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# GENETICS AND BREEDING

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# STUDY OF THE GROWTH AND FATTENING ABILITIES OF MALE LAMBS IN DIFFERENT TYPES OF BIRTH FROM THE ILE DE FRANCE BREED

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***Abstract***

*Subject of a scientific experiment were 16 male lambs of the Ile de France breed, divided into 2 groups of 8, with different types of birth, fattened for a period of 60 days. The aim of the experiment was to study the growth and fattening abilities of lambs with different types of birth. Live weight was recorded at birth, at 60, at 75, at 90, at 105 days and pre-slaughter live weight at 120 days. The average daily gain by groups and subperiods was calculated. The quantities of feed consumed and the residual quantities were reported daily. The data were processed by the methods of variation statistics with Data Analysis, EXCEL, 2016 by Microsoft. It was found that male twin lambs of the Ile de France breed started the experiment with 12.81% lower average live weight, but in 60 days achieved 8.74% significantly higher absolute gain of 25.312 kg, compared to the group of singles – 23.100 kg. The twins achieved a 7.42% higher average daily gain of 0.445 g compared to singles for the entire experimental period. The twin lambs achieved 1 kg increase with lower consumption, respectively by 5.57% of dry matter, 6.36% of energy and crude protein by 7.18% of the group of singles, which shows better feed utilization and the indication of compensatory growth in lambs born as twins.*

**Key words:** *average daily gain; live weight; sheep breed Ile de France; type of birth.*

## INTRODUCTION

The French meat breed Ile de France has valuable characteristics - high intensity of growth at an early age, excellent carcass conformation and taste of meat, polyestrous, high fertility and high milk yield in the first period of lactation. These qualities make it increasingly popular and widespread throughout the world and in our country.

The phenotypic manifestation of the genetic potential of the breed in our conditions have been studied after the first import in 1968 to 2005 by Bulgarian authors (Dimitrov, 1978; Dimitrov et al., 1982; Dimitrov et al., 1987; Tyankov et al., 2000; Slavov et al., 2004; Raycheva et al., 2005, etc.).

To establish the level of selection traits of the breed, experiments and comparative analyzes with other meat breeds were conducted by scientists in our country and abroad with different ages and with different types of birth (Laleva et al., 2006; Popova et al., 2019; Bianchi, G. et al. 2003). Laleva et al. (2021) conducted studies to establish the genetic parameters of selection traits.

The intensity of growth of the Ile de France offspring and the ability to achieve high gain at an early age are important for the economic results of rearing this breed.

The main revenues in meat breeds come from the sale of lambs for meat and breeding animals with high genetic potential according to the main selection traits. The selection limits for the Ile de France breed in Bulgaria were described by Dimitrov et al. (2016).

Assessment of the main productive traits of sheep of the Ile de France breed in Bulgaria and the effect of various sources of influence on live weight and daily gain were made by Achkakanova et al. (2019), and the fattening and slaughter qualities of lambs in our country and abroad were studied by a number of authors (Ivanova, 2021; Raicheva et al., 2010; Moreno et al., 2010, etc.).

The aim of the present study was to investigate the growth abilities and fattening qualities of male lambs of the Ile de France breed in different types of birth.

## MATERIALS AND METHODS

The scientific experiment was conducted in a livestock farm under real production conditions, to monitor the weight development of lambs of the Ile de France breed at different types of birth.

The practical part of the study was conducted in 2021 on a farm under the selective control of the breeding organization "Association for breeding the Ile de France breed in Bulgaria" (ABIDFBB) in the Dobrudja region. A total of 16 male lambs at 2 months were included in the experiment for a period of 60 days.

For this purpose, 2 groups of 8 male lambs were formed, equal in type of birth (singles, twins), age and live weight. The live weight trait was registered at birth, in the formation of groups at 60 days, at 75 days, at 90 days, at 105 days and pre-slaughter live weight at 120 days. Live weight was monitored in the morning before meals.

A total of 96 individual measurements of live weight of lambs were made, based on which the average daily gain by periods in different types of birth was calculated.

The animals have constant access to drinking water and salt for licking. The quantities of feed and residues were recorded daily from the start of the experiment until the end.

The concentrate mixture contained the following components in optimal proportions: corn, barley, oats, sunflower and soybean meal, alfalfa flour, corn germ, wheat bran, vitamin-mineral premix, calcium carbonate and sodium chloride. The average daily consumption of feed by types and groups is given in Table 1.

Table 1. Average daily feed consumption

| Ingredients, kg | 1st group: Singles | 2nd group: Twins |
|---|---|---|
| Alfalfa hay | 0.467 | 0.504 |
| Concentrate mixture | 0.739 | 0.725 |
| Straw | 0.060 | 0.063 |

Data on the chemical composition and nutritional value of the used feed are shown in Table 2.

The primary information was processed by the methods of variation statistics using Data Analysis, EXCEL, 2016 of Microsoft. The significance of the differences between the studied groups was established by the t-test of Student.

Table 2. Chemical composition and feeding value of feeds

| Items | Forages | | |
|---|---|---|---|
| | Concentrate mixture | Alfalfa hay | Straw |
| Dry matter, % | 89.22 | 85 | 90.2 |
| **% of the DM** | | | |
| Crude protein | 18.38 | 13.3 | 5.7 |
| Crude fibers | 5.66 | 17.5 | 22.43 |
| Crude fats | 2.43 | 1.85 | 1.11 |
| NFE | 56.54 | 40.2 | 38.03 |
| Ash | 6.47 | 7.5 | 8.87 |
| Ca | 0.76 | 1.7 | 1.4 |
| P | 0.7 | 0.9 | 0.32 |
| **1 kg DM contain:** | | | |
| ME, MJ | 12.11 | 8.35 | 6.05 |
| CP, g | 181.1 | 79.71 | 29.3 |
| FUG | 1.24 | 0.74 | 0.45 |

## RESULTS AND DISCUSSIONS

The data in Table 3 show that the group of twins started with 12.81% lower average live weight ($P < 0.05$) compared to singles. The same trend was maintained in the next live weight controls, as the differences in the mean values by groups move in a downward direction.

On the 15th day after starting the experiment, singles outperformed twins by 7.89%, on the 30th by 7.28%, on the 45th by only 2.79% and on the 60th by 3.11%. We observed a tendency for a compensatory effect, in which the group of twin lambs gradually narrows the differences in the average weight by reporting periods (subperiods) and shows a faster growth rate than the single ones. This trend was confirmed by the results for the realized absolute gain for the whole experimental period, which was on average 25.312 kg for twins and 23.100 kg for single lambs ($P < 0.05$). The group of twins gave a significantly higher increase in kg by 8.74% for a fattening period of 60 days.

Breeders of the Ile de France breed in our country present similar data from live weight controls. The annual edition of the Institut de l'Elevage (IDELE), INRAe, Races de France, (2020) reports close to our results for the weight of male lambs with different types of birth at 70 days, respectively 30.5 kg for those born as singles and slightly lower - 25 kg for those born as twins. Dimitrov et al. (1982) and Ivanova et al. (2017) reported lower average weights at weaning at 70 days.

