabolism, and physiological indices of juvenile *Arctic charr* underlined that over optimal temperature growth rate leads to: a decrease of energy intake, a higher demand of the metabolic system and changes in the ability cardiovascular system in sustaining the fish mechanism at higher degrees.

Another effect of water temperature is of about the fluctuations of chemical reactions, physiological processes, and metabolic steps. According Volkoff & Rønnestad' studies (2020), we can declare temperature as being very important because the affects the minimum and maximum metabolic rates and thereby the aerobic scope.

2. The influence of oxygen on the metabolic rate in fish

The most important process on Earth, photosynthesis, has as a result the diffusion of the oxygen from atmosphere, say Singh & Kumar, 2014. It also depends on chemical composition of water body which is very variable, depending on season, time of day, place and depth (Nimesh & Jain, 2016).

The main factor for keeping fish alive, for maintaining them a good health and keeping up the bacteria which decompose the waste produced by the fish, and to meet the biological oxygen demand within culture system is oxygen.

For assuring a fast oxygen intake from water into fish's blood, supposed to have an important availability of oxygen in water. This fact can be held by partial pressure gradient of oxygen, according to Mallya (2007).

The concentration of oxygen in the growth environment has a significant impact on the metabolic rate of fish (Tom, 1998; Ali et al., 2022). Through metabolism, food or stored energy is transformed into the energy needed to perform daily tasks.

Nimesh & Jain (2016) got to the conclusion that.

Many activities body (respiration, feeding) are slower when the availability of dissolved the oxygen is lower.

Other researches (Norin & Clark, 2016) entrechats the above idee that some fish can responded to hypoxia with a decrease in maximal metabolic rate, while individuals may differ greatly regarding their metabolic response.

Moss & Scott underlined through their study from 2011 standard metabolic rates for the bluegill, *Lepomis macrochirus*; largemouth bass, *Micropterus salmoides*; and the channel catfish, *Ictalurus punctatus*, at different degrees. These compared with other species was lower at the same temperature. But we have other two species like: bluegill and perch weighing more 15 grams than which responded indirectly proportion.

3. The impact of pollutants on the metabolic rate in fish

A team, Makiguchi et al. (2023) worked on the idea of comparing the metabolism and swimming performance of semi-wild and farmed fish (*Oncorhynchus masou*). There results were that semi-wild fish have a higher metabolic activity. Oher researches made by He et al., in 2023, was about the evaluate the different effect were regarding: growth's indicators, digestive catalysts, biochemical composition, metabolic factors and antioxidant activities of juvenile Yangtze sturgeon (*Acipenser dabryanus*). They conclusive that the with increasing levels activities feeding levels, higher amylase and lipase, and fish fed with 3% body weight day^{-1} led to a more intense trypsin activity.

Fish would be on the point to create a balance between their metabolism and growth by optimizing nutrient absorption and utilization when fed at different dietary levels.

Zhang et al. (2023) could demonstrate that damaged mitochondrial membrane potential and respiratory chain function through the exposer of female zebrafish to 0.05 mg/L and 0.5 mg/L florfenicol for 28 days. All this prove that toxicity and perturbed metabolic signaling in the F1 generation were related to the mitochondrial injury after exposing F0 female zebrafish to florfenicol.

A comparation upon metabolic effects caused by using copper oxide particles with two distinct structures: nanorods and nanosphere was done by Oliveira et al. (2022). They used the closed respirometry technique for analyses the metabolic rate, specific ammonia excretion, and swimming ability as biomarkers, the physiological effects on *Danio rerio.* This study showed that the metabolism of fish was affected by different morphological structures of the same copper oxide nanoparticle.

It is concluded that the characterization of nanoparticles is essential to understand their effects on fish, since their structural forms can cause different toxic effects on *D. rerio*.

Santos et al. (2023) in their study were investigated a non-target metabolomic vision to investigate changes in the metabolome of juvenile meagre (*Argyrosomus regius*) exposed to venlafaxine (20 μg/L). Venlafaxine conducted to significant alteration of endogenous metabolites tentatively identified in liver, brain and plasma, respectively, also variation metabolic profile where underlined.

Santos et al. (2023) discovered that another substance methamphetamine disturbance metabolism, physiology, behavior.

4. The influence of salinity on the metabolic rate in fish

The development, survival and the welfare condition of animals in general is influenced by several factors, one of the most important for fish being salinity. In terms of growth performance, each species has an optimal salinity range. This fact can be exemplified by the study carried out by Boeuf and Payan (2001) on the rohu species.

Thus, a temporary increase in salinity with a long acclimatization period has a minimal impact on growth and other physiological parameters.

CONCLUSIONS

This paper summarizes the most important effects of temperature, salinity, oxygen and pollutants on the metabolic rate of fish. Among them, temperature decreases the ability of fish to respond to chemical stress factors, through profound metabolic changes.

Thus, it reinforces the idea that temperature has a general effect on all levels of metabolism.

ACKNOWLEDGEMENTS

The present research was supported by the project An Integrated System for the Complex Environmental Research and Monitoring in the Danube River Area, REXDAN, SMIS code 127065, co-financed by the European Regional Development Fund through the Competitiveness Operational Programme 2014–2020, contract no. 309/10.07.2021.

The authors are grateful for the technical support offered by Reform - MoRAS through the Grant POSCCE ID 1815, cod SMIS 48745.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

INFLUENCE OF FEEDING TYPE ON GROWTH AND BLOOD PARAMETERS OF BLACK BARBUS, *Puntius nigrofasciatus*

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Abstract

A twenty-five weeks study of Black Barbus, P. nigrofasciatus fry was conducted to determine the effectiveness of various combinations of feed and its effect on growth, survival, and physiological and hematological parameters composition of fish blood. To achieve this goal, Black Barbus, P. nigrofasciatus fry were kept in three identical 120-liter aquariums, and fed different diets. The first diet included industrial feed in the form of flakes. The second diet included live microorganisms and crustaceans. The third diet was a 1:1 combination of industrial feed and live organisms. The results of the study showed a higher growth rate in Black Barbus, P. nigrofasciatus, which consumed combined feed, than peers consumed dry feed by 0.60 cm or 10.53% ($p<0.05$) and then peers consumed live feed by 0.20 cm or 3.28% ($p<0.05$). The content of hemoglobin in the blood was the same in fish of three diets. The level of erythrocytes was higher by 0.12 million×µl-1 in the blood of fry, whose diet was combined.

Key words: *aquaculture, feed, fry, hemoglobin, hydro-chemical parameters.*

INTRODUCTION

One of the important factors of effective fisheries, which combines high productivity with economically justified costs and high nutritional value of fish products is scientifically sound feeding of fish (Cottrell, 2022; James, 2013; Verma & Satyanarayan, 2016). Intensification fish farming involves the use of balanced and cost-effective feeds for feeding all age groups of fish. The main task in commodity fish farming is to ensure maximum growth of fish products in the shortest possible time. The solution of this problem is based on meeting the nutritional needs of fish (Brune et al., 2003; Kong et al., 2009). Scientifically based use of vitamins, minerals and enzymes in combination with other biologically active substances can significantly increase the efficiency of fish feeding by increasing the availability and digestibility of feed nutrients (Lall & Kaushik, 2021). The latest achievements in the field of biological sciences, combined with the growing capabilities of modern technology in the near future will contribute to the improvement of fish farming technologies, in which fish feeding will maintain a leading position (Pradeepkiran, 2019). With feed, the body of fish receives a variety of energy-rich substances, which are further broken down into simpler substances. The energy released in this case ensures the flow of various physiological processes. In addition, substances entering the body are used to repair worn out and build new cells and tissues, for the formation of hormones and enzymes (Bogdan, 2005).

It is important to choose the right quality feed with a huge variety of nutrients and biologically active substances, which at least slightly correspond to the natural diet of fish (Hamre et al., 2013; Prabhu et al., 2019). Mastering the principles of rational use of feed and modern methods of feeding fish opens up the possibility of significantly reducing the cost of feed per unit of fish production. This is not only an economically positive result, but also a circumstance that has a certain environmental significance, which logically follows from energy conservation, improving the environmental situation by significantly reducing the pressure on the environment (Velasco-Santamaría & Corredor-Santamaría, 2011). The process of growing high-quality standard fish stocking material, especially using the technologies of using fry, involves the use of live feed or starter feed mixtures enriched with biologically active substances (Krepych et al., 2021). Improperly selected feed can lead to various diseases and death of fish. For the normal functioning of fish, their feed must contain a complex of nutrients in certain quantities and ratios that will create its balance of feed. The need of fish in certain feed ingredients is not constant and depends on the age, size and sexual maturity of fish (Virtanen et al., 2008; Zhao et al., 2015), season, body weight, fatness and the quality of others environmental factors (Aubin et al., 2019; Tselu & Klei, 2017). Lack of nutrients (proteins, fats, carbohydrates, minerals and vitamins) that come with feed, leads to pathology in fish. Therefore, if possible, it is necessary to avoid the use of feed that is unbalanced in terms of basic nutrients (Vilain et al., 2016; Wang et al., 2016). In addition to the imbalance of feed, we should not forget about such a criterion as poor-quality feed and feed with violations of storage conditions of its ingredients. This is especially true of feed or its components that contain large amounts of fat, as this leads to its oxidation with the formation of highly toxic free fatty acids and peroxides. The use of such feeds can lead to the development of pathological processes and the death of fish (Mwihia et al., 2018; Oliveira & Vasconcelos, 2020; Pietsch, 2020).

The study of the influence of feed type on the growth and survival of fish in the laboratory allows the use of Black Barbus, *Puntius nigrofasciatus* (Günther, 1868), which is found both in natural waters of different regions and is widely used in aquaristics. Its homeland is the island of Sri Lanka. It lives in slow-flowing rivers and stagnant ponds with dense vegetation. The natural population of it is small, which was the reason for listing in the Red Book, but the fish is easily breeds in captivity (Carosi et al., 2017).

Mortality of Black Barbus, *P. nigrofasciatus* (Günther, 1868) during fattening and intensive growth under artificial conditions is associated with inappropriate feed (Rutaisire et al., 2015). Studies of the effect of feed on blood characteristics in Black Barbus *P. nigrofasciatus* have not been previously published. However, the results of the experiment (Prusińska et al., 2020) were found, which indicated that the content of neurophiles in the blood of Barbel Larvae *Barbus barbus* probably depended on feeding.

The behavior of Black Barbus, *P. nigrofasciatus* (Günther, 1868) during fattening, which will allow optimal survival and growth in artificial growing conditions, needs a clear understanding. It is necessary to study the intensity of growth and survival of fry in unnatural growing conditions. Barbus is usually limited to the river environment at the beginning of life, but adult fish are capable of high mobility and adapted to survive in both river and lake environments (Ondhoro et al., 2016**)**. From this it is possible to make assumptions about the possibility of applying different survival strategies taking into account the impact of feed on different age groups. Barbus is known to be an omnivorous fish species (Aruho et al., 2018; Corbet, 1961**)**, so its breeding requires knowledge of its diet and growth in a limited environment.

The study of the problem of efficient fish feeding is becoming more relevant than before with the growing feed demand for protein of fish origin and the high need to restore natural resources of fish farming. Studying the impact of different feeds on the growth of Black Barbus, *P. nigrofasciatus* (Günther, 1868) in aquarium conditions can transfer the experience gained to the process of breeding other related fish species in terms of their fish farming in natural and artificial reservoirs. The results will also be useful in the field of aquaristics, which is gaining popularity again and needs constant study to improve the conditions of fish, taking into account their physiological needs, adequate provision and humane treatment.

The aim of our study was to investigate the effect of different types of feeding on the growth of fish and their physiological using Black Barbus, *P. nigrofasciatus* (Günther, 1868) fry.

MATERIALS AND METHODS

This study was conducted for 25 weeks in 2022. The scientific experiment was staged at the Bila Tserkva National Agrarian University, Kyiv region, Ukraine (49.7631997,30.0764052).

Three 120-liter aquariums were used to determine the dynamics of Black Barbus, *P. nigrofasciatus* (Günther, 1868) growth. Ninety 0.7 cm Black Barbus, *P. nigrofasciatus* (Günther, 1868) fry were used for the experiment. Thirty fries were placed in each aquarium. Later they continued to be used as growing aquariums. The planting density of fry was based on the prospect of their growth. During the study, the hydrochemical conditions of water for the maintenance and cultivation of young Black Barbus, *P. nigrofasciatus* (Günther, 1868) were maintained at a constant level. Illumination in aquariums was in the range of 4-6watts per one liter of water. Duration of lighting was ten hours per day. For the reliability of the research, the parameters and conditions in the 3 aquariums were similar. Water for filling aquariums was used from the city watercourse, which had previously been defended and aerated for 4 days. Water samples for analysis were taken every day. The value of water index (pH), concentrations of biological elements (ammonium, nitrate and nitrate nitrogen), temporal hardness of water and the presence of dissolved oxygen were determined in the studied water.

Based on the purpose of the work, different types of feeding were used in each aquarium, namely (Table 1): the first aquarium included dry feed industrial production of the company Tetra, Sweden; the second aquarium included live Nauplii Cyclops, *Cyclops strenuus strenuous* (Fischer, 1851), live Artemis, *Artemia salina* (Linnaeus, 1758), live Ciliate Shoe, *Paramecium caudatum* (Ehrenberg, 1833), live Tubeworm, *Tubifex tubifex* (Müller, 1774), live Moth, *Chironomidae* (Newman, 1834), live Koretra, *Chaoborus* (Lichtenstein, 1800); the third aquarium included a combined type of feeding. Fish kept in the first aquarium were taken in group I, those kept in the second and third aquariums were classified according to II and III groups. Fish in the experiment received feed in quantities not exceeding 2.0% of the total weight of all fish in each aquarium twice a day in the morning and in the evening for 1 aquarium: 1-5 weeks – 21.42-61.2 g; 6-10 weeks – 61.2-91.8 g; 11-15 weeks – 91.8-134.6 g, 16-20 weeks – 134.6-162.18 g, 21-25 weeks – 162.18-174.42 g. Every seventh day of the week the fish did not receive feed at all.

The presented structure of the diet allows us to identify that in the first aquarium for feeding Black Barbus, *P. nigrofasciatus* (Günther, 1868) fry dry feed was used as a substitute, in the second was used live feed and in the third was used a combined type of feed (industrial feed and live feed).

Table 1. The use of feed depending on the fish age

Weeks	**Type of feed**		
	Group I	**Group II**	**Group III**
1-5	Dried Cyclops Nauplii, *Cyclops strenuus strenuus* (Fischer, 1851) – 35% Dried Artemia, *Artemia salina* (Linnaeus, 1758) – 35% Dried Rotifer, *Brachionus urceolaris* (Müller, 1773) – 30%	Live Nauplii Cyclops, *Cyclops strenuus strenuous* (Fischer, 1851) – 35% Live Artemis, *Artemia salina* (Linnaeus, 1758) – 35% Live Ciliate Shoe, *Paramecium caudatum* (Ehrenberg, 1833) – 30%	Dried Cyclops Nauplii, *Cyclops strenuus strenuous* (Fischer, 1851) – 25% Dried Artemia, *Artemia salina* (Linnaeus, 1758) – 25% Live Ciliate Shoe, *Paramecium caudatum* (Ehrenberg, 1833) – 50%
6-10	Dried Daphnia, *Daphnia magna* (Straus, 1820) – 50% 'Tetra Mikro Min' feed – 50%	Live Tubeworm, *Tubifex tubifex* (Müller, 1774) – 35% Live Moth, *Chironomidae* (Newman, 1834) – 35% Live Koretra, *Chaoborus* (Lichtenstein, 1800) – 30%	Live Tubeworm, *Tubifex tubifex* (Müller, 1774) – 25% Live Moth, *Chironomidae* (Newman, 1834) – 25% 'Tetra Mikro Min' feed – 50%
11-25	'Tetra Min' feed – 100%	Live Tubeworm, *Tubifex tubifex* (Müller, 1774) – 35% Live Moth, *Chironomidae* (Newman, 1834) – 35% Live Koretra, *Chaoborus* (Lichtenstein, 1800) – 30%	Live Tubeworm, *Tubifex tubifex* (Müller, 1774) – 25% Live Moth, *Chironomidae* (Newman, 1834) – 25% 'Tetra Min' feed – 50%

The biochemical composition and energy value of the fish diet for each individual period of retention was reflected in terms of its multicomponent composition (Table 2).