The results of Laleva et al. (2006) and Raycheva et al. (2005) are similar to ours, who found a lower average live weight at 70 days (20.750 kg) compared to our results. Dimitrov (1978) and Achkakanova et al. (2020) established values close to ours for the studied trait in lambs with different types of birth, and Achkakanova and Staykova (2019) reported observations and established average results for the live weight trait at 70 days - 23.736 kg with 1115 female lambs Ile de France.

Table 3. Live weight, kg

| Items | I$^{st}$ group: Singles, n = 8 | | | II$^{nd}$ group: Twins, n = 8 | | |
|---|---|---|---|---|---|---|
| | x | Sx | CV % | x | Sx | CV % |
| Live weight on admission, kg | 30.263 l | 1.188 | 11.1 | 26.388 l | 1.074 | 11.52 |
| First period - 15$^{th}$ day, kg | 36.9 | 1.266 | 9.7 | 33.988 | 1.453 | 12.09 |
| Second period - 30$^{th}$ day, kg | 42.038 | 1.107 | 7.45 | 38.975 | 1.678 | 12.18 |
| Third period - 45$^{th}$ day, kg | 47.138 | 1.354 | 8.13 | 45.825 | 1.75 | 10.8 |
| Live weight on the 60$^{th}$ day, kg | 53.363 | 1.231 | 6.52 | 51.7 | 1.73 | 9.47 |
| Total gain for the whole period, kg | 23.100 m | 0.629 | 7.71 | 25.312 m | 1.17 | 13.1 |

Significance of differences within rows – when symbols identical: A to Z - P< 0.001; a to k - P< 0.01; l to z - P< 0.05

The results in Table 4 are similar, where the data show a higher average daily gain by periods for lambs born as twins, except for the second reporting period (15-30 days), where the values are very close and without statistical significance.

The animals from the second group had a more significant advantage during the period 30-45 days, when they gave 25.46% higher average daily gain than the singles (P <0.01). The logical explanation for these results is related to the fact that the Ile de France breed is characterized by a significant reduction in the amount of mother's milk during this period and the offspring begins to rely more on feed consumption to achieve gain. With this adaptation, the twins are likely to be able to show their compensatory growth abilities. The variation is more significant in the group of singles, where it reached 28% in the second subperiod. The group of twins realized 7.42% higher average daily gain in total for the whole period of the experiment (P <0.05).

The annual edition of the Institut de l'Elevage (IDELE), INRAe, Races de France, (2020) reported lower than ours for the first control period, in terms of the average daily gain for single lambs - 0.333 kg. No results have been reported for lambs born as twins. Achkakanova and Staykova (2021) reported confirmed results for an average daily gain of male lambs from 30 to 70 days - 0.387 kg. Dimitrov, (1988) publicshed data on high intensity of 366-407 g average daily gain in individual fattening from 30 to 90 days of male lambs Ile de France in Bulgaria.

Studying the weight development of offspring of both sexes born in Bulgaria by mothers of French and Bulgarian reproduction, Dimitrov (1978) gives data on lower average daily gain of lambs Ile de France at 70 days (0.279 kg for male lambs and 0.251 kg for female lambs). Dimitrov et al. (1987) published values for the trait from 30 days to 70 days - 0.313 kg.

Ivanova and Raicheva (2017) published data on the average daily gain of male lambs Ile de France with different lineage from 0.240 kg to 0.261 kg, noting that singles gained more up until the 30 days, and twins increased the intensity of growth after the 30$^{th}$ day until the 70th day.

The results obtained for average daily gain confirmed the good adaptation of the breed and the ability to realize its potential for high intensity of growth at an early age.

Table 4. Average daily gain, kg

| Items | Ist group: Singles, n = 8 | | | IInd group: Twins, n = 8 | | |
|---|---|---|---|---|---|---|
| | X | Sx | CV % | x | Sx | CV % |
| Gain start - 15th day | 0.443 | 0.041 | 26.26 | 0.507 | 0.046 | 25.88 |
| Gain - 15th - 30th day | 0.367 | 0.036 | 28.05 | 0.356 | 0.028 | 22.62 |
| Gain 30th - 45th day | 0.319 a | 0.024 | 21.48 | 0.428 a | 0.027 | 17.91 |
| Gain 45th - 60th day | 0.508 | 0.03 | 16.85 | 0.514 | 0.023 | 12.83 |
| Average daily gain for the whole period | 0.412 l | 0.01 | 7.19 | 0.445 l | 0.2 | 12.54 |

Significance of differences within rows – when symbols identical: A to Z - P< 0.001; a to k - P< 0.01; l to z - P< 0.05

The results in Table 5 show that the group of twins achieved 1.94%, 1.12% and 0.26% higher intake of dry matter, metabolic energy and crude protein, respectively. Ivanova (2020) reported higher than our values for the indicators - respectively 1.349 kg DM, 12.69 MJ ME and 166.25 g. CP, in an experiment to test the effect of a supplement in the ration of female Ile de France lambs in a control group.

Regarding the utilization of the feed, the lambs born as twins managed to create 1 kg gain at the expense of less accepted nutrients and energy by 5.57% for the dry matter, 6.36% for the energy and 7.18% for the crude protein from the consumed feed.

Table 5. Intake of dry matter, energy and protein per day and utilization

| Items | Ist group | IInd group |
|---|---|---|
| | Singles, n = 8 | Twins, n = 8 |
| Dry matter intake - total, kg | 1.11 | 1.132 |
| Energy intake - total ME, MJ | 11.62 | 11.752 |
| Protein intake - CP, g | 152.566 | 152.956 |
| Dry matter per 1 kg gain, kg | 2.694 | 2.544 |
| Energy per 1 kg gain, ME, MJ | 28.204 | 26.409 |
| Crude protein per 1 kg gain, g | 370.306 | 343.721 |

## CONCLUSIONS

Male twin lambs from the Ile de France breed started the experiment with 12.81% lower average live weight but in 60 days realized 8.74% significantly higher absolute gain of 25.312 kg compared to the group of singles - 23.100 kg.

The twins achieved a 7.42% higher average daily gain of 0.445 g compared to singles - 0.412 g in total for the entire experimental period.

The twin lambs achieved 1 kg increase with lower consumption, respectively by 5.57% of dry matter, 6.36% of energy and crude protein by 7.18% of the group of singles, which shows better utilization of feed and the indication of compensatory growth in lambs born as twins.