Table 2. Biochemical composition and energy value of Black Barbus, *P. nigrofasciatus* (Günther, 1868) diet

Indicator	1-5 weeks	6-10 weeks	11-25 weeks
Group I (dry feed)			
Moisture content, %	9.00	9.00	8.00
Protein content, %	56.73	48.85	46.00
Fat content, %	13.67	10.20	12.20
Ash content, %	15.07	11.35	3.20
Nitrogen-free extractives content, %	14.53	29.60	42.00
Energy value of dry matter, kJ×g^{-1}	20.48	39.82	44.95
Group II (live feed)			
Moisture content, %	87.47	34.07	62.43
Protein content, %	57.70	51.30	49.70
Fat content, %	20.73	8.40	6.20
Ash content, %	12.37	6.30	6.80
Nitrogen-free extractives content, %	9.20	34.00	37.30
Energy value of dry matter, kJ×g^{-1}	23.54	36.30	39.45
Group III (combined feed)			
Moisture content, %	87.47	34.04	33.40
Protein content, %	57.70	51.30	51.30
Fat content, %	20.73	8.40	8.07
Ash content, %	12.37	6.30	6.30
Nitrogen-free extractives content, %	9.20	34.00	34.33
Energy value of dry matter, kJ×g^{-1}	45.19	40.83	40.66

Oxygen, temperature and hydro-chemical regimes were systematically monitored during the experimental works in the aquariums with the help of Tetra reagents. The water temperature during the study was kept within 24-25°C, which was provided by automatic heating. Water temperatures were within optimal limits for the growth and assimilation of Black Barbus, *P. nigrofasciatus* (Günther, 1868) feed. A pH meter PH-037 (0.00 to 14.00 pH, ±0,1 pH) (Kelilong Electron, China) and an oximeter AKT EZODO 7031 (0.0. ~ 20.00 mg×l^{-1} (ppm), 0.00 ~ 200.00%, ±0.20 mg×l^{-1} (ppm), ±2.00%) (EZODO, Taiwan) were used as control devices. The body length of fry at different ages was measured with a measuring tape (measuring range 0-100 mm, accuracy ±0.5) every 5 weeks. Body length was the length of the fish measured from the tip of the snout to the tip of the longest lobe of the caudal fin. This was a rectilinear measure that does not measure along the curve of the body. The rate of maturation was observed visually by behavior, changes in mating attire in males and physiological features in females were noted, and the influence of feed type on hematological parameters of Black Barbus, *P. nigrofasciatus* (Günther, 1868) was assessed. Every fish was weighed individually on electronic scales Radwag PS 200/2000.R1, (Axis, Poland) (measuring range 0.02-600.00 g, accuracy ±0.01). When weighing the fish, narcotization or anesthesia of the fry was not used. Water hardness was measured using Hach Method 8123 – Digital Titration using EDTA. Total ammonia nitrogen was measured using Hach Method 8038 – Nessler. Nitrite nitrogen was measured using Hach Method 8507 – Diazotization. Nitrate nitrogen was measured using Hach Method 8171 – Cadmium Reduction. Total nitrogen was measured using Hach Methods 10071, 10072 – Persulfate Digestion Method.

Fish feeding was stopped 24 hours before blood sampling. Anesthesia of the fish was done using tricaine methanesulfonate (MS-222; 0.3 g×l^{-1}) at the time before blood sampling. Blood samples were collected from the tail vein using sterile heparinized capillary tubes. The samples were then transferred to microtubes (Ningbo Siny Medical Technology Co., Ltd 0.6 ml Shanghai, China) containing ethylenediaminetetraacetic acid (EDTA, 1.26 mg (0.6 ml)$^{-1}$) for anticoagulation. The content of hemoglobin in the blood of Black Barbus, *P. nigrofasciatus* (Günther, 1868) was determined by hemoglobin-cyanide method. The principle of the method was that hemoglobin in interaction with iron-blue potassium is oxidized to methemoglobin, which forms with acetone cyanhydrin colored hemoglobin cyanide, the intensity of which is proportional to the hemoglobin content. Cyanmethemoglobin absorption was measured at 540 nm in a photoelectric calorimeter KFK-2 (Spectral range of the photocolorimeter from 315.00 to 980.00 nm, ±0.30 of the main error) (ZOMZ, Russia) against a standard solution (Bhaskaram et al., 2003). The number of erythrocytes in the blood of frys was counted in Goryaev's cell.

Data on the length of fry growth, hemoglobin content and erythrocyte content in the blood were presented as averages. In our experiment, a p-value of 5% is considered statistically significant. Significance of differences in the results of the length, weight and content of hemoglobin and erythrocytes in the blood was assessed using Kruskal-Wallis test. All statistical analyzes were processed using the standard package of statistical programs 'Microsoft Excel 2010'.

RESULTS AND DISCUSSIONS

The chemical values obtained in this study are presented in Table 1. According to the results of the study, the content of ammonium nitrogen did not exceed the normative limits and ranged from 0.03 to 0.64 $mg \times l^{-1}$ in the first aquarium, 0.03 to 0.55 $mg \times l^{-1}$ in the second aquarium and 0.03 to 0.43 $mg \times l^{-1}$ in the third aquarium (Table 3).

Table 3. Hydro-chemical parameters of water in aquariums for keeping Black Barbus, *P. nigrofasciatus*

Group	**NH_4^+ ($mg \times l^{-1}$)**	**pH**	**Hardness temporary (dH)**	**O_2 ($mg \times l^{-1}$)**	**NO_2 ($mg \times l^{-1}$)**	**NO_3 ($mg \times l^{-1}$)**
I (dry feed)	0.03-0.64	6.5-8.3	5-18	7.6-6.4	0.01-0.07	0.1-0.3
II (live feed)	0.03-0.55	6.5-8.0	5-14	7.6-8.0	0.01-0.06	0.1-0.2
III (combined feed)	0.03-0.43	6.5-7.4	5-10	7.6-8.9	0.01-0.06	0.1-0.2

The oxygen concentration did not fall below the normative values and averaged 6.4-8.9$mg \times l^{-1}$, which was ensured by continuous aeration of water in aquariums. The temporal hardness ranged from (dH) 5-18. The hydrogen index was optimal for biochemical processes and retention of young Black Barbus, *P. nigrofasciatus* (Günther, 1868) (6.5-8.3). The water was not contaminated with nitrites, which were present in small concentrations. The content of nitrites in water did not exceed the normative values and ranged from 0.01 to 0.07 $mg \times l^{-1}$ (up to 0.10 $mg \times l^{-1}$). Accordingly, the content of nitrates in water ranged from 0.1 to 0.3 $mg \times l^{-1}$, which does not exceed the normative values (up to 2.0 $mg \times l^{-1}$).

According to the determination of hydro-chemical parameters, in three aquariums where Barbus were kept, the condition of this species of fish was satisfactory, which indicates their individual and species adaptation to a given range of hydro-chemical parameters.

According to the research, it was found that the growth rates of fish for different types of feeding in each experimental aquarium using different types of feeding depending on the age of the fish differed from each other. During the study, Barbus fry were fed small portions and care was taken to ensure that uneaten feed did not accumulate in the aquarium.

Depending on the type of diet, we observed a tendency of different growth intensity of Black Barbus, *P. nigrofasciatus* (Günther, 1868) fry (Figure 1).

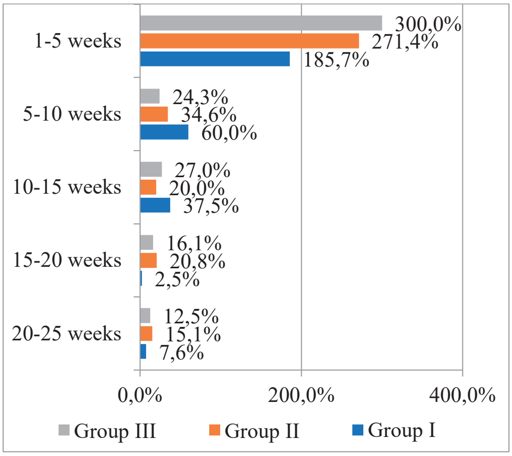

Figure1. The growth rate of the body length (cm) of Black Barbus, *Puntius nigrofasciatus* (Günther, 1868) fry every five weeks, %

Thus, Black Barbus, *P. nigrofasciatus* (Günther, 1868) fry grew the fastest from 1 to 5 weeks. Moreover, the most intensive growth was in fish that ate compound feed during this period. The slowest growth was from 20 to 25 weeks in all fish. Black Barbus, *P. nigrofasciatus* (Günther, 1868), which consumed dry feed, grew more intensively from 5 to 15 weeks. The fry, which consumed live feed, showed a higher growth rate from 15 to 25 week.

Analysis of fish growth dynamics showed that Barbus consuming live feed and compound feed had a higher growth rate than their counterparts consuming dry feed after the first 5 weeks by 0.60 cm or 30.00% (p<0.05), and 0.80 cm or 40.00% (p<0.05) (Table 4).

Table 4. The growth rate of the body length of the Black Barbus, *P. nigrofasciatus* (Günther, 1868) fry depending on the type of feeding, n = 90

Age (weeks)	Body length (cm)		
	Group I (dry feed)	Group II (live feed)	Group III (combined feed)
1	0.7±0.01[a]	0.7±0.02[a]	0.7±0.01[a]
5	2.0±0.04[a]	2.6±0.01[b]	2.8±0.02[b]
10	3.2±0.01[a]	3.5±0.05[b]	3.7±0,06[b]
15	4.4±0.09[a]	4.2±0.01[a]	4.7±0.04[b]
20	5.3±0.07[a]	5.3±0.03[a]	5,6±0,09[b]
25	5.7±0.11 [a]	6.1±0.10[b]	6.3±0.02[b]

The difference between the averages shown with different letters in the same row is significant (p<0.05).

The predominance of fish of the experimental groups over the fish of the control in terms of body length at 10 weeks of retention was slightly less and amounted to 0.30 cm or 9.38% (p<0.05) in II group and 0.05 cm 15.63% (p<0.05) in III group. At week 15, there was no significant difference between dry-fed and live-fed fish, but mixed-fed fish significantly outperformed group I peers by 0.30 cm 6.82% and group II peers by 0.50 cm or 11.90%. (p<0.05). At the end of the 20th week of detention, fish consumed compound feed were longer than their counterparts consuming both dry and live feed by 0.30 cm or 5.66%.

Using different types of feeding, it can be observed that on the 25th week in the III aquarium (combined type of feeding) Black Barbus, *P. nigrofasciatus* (Günther, 1868) fry reached a maximum size of 6.3 cm, which is more than peers of the first control group by 0.60 cm or 10.53% (p<0.05) and peers of the second experimental group by 0.20 cm or 3.28% (p<0.05). It was also interesting that during the feeding of live feed (II aquarium) the growth rate of fry at 25 weeks (6.1cm), although higher than the fry in the first aquarium (5.70 cm) by 0.40 cm or 7.02% (p<0.05), but in the period from 10 by week 15 they had growth retardation. Thus, the use of a combined type of feeding contributed to the best results of growth of Black Barbus, *P. nigrofasciatus* (Günther, 1868).

Live weight of fish during the first week of rearing was the same in all groups. At the end of the 5th week, the excess weight of the fry that consumed the combined feed was found to be higher than the counterparts that received live feed by 0.2 g or 8.0% (p<0.05) and over the counterparts that received dry commercial feed by 0.7 g or 28.0% (p<0.05). Weighing the fry at the end of the 10th week showed that they weighed the same in group II and group III, but the fish from group I that consumed dry food weighed less than their peers that consumed live food by 0.3 g or 10.71% (p<0.05) and less than their counterparts, who consumed combined feed by 0.4 g or 14.29% (p<0.05). The analysis of the weight of Black Barbus fry *P. nigrofasciatus* (Günther, 1868) on the 15th week of research showed that fish from group I exceeded fish from group II by 0.2 g or 5.13% (p<0.05), but weighed less than fish from group III by 0.3 g or 7.69% (p<0.05). On the 20th week of rearing fry, their weight in group I and group II was equal, however, fish from group III that consumed combined feed equally exceeded their counterparts that consumed dry and live feed by 0.3 g or 6.52% (p<0.05). The end of the experiment at the 25th week showed the lowest weight in Black Barbus fish that consumed dry food, which was less than that of counterparts receiving combined feed by 0.5 g or 9.09% (p<0.05) and less than that of counterparts given live food by 0.3 g or 6.0% (p<0.05) (Table 5).

Table 5. The growth rate of the body weight of the Black Barbus, *P. nigrofasciatus* (Günther, 1868) fry depending on the type of feeding, n = 90

Age (weeks)	Body weight (g)		
	Group I (dry feed)	Group II (live feed)	Group III (combined feed)
1	0.6±0.01[a]	0.6±0.01[a]	0.6±0.02[a]
5	1.8±0.02[a]	2.3±0.03[b]	2.5±0.02[c]
10	2.8±0.02[a]	3.1±0.04[b]	3.2±0.03[b]
15	3.9±0.06[a]	3.7±0.03[b]	4.1±0.05[c]
20	4.6±0.05[a]	4.6±0.06[a]	4.9±0.06[b]
25	5.0±0.09[a]	5.3±0.09[b]	5.5±0.08[b]

The difference between the averages shown with different letters in the same row is significant (p<0.05).

The study of hematological parameters did not show a significant difference in the number of erythrocytes and hemoglobin content in the blood of Black Barbus, *P. nigrofasciatus* (Günther, 1868) from the first and second aquariums, which indicates the normal physiological development of fish when fed all types of feed. In the blood of fish of the third aquarium there was a tendency to increase the number of erythrocytes, which was 1.132±0.053 million×μl^{-1}, which is 12.08% (p<0.05) more than in the first aquarium. No differences in the blood of fish relative to the second aquarium were found (Table 6).

Table. 6. Physiological and hematological parameters of Black Barbus, *P. nigrofasciatus* (Günther, 1868), n = 90

Group	Hemoglobin content, g%	The number of erythrocytes, million×μl^{-1}
I (dry feed)	5.97±0.812^{a}	1.010±0.041^{a}
II (live feed)	6.04±0.531^{a}	1.101±0.046^{a}
III (combined feed)	5.91±0.627^{a}	1.132±0.039^{b}

The difference between the averages shown with different letters in the same row is significant (p<0.05).

The survival of the fry is the most urgent thing in the cultivation and breeding of any species of fish. Very often in the fry of schooling fish, including Black Barbus, *P. nigrofasciatus* (Günther, 1868), is different growth rates of representatives. Therefore, larger and stronger specimens suppress or completely kill smaller and weaker members of the pack. One of the stages of our research was to analyze the survival of Black Barbus, *P. nigrofasciatus* (Günther, 1868) in different types of feeding (Table 7).

Table 7. Survival of Black Barbus, *P. nigrofasciatus* (Günther, 1868) depending on the type of feeding for 25 weeks

Group	Quantity at the beginning of the experiment	Including		Quantity at the end of the experiment	Survival,%
		males	females		
I (dry feed)	30	10	20	21	70
II (live feed)	30	12	18	25	83
III (combined feed)	30	5	25	29	97

Thus, according to our research, when feeding Black Barbus, *P. nigrofasciatus* (Günther, 1868) fry was using only dry feed, the greatest death of fry was observed. This fact indicates that very often this type of feeding in fish is obese and liver degeneration, which in the future leads to their death. The best results were with the use of a combined type of feeding. Only one specimen died in the third aquarium.

Modern fish farming and aquaristics offer fish-balanced artificial feeds. The results of our research on the influence of the type of feed on the physiological state of fish did not coincide with the reports of other authors, who indicated that feeding fish only dry feed is impractical because long-term feeding can worsen the physiological condition of fish and the ability of broodstock to reproduce (Davidson et al., 2013; Bayrak et al., 2009).

The results of our experiment coincided with the reports of other authors who reported that Black Barbus, *P. nigrofasciatus* (Günther, 1868) are quite unpretentious in terms of diet, in the aquarium they are actually omnivorous aquatic organisms, happy to consume both live and dry feed (Viskushenko, 2021).

The published results show a significant effect of feed types on the relative growth of Javanese Barbus, *P. orphoides* (Valenciennes, 1842). It also became known the highest increase was given by the use of feed types in combination with 35.0% soybean feed, 35.0% *I. Aquatica* (Forssk) leaf and 30.0% dry feed pellets.

We found results similar to those previously published data (Jagtap & Kulkarni, 2013), who says the growth rate was higher in fish using a compound feed that included pellets and mosquito larvae. However, our results did not coincide with this report on the survival of 100% of fish, as we got reduced survival of fish kept on all types of feed.

Our results did not coincide with reports (Ortega-Salas et al., 2009) indicating that a diet of live ingredients improves the growth and survival of aquarium fish compared to a dry or combined diet.

The data we found coincided with conclusions of experiment that showed fish fry fed with the combined diet showed significantly better survival rate (54.80±2.43%) than those fed with other feed types (p<0.001) (Alavi et al., 2009; Kami´nski et al., 2010).

Scientists report (Thandile & Akewake, 2022) that live feed helps reduce the time required for the process of organogenesis of aquarium fish, and allows the early formation of a functional digestive system, which then optimizes the growth of fry. However, we found that the best feed for Black Barbus, *P. nigrofasciatus* (Günther, 1868) was live feed in combination with industrial dry feed.

Our findings also coincided with a report (David & Aaron, 2022) indicating that commercial pelleted feeds showed lower fish growth outcomes than natural feeds in terms of growth on a fixed diet.

The authors (Kim et al., 2016) report that fish fed diets with a protein content of 45.0%, 50.0% and 60.0% had higher feed efficiencies and specific growth rates than fish fed diets with a protein content of 40.0% and below. Our result was similar to these data and shows Barbus fish, that had higher protein content in the third aquarium using the combined type of feeding, showed the best growth result.

It is known that environmental parameters affect the number, morphological composition and structure of blood cell distribution (Srivastava & Choudhary, 2010). The published results (Haghbayan & Mehrgan, 2015) showed that growing fish on an artificial diet with high bone meal had a positive effect on growth rates ($p<0.001$), but it reduced hematocrit and hemoglobin. Our studies did not show a difference in the level of hemoglobin in the blood of fish when using artificial, live and combined feed.

The number of erythrocytes is closely related to the activity of fish, water temperature fluctuations and the concentration of dissolved oxygen in the aquarium. Other environmental factors have a definite impact and are manifested along with seasonal variability. The number of erythrocytes also depends on age, sex, diet and reproductive status and can range from $0.5–1.5\times10^6$ $(mm^3)^{-1}$ in less active species to $3.0–4.2\times10^6$ $(mm^3)^{-1}$ in more active species (Witeska 2013). But in our experimental aquariums, the oxygen content was the same throughout the study period, but the level of erythrocytes was higher in Barbus fry, which consumed compound feed. We attribute this to an increase in the metabolic rate of fish on the combined feed.