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# THE INFLUENCE OF HYPODYNAMICS ON SOME PARTICULARITIES INTERIOR OF SHEEP KARAKUL

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***Abstract***

*The aim of this research was to identify the impact of hypodynamics on the particularities of the interior of Karakul sheep, raised in different conditions of maintenance. The research was conducted on three similar batches of Karakul sheep, 150 heads in each batch. They were raised in different conditions, from the age of 3 months to 32 months. During the winter (December-March), the sheep from all batches were traditionally kept in stables in paddocks and fed with a mixture of chopped fodder, according to the zootechnical norms. During the summer (April-November), the sheep from Batch I (control) were kept grazing with daily movement at a distance of up to 10-15 km. The experimental Batch II sheep were maintained in the summer at the stable with daily active walking at a distance of 2-3 km. The sheep from Batch III were maintained throughout the experiment, at the stable under hypodynamic conditions. Sheep from batches II and III during the summer were fed green mangers, according to the zootechnical norms. It was found that in sheep in Batches III and II, the degree of oxygen saturation of arterial blood was significantly lower, compared to that of sheep in Batch I, by 7.4 and 2.8% ($t_d$ = 5, 81 and 2.89; P <0.001 and P <0.01). At the same time, in sheep in Batches III and II, the degree of oxygen saturation of venous blood was significantly higher, compared to that of sheep in control Batch I, with 64.6 and 29.2% ($t_d$ = 5.52 and 2.35; P <0.001 and P <0.05). In Batches III and II sheep, the level of oxygen utilization by body tissues was significantly lower compared to that of Batch I sheep, respectively by 40.1 and 17.4% ($t_d$ = 7.43 and 3.06; P <0.001 and P <0.01). Sheep in Batch III significantly outnumbered their contemporaries in Batch I by 1.04 kg or 41.1% ($t_d$ = 9.15; P <0.001) of gross internal fat deposited in the body after slaughter, by 1.80 kg or 7.5% ($t_d$ = 2.61; P <0.05), after cutting yield, by 3.17 or 6.6% ($t_d$ = 2.82; P <0.05) and after mass of the omasum, with 28 g or 29.2% ($t_d$ = 5.65; P <0.01). At the same time, the sheep from Batches II and III, yielded significantly to the contemporaries from Batch I, after the development of internal organs, such as: liver, heart, lungs, kidneys, stomach, rumen, abomination and small intestine, by 14.4-37.9% (P <0.05 - 0.01). In the Batch III sheep, there was an obvious tendency to decrease, compared to the control Batch, the quality of the furskins, expressed by the weight of the furskins of Sort I, by 12.9% ($t_d$ = 1.88; P <0.1).*

***Key words****: blood, furskin Karakul, hypodynamics, internal organs, oxygen, sheep.*

## INTRODUCTION

In the Republic of Moldova, sheep of all races, including the Karakul race, are traditionally maintained in the winter (December - March) - in the stable, and in the summer (April - November) - in the pasture.

At the same time, in connection with the intensification of agriculture, the widening of arable land and the reduction of natural pastures, some farm managers are addressing the problem of maintenance sheep in paddy fields and feeding them in mangers with mixtures of shredded fodder - summer.

Stable sheep maintenance system is well known in developed countries for the intensive rearing of young sheep for fattening the meat.

Thus, according to information from France (Le Hen, 1983), an analysis was made of 500 households in which four systems of maintenance and rearing of young sheep were practiced: 1 - grazing; 2 - grazing with the mothers and final fattening at the stable; 3 - increase in stabling during lactation and subsequent fattening during grazing; 4 - at the stable. It was found that the highest production yield was obtained by the rational combination of maintenance of the sheep during the period of lactation at the stable with that of grazing at fattening.

Weis Kassel (1983), in the FRG, performed experiments on two batches of sheep maintenance on meadows grown in plots (with additional feeding of concentrates) and in the stable. For weaning, 123 and 137 lambs were raised for every 100 sheeps. The cost of feed for the production of 1 kg of meat amounted to 1.94 and 1.77 marks, respectively.

Such experiences of raising young sheep in various conditions of maintenance both year-round at the stable and mixed - during the winter at the stable, and in the summer at grazing, were also carried out by Ожигов et al (1982), Алексиев (1982), Конурбаев (1982). They concluded that sheep raised both year-round and mixed (stable in winter and grazing in summer), with good nutrition, achieve a fairly good development, without essential differences in productive performance and development of internal organs.

At the same time, information on special experiences of maintenance of Karakul sheep all year round in stable conditions for a long period of several years is not known in the profile literature. However, taking into account the fact that classical researchers in the Karakul race (Иванов, 1964; Кошевой, 1975; Дъячков, 1980; Аверьянов et al, 1968; Taftă et al, 1997) states that the conditions of maintenance and nutrition can influence the quality of the furskins obtained from newborn lambs, then the issue addressed requires further study.

We believe that one of the main factors that can influence the organism of sheep in stable conditions is hypodynamics, expressed by limiting physical movement in the paddock. We assume that the presence of sheep in the conditions of hypodynamics at the stable can have an impact on physiological processes, the development of internal tissues and organs. So, it is about some changes or adaptations of the inner particularities, of the organism metabolism, such as the catabolic and anabolic processes.

Some researchers (Onmaz et al., 2009; Celi, 2010; Varghese et al., 2017; Izer et al., 2020) state that the catabolic and anabolic processes of metabolism in the organism can be monitored by the level of saturation with oxygen from the arterial blood compared to the venous one.

Genes (2021) states that lack of exercise (hypodynamics) causes a lack of sufficient oxygen in the blood, which can be transported to the cells of tissues and organs, which leads to decreased immunity of the organism and decreased resistance to various weather conditions.

Jordán M.J. (2020) considers that sheep grazing improves their enzymatic antioxidant defense during the stressful period of lamb weaning and also the antioxidant status of sheep plasma in both physiological stages: lactation and after weaning the lamb.

According to Hohimer et al (1984), the oxygen content in the arterial blood in goats, after running in a special trainer, increases, compared to the state of rest, by 12.8 - 14.6%. At the same time, there is a certain increase in the hemoglobin content in the blood from 9.7 to 10.7%.

In the research of Lotgering et al. (1983), it found that, after physical load (running), oxygen consumption in sheep increased from 5.8 to 32.1 ml/min/kg, while increasing the value of the difference in oxygen content in the arterial blood and venous from 3.9 to 8.0 ml/L, and the oxygen content in the venous blood suddenly decreased from 70 to 26%.

Фазульзянов (1979), in repeated experiments, showed that the long-term stabilization of fine wool sheep leads to their overweight fattening and impaired reproductive rates.

Considering that special research on the long-term growth of Karakul sheep in hypodynamic (in stable) conditions for several years is missing in the profile literature, it is particularly relevant to elucidate the influence of this maintenance system on the organism of sheep.

In this context, the aim of this scientific paper was to identify the impact of hypodynamics on the particularities of the interior of Karakul sheep, raised in different conditions of maintenance.

## MATERIALS AND METHODS

The research was conducted on three similar batches of Karakul sheep, 150 heads in each batch. They were raised in different conditions, from the age of 3 months to 32 months.

During the winter (December - March), sheep from all batches were traditionally maintenance in stables in paddocks and fed with a mixture of shredded fodder (hay, hay, silage, straw) and 0.3 kg of granulated concentrate (whole grain plant), distributed in mangers, according to the zootechnical norms.