CONCLUSIONS

Our results showed that the combined feeding allowed us to produce in the 25th week of the experiment Black Barbus, *P. nigrofasciatus* (Günther, 1868), with a maximum body length of 6.30 cm and a body weight of 5.5 g, which was more compared to the analogues that consumed dry industrial feed and live feed of microorganisms and crustaceans. The haemoglobin content in the blood of the fish did not differ significantly among the different diets, but the erythrocyte content was 12.08% higher in the fish that consumed combined diets. The survival rate of Black Barbus, *P. nigrofasciatus* (Günther, 1868), was also higher in fish fed combined diets and was 97.00%.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

EFFECTS OF KOMBUCHA AND MILK KEFIR DIETARY SUPPLEMENTS ON THE MEAT BODY COMPOSITION OF SIBERIAN STURGEON (*Acipenser baerii*)

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Abstract

This paper aimed to evaluate the effect of a diet supplemented with fermented products on the meat quality of Siberian sturgeon (Acipenser baerii) reared in a recirculation aquaculture system. The fermented product was made by combining artisanal cultures of Kombucha and milk kefir granules grown up in sucrose, black tea, and bovine colostrum. Four experimental groups were established: V1 - a control group that received a commercial diet with 54% crude protein, and V2 to V4 groups, which received the same diet supplemented with 1 g/kg, 2 g/kg, and 3 g/kg of fermented product, respectively. After 35 days of diet administration, the biochemical composition of fresh meat was analysed. The results showed that the addition of fermented products significantly influenced the water, ash, and lipid content of A. baerii meat ($p < 0.05$), while the protein content was not influenced ($p > 0.05$) by the administrated diet. In conclusion, it is evident that adding the fermented product in A. baerii meat has an improving effect on body composition

***Key words**: bioactive fermented products, meat composition, sturgeon.*

INTRODUCTION

The overexploitation of sturgeons for caviar and meat, corroborated with the habitat deterioration, led to drastic declines in natural populations. The high demand for these products has driven the development of sturgeon aquaculture, which has become an important industry in many countries.

Siberian sturgeon (*Acipenser baerii*) is a species of fish that has been farmed for thousands of years for its valuable meat, caviar, and other by-products, showing a rapid growth rate (Pyka et al., 2003; Babaei et al., 2017; Williot et al., 2018), higher resistance to pathogens (Kayiş et al., 2016), and can be reared using a wide range of diets and environmental conditions (Bronzi et al., 2011; Xu et al., 2019).

The production of high-quality sturgeon meat requires a balanced and nutritious diet, which is a major challenge for producers. Over the past few years, there has been a huge interest in supplementing the sturgeon's diet with different feed additives, as this has been proven to exhibit a significant impact on the growth performance and biochemical composition of fish meat (Palmegiano et al., 2008; Sayed et al., 2020; Mocanu et al., 2022). Generally, feed additives typically contain a range of beneficial substances, such as probiotics, enzymes, minerals, and vitamins, that can have a positive effect on the growth and well-being of fish. The quality of sturgeon meat is of significant interest to both researchers and producers since it has a significant influence on the overall quality and value of the obtained product.

Kombucha, also known as SCOBY (Symbiotic Culture of Bacteria and Yeasts), is made up of several organic acids, sugars, vitamins, amino acids, biogenic amines, purines, pigments, lipids, proteins, hydrolytic enzymes, ethanol, caffeine, carbon dioxide, polyphenols, anions, minerals, and bacterial metabolites, such as D-saccharic acid-1, 4-lactone (DSL), according to Jayabalan et al. (2014).

Kefir grains consist of a symbiotic culture of bacteria, yeast, and acetic acid bacteria that adhere to a matrix of polysaccharides (Chen et al., 2015). The mixture of kombucha culture and milk kefir grains gives rise to various consortia of naturally occurring microorganisms, such as lactic acid, acetic acid, and yeast. Through their fermentative activity, these microorganisms produce a diverse array of bioactive compounds, including prebiotics, probiotics, postbiotics, and para-probiotics, which have been found to offer *in vitro* and *in vivo* benefits (Pihurov et al., 2021).

Studies conducted in the past have revealed that the inclusion of fermented products in the diet can enhance the sensory characteristics and extend the shelf life of the final product (Ndaw et al., 2008). Fermentation can also lead to the synthesis of metabolites that exhibit antitoxic properties, thereby reducing the bioaccumulation of heavy metals in water and fish tissues, and offering direct protection against oxidative stress induced by metals (Giri et al., 2018). Moreover, these products can serve as a viable alternative to antibiotics, while still promoting the growth and well-being of fish (Choi et al., 2022).

While there is a growing interest among sturgeon farmers to cultivate healthy fish and optimize feed efficiency, there is a lack of available information regarding the nutritional requirements and feeding practices specific to Siberian sturgeon. In this context, the objective of this study was to assess how the dietary inclusion of fermented products affects the body composition of Siberian sturgeon meat.

MATERIALS AND METHODS

Experimental design. The experiment took place at the University Dunărea de Jos, Galați, Romania, at the Romanian Centre for the Modelling of Recirculating Aquaculture Systems (www.moras.ugal.ro, accessed on February 9th, 2021). Four different experimental groups were established, including the control group (V1), which was fed with a commercial diet containing 54% crude proteins and 15% lipids; the V2 group, which received the same diet supplemented with 1 g/kg of the fermented product; the V3 group, which received 2 g/kg of the fermented product; and the V4 group, which received 3 g/kg of fermented product. The fermented product used in our study was obtained in the Microbiology Laboratory of the Faculty of Food Science and Engineering. The product was obtained by combining artisanal cultures of kombucha and milk kefir granules, which were grown in black tea, sucrose, and bovine colostrum.

Fish body composition analysis. After 35 days of diet administration, the proximate composition analyses of the fish body were determined by the standard methods of AOAC, 2003. The fish were eviscerated and filleted, and only the muscular tissue was blended and utilized for subsequent analysis.

Moisture content (%) was assessed by subjecting the samples to a constant temperature of 105 °C in a convection oven (Jeiotech, Jeio Tech Co., Inc, Korea) until a constant weight was achieved (AOAC, 2016). The dry samples were then finely ground and utilized further for the evaluation of protein, fat, and ash levels.

Crude protein content (%). The Dumas method was used to quantify the nitrogen content of the dry samples, which was then converted into crude protein content (%) using the common conversion factor of N×6.25. The combustion of samples was performed at 1100°C using a Primacs SNC 100 (Skalar Analytical B.V., The Netherlands).

The lipid content (%) was determined using the Soxhlet extraction method with petroleum ether as the solvent (C. Gerhardt GmbH & Co. KG, Germany), following the AOAC method from 1997.

The ash content (%) was determined by heating the sample in a muffle furnace (Nabertherm, Applied Scientific Instruments Co., Ltd. Thailand) at 525±25°C for 8 hours. All calculations for protein, fat, and ash content were reported based on the dry weight.

The fish experiments were conducted with the approval of the Ethics Committee of the University Dunărea de Jos from Galați.

Statistical analysis. The statistical analysis of the fish's proximate composition was conducted using SPSS software for Windows, version 21.0 (SPSS Inc., Chicago, United States). Results were considered statistically significant at $p<0.05$. The mean values and standard deviations (S.D) of each species were calculated and presented. Differences between the mean values were determined by One-factor analysis of variance (ANOVA), and Duncan's test was used to identify specific groups with significantly different means.

RESULTS AND DISCUSSIONS

The biochemical composition of fish is crucial for fish preservation. Typically, fish meat comprises 70-84% water, 15-24% protein, 0.1-22% fat, 1-2% minerals, and 0.1-1% carbohydrates. The protein content is particularly important since it contributes to the nutritional value and sensory characteristics of the final product. The fat content also plays a significant role in the taste, texture, and shelf life of fish products (Love, 1970; Desta et al., 2019; Nicolae, 2020; Ahmed et al., 2022). Body composition is a good indicator of the physiological condition of fish (Ali et al., 2005).

The proximate composition of fish can vary greatly between and within species and is influenced by various factors such as food composition, feeding rate and frequency, age, size, sex, genetics, and season/migration (Morris, 2001; Kestin et al.,2001; Mazumder et al., 2008; Begum et al., 2016).

The body composition analysis results of the experimental fish are illustrated in Figures 1-4. There was no significant difference found in the protein content ($p>0.05$) of *A. baerii* meat among the experimental variants. However, significant differences were observed in the water, ash, and lipid content of the meat ($p<0.05$).

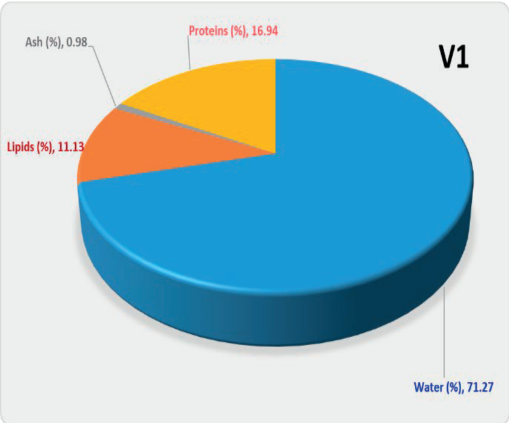

Figure 1. Proximate composition analysis of fish meat in V1 Variant

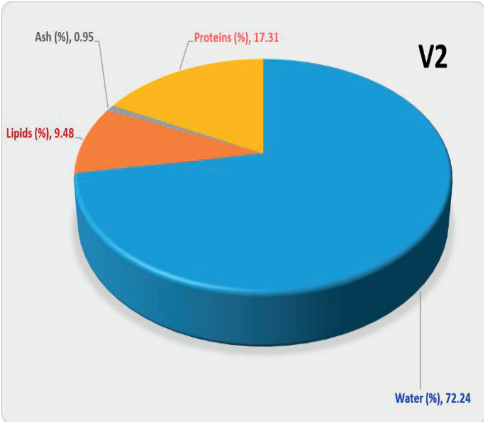

Figure 2. Proximate composition analysis of fish meat in V2 Variant

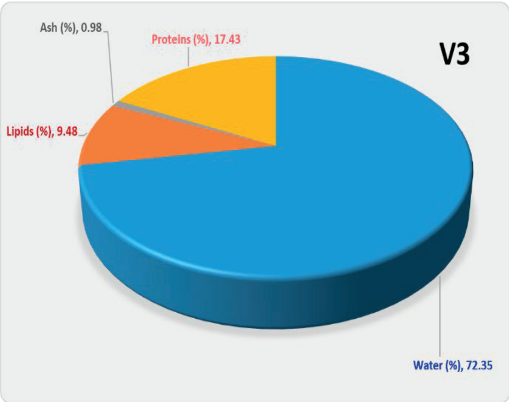

Figure 3. Proximate composition analysis of fish meat in V3 variant

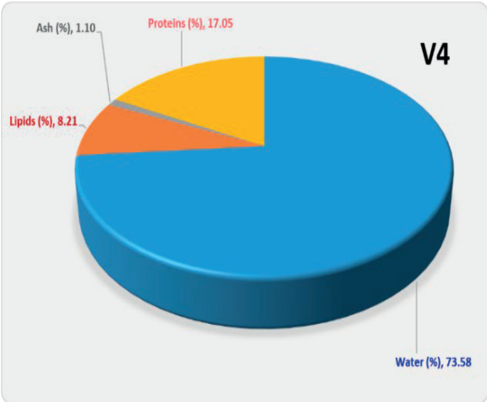

Figure 4. Proximate composition analysis of fish meat in V4 Variant

The inclusion of different levels of the fermented product in *A. baerii* food leads to a significant ($p<0.05$) reduction of the lipid content. The values of the lipids content showed a significantly higher value in the control variant, (11.13±1.29%), with no significant differences between fish fed with feed fermented product at 1 g/kg feed (9.48±0.96%), and 2 g/kg (9.48±0.31%). A significant ($p<0.05$) lower lipid content (8.21±0.87%) was registered in the variant V4 (3 g/kg fermented product). In conclusion, the inclusion of probiotics in the diets of fish resulted in lower levels of crude lipids compared to the control group. This indicates that the fish had a higher protein-to-fat ratio, which is highly desirable in aquaculture practices.

Moisture content and lipid content were found to have an inverse relationship. The water content showed significantly lower ($p<0.05$) moisture contents in the V1 group (71.27±1.03%), followed by fish fed with 1 g/kg fermented product (72.24±1.146%), and 2 g/ kg respectively (72.35±1.44%), with no differences ($p>0.05$), and by the V4 variant, with the highest water content (73.58±1.44%).

The results obtained by us were similar to those reported by Ayoola et al. (2013), for African catfish *Clarias gariepinus,* who registered after 90 days of feeding diets supplemented with the probiotic *Lactobacillus* and *Bifidobacterium,* an increase of water content with the probiotic dose (0; 0.5; 1; 1.5, respectively 2 g probiotic), respectively a linear decrease of the lipids content.

Although there were no statistically significant differences ($p>0.05$) observed in the protein content between the variants, slightly higher values were recorded in variants V2, V3, and V4. Fish meat with higher protein content and lower fat is desirable in aquaculture. The same results were obtained by Opiyo et al., 2019, who supplemented the diet of Nile tilapia with different levels of *Saccharomyces cerevisiae* (1×10^{10} CFU g^{-1}) or *Bacillus subtilis* (1×10^{9} CFU g^{-1}).

The ash content of the samples was found to be significantly higher ($p<0.05$) in the V4 variant (1.10±0.13%) compared to V1, V2, and V3, which showed no significant differences ($p>0.05$) in terms of ash content.

There are reports in the literature indicating that the addition of probiotics does not cause significant changes in the protein, lipid, or ash content of fish meat (Merrifield et al., 2010). A study conducted by Mocanu et al. (2012) also state that the addition of probiotics (BioPlus®2B) to fish feed at excessively high doses does not have a significant impact on the biochemical composition of meat. However, when probiotics are administered at an appropriate dosage, they can potentially improve the nutritional qualities of the meat.

In contrast, some studies have reported a significant increase in the protein content of fish meat, which could be attributed to increased nutrient deposition or higher secretion of proteins by probiotics in the gastrointestinal tract (Lara-Flores & Olvera-Novoa, 2013).

According to a study conducted by Opiyo et al., in 2019, it was found that fish fed with diets supplemented with probiotics, specifically *S. cerevisiae,* exhibited a notable increase in protein content and a decrease in lipid content compared to the control group. The observed increase in protein content may be attributed to enhanced nutrient deposition within the fish.

In conclusion, it is evident that adding the fermented product in *A. baerii* meat has an improving effect on body composition. However, it is important to consider that the effects of probiotics on the composition of fish meat may vary depending on the fish species, type of probiotic, as well as other factors such as diet and environmental conditions.

CONCLUSIONS

The current study examined the impact of dietary fermented products made from a combination of kombucha and milk kefir granules grown in sucrose, bovine colostrum and black tea, on the crude protein and lipid content of fish meat compared to a control group. The findings indicate that the inclusion of fermented products led to an increase in crude protein and a decrease in lipid content, which could be advantageous for fish meat.

However, the effects of the fermented product on fish meat composition may be influenced by various factors such as microorganism type, administration dose and duration, fish species and age, and environmental conditions.

Further research is required to better understand the potential benefits and mechanisms of this product in aquaculture, with the aim of enhancing the proximate composition of *A. baerii* meat.

ACKNOWLEDGEMENTS

The principal author thanks the "Dunărea de Jos" University of Galați, which through the University Degree Program, the doctoral studies contract has supported the achievement. Also, the authors are grateful for the technical support offered by MoRAS through the Grant POSCCE ID 1815, cod SMIS 48745 (www.moras.ugal.ro).

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

ESTIMATION OF GROWTH AND MORTALITY OF SOME COMMERCIAL CYPRINIDS FROM THE DANUBE DELTA

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Abstract

Common carp and Prussian carp are two of the main exploited freshwater fish from the Danube Delta, that`s why obtaining information regarding the stock assessment has great importance for population structure. Our study aimed to investigate the parameters of growth and mortality among these populations. From the result of our study, the correlation between length and weight was W = 0.04×Lt2.70 for common carp and W = 0.10×Lt2.47 for Prussian carp. The calculated parameters for mortality were: total mortality (Z) was 1.74 for Common carp and 2.29 for Prussian carp, the natural (M) was 0.82 for Common carp, respectively 1.03 for Prussian carp, while the rate of exploitation reached a value of 0.53 for common carp and 0.55 for Prussian carp. In conclusion, the study analysed mortality due to natural causes, fishing, and overfishing and concluded that both species are currently being overexploited.

Key words*: freshwater fish, inland fishing, Length-Weight ratio, von Bertalanffy's equation.*

INTRODUCTION

Carp is by far the most representative fish in Romanian fisheries. The carp as a wild species was the basic fishery production in the lower Danube basin. It was and it will remain, at least as goal, the main fish species in Romania (Nicolae et al., 2012; 2018).
The common carp, scientifically known as *Cyprinus carpio* L. 1758, is widely distributed across the globe, inhabiting freshwater, brackish waters, and large lakes with dense vegetation (Kottelat & Freyhof, 2007). The Prussian carp (*Carassius gibelio*) is a freshwater species that can be found in various aquatic habitats ranging from plains to hills. However, it does not thrive well in areas with excessive overgrown vegetation (Gheorghe et al., 2012). In Romania, the common carp and the Prussian carp are one of the most preferred fish by Romanian consumers due to its texture and pleasant flavour (Stroe et al., 2022).
In Romania, over 70%, of inland fishing is represented by fishing in the Danube Delta, even though, in the last years, the Danube Delta fisheries have diminished because of the decline of fish stocks, mostly due to habitat loss to floodplain and impoundments (Năstase et al., 2017).
From one year to another, the average size and structure of fish captured from the Danube Delta changed, decreasing from 1747 tons between 1963 and 1974 to 252 tons in the period 1992 and 2003 to 158 tons (2018-2022) in Gorgova-Uzlina lake-complex (Năvodaru & Cernișencu, 2006).
In the given situation, an investigation was carried out in the Danube Delta's Gorgova-Uzlina Lake complex during the year 2022 in order to determine certain population traits of common carp and Prussian carp, including their distribution of length and weight, the relationship between length and weight, as well as various growth parameters and mortality rates.

MATERIALS AND METHODS

Study area, fish sampling. Specimens of *C. carpio* and *C. gibelio* were sampled in 2022 in the Gorgova-Uzlina Lake complex (Figure 1).