During the summer (April-November), the sheep from Batch I, which served as a witness,

were maintenance grazing with daily commute (round trip) at a distance of up to 10-15 km.
The experimental Batch II sheep were maintenance in the summer at the stable with daily active walking (active walking) at a distance of 2-3 km.
The sheep from Batch III, during the whole period of the experiment, were maintained and permanently raised at the paddock in conditions of hypodynamics.
Sheep from batches II and III during the summer were fed on mangers with green mass of mown fodder plants (mixture of grasses and legumes, peas + oats, ryegrass, alfalfa, asparagus, etc.), according to the zootechnical norms.
In order to elucidate the particularities of the development of the interior in sheep, the following were researched:
* the degree of oxygen saturation of arterial and venous blood, as well as the level of oxygen utilization by organism tissues;
* the degree of development of some internal organs of the sheep;
* the quality of Karakul furskins obtained from newborn lambs.
The degree of oxygen saturation of the blood was determined by the cuvette method on the combined Oxyhemometer model 057. To determine the level of oxygen consumption by the organism tissues, at the age of 32 months, arterial blood samples were taken and venous from 20 representative sheep from each of the 3 experimental batches.
Arterial blood samples were taken in a syringe coupled with a rubber hose with a puncture needle from the abdominal aorta, by inserting it into the last intervertebra to the lower level of the spinal cord, at an angle of 45$^{o}$, according to the method of Воронин (2015).
Venous blood samples were taken from the jugular vein by the traditional method. The collected blood was immediately placed under vaseline oil to prevent it from coming into contact with atmospheric oxygen.
Prior to the determination of oxygen saturation, the blood under the oil was previously diluted 1 to 1 in a special solution of 0.3 g of sodium salicylate ($C_7H_5O_3Na$) and 2.0 g of sodium chloride (NaCl) to 100 ml of distilled water. The blood sample was placed in the cuvette and placed in the device. The device's oxygen saturation read on the screen. The difference between the degrees of oxygen saturation of arterial and venous blood was the level of oxygen utilization by the organism tissues.
In order to research the development of the internal organs and the particularities of the deposition of the raw internal fat, at the age of 32 months, the control slaughter of 3 representative sheep from each of the three experimental batches was performed. In slaughtered sheep were studied: carcass mass, raw internal fat, slaughter yield, mass of internal organs (liver, heart, lungs, kidneys, stomach, rumen, omasum, abomasum and small intestine).
The qualities of the furskins were assessed according to the methods of the Union Institute for Scientific Research for Karakulture (Дъячков et al., 1963), as well as according to the provisions of ГОСТ (1984) in force for purrace Karakul furskins.
The data obtained as a result of the research were statistically processed with the help of the computer software "STATISTICS - 12" and their certainty was assessed, according to the biometric variational statistics, according to the methods of Плохинский (1989).

## RESULTS AND DISCUSSIONS

It is known that the main function of the respiratory system is to remove carbon dioxide ($CO_2$) from the systemic venous blood that reaches the lungs and to add oxygen ($O_2$) to the arterial blood. Gaseous exchange in the respiratory system refers to the diffusion of oxygen and carbon dioxide into the lungs and peripheral tissues. Thus, in the arterial and venous blood is found oxygen and carbon dioxide, the amount of which varies depending on the physiological state of the animal (Chiuțu, 2012).
Раушенбах (1976) shows that the general level of oxidative processes in the organism depends on the percentage of oxygen utilization in the inspired air. In this way, the animals have a certain reaction to adapt to changing environmental conditions.
The results of our research have shown that sheep maintenance conditions have significantly influenced the physiological processes of metabolism in the organism, in particular, the function of blood respiration, confirmed by the variability in the degree of oxygen saturation of

arterial and venous blood, and the level of use of oxygen by the tissues of the organism (Table 1).

Table 1. Degree of oxygen saturation of arterial and venous blood in experimental sheep

| Batch | N | M ± m, % | The difference compared to Batch I | | $t_d$ |
|---|---|---|---|---|---|
| | | | d | % | |
| Arterial blood | | | | | |
| Batch I | 20 | 97.02 ± 0.44 | - | - | - |
| Batch II | 20 | 94.30 ± 0.83 | -2.72 | 2.8 | 2.89** |
| Batch III | 20 | 89.87 ± 1.15 | -7.15 | 7.4 | 5.81*** |
| Venous blood | | | | | |
| Batch I | 20 | 30.35 ± 3.14 | - | - | - |
| Batch II | 20 | 39.22 ± 2.09 | +8.87 | 29.2 | 2.35* |
| Batch III | 20 | 49.95 ± 1.66 | +19.60 | 64.6 | 5.52*** |
| Arterio-venous difference | | | | | |
| Batch I | 20 | 66.67 ± 3.16 | - | - | - |
| Batch II | 20 | 55.07 ± 2.08 | -11.60 | 17.4 | 3.06** |
| Batch III | 20 | 39.92 ± 1.72 | -26.75 | 40.1 | 7.43*** |

*Remark: * P < 0,05; ** P < 0,01; *** P < 0,001.*

It was found that the degree of oxygen saturation of the arterial blood varied on average from 89.87% in sheep in Batch III to 97.02% in sheep in Batch I. The arterial blood of the sheep from Batch I, which were traditionally maintained during the summer (April-November) when grazing at a distance of 10-15 km, was more saturated with oxygen, compared to the blood of sheep from batches II and III, which were maintained during this period at the stable, respectively, with 2.72 and 7.15 units or 2.8 and 7.4% ($t_d$ = 2.89 and 5.81; P <0.01 and P <0.001).

The level of residual oxygen in venous blood varied on average from 30.35% in Batch I sheep to 49.95% in Batch III sheep. It was found that the level of residual oxygen in the venous blood of the sheep in Batch I, which were traditionally maintained during the summer grazing at an essential distance, was, on the contrary, lower compared to the blood of the sheep in the batches II and III, which were maintained during this period at the stable, respectively, with 8.87 and 19.6 units or 29.2 and 64.6% ($t_d$ = 2.35 and 5.52; P <0.05 and P <0.001).

The arterio-venous difference in the degree of oxygen saturation of the blood varied on average from 39.92% in sheep in Batch III, to 66.67% in sheep in Batch I.

Judging by the arterio-venous difference in blood saturation with oxygen, it was found that the level of oxygen utilization by the organism tissues in Batch I sheep, which were traditionally maintained, during the summer grazed at an essential distance, was higher than that of the sheep in batches II and III, which were maintained during this period at the stable, respectively, by 11.60 and 26.75 units or 17.4 and 40.1% ($t_d$ = 3.06 and 7.43; P <0.01 and P <0.001).

The results obtained by us regarding the degree of oxygen saturation of arterial and venous blood, as well as the level of oxygen utilization by the organism tissues, are consistent with the conclusions drawn by Сидоров (1975), Раушенбах (1976), Lotgering et al. (1983), Hohimer et al. (1984).

These data allow us to consider that, as a result of active walks, the organism of sheep maintained for grazing adapts to certain conditions of physical activity and produces a significant increase in oxygen consumption. The degree of dissociation of oxygen and its diffusion into tissue cells occurs at a faster rate. Oxygen is used more intensively for metabolic processes. Therefore, the catabolic processes of metabolism prevail in the organism of these sheep.

At the same time, the conditions of hypodynamics, in which the sheep from batches II and III were maintained, caused a decrease in the oxygen saturation of the arterial blood and the rate of its use by the organism tissues. The degree of dissociation of oxygen and its diffusion into tissue cells occurs at a slower rate. Anabolic processes of metabolism prevail in the organism of sheep maintained in stable conditions under hypodynamics.

Some researchers also tell us about the influence of sheep maintenance systems on some interior features.

According to Яшунин et al. (1980), sheep increased in stable conditions, exceeded their contemporaries increased in grazing, by carcass mass by 37.8-44.4%, lean meat mass by 52.7 - 61.6% and slaughter yield by 6.2-8.6%.

In the experiments of Султанов (1979), young ewes bred at stable had advantages, compared to those bred at grazing, at the index of carcass mass, by 43.0 - 81.4%, at the cutting yield, by 4.8-12.2% and the yield of lean meat, with 3.2-7.9%.

Our data, obtained as a result of the control slaughter of the representative sheep from the researched batches, showed that the conditions of physical activity at grazing and hypodynamics at stables had a different influence on the development of their internal tissues and organs (Table 2).

Table 2. Level of slaughterhouse and development indices of internal organs of sheep of 32 months

| Batches | N | M ± m | The difference compared to Batch I | | $t_d$ |
|---|---|---|---|---|---|
| | | | d | % | |
| Housing mass, kg | | | | | |
| Batch I | 3 | 21.60 ± 0.49 | - | - | - |
| Batch II | 3 | 22.23 ± 0.23 | +0.63 | 2.9 | 1.16 |
| Batch III | 3 | 22.37 ± 0.38 | +0.77 | 3.6 | 1.24 |
| Raw internal fat, kg | | | | | |
| Batch I | 3 | 2.53 ± 0.10 | - | - | - |
| Batch II | 3 | 3.33 ± 0.57 | +0.80 | 31.6 | 1.39 |
| Batch III | 3 | 3.57 ± 0.04 | +1.04 | 41.1 | 9.15*** |
| Cutting mass, kg | | | | | |
| Batch I | 3 | 24.13 ± 0.57 | - | - | - |
| Batch II | 3 | 25.57 ± 0.76 | +1.44 | 6.0 | 1.51 |
| Batch III | 3 | 25.93 ± 0.39 | +1.80 | 7.5 | 2.61* |
| Cutting yield, % | | | | | |
| Batch I | 3 | 48.23 ± 1.05 | - | - | - |
| Batch II | 3 | 50.83 ± 1.31 | +2.60 | 5.4 | 1.55 |
| Batch III | 3 | 51.40 ± 0.40 | +3.17 | 6.6 | 2.82* |
| Liver, g | | | | | |
| Batch I | 3 | 724 ± 25 | - | - | - |
| Batch II | 3 | 539 ± 10 | -185 | 25.6 | 6.83** |
| Batch III | 3 | 594 ± 19 | -130 | 18.0 | 4.11** |
| Heart, g | | | | | |
| Batch I | 3 | 160 ± 5.4 | - | - | - |
| Batch II | 3 | 134 ± 1.7 | -26 | 16.3 | 4.67** |
| Batch III | 3 | 137 ± 5.4 | -23 | 14.4 | 3.01* |
| Lungs, g | | | | | |
| Batch I | 3 | 629 ± 30 | - | - | - |
| Batch II | 3 | 514 ± 21 | -115 | 18.3 | 3.10* |
| Batch III | 3 | 488 ± 36 | -141 | 22.4 | 2.98* |
| Kidneys, g | | | | | |
| Batch I | 3 | 117.6 ± 3.3 | - | - | - |
| Batch II | 3 | 93.3 ± 7.4 | -24.3 | 20.7 | 2.99* |
| Batch III | 3 | 98.6 ± 2.6 | -19.0 | 16.2 | 4.45** |
| Stomach, kg | | | | | |
| Batch I | 3 | 1.49 ± 0.02 | - | - | - |
| Batch II | 3 | 1.18 ± 0.05 | -0.31 | 20.8 | 5.52** |
| Batch III | 3 | 1.25 ± 0.03 | -0.24 | 16.1 | 6.15** |
| Rumen g | | | | | |
| Batch I | 3 | 983 ± 24 | - | - | - |
| Batch II | 3 | 752 ± 51 | -231 | 23.5 | 4.08* |
| Batch III | 3 | 785 ± 18 | -198 | 20.1 | 6.60** |
| Omasum, g | | | | | |
| Batch I | 3 | 96 ± 3 | - | - | - |
| Batch II | 3 | 115 ± 4 | +19 | 19.8 | 3.89* |
| Batch III | 3 | 124 ± 4 | +28 | 29.2 | 5.65** |
| Abomasum, g | | | | | |
| Batch I | 3 | 244 ± 27 | - | - | - |
| Batch II | 3 | 183 ± 18 | -61 | 25.0 | 1.83 |
| Batch III | 3 | 196 ± 18 | -48 | 19.7 | 1.47 |
| Small intestine, kg | | | | | |
| Batch I | 3 | 1.03 ± 0.13 | - | - | - |
| Batch II | 3 | 0.65 ± 0.04 | -0.38 | 36.9 | 2.70* |
| Batch III | 3 | 0.64 ± 0.03 | -0.39 | 37.9 | 2.86* |

It has been found that the maintenance of sheep in Batch III in the stable under hypodynamic conditions, without active exercise for a long period (from birth to 32 months), leads to excessive development of tissues, slaughter indices and parts of the stomach, and, at the same time, to the decrease of the level of development of some internal organs.

Thus, the sheep in Batch III, which were permanently maintenance in the stable under hypodynamic conditions, significantly outperformed their contemporaries in Batch I (control), which were maintenance in the winter during the stable and during the summer - during grazing. after the amount of crude internal fat by 1.04 kg or 41.1% ($t_d$ = 9.15; P <0.001), after cutting mass by 1.80 kg or 7.5% ($t_d$ = 2.61; P <0.05), after cutting yield, by 3.17 or 6.6% ($t_d$ = 2.82; P <0.05) and mass of omasum, by 28 g or 29.2% ($t_d$ = 5.65; P <0.01).

In the Batch II sheep, which were maintenance for the whole period also in the stable, especially since, in the summer, they had an active daily walk at a distance of 2-3 km without grazing, only a trend was observed. weaker than the contemporaries in Batch I, according to the amount of raw internal fat, mass and yield at cutting.

Sheep from the experimental Batch II significantly exceeded their contemporaries from the control Batch I after the omasum meal, by 19g or 19.8% ($t_d$ = 3.89; P <0.05).

At the same time, the sheep from groups II and III, which were maintenance in the stable under hypodynamic conditions, yielded significantly to the contemporaries from the control group, which were kept during the summer grazing, after the development of the liver, respectively, with 185 g and 130 g or 25 g, 6 and 18.0% ($t_d$ = 6.83 and 4.11; P <0.01), after heart development, with 26 and 23 g or 16.3 and 14.4% ($t_d$ = 4.67 and 3.01; P <0.01 and P <0.05), after lung development, with 115 and 141 g or 18.3 and 22.4% ($t_d$ = 3, 10 and 2.98; P <0.05), after kidney development, with 24.3 and 19.0 g or 20.7 and 16.2% ($t_d$ = 2.99 and 4.45; P <0.05 and P <0.01), after stomach development, with 0.31 and 0.24 kg or 20.8 and 16.1% ($t_d$ = 5.52 and 6.15; P <0.01), after development rumen, with 231 and 198 g or 23.5 and 20.1% ($t_d$ = 4.08 and 6.60; P <0.05 and P <0.01) and small intestine, with 0.38 and 0.39 kg or 36.9 and

37.9%, also having a lower tendency to develop abomasum.

In the context of the above findings, it is particularly important for the Karakul race to elucidate the impact of the maintenance system in the conditions of hypodynamics on the furskin qualities of newborn lambs.

The results of the research showed that the essential differences between the furskin qualities of the lambs of the newborn lambs of the sheep in the control batch, maintenance during the summer grazing and the lambs born of the sheep of the Batch II, maintenance during the summer period in the stable with walking active, practically, does not to ascertain (Table 3).