The Gorgova lake complex is situated on the depression of the same name (about 26,000 ha) and includes important lakes such as Gorgova, Isac, Uzlina, Isacel, Cuibeda, Obretinul Mic, Obretinciuc, Potcoava, Gorgovat, Cruglic. This complex is crossed by the Litcov Canal, which is the most important waterway in this part of the Danube Delta.

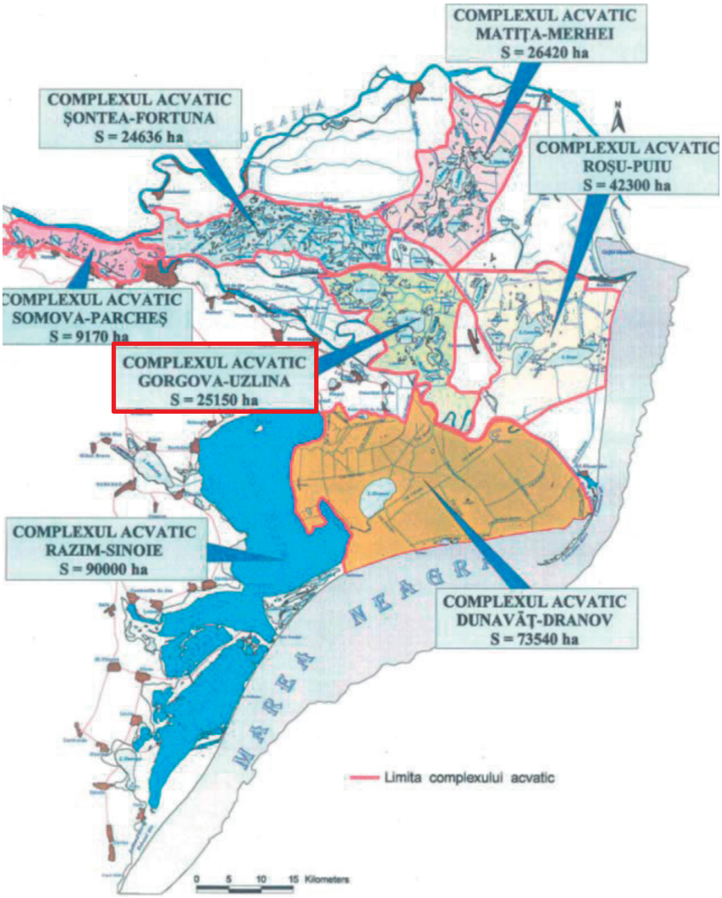

Figure 1. Map of the natural lakes from the Danube Delta (Photo after Năvodaru, 2008)

402 specimens, including 182 Prussian carp and 220 common carp were randomly sampled from commercial catches beginning in April till October 2022. Using an ichtyometer, we measured the fish height (H, cm), total length (L_T, in cm), and fork length (L_F, in cm). The body weight was determined with an electronic scale.

The growth and mortality parameters estimation. For data analysis, we used the average values of the length and body weight of classes. For the determination of the fish growth, we used the Von Bertalanffy equation (Gayanilo et al., 2005). Using the curve analysis of the length converted catch (Ricker, 1975) from the FISAT II software, we estimated the natural mortality (M) at the annual average water temperature of 14°C. Fishing mortality (F) was calculated with the formula described by Pauly (1984): F=Z-M (where: Z represents the total mortality and M – is the natural mortality). The exploitation level (E) was calculated with the formula: E=F/Z (Gulland, 1971). Information regarding the condition of fish stocks can be obtained through the exploitation ratio. Fish stocks with exploitation ratio values less than 0.5 are considered to be easily exploitable, while those with ratios between 0.5 and 1 are heavily exploited.

Total length-weight (TL-W) correlations, were determined after Ricker (1975), taking into consideration the total length and body weight of all the measured fish. The equations resulted as follows: $W = a \times L_T^b$ (W - the individual weight of fish; L_T - the total length, a-the intercept; b -the slope of the regression. Ricker, 1975 states that values of the slope "b" under the value of 3 indicate isometric growth (when different parts of an organism grow at the same rate relative to each other), while a value of "b" higher than 3 indicates an allometric growth (when different parts of an organism grow at different rates relative to each other).

Data analysis. To analyse the length-frequency data, the data were grouped into intervals of 3 m using Microsoft Excel 2019 and the software package FiSAT II.

RESULTS AND DISCUSSIONS

Understanding fish population dynamics (i.e., how mortality, growth, and exploitation rate) is mandatory for knowledge of fisheries management.

In the Table 1 are presented the average values of the f of the carp and Prussian carp population from the Gorgova-Uzlina complex from the year 2022.

Table 1. Biometric parameters for common carp and Prussian carp (Gorgova-Uzlina area, 2022)

Fish species	Statistical parameters, limits	W (g)	LT (cm)	LF (cm)	H (cm)
C. carpio	Mean±Sdev	2209.98±1276.79	52.78-9.96	45.51±8.68	14.56-2.31
	Min-max	650-7500	40-82	25-71	10-23
C. gibelio	Mean±Sdev	604.81±262.26	31.56±5.51	25.64±3.98	11.02±1.54
	Min-max.	200-1400	21-50	17.5-34	8-17

Note: W - body weight; LT - total length; LF - fork length; H - height

The average total length of common carp was determined to be 52.78 ± 9.96 cm, with a range of 40 cm to 82 cm, while the total length for Prussian carp was found between 21 and 50 cm, with an average value of 31.56 ± 5.51 cm.

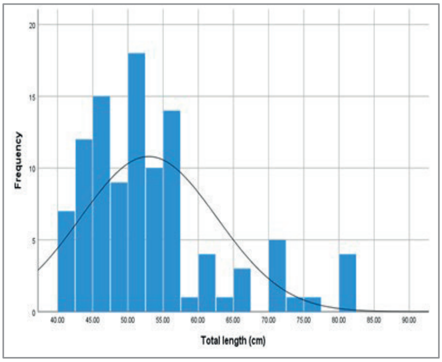

Figure 2. Distribution of total length-frequency for common carp population from Gorgova-Uzlina lake complex, during the year 2022

The overall histograms present a higher frequency at a total length range from 47.2 to 54.4 cm for common carp (Figure 2), while Prussian carp present a higher frequency at a total length range from 29.2 to 33.3 cm LT (Figure 3).

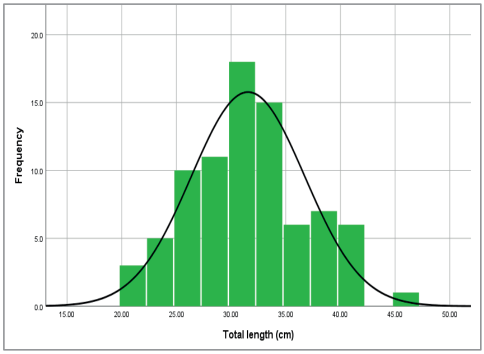

Figure 3. Distribution of length-frequency of Prussian carp population from Gorgova-Uzlina lake complex, during the year 2022

The Lt-W relation was calculated as follows: $W=0.04\times Lt^{2.70}$ ($r^2=0.95$) for common carp (Figure 4), and $W=0.10\times Lt^{2.48}$ ($r^2=0.92$) for Prussian carp, respectively (Figure 5).

The regression coefficient "b" showed negative allometric growth, for both species (b=2.70 for common carp, and b=2.48 for Prussian carp respectively). The value of "b" for the common carp is close to values reported by Gheorghe et al. (2011) (b=2.84), for the period 2006-2009 in the northern part of Brăila Natural Park (Fundu Mare Island, Cravia, and Calia branch).

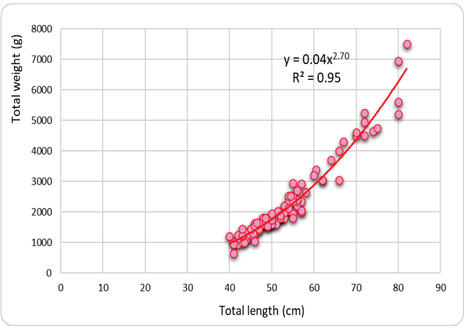

Figure 4. Total length-Weight correlations for common carp (Gorgova-Uzlina lake complex, the year 2022)

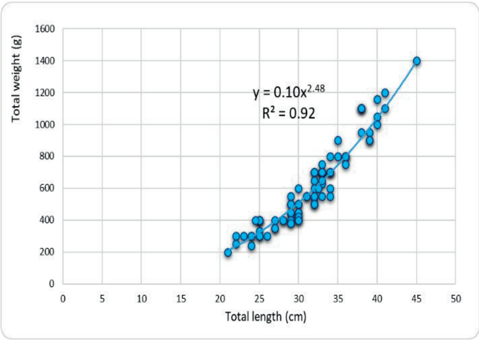

Figure 5. Total length-Weight correlations for Prussian carp (Gorgova-Uzlina Lake complex, the year 2022)

For Prussian carp, fished in the Danube River, between Brăila (km 170) and Gropeni (km 196) during the period 2006-2009, Gheorghe et al., 2012, reported a higher value of "b" (b=2.86). Generally, the values of the "b" coefficient is affected by several factors, such as: sex, age, physiological conditions, food availability, life span, or growth increments (Sinovčić, 2000).

Growth parameters. Estimates of population parameters are essential for understanding the biological characteristics of fish species (Camargo et al., 2015; Talet et al., 2019). In this context, length frequency data and growth curves were plotted with the help of FISAT II for each specie (Table 2).

The asymptotic length ($L\infty$) and the growth rate coefficient (k) determined by the direct-fit of length frequency (ELEFAN I) was 70.35 cm, and 0.71 year^{-1} for common carp, while for Prussian carp, the $L\infty$ and k were estimated as 47.25 cm and 0.85 year^{-1}.

Hypothetical age (t_0) was predicted as -0.71/year for carp, respectively -0.85/year for Prussian carp, which gave the Von Bertalanffy growth equations: Lt = 70.35 (1-exp [-0.71(t+0.49)]) for common carp, respectively Lt = 47.25 (1-exp [-0.85(t+0.50)]) for Prussian carp.

Table 2. Von Bertalanffy growth equation parameters for *Cyprinus carpio* and *Carassius gibelio* species at the Gorgova-Uzlina, during the year 2022

Fish species	Asymptotic length	Growth rate coefficient of Von Bertalanffy	Hypothetical age	Total mortality	Natural mortality	Fishing mortality	Exploitation rate
Common carp	70.35	0.71	-0.49	1.74	0.82	0.92	0.53
Prussian carp	47.25	0.85	-0.50	2.29	1.03	1.26	0.55

The values of the asymptotic length and the growth coefficient obtained for the Prussian carp were found to be lower than those reported in other studies.

Gheorghe et al., 2012 reported an asymptotic length of 39.38, for Prussian carp fishing in the Danube River, Braila (km 170) - Gropeni (km 196). However, the values are similar to those reported at www.fishbase.org, from other parts of Romania country (Danube Delta at Puiu-Roşu, 47.8 cm, Somova - 40 cm, Isacova 45.7 cm).

It is generally assumed that the differences between the values of $L\infty$ and K can be influenced by factors such as population size, environmental conditions, and species density, as noted by Adams (1980).

Thus, these factors may be related to the observed variances.

Mortality coefficients and exploitation ratio. The rate of natural mortality (M) for the common carp was predicted to be 0.82 year^{-1} using Pauly`s (1980) equation, at a mean temperature of 14°C. Using the length-converted catch curve (Figure 6) we determined a total mortality of 1.74 year^{-1}, while the fishing mortality was predicted to be 0.92 year^{-1}, and the exploitation ratio (E) was approximated to 0.53 year^{-1}.

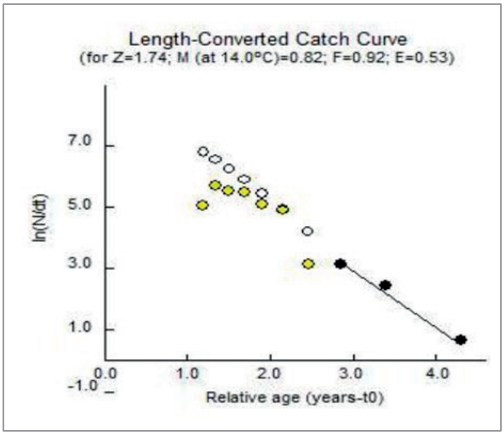

Figure 6. Length converted catch curve of common carp (lake complex Gorgova-Uzlina, 2022)

For Prussian carp, the natural mortality was predicted to be 1.03 year^{-1}, while the total mortality is Z=1.74 year^{-1}. The fishing mortality registered a value of 1.26 year^{-1}, and the exploitation rate recorded a value of 0.55 year^{-1} (Figure 7). In our study, the mortalities due to natural and environmental causes (M) registered lower values than those caused by fishing, for both species, but higher in comparison with those registered in 2011 and 2012 by Gheorghe et al.

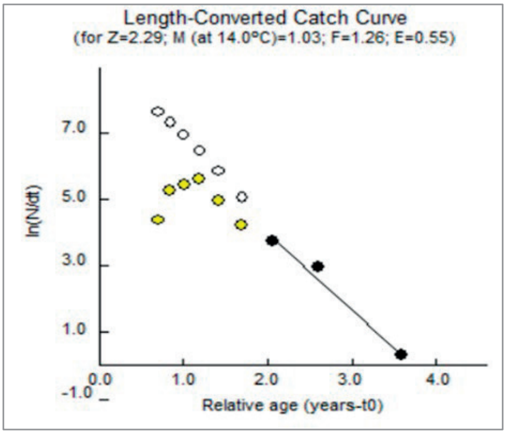

Figure 7. Length converted catch curve of Prussian carp (lake complex Gorgova-Uzlina, 2022)

According to Pauly (1983), high values of natural mortality and fishing mortality can indicate an overfished condition. Also, from the results of the exploitation rate, it appears that the stocks of common carp and Prussian carp populations are overexploited (E=0.53 for common carp and E=0.55 for Prussian carp). Therefore, according to the affirmation of Gulland, 1971, an exploitation rate bigger than 0.5 is a statement for an overfished stock, we can conclude that the common carp and Prussian carp populations are overexploited in this area of the Danube Delta.

CONCLUSIONS

The aim of the present study was to collect new data regarding the growth and mortality of common carp and Prussian carp, from the Gorgova-Uzlina Lake complex.

The growth rate of fish has significant implications for their ecological dynamics, including factors such as susceptibility to predation and timing of sexual maturation, as well as their participation in fisheries.

As a result of our study, it can be established that the total length of carp fishes' ranges between 40-82 cm and of the Prussian carp between 21-50 cm.

In terms of correlations between the total length-weight, we observed negative allometric growth patterns for both species.

Also, the study analysed mortality due to natural causes, fishing, and overfishing and concluded that both species are currently being overexploited.

ACKNOWLEDGEMENTS

The authors are grateful for the technical support offered by D.D.B.R.A. Romania through contract no. 29/23.05.2022.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

OCCUPATION RATES OF ARTIFICIAL NEST BOXES BY LESSER KESTREL IN SPA "SAKAR" (BG0002021), BULGARIA

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Abstract

Lesser Kestrel often nests in urban areas, where is provided nesting sites and the level of threat of predation is lowland. Demolition of older buildings where the birds nested is the problems of the breeding range. Due to the drastic reduction of natural habitats, the placement of artificial nest boxes provides reliable nesting sites with a low risk of predation. Over 70 artificial nest boxes were installed on the territory of SPA `Sakar` part of NATURA 2000 where the Lesser Kestrel has been successfully recovered as a breeder. The installed artificial nest boxes are different types providing more breeding opportunities. In this survey our goal is to process which factors affect the occupation rate of provided artificial nest boxes. The results showed that artificial nest boxes performances (type of the nest boxes, height above ground and etc.) significantly influenced the occupancy. We conclude that artificial nest boxes are of great importance in providing safe nesting sites.

Key words: *endangered species, Falco naumanni, Natura 2000, nest boxes.*

INTRODUCTION

Lesser Kestrel (*Falco naumanni*, Fleischer, 1818) often nests in urban areas, where is provided nesting sites and the level of threat of predation is lowland.

In Europe in each decade since 1950, have occurred declines equivalent to 46% and in South Africa on the wintering grounds, in each decade since 1971 there have been declines equivalent to 25% (BirdLife International, 2004).

The problems in the breeding range are include demolition of older buildings where the birds nested, intensification of agriculture, loss of habitat through afforestation, human persecution and urbanization, pesticide poisoning and interspecific competition (Biber, 1996). In South Africa the principal threats are the loss of grassland habitat to overgrazing and pesticide effects. When the birds are attracted to outbreaks of locusts or crickets, which are sprayed by farmers (Pepler, 2000).

The best method in birds' conservation is preserving suitable habitats by restoring degraded habitats or maintaining proper management practices. This method is contributed to the increase of population size and efficient in the conservation (Newton, 1994; Avilés & Parejo, 2004; Gottschalk et al., 2011; Olah et al., 2014).

Providing artificial nesting places is therefore conservation programmes need to evaluate their efficiency and costly (Korpimäki, 1985; Lowther, 2012; Lambrechts et al., 2012; MØller et al., 2014).

The critically low number of Lesser Kestrel populations and isolation, which do not allow the species to recover naturally, is the most serious problem today. Additional conservation efforts are necessary to preserve and ensure the sustainable existence of the recovered colony. The placement of artificial nest boxes provides reliable nesting sites with a low risk of predation, due to the drastic reduction of natural habitats. The artificial nest boxes for Lesser Kestrel are common practice in Europe. Countries like Bulgaria, Croatia, France, Greece, Italy, Spain, Portugal uses them for recovery as a breeding species and strengthening existing colonies (Yaneva et al., 2022a).

Lesser Kestrel can be described as representative of farmland birds. Given the food habitats it uses is a top predator in these territories, feeding with rats, amphibians, reptiles, etc. This defines it as particularly sensitive to changes in agricultural territory, where with the intensification, the capacity of ecosystem services from these sources has significantly decreased.

MATERIALS AND METHODS

The field studies were fulfilled on the territory of Lesser Kestrel Release and Adaptation Module in village Levka SPA "Sakar" (BG0002021) part of European Ecological Network NATURA 2000. In this territory for the species are laid targeted conservation activities by a team of "Green Balkans - Stara Zagora" NGO within a project "Better Life for Lesser Kestrel in South-East Balkans" LIFE19 NAT/BG/001017.
From 2020 until 2022 was monitored of the occupied artificial nest boxes. When is the breeding season of Lesser Kestrel was carried out the monitoring in the period from March to September. Except standard methods we are used additionally observation with follow-up: binoculars; field scope tube; camera and video surveillance (Yaneva et al., 2022b).
Through field standard observation methods audition have been carried out direct inspections of artificial nest boxes to following the occupied range and breeding success. Which are implemented during a certain period in order to determine the exact number of hatched chicks.
All information from the monitoring of observation from breeding seasons is filled in electronic data base of "Green Balkans - Stara Zagora" NGO.
With specialized electronic tape measure were measured all the artificial nest boxes placed near the Lesser Kestrel colony in village Levka SPA "Sakar" (BG0002021). The data are recorded in a specially developed form. In the artificial nest boxes form fill in number, type, type of the building on which it is installed and height above ground.

RESULTS AND DISCUSSIONS

At the territory of the Lesser Kestrel colony in village Levka SPA "Sakar" (BG0002021) have been identified three types of artificial nest boxes:

- Type 1 - Classical wall artificial nest box;
- Type 2 - Cavity wall artificial nest box;
- Type 3 - Under-roof artificial nest box.

After data processing it was found that a total of 76 artificial nest boxes were installed as follows:

- Type 1 were installed 30 artificial nest boxes;
- Type 2 were installed 20 artificial nest boxes;
- Type 3 were installed 26 artificial nest boxes (Figure 1).

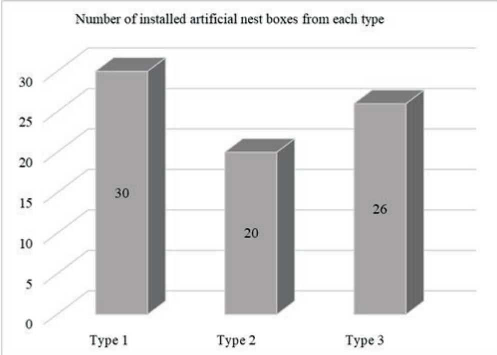

Figure 1. Number of installed artificial nest boxes from each type

All 76 installed artificial nest boxes in the territory of Lesser Kestrel colony were measured the height above ground in meters at which they are mounted. We obtain the following results:

- 4 artificial nest boxes are mounted at a height between 3.00-3.50 m;
- 7 artificial nest boxes are mounted at a height between 3.50-4.00 m;
- 32 artificial nest boxes are mounted at a height between 4.00-4.50 m;
- 16 artificial nest boxes are mounted at a height between 4.50-5.00 m;
- 17 artificial nest boxes are mounted at a height between 5.00-5.50 m (Figure 2).

From the field studies and the monitoring carried out during the breeding seasons of Lesser Kestrel in 2020, 2021 and 2022 are established 27 successful breeding pair in artificial nest boxes. These are 35.53% of the total number of all installed artificial nest boxes for Lesser Kestrel (Figure 3).

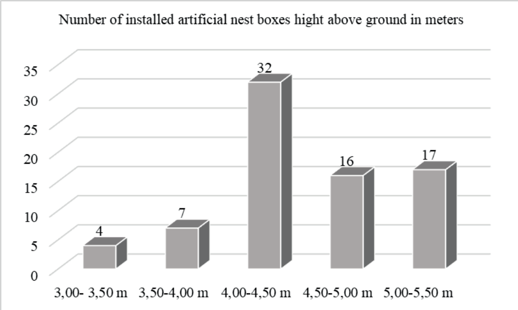

Figure 2. Number of installed artificial nest boxes height above ground in meters

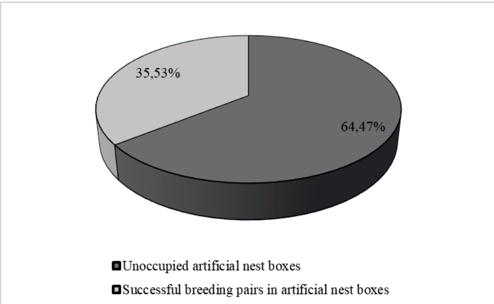

Figure 3. Successful breeding pairs and unoccupied artificial nest boxes in percents

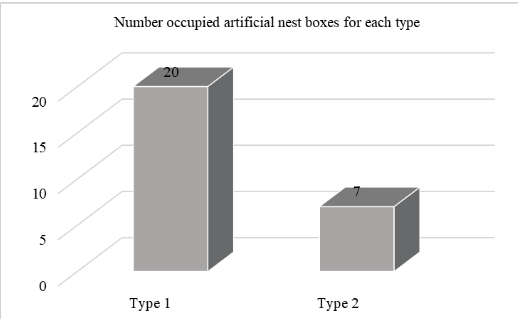

Figure 4. Number occupied artificial nest boxes for each type

During the study period we didn`t recorded successful breeding pair in Type 3 (Under-roof nest box). In that reason we continued to consider only the two types that have been registered successful breeding pairs.
27 successful breeding pair are 54% of the total number of 50 installed artificial nest boxes of Type 1 and Type 2 for Lesser Kestrel (Figure 5).

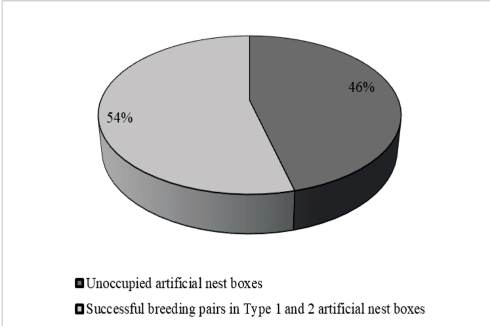

Figure 5. Successful breeding pairs and unoccupied artificial nest boxes of Type 1 and Type 2

All successful occupied artificial nest boxes we classified into three categories as occupied once, occupied twice and occupied three times.
For Type 1 - Classical wall nest box:

- Occupied once - 12 times;
- Occupied twice - 6 times;
- Occupied three times - 2 times.

For Type 2 - Cavity wall nest box:

- Occupied once - 3 times;
- Occupied twice - 3 times;
- Occupied three times - 1 time (Figure 6).

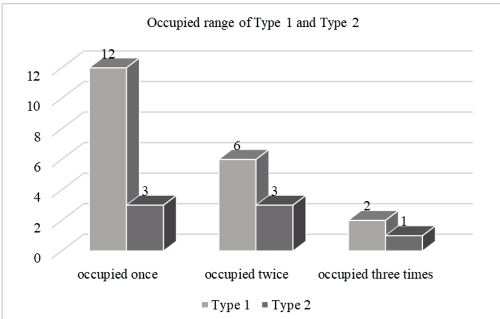

Figure 6. Occupied range of Type 1 and Type 2

According to the occupancy of the artificial nest boxes height above the ground during the study we found the following results (Figure 7):

- 3.00-3.50 m were occupied 1 time;
- 4.00-4.50 m were occupied 4 times;
- 4.50-5.00 m were occupied 10 times;
- 5.00-5.50 m were occupied 12 times

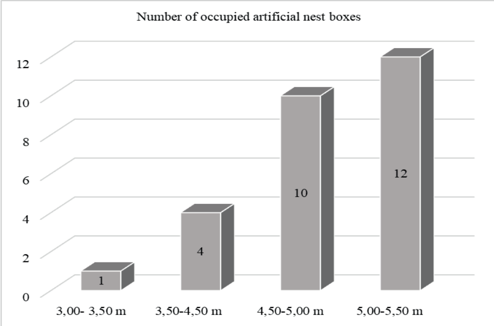

Figure 7. Number of occupied artificial nest boxes

For the parameters Fledging success, Breeding success and Eggs laid we obtain the following results:
For breeding season 2020 (Table 1) (Figure 8):

Table 1. Occupied rate per height above ground 2020

Breeding Season 2020			
Height above the ground	Fledging success	Breeding success	Eggs laid
3.00-3.50 m	0	0	0
3.50-4.00 m	5	5	5
4.50-5.00 m	22	24	28
5.00-5.50 m	32	35	41

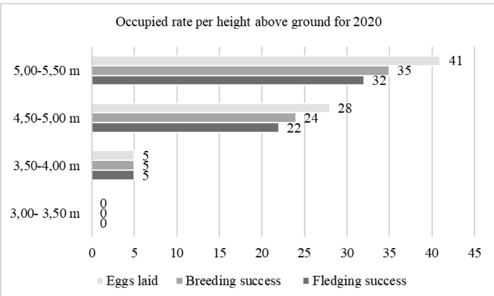

Figure 8. Occupied rate per height above ground 2020

For breeding season 2021 (Table 2) (Figure 9):

Table 2. Occupied rate per height above ground 2021

Breeding Season 2021			
Height above the ground	Fledging success	Breeding success	Eggs laid
3.00-3.50 m	0	0	0
3.50-4.00 m	2	2	3
4.50-5.00 m	16	16	18
5.00-5.50 m	15	16	28

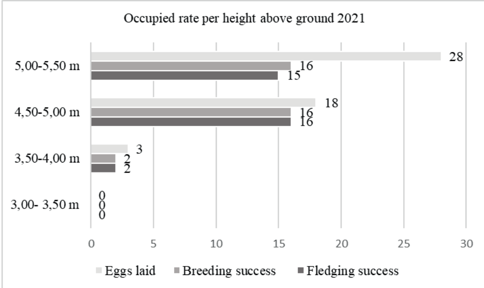

Figure 9. Occupied rate per height above ground 2021

For breeding season 2022 (Table 3) (Figure 10)

Table 3. Occupied rate per height above ground 2022

Breeding Season 2022			
Height above the ground	Fledging success	Breeding success	Eggs laid
3.00-3.50 m	4	4	5
3.50-4.00 m	12	13	14
4.50-5.00 m	18	19	24
5.00-5.50 m	20	22	27

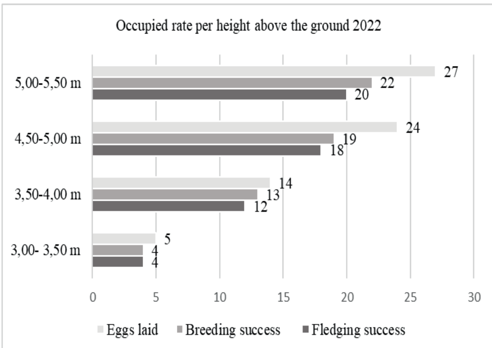

Figure 10. Occupied rate per height above ground 2022

For all the period of the study (Table 4) (Figure11)

Table 4. Occupied rate per height above ground 2020-2022

Breeding Season 2020-2022			
Height above the ground	Fledging success	Breeding success	Eggs laid
3.00-3.50 m	4	4	5
3.50-4.00 m	19	20	22
4.50-5.00 m	56	59	70
5.00-5.50 m	67	73	96

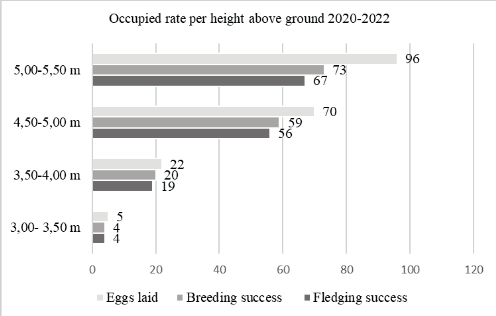

Figure 11. Occupied rate per height above ground 2020-2022

CONCLUSIONS

The current study establishes that 35.53 % from the total 76 installed artificial nest boxes Lesser Kestrel breeding successful. It was found that 54 % of the total number of 50 installed artificial nest boxes of Type 1 and Type 2 are used successful from Lesser Kestrel.

From each parameters the height between 4.50-5.50 m was the most preferred for occupied rate.

Lesser Kestrel adapts extremely successfully to artificial nest boxes and this is a major way to conserve the species as well as increase its numbers all studies conducted show that.

ACKNOWLEDGEMENTS

This study was supported by Agricultural University of Plovdiv in the frame of PhD thesis program D -141.

All field research was implemented with the support of “Green Balkans – Stara Zagora” NGO within a project “Better Life for Lesser Kestrel in South-East Balkans” LIFE19 NAT/BG/001017.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

PARASITES AND PARASITE COMMUNITIES OF *Squalius orpheus* Kottelat & Economidis, 2006 FROM THE LUDA YANA RIVER

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Abstract

The present study presents new data on the helminths and helminth communities of fish from the freshwater ecosystem of the Luda Yana River as part of the Maritsa River Basin in Bulgaria, Eastern Aegean Water Basin. In connection with this, 32 specimens of Orpheus dace (Squalius orpheus Kottelat & Economidis, 2006) were collected and examined. The specimens were collected in the autumn of 2022 in the vicinity of the Popintsi village, located in the middle section of the Luda Yana River. The helminthological examination was carried out according to standard methods. Invasion with 3 species of helminths was found - one cestode species (Caryophyllaeides fennica (Schneider, 1902) Nybelin, 1922); one acanthocephalan species (Acanthocephalus lucii (Müller, 1776) Lühe, 1911) and one nematode species (Rhabdochona denudata (Dujardin, 1845) Railliet, 1916). Ac. lucii is reported for the first time from Sq. orpheus in Bulgaria.

Key words*: Aegean Water Basin, Bulgaria, helminths, helminth communities, Orpheus dace.*

INTRODUCTION

The East Aegean region covers the catchment basins of four large rivers in Southern Bulgaria - the Maritsa River, the Arda River, the Tundzha River, and the Byala Reka River (Ministry of environment and waters. East Aegean River Basin Directorate, Plovdiv). The Maritsa River ranks fourth in length in Bulgaria (with 321.6 km on Bulgarian territory) (Chunchukova et al., 2019a; 2019b). The catchment basin of the river in Bulgaria is 21,084 km^2, including over 100 tributaries (Ministry of environment and waters. East Aegean River Basin Directorate, Plovdiv). The Luda Yana River, a left tributary of the Maritsa River, is 74 km long. The river begins from the Sredna Gora Mountains, flows through the Upper Thracian Plain, and flows into the Maritsa River in the area of the village of Sinitovo (Kolev, 2013; 2020). Along its entire course, the Luda Yana River is exposed to anthropogenic pollution (ore mining, industrial waste water, sewerage, irrigation, and others) (Kirin et al., 2019a). Different authors study the anthropogenic impact on the water quality and biodiversity of the river (Rabadjieva et al., 2009; Georgieva et al., 2014; Nam & Tamburadzhiev, 2019; Gartsiyanova et al., 2020; Gartsiyanova et al., 2021; Radeva & Seymenov, 2021; Gartsiyanova et al., 2022). There are a number of studies on the anthropogenic pressure (pollution with heavy metals) on a number of water bodies being part of the Aegean Water Basin - the Sazliyka River (Mn contamination; Atanasov et al., 2013; Cd contamination, Valkova et al., 2015; Pb contamination, Valkova et al., 2016); the Zhrebchevo Dam (Mn contamination, Atanasov et al., 2013; Fe, Ni, Pb, Mn, Cu, Cr, Cd, Zn contamination, Zhelyazkov et al., 2014; Cd contamination, Valkova et al., 2015; Pb contamination, Valkova et al., 2016; Cd, Ni, Pb, Zn contamination, Zhelyazkov et al., 2018); the Tundzha River (Mn contamination, Atanasov et al., 2013; Cd contamination, Valkova et al., 2015; Pb contamination, Valkova et al., 2016); the Bedechka River (Mn contamination, Atanasov et al., 2013; Cd contamination, Valkova et al., 2015; Pb contamination, Valkova et al., 2016); the Zagorka Lake (Fe, Mn, Cu, Cr, Ni, Zn, Pb, Cd contamination, Atanasov et al., 2012); the Ovcharitsa Dam (Fe, Mn, Cu, Cr, Ni, Zn, Pb, Cd contamination, Atanasov et al., 2012; Fe, Mn contamination, Valkova et al., 2020; Cu, Cd contamination, Valkova et al., 2021); the Chepelarska River (Pb, Cd contamination, Dospatliev et al., 2015); others. Kolev (2013) studied the ichthyofauna of rivers entering the Maritsa River and reported the following species for the Luda Yana River: *Gobio bulgaricus* Drensky, 1926; *Pseudorasbora parva* Temminck & Schlegel, 1846; *Barbus cyclolepis* Heckel, 1837; *Carassius gibelio* Bloch, 1782; *Alburnus alburnus* Linnaeus, 1758; *Rutilus rutilus* Linaeus, 1758; *Squalius orpheus* Kottelat & Economidis, 2006; *Cobitis strumicae* Karaman, 1955; *Salmo* sp.; *Lepomis gibbosus* Linnaeus, 1758; *Perca fluviatilis* Linnaeus, 1758. Fish are directly affected by the pollutants in the aquatic environment and indicate the condition of the environment. They are an important part of the monitoring systems of aquatic ecosystems (Authman et al., 2015). Parasites can also reflect changes in the environment because their life cycles involve a number of intermediate and definitive hosts (Kirin, 2013). Studies on parasites and parasite communities of fish from the Luda Yana River were carried out by Kirin (2002b); Kirin et al. (2019a).

The purpose of this study is to provide new data on the species composition of helminths and the structure of the helminth communities of Orpheus dace from the ecosystem of the Luda Yana River, part of the Maritsa River Basin in Bulgaria.