Table 3. Furskin qualities obtained from lambs newborns in the experimental batches

| Batches | N | M ± m | The difference compared to Batch I | | $t_d$ |
|---|---|---|---|---|---|
| | | | d | % | |
| Furskin share of Sort I, % | | | | | |
| Batch I | 80 | 88.7 ± 3.6 | - | - | - |
| Batch II | 76 | 85.5 ± 4.1 | -3.2 | 3.6 | 0.59 |
| Batch III | 75 | 77.3 ± 4.9 | -11.4 | 12.9 | 1.88 |
| Furskin share valuable jacket group, % | | | | | |
| Batch I | 80 | 62.5 ± 5.4 | - | - | - |
| Batch II | 76 | 61.8 ± 5.6 | -0.7 | 1.1 | 0.09 |
| Batch III | 75 | 52.0 ± 5.8 | -10.5 | 16.8 | 1.25 |
| Furskin share of Sort II, % | | | | | |
| Batch I | 80 | 11.3 ± 3.6 | - | - | - |
| Batch II | 76 | 10.5 ± 3.5 | -0.8 | 7.1 | 0.16 |
| Batch III | 75 | 16.0 ± 4.3 | +4.7 | 41.6 | 0.84 |

At the same time, there was an obvious trend of decreasing the share of Sort I furskins in the furskins of newborn lambs from ewes in Batch III, which were permanently maintained at the stable until the age of 32 months, compared to the furskins of new-born lambs from ewes of the control batch, which were maintained during the summer grazing, with 12.9% ($t_d$ = 1.88; P < 0.1).

There is also a weaker trend of decreasing the share of valuable furskins in the Jacket Group and increasing the share of less valuable furskins from Sort II, obtained from the lambs from Batch III, whose mothers were permanently maintained at the stable.

Generalizing the results of the research, we can conclude that the more intensive use of oxygen by the tissues of the organism of sheep maintenance grazing results in a higher level of catabolic processes, which contributed to the greater development of internal organs and, at the same time, decreased deposition in the organism of raw internal fat. At the same time, the hypodynamics in the conditions of sheep maintenance at the stable, leads to a decrease in the level of oxygen utilization by the organism tissues, to the weaker development of some internal organs and, at the same time, to the accentuation of the process of deposition of raw internal fat.

Therefore, the maintenance conditions of the sheep (at the stable or grazing) cause a clear influence on the interior of the organism as a whole, including certain organs and tissues in particular.

## CONCLUSIONS

The degree of oxygen saturation of the arterial blood in Batch III sheep, permanently maintained at the stable under hypodynamic conditions, as well as in Batch II sheep, maintained at the stable with daily active walking during the summer, was significantly lower compared with that of the control sheep in the first batch, maintained during the summer grazing period, by 7.4 and 2.8%, respectively ($t_d$ = 5.81 and 2.89; P <0.001 and P <0.01).

At the same time, the degree of oxygen saturation of the venous blood in the sheep in Batch III, permanently maintained at the stable in hypodynamic conditions, as well as in the sheep in Batch II, maintained in the stable with daily active walking during the summer, on the contrary, was significant. higher compared to the control sheep in the first batch, maintained during the summer grazing, respectively, by 64.6 and 29.2% ($t_d$ = 5.52 and 2.35; P <0.001 and P <0.05).

The level of oxygen utilization by the tissues of the organism, defined by the arterio-venous difference in the degree of oxygen saturation of the blood in Batch III sheep, permanently maintained at the stable under hypodynamic conditions, as well as in the Batch II sheep, maintained at the stable with daily active walking during the summer, was significantly lower than that of the sheep in the control Batch I, maintained during the summer grazing, respectively, by 40.1 and 17.4% ($t_d$ = 7.43 and 3.06; P <0.001 and P <0.01).

Sheep in Batch III, which were permanently maintained at the stable under hypodynamic conditions, significantly outperformed their contemporaries in Batch I (control), which were maintained during the summer grazing, according to the amount of crude internal fat, by 1.04 kg or 41.1% ($t_d$ = 9.15; P <0.001), after cutting mass, by 1.80 kg or 7.5% ($t_d$ = 2.61; P <0.05), after yield at cut, by 3.17 or 6.6% ($t_d$ = 2.82; P <0.05) and omasum mass, by 28 g or 29.2% ($t_d$ = 5.65; P <0.01).

At the same time, the sheep from batches II and III, which were kept in the stable under hypodynamic conditions, yielded significantly to the contemporaries from the control group, which were maintained during the summer grazing, after the development of a series of internal organs, such as: liver, heart, lungs, kidneys, stomach, rumen, abomasum and small intestine, with 14.4 - 37.9% (P <0.05 - 0.01).

In Batch III sheep, which were permanently maintained at the stable under hypodynamic conditions, there was an obvious tendency to decrease the quality of the furskins obtained from newborn lambs, expressed by the share of the furskins of Sort I, compared to the furskins from Batch control, obtained from lambs maintained in summer grazing, by 12.9% ($t_d$ = 1.88; P <0.1).

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# CONSERVATION AND VALORISATION OF BEE SPECIES *APIS MELLIFERA CARPATICA* IN CONTEXT CLIMATE CHANGE

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***Abstract***

*The aim of this scientific paper was to identify and highlight the innovative technology of conservation and valorisation of the bee species Apis mellifera Carpatica in the conditions of climate change. Scientific research has been conducted on bee populations growth in the experimental apiary of the Institute of Zoology. Research results have shown that the sustainable selection of purebred bee families, with the application of innovative methods of genetic amelioration in climate change, contributes to the conservation of the population with an appropriate level of development of morpho-productive traits. The conservation of bee populations requires their protection from pesticide residues, which are more and more common in the flowers of some entomophilous agricultural plants. Knowledge of the most dangerous and widespread pesticide residues, identification of ecological biotopes, are necessary actions of technology for the conservation of bee populations. The maintenance of bee families in comfortable and ecological hives is one of the technological methods that ensure the conservation of the species and race of bees Apis mellifera Carpatica. The hives of vertical models have comfort advantages for bees, compared to the horizontal ones, and ensure an increase of the prolificacy of the queens - by 3.5% (td = 2.07; P <0.05), of the family power - by 6.0% (td = 2.41; P <0.05) and honey production by 19.1% (td = 5.33; P <0.001) and an economic efficiency of at least 23.8 euros per family of bees. Feeding bee families during poor harvesting periods in the wild with nutritious supplements, enriched with biologically active substances of different organic origin, contributes to strengthening the vital activity of bee families, ensuring the increase of queen prolificacy and the number of brood capacity by 7.7-45.9%; family power by 9.3-16.9%; flight intensity of bees by 6.8-7.7%; disease resistance by 5.0-8.4%; winter hardiness by 10.5%; the amount of wax raised in the nest by 36.7-39.3%; the amount of pasture with 23.3-27.6% and the amount of honey accumulated in the nest with 19.6-38.9%. Rational use of Apis mellifera Carpatica species and bee race can be achieved by exploiting bee families not only for obtaining bee products, but also for their use in the directed pollination of entomophilous agricultural crops that contribute to the increase of fruit harvest in orchards by 15-30 % and sunflower seeds with 21.3-36.3%.*

**Key words**: *Apis mellifera Carpatica, genetic conservation, organic food, pesticides, protection, selection.*

## INTRODUCTION

Conservation of the bee species *Apis mellifera* in the 21st century has become a particularly current issue due to global climate change, intensifying the impact of anthropogenic activity, especially in modern agriculture (Cebotari et al., 2019b; Cebotari et al., 2021b). A European source (Consequences of climate change, 2018) is mentioned that climate change on Earth is occurring so rapidly that the survival of many plant and animal species is threatened. Many species of terrestrial, freshwater and marine animals have already migrated. Some plant and animal species are at risk of extinction if global average temperatures continue to rise uncontrollably, contrary to the Paris Agreement - the United Nations Framework Convention on Climate Change (2016) and Council Decision (EU) 2016/1841 (2016).