MATERIALS AND METHODS

In the autumn of 2022, 32 specimens of Orpheus dace (*Squalius orpheus* Kottelat & Economidis, 2006) from the Luda Yana River were examined. The fish were collected from the river in the vicinity of the village of Popintsi (denoted as a biotope), Pazardzhik Province (Figure 1).

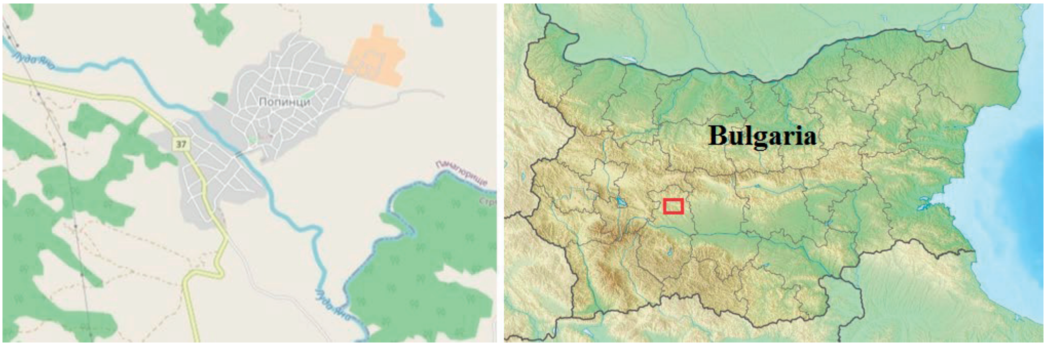

Figure 1. Location of the studied biotope from the Luda Yana River (Caynax Sports Tracker GPS; https://bg.wikipedia.org/wiki/Попинци with changes)

The scientific name of the species is given by Fröse & Pauly (2022). The body surface as well as the organs of the fish were examined for parasites immediately after capture. The ecologoparasitological study was completed in a laboratory at the Agricultural University - Plovdiv (Department of Agroecology and Environmental Protection) according to standard methods (Zashev & Margaritov, 1966; Moravec, 2013; and others). Permanent microscopic preparations (for the representatives of class Cestoda; Scholz & Hanzelová, 1998) and temporary microscopic preparations (for the representatives of classes Acanthocephala and Nematoda; Moravec, 2013) were prepared. Species belonging to the helminths ware determined according to Petrochenko (1956); Kakacheva-Avramova (1983); Moravec (2013), and others. Helminth communities are examined at the level of component communities and the level of infracommunities. Concerning the prevalence (Kennedy, 1993), species are divided into accidental (P% < 10), component (10 < P% < 20), and core (P% > 20). Basic ecological indices (mean intensity (MI), mean abundance (MA) and prevalence (P%), as well as Brillouin's diversity index (HB), Pielou's evenness index (E), Simpson's dominance index (C) were calculated (Margolis et al., 1982; Magurran, 1988; Kennedy, 1993).

RESULTS AND DISCUSSIONS

Model fish species

Sq. orpheus was selected as a model fish species (reported as *Squalius cephalus* (Linnaeus, 1758); *Leuciscus cephalus* (Linnaeus, 1758) in the Aegean Water Basin until 2006); genus *Squalius* Bonaparte 1837; family Cyprinidae. Orpheus dace is a freshwater, pelagic, omnivorous fish. The species is distributed in the rivers part of the Aegean Water Basin on the territory of Bulgaria, Greece, and Turkey. It is found in streams and rivers where the current is moderate or the water is standing (Fröse & Pauly, 2022).

Structure of the helminth communities

During the study of the 32 specimens Orpheus dace from Popintsi biotope, three taxa of helminths were found - *Caryophyllaeides fennica* (Schneider, 1902) Nybelin, 1922 (class Cestoda); *Acanthocephalus lucii* (Müller, 1776) Lühe, 1911 (class Acanthocephala) and *Rhabdochona denudata* (Dujardin, 1845) Railliet, 1916 (class Nematoda).

Component community

In the component community of Orpheus dace, the representatives of class Acanthocephala (1 species with 15 specimens) had the largest number of specimens, and the representatives of class Cestoda (1 species with 1 specimen) had the smallest number. In the helminth community of *Sq. orpheus*, one component helminth species (*Ac. lucii*) was found, with a prevalence P% = 15.63. While *C. fennica* and *Rh. denudata* were accidental helminth species, with prevalence P% = 3.13 and P% = 6.25, respectively. *Ac. lucii* also had the highest values for MI and MA (Table 1).

Table 1. Species diversity and ecological indices in the helminth community of *Squalius orpheus* from the Luda Yana River (N - number of investigated fish; n - number of infected fish; p - number of fish parasites; MI - mean intensity; MA - mean abundance; P% - prevalence; R - range)

***Squalius orpheus* (N = 32 / Popintsi biotope)** **Parasite species**	**n**	**p**	**MI**	**MA**	**P%**	**R**
Caryophyllaeides fennica (Schneider, 1902) Nybelin, 1922	1	1	1.00	0.03	3.13	1
Acanthocephalus lucii (Müller, 1776) Lühe, 1911	5	15	3.00	0.09	15.63	1-5
Rhabdochona denudata (Dujardin, 1845) Railliet, 1916	2	3	1.50	0.05	6.25	1-2

Infracommunity

In the present study of 32 *Sq. orpheus* specimens, parasites were found in eight of them (25%). All specimens Orpheus dace were infected with only one helminth species. In one specimen of *Sq. orpheus*, one specimen of *C. fennica* was found. In 1, 3, and 1 *Sq. orpheus* specimens, 1, 3, and 5 specimens of *Ac. lucii* were found, respectively. Two Orpheus dace specimens were infected with 1 and 2 specimens of *Rh. denudata*, respectively. The helminthological examination showed infection with 3 species and a total of 19 helminth specimens. The number of the found helminth specimens in one Orpheus dace specimen varied from 1 to 5. The index of diversity and evenness are close (HB = 0.51 and E = 0.57, respectively). The dominance index, C = 0.65 is highly associated with the dominance of *Ac. lucii* (Table 2).

Table 2. Infracommunity of *Squalius orpheus* from the Luda Yana River

	Number of parasite species	
	0	**1**
Number of specimens *Sq. orpheus*	24	8
Total number of species (Mean number of species ± SD)	3 (0.25±0.44)	
Total number of specimens (Mean number of specimens ± SD)	19 (0.59±1.32)	
Range	1-5	
Brillouin's diversity index (HB)	0.51	
Pielou's evenness index (E)	0.57	
Simpson's dominance index (C)	0.65	

For the last 22 years, a number of studies on the species composition and ecological indices of parasites of *Sq. orpheus* from the Aegean Water Basin in Bulgaria have been conducted. Studies on Orpheus dace parasites from Luda Yana River have not been conducted (Table 3).

Table 3. Species composition of *Squalius orpheus* parasites from the Aegean Water Basin in Bulgaria

Authors	Localities	Species composition of ***Squalius orpheus*** parasites
Kakacheva-Avramova, 1965	reservoirs of Thrace	*Rh. Denudate*
Margaritov, 1964	Maritsa, Vacha, Chepinska rivers	*Rh. denudata*
Kirin, 2000	Maritsa River – Plovdiv	*Allocreadium isoporum* (Looss, 1894) Looss, 1902 (syn. *Allocreadium isoporum macrorchis* Koval & Kulakowskaya in Koval, 1957); *Clinostomum complanatum* (Rudolphi, 1814) Braun, 1899 (metacercaria); *Caryophyllaeus brachycollis* (Janiszewska, 1951); *Bathybothrium rectangulum* (Bloch, 1782) Lühe, 1902; *Acanthocephalus tenuirostris* (Achmerov & Dombrovskaja-Achmerova, 1941) Yamaguti, 1963; *Acanthocephalus anguillae* (Müller, 1780) Lühe, 1911; *Pomphorhynchus laevis* (Zoega in Müller, 1776) Porta, 1908; *Philometra ovata* (Zeder, 1803) Skrjabin, 1923 (syn. *Philometra abdominalis* Nybelin, 1928)
Kirin, 2001a	Kardzhali Rezervoir	*All. isoporum*; *Cl. complanatum* (metacercaria); *Ichthyocotylurus pileatus* (Rudolphi, 1802) Odening, 1969 (metacercaria); *C. brachycollis*; *B. rectangulum*; *Rh. denudata*
Kirin, 2001b	Maritsa River – Pazardzhik	*All. isoporum*; *Cl. complanatum* (metacercaria); *C. brachycollis*; *B. rectangulum*; *Ac. anguillae*; *P. laevis*
Kirin, 2001c	Mesta River	*Cl. complanatum* (metacercaria); *Ichth. pileatus* (metacercaria); *C. brachycollis*; *Caryophyllaeides fennica* (Schneider, 1902) Nybelin, 1922; *Ac. tenuirostris*; *Ac. anguillae*; *Rh. Denudate*
Kirin, 2002a	Chepelarska River – between Asenovgrad and Bachkovo	*B. rectangulum*; *Ac. anguillae*; *Ac. tenuirostris*; *Contracaecum microcephalum* (Rudolphi, 1809) Baylis, 1920 (syn. *Contracaecum squalii* Linstow, 1907 Skrjabin, 1917 (larvae)); *Rh. Denudate*
Kirin, 2002c	Arda River – cascade Gorna Arda	*All. isoporum*; *Cl. complanatum* (metacercaria); *Ichth. pileatus* (metacercaria); *C. fennica*; *C. brachycollis*; *B. rectangulum*; *Ac. anguillae*; *Ac. tenuirostris*; *Rh. denudata*
Kirin et al., 2002; Kuzmanov et al., 2002	Arda River – Rabovo	*C. fennica*; *Rh. Denudate*
	Arda River – Madzharovo	*Ichth. pileatus* (metacercaria); *C. fennica*; *C. brachycollis*
Kirin et al., 2003; Kuzmanov et al., 2003	Arda River – Huhla	*Ichth. pileatus* (metacercaria); *Cl. complanatum* (metacercaria); *C. fennica*; *C. brachycollis*; *Schyzocotyle acheilognathi* (Yamaguti, 1934) Brabec, Waeschenbach, Scholz, Littlewood & Kuchta, 2015 (syn. *Bothriocephalus acheilognathi* Yamaguti, 1934); *Ligula intestinalis* (Linnaeus, 1758) Gmelin, 1790 (plerocercoid); *Ac. anguillae*; *Rh. Denudate*
Kirin et al., 2005	Stryama River – from 9 biotopes	*All. isoporum*; *C. fennica*; *C. brachycollis*; *B. rectangulum*; *P. laevis*; *Ac. anguillae*; *Ac. tenuirostris*; *Rh. denudata*
Kirin, 2006	Arda River – Rabovo	*C. fennica*; *Rh. Denudate*
	Arda River – Madzharovo	*Ichth. pileatus* (metacercaria); *C. fennica*; *C. brachycollis*
	Arda River – Huhla	*Ichth. pileatus* (metacercaria); *Cl. complanatum* (metacercaria); *C. fennica*; *C. brachycollis*; *Sch. acheilognathi*; *L. intestinalis* (plerocercoid); *Ac. anguillae*; *Rh. Denudate*
	Arda River – Slaveevo	*Ichth. pileatus* (metacercaria); *Posthodiplostomum cuticola* (von Nordmann, 1832) Dubois, 1936 (metacerc); *C. fennica*; *C. brachycollis*; *Sch. acheilognathi*; *L. intestinalis* (plerocercoid); *Paradilepis scolecina* (Rudolphi, 1819) (cysticerc); *Ac. anguillae*; *Rh. denudata*; *Raphidascaris acus* (Bloch, 1779) Railliet & Henry, 1915 (larvae)
Kirin & Shukerova, 2006	Arda River – Slaveevo	*Ichth. pileatus* (metacercaria); *P. cuticola* (metacerc); *C. fennica*; *C. brachycollis*; *Sch. acheilognathi*; *L. intestinalis* (plerocercoid); *P. scolecina* (cysticerc); *Ac. anguillae*; *Rh. denudata*; *R. acus* (larvae)
Kirin & Shukerova, 2007	Arda River	*Ichth. pileatus* (metacercaria); *Cl. complanatum* (metacercaria); *C. fennica*; *C. brachycollis*; *Sch. acheilognathi*; *L. intestinalis* (plerocercoid); *P. scolecina* (cysticerc); *Ac. anguillae*; *Rh. denudata*; *R. acus* (larvae)
Kirin et al., 2012, 2013a	Tundzha River	*Ichth. pileatus* (metacercaria); *Cl. complanatum* (metacercaria); *C. fennica*; *C. brachycollis*; *Sch. acheilognathi*; *L. intestinalis* (plerocercoid); *Ac. anguillae*; *Rh. Denudate*
Kirin et al., 2013b	Arda River	free of parasites
Kirin et al., 2019b	Stryama River	*All. isoporum*; *C. brachycollis*; *P. laevis*; *Rh. denudata*
Chunchukova et al., 2020a	Topolnitsa River	*P. laevis*

The found three helminth species of Orpheus dace, from the present study, have been reported for a large number of fish host species from different water bodies on the territory of Bulgaria (Table 4).

Table 4. Fish hosts of *Caryophyllaeides fennica*, *Acanthocephalus lucii*, and *Rhabdochona denudata* in Bulgaria

Authors	Localities	Host
	studies on *CARYOPHYLLAEIDES FENNICA*	
Margaritov, 1959	Iskar River – Vrazhdebna; Tundzha River – Vetren, Kazanlak Municipality; Iskar River – Vrazhdebna, Kalkovo, Samokov Municipality; Palakaria River, Shiroki dol, Samokov Municipality Iskar River – Vrazhdebna	*Barbus barbus* (Linnaeus, 1758) *Barbus petenyi* Heckel, 1852 *Squalius cephalus* (Linnaeus, 1758) (syn. *Leuciscus cephalus*)
Margaritov, 1964	Batak Reservoir	*Sq. cephalus*
Margaritov, 1965	Maritsa River	*B. cyclolepis*; *Sq. cephalus*; *V. melanops*
Kakacheva-Avramova, 1965	Asenitsa, Harmanliyska, Topolnitsa, Syutliyka, Bedechka rivers	*B. cyclolepis*; *Sq. cephalus*; *V. melanops*
Margaritov, 1966	Danube River – from the sections Svishtov – Ruse and the mouth of the Timok River – Novo Selo	*Barbus barbus* (Linnaeus, 1758)
	Danube River, between the mouth of the Timok River and Novo Selo	*Sander lucioperca* (Linnaeus, 1758) (syn. *Lucioperca lucioperca*)
Kakacheva-Avramova, 1969	Nishava, Ogosta, Vodomerka, Buchinska, Vrabnishka, Burzia, Chuprenska, Iskretska, Botunya, Bebresh rivers Bogovina River Bogovina, Nishava, Ogosta, Vodomerka, Burzia, Botunya, Bebresh rivers Botunya, Bebresh rivers Ogosta River Leva River	*B. petenyi* *B. barbus* *Sq. cephalus* *Gobio gobio* (Linnaeus, 1758); *Chondrostoma nasus* (Linnaeus, 1758) *Alb. alburnus*
Margaritov, 1977	Shiposhnitsa River, Iskar Reservoir	*Sq. cephalus*, *R. rutilus*
Kakacheva-Avramova et al., 1978	Danube River – Vidin, Lom, Svishtov, Silistra	*Abramis brama* (Linnaeus, 1758); *Alb. alburnus*; *Ballerus sapa* (Pallas, 1814) (syn. *Abramis sapa*); *Ballerus ballerus* (Linnaeus, 1758) (syn *Abramis ballerus*); *B. barbus*; *Blicca bjoerkna* (Linnaeus, 1758); *Pelecus cultratus* (Linnaeus, 1758); *S. lucioperca* (syn. *Stizostedion lucioperca*); *Scardinius erythrophthalmus* (Linnaeus, 1758); *Vimba vimba* (Linnaeus, 1758) (syn. *Vimba vimba carinata*)
Kakacheva-Avramova, 1973	Vit, Cherni Vit rivers	*Sq. cephalus*
Margaritov, 1977	Shiposhnitsa River, Iskar Reservoir	*Sq. cephalus*
Kakacheva-Avramova, & Menkova, 1978	Palakaria River	*Sq. cephalus*
Kakacheva-Avramova, & Nedeva-Menkova, 1981	Struma, Zheleznitsa rivers	*B. barbus*
Kirin, 2001c	Mesta River	*Sq. orpheus*; *Barbus meridionalis petenyi* Heckel, 1847
Kirin, 2002c	Arda River – from the confluence of the Cherna, Rodozemska and Madanska rivers to the Kardzhali Rezervoir / cascade Gorna Arda	*Sq. orpheus*
Kirin et al., 2002; Kuzmanov et al., 2002	Arda River – Rabovo, Madzharovo	*Sq. orpheus*
	Arda River – Rabovo	*Alb. alburnus*
Kirin, 2003	Arda River – from the confluence of the Cherna, Rodozemska and Madanska rivers to the Kardzhali Rezervoir	*B. cyclolepis*; *Alb. alburnus*
Kirin et al., 2003; Kuzmanov et al., 2003	Arda River – Huhla	*Sq. orpheus*
Kirin et al., 2005	Stryama River	*Sq. orpheus*
Kirin, 2006	Arda River – Rabovo, Madzharovo, Huhla, Slaveevo	*Sq. orpheus*
Kirin & Shukerova, 2006; 2007	Arda River – Slaveevo	*Sq. orpheus*
Atanasov, 2012	Danube river – from the region of Dobri dol	*B. barbus*
Kirin et al., 2012; 2013a	Tundzha River	*Sq. orpheus*
Shukerova, 2010	Srebarna Lake	*P. fluviatilis*
Kirin et al., 2019a	Luda Yana River – Popintsi	*R. rutilus*

Authors	Localities	Host
	studies on *CARYOPHYLLAEIDES FENNICA*	
Kirin et al., 2020	Tamrashka River – between Hrabrino and Parvenets	*B. cyclolepis*
studies on *ACANTHOCEPHALUS LUCII*		
Margaritov, 1959	Danube River – Svishtov	*Sillurus glanis* Linnaeus, 1758
	Iskar River – Vrazhdebna, Kalkovo	*Sq. cephalus*
Margaritov, 1966	Danube River – between Svishtov and Ruse and between the Timok River and Novo Selo	*P. fluviatilis*
Kakacheva-Avramova et al., 1978	Danube River – Svishtov, Silistra and in the region of Vidin	*B. sapa*; *Sq. cephalus*; *R. rutilus*; *S. glanis*; *P. fluviatilis*; *Lota lota* (Linnaeus, 1758); *Gymnocephalus schraetser* (Linnaeus, 1758) (syn. *Acerina schraetser*); *Benthophilus stellatus* (Sauvage, 1874); *Proterorhinus marmoratus* (Pallas, 1814)
Atanasov, 2012	Danube River – Archar and Kozloduy	*L. lota*; *Zingel zingel* (Linnaeus, 1766)
Chunchukova, 2017; Chunchukova et al., 2016; Chunchukova et al., 2017; Chunchukova et al., 2020b	Danube River – Vetren	*Abr. brama*
Chunchukova, 2017; Chunchukova et al., 2018	Danube River – Vetren	*Alb. alburnus*
Shukerova, 2010; Shukerova et al., 2010	Srebarna Lake	*P. fluviatilis*
Chunchukova et al., 2020c	Ogosta River	*Sq. cephalus*
Kirin et al., 2019a	Luda Yana River – Popintsi	*R. rutilus*
Kirin & Chunchukova, 2021	Tundzha River	*S. glanis*
	studies on *RHABDOCHONA DENUDATA*	
Margaritov, 1959	Iskar River – Vrazhdebna	*B. barbus*; *Sq. cephalus*
Kakacheva-Avramova, 1962	Strumeshnitsa River	*Scardinius erythrophthalmus* (Linnaeus, 1758)
Margaritov, 1964	Maritsa, Vacha, Chepinska rivers Maritsa River Maritsa, Chepinska rivers Maritsa, Vacha, Chepinska, Topolnitsa rivers	*Sq. orpheus* *V. melanops* *Alb. alburnus* *B. cyclolepis*
Kakacheva-Avramova, 1965	reservoirs of Thrace	*Sq. orpheus*; *Alb. alburnus*; *L. aspius*; *B. cyclolepis*
Kakacheva-Avramova, 1969	Ogosta, Vrabnishka, Burzia, Nishava, Botunya, Leva, Archar, Berkovska, Vrabnishka, Chuprenska rivers Chuprenska, Burzia, Leva rivers Leva River Burzia River Ogosta, Lom, Leva rivers	*Sq. cephalus* *B. petenyi* *B. barbus* *G. gobio* *Alb. alburnus*
Margaritov, 1977	Shiposhnitsa River, Iskar Reservoir	*Sq. cephalus*
Kakacheva-Avramova & Menkova, 1978	Palakaria River	*Sq. cephalus*
Kakacheva-Avramova et al., 1978	Danube River – Vidin	*Z. zingel* (syn. *Aspro zingel*); *Zingel streber* (Siebold, 1863) (syn. *Aspro streber*); *Alb. alburnus*
Kakacheva-Avramova & Nedeva-Menkova, 1981	State Fisheries Blagoevgrad Zheleznitsa,Blagoevgradska Bistritsa, Gradevska, Struma rivers	*Cobitis taenia* Linnaeus, 1758 *Sq. cephalus*
Kirin, 2001a	Kardzhali Rezervoir	*Sq. orpheus*
Kirin, 2001c	Mesta River	*Sq. orpheus*
Kirin, 2001d	Mesta River	*Cyprinus carpio* Linnaeus, 1758
Kirin, 2001e	Veleka, Rezovska rivers	*Alb. alburnus*
Kirin, 2002a	Chepelarska River – between Asenovgrad and Bachkovo	*Sq. orpheus*
Kirin, 2002c	Arda River – from the confluence of the Cherna, Rodozemska and Madanska rivers to the Kardzhali Rezervoir / cascade Gorna Arda	*Sq. orpheus*
Kirin, 2003	Arda River – from the confluence of the Cherna, Rodozemska and Madanska rivers to the Kardzhali Rezervoir	*Alb. alburnus*
Kirin et al., 2002; Kuzmanov et al., 2002	Arda River – Rabovo, Madzharovo	*Alb. alburnus*

Authors	Localities	Host
	studies on ***RHABDOCHONA DENUDATA***	
Kirin et al., 2002; Kuzmanov et al., 2002	Arda River – Rabovo, Madzharovo	*Alb. alburnus*
Kirin et al., 2002; Kuzmanov et al., 2002; Kirin, 2006	Arda River – Rabovo	*Sq. orpheus*
Kirin et al., 2003; Kuzmanov et al., 2003; Kirin, 2006	Arda River – Huhla	*Sq. orpheus*
Kirin et al., 2005	Stryama River	*Sq. orpheus*
Kirin, 2006; Kirin & Shukerova, 2006	Arda River, Slaveevo	*Sq. orpheus*
Kirin & Shukerova, 2007	Arda River	*Sq. orpheus*
Shukerova & Kirin, 2008	Srebarna Lake	*Sc. erythrophthalmus*
Shukerova, 2010	Srebarna Lake	*L. aspius*; *Sc. erythrophthalmus*
Atanasov, 2012	Danube River – Archar	*B. barbus*; *Sc. erythrophthalmus*; *Sq. cephalus*
Kirin et al., 2012, 2013a	Tundzha River	*Sq. orpheus*
Chunchukova et al., 2019a	Maritsa River	*Alb. alburnus*
Chunchukova et al., 2019b	Maritsa River	*Sc. erythrophthalmus*
Kirin et al., 2019a	Luda Yana River – Popintsi	*R. rutilus*
Kirin et al., 2019b	Stryama River	*Sq. orpheus*
Kuzmanova et al., 2019	Osym River – Lovech	*Sq. cephalus*
Chunchukova & Kirin, 2020a	Danube River – Silistra	*Abr. brama*
Chunchukova & Kirin, 2020b	Tundzha River – Yambol	*L. aspius*
Chunchukova et al., 2020c	Ogosta River	*Sq. cephalus*

CONCLUSIONS

In the autumn of 2022, 32 specimens of *Sq. orpheus* from the Luda Yana River (Popintsi biotope) were examined for parasites. Infection was found in 25% of the examined specimens. Three helminth species were found (*Caryophyllaeides fennica* (Schneider, 1902) Nybelin, 1922; *Acanthocephalus lucii* (Müller, 1776) Lühe, 1911; *Rhabdochona denudata* (Dujardin, 1845) Railliet, 1916). In the component community of Orpheus dace, *Ac. lucii* (MI = 3.00; MA = 0.09; P% = 15.63) had the highest ecological indices. *C. fennica*, *Ac. lucii* and *Rh. denudata* were reported of *R. rutilus* from the Luda Yana River (Popintsi biotope), but were not reported of *Sq. orpheus* from the Luda Yana River. *Sq. orpheus* is a new host for *Ac. lucii* in Bulgaria. The parasite species found in the present study are not dangerous for humans and fish. Studies on parasites are important for conservation of the fish resources.

ACKNOWLEDGEMENTS

This research is supported by the Bulgarian Ministry of Education and Science under the national Program” Young Scientists and Postdoctoral Students-2”. I thank the leadership of the Agricultural University – Plovdiv for the opportunity to participate in this project, a part of the national program” Young Scientists and Postdoctoral Students-2”.

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Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

PARASITES AND PARASITE COMMUNITIES OF *Squalius orpheus* Kottelat & Economidis, 2006 FROM THE CHEPELARSKA RIVER

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Abstract

In the autumn of 2022, 37 specimens of Orpheus dace (Squalius orpheus Kottelat & Economidis, 2006) were subjected to helminthological examination. The fish were caught from the lower section of the Chepelarska River (in the area of Katunitsa village). Three endohelminth species were isolated - Acanthocephalus tenuirostris (Achmerov & Dombrovskaja-Achmerova, 1941) Yamaguti, 1963 (class Trematoda), Contracaecum sp., Rhabdochona denudata (Dujardin, 1845) Railliet, 1916 (class Nematoda). The component community and infracommunity of Orpheus dace were reviewed. One core species (Contracaecum sp.; P% = 32.43) was found in the component community of Sq. orpheus. Brillouin's diversity index (HB), Pielou's evenness index (E), and Simpson's dominance index (C) were calculated. The research aims to provide data on the helminths and helminth communities of Orpheus dace from the freshwater ecosystem of the Chepelarska River. The studied biotope (Katunitsa) is a new habitat for the found helminth species of Orpheus dace.

***Key words**: Acanthocephalus tenuirostris, Bulgaria, Contracaecum sp., Maritsa River Basin, Rhabdochona denudata.*

INTRODUCTION

The Maritsa River (Έβρος; Meriç) is the longest river on the Balkan Peninsula (539 km) (Chunchukova et al., 2019a). On Bulgarian territory, it flows for 321.6 km (Chunchukova et al., 2019a; 2019b). The river begins from the Rila Mountains, flows through the Upper Thracian Plain, and enters the Aegean Sea (Chunchukova et al., 2019a). The river partially forms the Bulgarian-Greek border and the Greek-Turkish border (Kirin, 2013; 2014). On Bulgarian territory, the Maritsa River has over 100 tributaries. The left tributaries spring from the Balkan Mountains, the Sredna Gora Mountains, the Sarnena Gora Mountains, and the right – from the Rhodope Mountains. The number of left and right tributaries of the river is approximately equal. The longest tributaries of the river flowing into it on Bulgarian territory are the Topolnitsa River with a length of 155 km, the Rakitnitsa River with a length of 145 km, the Vacha River with a length of 112 km, the Stryama River with a length of 110 km, the Chepelarska River with a length of 86 km, etc. (Ministry of environment and waters). The Chepelarska River begins from the Rhodope Mountains (in the area of the Pamporovo resort complex), passes through the Upper Thracian Plain, where it flows into the Maritsa River between the towns of Plovdiv and Sadovo. Several settlements are located along the river (the town of Chepelare, the village of Hvoyna, the village of Narechenski bani, the village of Bachkovo, the town of Asenovgrad, the village of Katunitsa) (Assenovgrad Municipality Program, 2018).

The Chepelarska River is anthropogenically influenced as a result of urbanization, sewerage, the entry of waste water from industrial enterprises and enterprises from the food industry (dairy farm), the construction of dams, changes in the river bed, extraction of aggregates, construction of barriers, etc. (Project PURB, 2016-2021).

Dospatliev et al. (2015) studied the pollution of the Chepelarska River with Cd and Pb. The authors reported that the source of Cd pollution is the Non-ferrous Metals Smelter (KCM S.A.), and of Pb - a tailings pond of Gorubso-Lucky. Anthropogenic pollution affects aquatic organisms (Juhásová et al., 2019). Various authors investigated heavy metal pollution in water, sediments, or aquatic organisms from rivers being part of Eastern Aegean Sea River Basin (Atanasov et al., 2012; Atanasov et al., 2013; Zhelyazkov et al., 2014; Valkova et al., 2015; Valkova et al., 2016; Zhelyazkov et al., 2018; Valkova et al., 2020; Valkova et al., 2021; others). Fish and their parasites are basic biological elements of the freshwater ecosystem, used for ecological assessment of the condition of the aquatic environment (Kirin, 2013; Kirin & Kuzmanova, 2014). Few authors study parasites of fish from the Chepelarska River (Kakacheva-Avramova, 1965; Kirin, 2002a; Chunchukova, 2020). Most of the existing studies focus on the parasite fauna of fish from the Maritsa River (Kirin, 2000; 2001b; 2013; 2014; Chunchukova et al., 2019a; 2019b) and its tributaries - the Arda River (Kirin, 2002b; 2003; 2006; Kirin et al., 2002; Kuzmanov et al., 2002; Kirin et al., 2003; Kuzmanov et al., 2003; Kirin & Shukerova, 2006; 2007; Kirin et al., 2013b); the Stryama River (Kirin et al., 2005; Kirin et al., 2019b); the Tundzha River (Kirin et al., 2012; 2013a; Chunchukova & Kirin, 2020b; Kirin & Chunchukova, 2021; 2022); the Topolnitsa River (Chunchukova et al., 2020a); others.

The present study aims to provide new data on the helminths and helminth communities of Orpheus dace (*Squalius orpheus* Kottelat & Economidis, 2006) from the freshwater ecosystem of the Chepelarska River.

MATERIALS AND METHODS

A total of 37 specimens of Orpheus dace (*Squalius orpheus* Kottelat & Economidis, 2006) were examined for the presence of helminths. The fish were collected from the Chepelarska River in the vicinity of the village of Katunitsa (designated as Katunitsa biotope); in the autumn of 2022. Biotope Katunitsa (42°06'05.7"N, 24°51'58.1"E) is located on the right bank of the river, about 8 km from the mouth of the Chepelarska River in the Maritsa River (Figure 1).

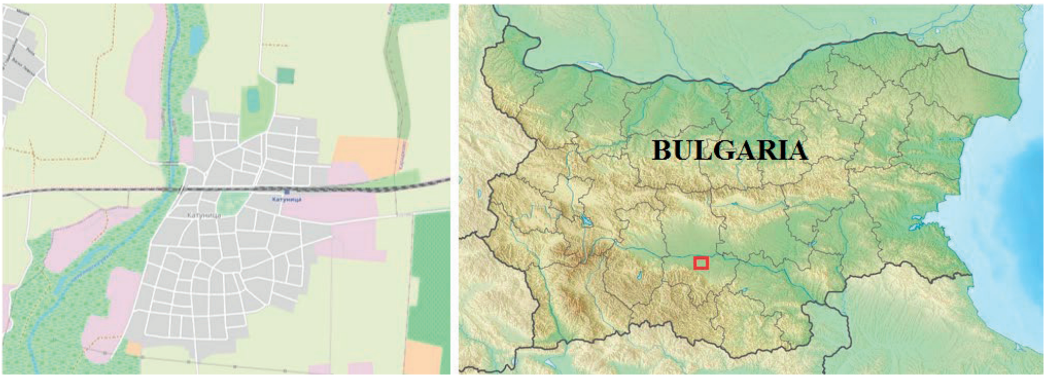

Figure 1. Location of the studied biotope from the Chepelarska River (Caynax Sports Tracker GPS; https://bg.wikipedia.org/wiki/Катуница with changes)

The fish species are recorded according to Fröse & Pauly (2022). Immediately after capture (in the field), a visual inspection of the fish was carried out. The helminthological examination of the fish was completed in laboratory conditions according to standard methods (Zashev & Margaritov, 1966; Bauer (Ed.), 1987; Moravec, 2013; others). The isolated helminth specimens were stored in 70% ethyl alcohol. Temporary preparations were prepared from the stored helminths of classes Acanthocephala and Nematoda, according to standard methods (Zashev & Margaritov, 1966; Moravec, 2013; others). Found helminth taxa were noted according to commonly accepted taxonomy (Bauer (Ed.), 1987; Moravec, 2013; others). The structure of helminth communities was examined at two levels: component communities and infracommunities. The component communities were represented by the indices: mean intensity (MI); mean abundance (MA); prevalence (P%), and the infracommunities - by the indices: total number of species; mean number of species; the total number of specimens; mean number of specimens;

Brillouin's diversity index (HB); Pielou's evenness index (E); Simpson's dominance index (C) (Magurran, 1988).

RESULTS AND DISCUSSIONS

Model fish species

The object of study is 37 specimens of *Sq. orpheus* (syn. *Squalius cephalus* (Linnaeus, 1758); *Leuciscus cephalus* (Linnaeus, 1758) in the Aegean catchment basin until 2006); family Cyprinidae. Orpheus dace is a freshwater, pelagic fish. The species is omnivorous and uses mainly insects for food (Fröse & Pauly, 2022). Orpheus dace is included in the IUCN Red List with the category "LC=Least Concern"; endemic of Europe (Freyhof & Brooks, 2011); endemic of the rivers from the Aegean catchment basin (Economidis et al., 2009).

Structure of the helminth communities

The present study of Orpheus dace revealed infection with three taxa of helminths – 1 species of class Acanthocephala (*Acanthocephalus tenuirostris* (Achmerov & Dombrovskaja-Achmerova, 1941) Yamaguti, 1963) and 2 species of class Nematoda (*Contracaecum* sp.; *Rhabdochona denudata* (Dujardin, 1845) Railliet, 1916).

Component community

During the study on the component community of Orpheus dace, it was found that the representatives of class Nematoda (2 species with 35 specimens) had the largest number of specimens. In the component community of Orpheus dace, one core species was found - *Contracaecum* sp. with prevalence P% = 32.43, as well as two accidental helminth species – *Ac. tenuirostris* and *Rh. denudata* with prevalence P% = 5.41 and P% = 2.70, respectively, according to the selected criteria (Kennedy, 1993). *Contracaecum* sp. also had the highest values for MI and MA (MI = 2.75 and MA = 0.07). The number of detected specimens of *Ac. tenuirostris* in one specimen Orpheus dace varied from 1 to 2 specimens; of *Contracaecum* sp. - from 2 to 5 specimens, and of *Rh. denudata* had 2 specimens (Table 1).