The Bee Decline - Greenpeace Research Laboratories Report (2013) is mentioned that “climate change, such as rising temperatures, changing rainfall patterns, and extreme or more irregular weather events have an impact on pollinator populations. Some of these changes may affect them individually, eventually affecting their communities, which is reflected in the increasing rate of extinction of pollinator species”.

For example, it is documented that honey bees in Poland react to climate change by performing the cleaning flight (the time of "waking up" after winter) earlier than usual, in line with the phenomenon generally known as "changing seasons". The bee clearing flight took place a month earlier than the average 25-year observation, which was attributed to increases in atmospheric temperature (Sparks et al., 2010).

Climate change can lead to a change in flowering patterns, a shift in the flowering period of honey plants, which is a major source of food for bees, or a change in the season, in which case the flowering period no longer corresponds to the time when the bees "wake up" spring (Kremen et al., 2007). Due to changing flowering times and patterns of plants, climate change affects the action between pollinators and their food source. Thus, the research of some authors (Memmott et al., 2007) shows that 17-50% of pollinator species suffer from lack of food in the case of realistic climate change scenarios that cause changes in plant flowering patterns. The authors anticipate that the effect of these effects may lead to the potential extinction of both some pollinators and some plants, resulting in disruption of the essential interaction between them.

The invasion of the mite *Varroa destructor* is one of the most dangerous parasitic diseases, which attacks exacerbated the most valuable, from a productive point of view, useful species of insects, such as the bee *Apis mellifera* L., having a fairly accelerated character, with a destructive impact extremely harmful, endangering the existence of bee families (Cebotari et al., 2013). In this context, the protection of honey bees from this invasion by applying targeted selection after natural antivarous resistance and the index of low fertility mites, called SMR - "Suppression of mite reproduction", due to the preferential elimination by bees of breeding mites, renamed and “Varroa sensitive hygiene - VSH”, as an inherited trait from parental families, is another important issue in *Apis mellifera* conservation technology (Cebotari et al., 2019c; Mondet et al., 2020).

In this context, research on the identification of bee populations adapted to local climate change conditions, their conservation through methods of selection and genetic improvement of bee families according to the main morpho-productive characters in order to increase their productivity and disease resistance, become scientific approaches particularly important and current (Cebotari, 2006; Cebotari et al., 2015a; Cebotari et al., 2021a).

Of particular concern, both in Europe and around the world, has recently been the main concern for systemic pesticides, which are used in agriculture to treat seeds and spray crops to control pests and weeds.

According to "Beyond Pesticides" (2014), neonicotinoid pesticides have neurotoxic, reproductive and mutagenic harmful effects on insects, birds, fish, freshwater snails, earthworms, dragonflies, mosquitoes, and vertebrates, noting that "neonicotinoids could represent the new contemporary ecological disaster, being a threat to nature."

According to other sources (Gill et al., 2012), some pesticides, such as the organochlorine insecticide Fipronil, are one of the main chemical factors that are causing the collapse of bee colonies.

Several researchers (Alaux et al., 2010; Henry et al., 2012; Oliveira et al., 2013) have shown that there is a synergism of additive action when pesticides are applied in combination. For example, the neonicotinoid Tiacloprid becomes about twice as toxic to honey bees when used in combination with the fungicide Propiconazole, and three times as toxic - in combination with Trifumizole (Henry et al., 2012). Other research has shown that there is a significant synergy between fungicides, neonicotinoid insecticides and pyrethroids, as well as the acaricides Flumetrin, Cumafos and Fluvalinat (Oliveira et al., 2013).

Along with the interactions of different pesticides, insecticides also show synergies with other stressors, such as parasite infestations. For example, honey bee mortality was higher in those infested with the *Nosema* parasite and a synergistic interaction of factors was found, which reduce the enzymatic activity related to the sterilization of colony food (Alaux et al., 2010).

A particularly important role in the conservation technology of *Apis mellifera* belongs to the conditions of maintenance of bee families. The hive housing bees creates their comfort or discomfort that directly affects the vitality of the colony and, as a result, its productivity (Cebotari et al., 2012). Therefore, the creation of optimal conditions of comfort in ecological hives of vertical type, is an indispensable condition of the technology of conservation of bee populations.

A particularly specific problem is the feeding of bees during poor harvesting periods in the wild. To compensate for the lack of nutrients in the diet of bees during poor harvesting periods in nature, most beekeepers feed bee families with sugar syrup, which, in addition to carbohydrates, lacks a significant number of biologically active substances. Under these conditions, the consolidation of their power and vigor through balanced feeding methods with nutriational supplements enriched with biologically active organic substances is of major importance (Toderaș et al., 2012a, 2012b, 2012c).

The technology of conservation and valorisation of the species *Apis mellifera* becomes complex only if it (the species) is rationally used to pollinate entomophilous crops, contributing substantially to increasing the harvest and economic efficiency of the branches of phytotechnics and beekeeping (Cebotari et al., 2015b, 2015c, 2017). Thus, the development of innovative methods and techniques for bee-directed pollination of entomophilous agricultural crops is a current scientific issue of species valorisation.

Therefore, the targeted selection of bee families in order to increase their adaptability to local environmental conditions, increase their productivity and disease resistance (especially in *Varroa*), protect bee families from pesticide residues, maintain bee families in comfortable vertical beehives, organic feeding of bees during poor harvesting periods in nature, as well as the use of bee families in the directed pollination of entomophilous agricultural crops present, as a whole, the innovative technology of conservation and valorisation of *Apis mellifera* L.

## MATERIALS AND METHODS

Scientific research on the selection of bee families has been carried out on the *Apis mellifera Carpatica* bee population grown in the experimental apiary of the Institute of Zoology.

The selection conservation of the local bee population was carried out by the progressive targeted method according to the independent limits of some important morpho-productive characters of the bee families. The selection was made according to the level of development of the following characters: queen prolificacy, family strength, winter hardiness, viability of brood, disease resistance, honey production accumulated in the nest, rating class. The evaluation of the level of development of morpho-productive characters was performed, according to the methods developed by us (Cebotari et al., 2010) for the Zootechnical Norm regarding the rating of bee families, breeding and certification of beekeeping breeding material, approved by Government Decision of the Republic of Moldova no. 306 of 28.04.2011 (2011).

Taking into account the biological peculiarities of the bee population (Cebotari et al., 2011), after the results of the assessment of all bee families in the apiary, the best performing families were selected annually to complete the breeding lot, which was later used to produce breeding used for reproduction.