Table 1. Component community of *Squalius orpheus* from the Chepelarska River
(N - number of investigated fish; n - number of infected fish; p - number of fish parasites; MI - mean intensity; MA - mean abundance; P% - prevalence; R - range)

***Squalius orpheus* (N = 37 / Katunitsa biotope)** **Parasite species**	**n**	**p**	**MI**	**MA**	**P%**	**R**
Acanthocephalus tenuirostris (Achmerov & Dombrovskaja-Achmerova, 1941) Yamaguti, 1963	2	3	1.50	0.04	5.41	1-2
Contracaecum sp.	12	33	2.75	0.07	32.43	2-5
Rhabdochona denudata (Dujardin, 1845) Railliet, 1916	1	2	2.00	0.05	2.70	2

Infracommunity

From the 37 examined specimens of *Sq. orpheus*, it was found that 15 specimens (40.54%) were infected with 1 helminth species, and 22 specimens (59.46%) were not infected. In the infracommunity of Orpheus dace from the Chepelarska River, the number of the found helminth specimens varied from 1 to 5. Thirty-eight helminth specimens were isolated. In 2 Orpheus dace specimens, 1 and 2 specimens of *Ac. tenuirostris* was found; in 7, 3, and 2 *Sq. orpheus* specimens, 2, 3, and 5 specimens of *Contracaecum* sp. were found, respectively; in 1 Orpheus dace specimen, 2 specimens of *Rh. denudata* were found. Brillouin's diversity index HB = 0.65, and the Pielou's evenness index E = 0.73. The dominance index is low, associated with the dominance of a taxon - *Contracaecum* sp. (Table 2).

Kakacheva-Avramova (1965) studied parasites of fish (*Barbus cyclolepis* Heckel, 1837, *Gobio gobio* (Linnaeus, 1758), *Sq. cephalus*) from the Chepelarska River and reported infection with *Allocreadium isoporum* (Looss, 1894) Looss, 1902 (syn. *Allocreadium isoporum macrorchis* Koval & Kulakowskaya in Koval, 1957); *Caryophyllaeus brachycollis* (Janiszewska, 1951); *Caryophyllaeides fennica* (Schneider, 1902) Nybelin, 1922; *Bathybothrium rectangulum* (Bloch, 1782) Lühe, 1902; *Acanthocephala* gen. sp.; *Rh. denudata*.

Table 2. Infracommunity of *Squalius orpheus* from the Chepelarska River

	Number of parasite species	
	0	1
Number of specimens *Sq. orpheus*	22	15
Total number of species (Mean number of species ± SD)	3 (0.42±0.50)	
Total number of specimens (Mean number of specimens ± SD)	38 (1.03±1.37)	
Range	1-5	
Brillouin's diversity index (HB)	0.65	
Pielou's evenness index (E)	0.73	
Simpson's dominance index (C)	0.54	

Chunchukova (2020) studied the parasite fauna of *B. cyclolepis* from the Chepelarska River (Bachkovo biotope) and reported two helminth species - *Pomphorhynchus laevis* (Zoega in Müller, 1776) Porta, 1908 and *Rhabdochona hellichi* (Sramek, 1901). Kirin (2002a) studied the helminth fauna of *Sq. orpheus* from the Chepelarska River (between the town of Asenovgrad and the village of Bachkovo), and reported 5 endohelminth species - *B. rectangulum*; *Acanthocephalus anguillae* (Müller, 1780) Lühe, 1911; *Ac. tenuirostris*; *Contracaecum squalii* (Linstow, 1907) Skrjabin, 1917 (larvae); *Rh. denudata*.

There are a number of studies on the parasite fauna of Orpheus dace from the Maritsa River and its tributaries on the territory of Bulgaria. During ecologoparasitological studies of *Sq. orpheus* from the Maritsa River, *All. isoporum*; *Clinostomum complanatum* (Rudolphi, 1814) Braun, 1899 (metacercaria); *C. brachycollis*; *B. rectangulum*; *Ac. tenuirostris*; *Ac. anguillae*; *P. laevis* and *Philometra ovata* (Zeder, 1803) Skrjabin, 1923 (syn. *Philometra abdominalis* Nybelin, 1928) were reported in the region of the city of Plovdiv (Kirin, 2000); *All. isoporum*; *Cl. complanatum* (metacercaria); *C. brachycollis*; *B. rectangulum*; *Ac. anguillae* and *P. laevis* in the area of the city of Pazardzhik (Kirin, 2001b). The study of Orpheus dace from the Arda River found infection with – *All. isoporum*;*Cl. complanatum* (metacercaria); *Ichthyocotylurus pileatus* (Rudolphi, 1802) Odening, 1969 (metacercaria); *C. fennica*; *C. brachycollis*; *B. rectangulum*; *Ac. anguillae*; *Ac. tenuirostris* and *Rh. denudata* from the confluence of the Cherna, Rodozemska and Madanska rivers to the Kardzhali Rezervoir (Kirin, 2002b); *C. fennica* and *Rh. denudata* in the region of the village of Rabovo; *Ichth. pileatus* (metacercaria); *C. fennica* and *C. brachycollis* in the area of the town of Madzharovo (Kirin et al., 2002; Kuzmanov et al., 2002; Kirin, 2006); *Ichth. pileatus* (metacercaria); *Cl. Complanatum* (metacercaria); *C. fennica*; *C. brachycollis*; *Schyzocotyle acheilognathi* (Yamaguti, 1934) Brabec, Waeschenbach, Scholz, Littlewood & Kuchta, 2015 (syn. *Bothriocephalus acheilognathi* Yamaguti, 1934); *Ligula intestinalis* (Linnaeus, 1758) Gmelin, 1790 (plerocercoid); *Ac. anguillae* and *Rh. denudata* in the region of the village of Huhla (Kirin et al., 2003; Kuzmanov et al., 2003; Kirin, 2006); *Ichth. pileatus* (metacercaria); *Posthodiplostomum cuticola* (von Nordmann, 1832) Dubois, 1936 (metacerc); *C. fennica*; *C. brachycollis*; *Sch. acheilognathi*; *L. intestinalis* (plerocercoid); *Paradilepis scolecina* (Rudolphi, 1819) (cysticerc); *Ac. anguillae*; *Rh. denudata* and *Raphidascaris acus* (Bloch, 1779) Railliet & Henry, 1915 (larvae) in the area of the village of Slaveevo (Kirin, 2006; Kirin & Shukerova, 2006); *Ichth. pileatus* (metacercaria); *Cl. Complanatum* (metacercaria); *C. fennica*; *C. brachycollis*; *Sch. acheilognathi*; *L. intestinalis* (plerocercoid); *P. scolecina* (cysticerc); *Ac. anguillae*; *Rh. denudata* and *R. acus* (larvae) (Kirin & Shukerova, 2007). Few studies on helminths of *Sq. orpheus* from the Stryama River, indicated the species as a host of *All. isoporum*; *C. fennica*; *C. brachycollis*; *B. rectangulum*; *P. laevis*; *Ac. anguillae*; *Ac. tenuirostris* and *Rh. denudata* (Kirin et al., 2005); *All. isoporum*; *C. brachycollis*; *P. laevis* and *Rh. denudata* (Kirin et al., 2019b). Some studies on Orpheus dace from the Tundzha River and the Topolnitsa River, found infection with *Ichth. pileatus* (metacercaria); *Cl. Complanatum* (metacercaria); *C. fennica*; *C. brachycollis*; *Sch. acheilognathi*; *L. intestinalis* (plerocercoid); *Ac. anguillae*; *Rh. denudata*

(Kirin et al., 2012; 2013a) and *P. laevis* (Chunchukova et al., 2020a), respectively. The three helminth species of Orpheus dace detected in the present study were reported in the same species as well as other fish species in Bulgaria (Table 3).

Table 3. Fish hosts of *Acanthocephalus tenuirostris*, *Contracaecum* sp., and *Rhabdochona denudata* in Bulgaria

Authors	Localities	Host
STUDY ON *ACANTHOCEPHALUS TENUIROSTRIS*		
Kakacheva-Avramova & Menkova, 1978	Palakariya River	*Barbus petenyi* Heckel, 1852 (syn. *Barbus meridionalis petenyi* Heckel, 1847), *Sq. cephalus*
Kirin, 2000	Maritsa River, Plovdiv	*Sq. orpheus*
Kirin, 2001c	Mesta River	*Sq. orpheus*, *B. petenyi*
Kirin, 2002a	Chepelarska River, between Asenovgrad and Bachkovo	*Sq. orpheus*
Kirin, 2002b	Arda River, cascade Gorna Arda	*Sq. orpheus*
Kirin et al., 2005	Stryama River	*Sq. orpheus*
Chunchukova & Kirin, 2020b	Tundzha River, Yambol	*Leuciscus aspius* (Linnaeus, 1758) (syn. *Aspius aspius* (Linnaeus, 1758))
STUDY ON *CONTRACAECUM* SP. (syn. *Contracaecum bidentatum* (Linstow, 1899); *Hysterothylacium aduncum* (Rudolphi, 1802) Deardorff & Overstreet, 1981; *Contracaecum microcephalum* (Rudolphi, 1809) Baylis, 1920; *C. squalii*)		
Margaritov, 1959	Danube River, Krivina	*Acipenser ruthenus* Linnaeus, 1758
	State Fisheries Yambol	*Cyprinus carpio* Linnaeus, 1758
Margaritov, 1966	Danube River, between the mouth of the Timok River and Novo Selo	*Ac. ruthenus*, *Zingel zingel* (Linnaeus, 1766) (syn. *Aspro zingel* (Linnaeus, 1758)), *Zingel streber* (Siebold, 1863) (syn. *Aspro streber* Siebold, 1863), *Gymnocephalus cernua* (Linnaeus, 1758) (syn. *Acerina cernua* (Linnaeus, 1758)), *Gymnocephalus schraetser* (Linnaeus, 1758) (syn. *Acerina schraetser* (Linnaeus, 1758)), *Neogobius fluviatilis* (Pallas, 1814) (syn. *Gobius fluviatilis* Pallas, 1814), *Ponticola constructor* (Nordmann, 1840) (syn. *Gobius cephalarges constructor* Nordmann, 1840)
	Danube River, between the mouth of the Timok River and Novo Selo; between Svishtov and Ruse	*Alosa immaculata* Bennett, 1835 (syn. *Alosa kessleri pontica* (Eichwald, 1838))
Kakacheva-Avramova et al., 1978	Danube River, Svishtov, Vidin, Silistra	*Lota lota* (Linnaeus, 1758), *Ponticola kessleri* (Günther, 1861) (syn. *Gobius kessleri* Günther, 1861), *N. fluviatilis*, *Perca fluviatilis* Linnaeus, 1758, *Barbus barbus* (Linnaeus, 1758)
Kirin, 2001d	Mesta River	*C. carpio*
Shukerova, 2010	Srebarna Lake	*Alburnus alburnus* (Linnaeus, 1758), *L. aspius*
Shukerova, 2005; 2010	Srebarna Lake	*Carassius gibelio* (Bloch, 1782)
Shukerova, 2006; 2010	Srebarna Lake	*C. carpio*
Shukerova, 2010; Shukerova et al., 2010	Srebarna Lake	*P. fluviatilis*
Chunchukova, 2017; Chunchukova et al., 2016	Danube River, Vetren	*Alb. alburnus*, *Abramis brama* (Linnaeus, 1758)
Chunchukova, 2017	Danube River, Vetren	*B. barbus*
Shukerova, 2010; Shukerova & Kirin, 2019	Srebarna Lake	*Rutilus rutilus* (Linnaeus, 1758)

Authors	Localities	Host
STUDY ON *CONTRACAECUM* SP. (syn. *Contracaecum bidentatum* (Linstow, 1899); *Hysterothylacium aduncum (*Rudolphi, 1802) Deardorff & Overstreet, 1981; *Contracaecum microcephalum* (Rudolphi, 1809) Baylis, 1920; *C. squalii*)		
Kirin, 2002a	Chepelarska River, between Asenovgrad and Bachkovo	*Sq. orpheus*
Kirin et al., 2013b	Danube River, Vetren	*Al. immaculata*
Kirin & Kuzmanova, 2014	Ivaylovgrad Reservoir	*Silurus glanis* Linnaeus, 1758
Stoyanov et al., 2018	Atanasovsko Lake	*Lepomis gibbosus* (Linnaeus, 1758), *Knipowitschia caucasica* (Berg, 1916), *Gasterosteus aculeatus* Linnaeus, 1758
Kirin & Chunchukova, 2021	Tundzha River	*S. glanis*
Kirin & Chunchukova, 2022	Tundzha River	*C. gibelio*
Nachev et al., 2022	Danube River	*Al. immaculata*
STUDY ON *RHABDOCHONA DENUDATA*		
Margaritov, 1959	Iskar River, Vrazhdebna	*B. barbus*, *Sq. cephalus*
Kakacheva-Avramova, 1962	Strumeshnitsa River	*Scardinius erythrophthalmus* (Linnaeus, 1758)
Margaritov, 1964	Maritsa River, Vacha River, Chepinska River Maritsa River Maritsa River, Chepinska River Maritsa River, Vacha River, Chepinska River, Topolnitsa River	*Sq. orpheus* *Vimba melanops* (Heckel, 1837) *Alb. alburnus* *B. cyclolepis*
Kakacheva-Avramova, 1965	reservoirs of Thrace	*Sq. orpheus*, *Alb. alburnus*, *L. aspius*, *B. cyclolepis*
Kakacheva-Avramova, 1969	Ogosta River, Vrabnishka River, Burzia River, Nishava River, Botunya River, Leva River, Archar River, Berkovska River, Vrabnishka River, Chuprenska River Chuprenska River, Burzia River, Leva River Leva River Burzia River Ogosta River, Lom River, Leva River	*Sq. cephalus* *B. petenyi* *B. barbus* *G. gobio* *Alb. alburnus*
Margaritov, 1977	Shiposhnitsa River, Iskar Reservoir	*Sq. cephalus*
Kakacheva-Avramova & Menkova, 1978	Palakariya River	*Sq. cephalus*
Kakacheva-Avramova et al., 1978	Danube River, Vidin	*Z. zingel, Z. streber, Alb. alburnus*
Kakacheva-Avramova & Nedeva-Menkova, 1981	State Fisheries Blagoevgrad Zheleznitsa River Blagoevgradska Bistritsa River, Gradevska River, Struma River	*Cobitis taenia* Linnaeus, 1758 *Sq. cephalus*
Kirin, 2001a	Kardzhali Rezervoir	*Sq. orpheus*
Kirin, 2001c	Mesta River	*Sq. orpheus*
Kirin, 2001d	Mesta River	*C. carpio*
Kirin, 2001e	Veleka River, Rezovska River	*Alb. alburnus*
Kirin, 2002a	Chepelarska River, between Asenovgrad and Bachkovo	*Sq. orpheus*
Kirin, 2002b	Arda River, from the confluence of the Cherna, Rodozemska and Madanska rivers to the Kardzhali Rezervoir / cascade Gorna Arda	*Sq. orpheus*
Kirin, 2003	Arda River, from the confluence of the Cherna, Rodozemska and Madanska rivers to the Kardzhali Rezervoir	*Alb. alburnus*

Authors	Localities	Host
STUDY ON *CONTRACAECUM* SP. (syn. *Contracaecum bidentatum* (Linstow, 1899); *Hysterothylacium aduncum (*Rudolphi, 1802) Deardorff & Overstreet, 1981; *Contracaecum microcephalum* (Rudolphi, 1809) Baylis, 1920; *C. squalii*)		
Kirin et al., 2002; Kuzmanov et al., 2002	Arda River, Rabovo, Madzharovo	*Alb. alburnus*
Kirin et al., 2002; Kuzmanov et al., 2002; Kirin, 2006	Arda River, Rabovo	*Sq. orpheus*
Kirin et al., 2003; Kuzmanov et al., 2003; Kirin, 2006	Arda River, Huhla	*Sq. orpheus*
Kirin et al., 2005	Stryama River	*Sq. orpheus*
Kirin, 2006; Kirin & Shukerova, 2006	Arda River, Slaveevo	*Sq. orpheus*
Kirin & Shukerova, 2007	Arda River	*Sq. orpheus*
Shukerova & Kirin, 2008	Srebarna Lake	*Sc. erythrophthalmus*
Shukerova, 2010	Srebarna Lake	*L. aspius*, *Sc. erythrophthalmus*
Atanasov, 2012	Danube River, Archar	*B. barbus, Sc. erythrophthalmus, Sq. cephalus*
Kirin et al., 2012, 2013a	Tundzha River	*Sq. orpheus*
Chunchukova et al., 2019a	Maritsa River	*Alb. alburnus*
Chunchukova et al., 2019b	Maritsa River	*Sc. erythrophthalmus*
Kirin et al., 2019a	Luda Yana River, Popintsi	*R. rutilus*
Kirin et al., 2019b	Stryama River	*Sq. orpheus*
Kuzmanova et al., 2019	Osym River, Lovech	*Sq. cephalus*
Chunchukova & Kirin, 2020a	Danube River, Silistra	*Abr. brama*
Chunchukova & Kirin, 2020b	Tundzha River, Yambol	*L. aspius*
Chunchukova et al., 2020b	Ogosta River	*Sq. cephalus*

CONCLUSIONS

In the autumn of 2022, 37 specimens of *Sq. orpheus* from the Chepelarska River were examined for the presence of helminths. Infection was found in 40.54% of the examined Orpheus dace specimens. Thirty-eight specimens of helminths were found, belonging to 3 species and 2 classes. One core parasite species and two accidental species were found in the component community of Orpheus dace. The present study provides new data on helminths and helminth communities of Orpheus dace from the Chepelarska River. Katunitsa biotope is a new habitat for the found helminths (*Ac. tenuirostris*; *Contracaecum* sp. and *Rh. denudata*) of Orpheus dace. In the present study, a pathogenic parasite species (*Contracaecum* sp.) to fish and humans was found. Studies on parasites have significance for the conservation of the fish populations.

ACKNOWLEDGEMENTS

This research is supported by the Bulgarian Ministry of Education and Science under the national Program" Young Scientists and Postdoctoral Students-2". I thank the leadership of the Agricultural University – Plovdiv for the opportunity to work on this project. The helminthological studies were carried out in the laboratory of the Department of Agroecology and Environmental Protection at the university.

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Volume LXVI, No. 2

2023
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