The protection of bee families from pesticide residues was achieved by placing bee families stationary and harvesting in areas (sites) safe and harmless to bees.

Previously, the honey flora in the area intended for location was tested for the residual content of the most dangerous 46 pesticides, of which 22 pesticides monitored by the EU (such as: ά-HCH, β-HCH, Lindan, DDT-total, PSV, Aldrin, Dieldrin, Endrin, Hexachlorobenzol, Phenol, Ethylenedibromide, Coumafos, Fluvalinate, Flumetrin, Chlordimeform, Carbandazine, Naphthalen, Amitraz, Cymiazole, Tetramethrin, Glyphosate, Fipronil) and 24 more common pesticides in our country, such as: organochlorine insecticide (Spirodiclofen), organophosphorus insecticides (Azoxystrobin, Carbendazim, Dimethoate, Chlorpyrifos), neonicotinoid insecticides (Imidacloprid, Tiacloprid, Tiametoxam, Clotianidin), pyrethroid insecticides (Tau-fluvalinate, Deltamethrin, Cypermethrin, Pyrethrin), triazole fungicides (Bitertanol, Fenhexamide, Diphenoconazole, Mepanipyrim, Cyproconazole), dicarbosimidic fungicides (Cyprinodyl), herbicides (Sulfosulfurol, Amidosulfuron, Amitrol, Petoxamide, Pendimethalin). For this, at the beginning of the flowering period of the honey plants, mixed flower samples were taken from the main honey plants, located in the site where the apiary was expected to be placed at harvest. From the flight area of the bees, 5-7 flower samples were taken from different places, at different distances from the place planned for the location of the apiary. Each sample weighed at least 100 g of flowers. The samples were packed in plastic bags and transported the same day for analysis to the accredited laboratory of the U.S. "Center for Applied Metrology and Certification", in accordance with the Sanitary-Veterinary Norms on the methodology of sampling, processing, packaging and transport of samples for laboratory examinations (2010). The results of the laboratory analyzes, regarding the pesticide residues in flowers, were examined by comparing the data obtained with the maximum admissible limits, according to the Sanitary Regulation on the maximum permitted limits of pesticide residues, approved by Government Decision no. 1191 of 23.12.2010 (2010). If the concentrations of pesticide residues did not exceed the maximum permissible limits, then the environment was considered unpolluted and suitable for organic beekeeping.

In order to determine the comfort level of bees in hives of different types, experimental tests were performed for the maintenance of bee families in hives of horizontal and vertical type (Cebotari et al., 2012).

For the organic feeding of bees during the deficient periods of harvesting in nature by us, a series of nutritional supplements and new procedures for feeding bee families were tested and developed (Toderaș et al., 2012a; 2012b; 2012c; Toderaș et al., 2014; Toderaș et al., 2016a; 2016b; 2016c; 2016d), which ensures the increase of the morpho-productive performances of bee families.

In order to identify the methods of rational use of *Apis mellifera* in the pollination of entomophilous agricultural crops, a series of scientific researches have been undertaken and several techniques (methods) of bee-directed pollination of plum and apple fruit crops have been developed (Cebotari et al., 2015b), as well as sunflower (Cebotari et al., 2015c; Cebotari et al., 2017).

The data obtained as a result of the research were statistically processed using the computer software "STATISTICS - 12" and their certainty was assessed, according to the biometric variational statistics, according to the methods of Плохинский Н.А. (1989).

## RESULTS AND DISCUSSIONS

As a result of the multi-annual scientific research carried out in the Apiculture Laboratory of the Institute of Zoology, a series of innovative procedures (techniques) for genetic amelioration, organic feeding, maintenance and ecological care of bee families, as well as rational use of to the pollination of entomophilous crops, which is, as a whole, the innovative technology of conservation and valorisation of the species *Apis mellifera*.

**Conservation of the species *Apis mellifera*.** Among the conservation technologies of the species *Apis mellifera* are part of it: selection and breeding of bees from the local *Apis mellifera Carpatica* purebred population, protection of bee families from pesticide residues, maintenance of bee families in comfortable ecological hives, innovative organic nutrition of bee families in periods of inadequate harvesting, prophylaxis and treatment by ecological methods of diseases and pests of bees.

**Conservation by selection of the local population *Apis mellifera Carpatica*.** The technology of conservation of the local genetic fund of bees is realized, according to the National Program for conservation and genetic amelioration of the local bee population in the Republic of Moldova, by selecting and raising them in purebred (Cebotari, 2006).

The selection process provides for a systematic and sustainable annual assessment, over several years, of the level of development of a series of morpho-productive characters. Following the results of the evaluation, the best performing bee families were revealed and the selected batches of breeding were created, intended for the reproduction of beekeeping breeding material.

For reproduction, selected queens of the solocyte type were bred by innovative methods and tested according to the qualities of the descendants (Cebotari et al., 2015d; Cebotari et al., 2018). For the directed mating of queens, the most efficient innovative method was used, such as the instrumental insemination with semen of drones selected from the best performing paternal families.

Research results (Cebotari et al., 2021a) showed that instrumentally inseminated queens have a higher prolificacy compared to their contemporaries naturally mated in the nuptial flight, with 164 eggs / 24 hours, or 10.0% (td = 6.1; P <0.001). Working bees in the families of instrumentally inseminated queens had a higher development of external morphometric indices compared to their contemporaries in the families of queens naturally mated in the nuptial flight, as shown in: tube length - by 0.19 mm, or 2.9% (td = 6.71; P <0.001), ulnar index of the anterior right wing - 4.3 absolute units, or 10.2% (td = 5.00; P <0.001) and the share of bees with positive discoidal dislocation - by 11.0 absolute units, or by 15.4% (td = 3.10; P <0.01). As a result, the bee families of instrumentally inseminated queens had a significantly higher level of development of morpho-productive characters, compared to their contemporaries of queens naturally mated in nuptial flight, as follows: colony power - 0.17 kg, or by 6.5% (td = 6.07; P <0.001), disease resistance - by 3.4 absolute units, or by 4.1% (td = 3.15; P <0.01), viability brood - by 4.0 absolute units, or by 4.7% (td = 3.70; P <0.001) and honey production - by 8.39 kg, or by 19.9% (td = 5.31; P <0.001).

An eloquent example of the sustainable selection of bee families can serve the result obtained in the genetic amelioration and purebred conservation of the *Apis mellifera Carpatica* bee population raised in the experimental apiary of the Institute of Zoology during the years 2015-2021 (Table 1).

Table 1. Dynamics of the development level of the morpho-productive characters of the bee families from the experimental apiary in the period 2015-2021

| Name of morpho-productive characters | Level of development of morpho-productive characters, M ± m | | | | Standard of the race | Mediate 2015 - 2021 | 2021, % compared to the standard |
|---|---|---|---|---|---|---|---|
| | 2015 N=50 | 2017 N=50 | 2019 N=50 | 2021 N=50 | | | |
| Prolificity of queens, eggs / 24 hours | 1795±17 | 1678±16 | 1716±20 | 1644±17 | 1600 | 1708 | 102,8 |
| Family power, kg | 3.04±0.03 | 2.36±0.03 | 2.38±0.03 | 2.34±0.02 | 2.33 | 2.53 | 100.4 |
| Winter hardiness, % | 88.6±0.4 | 86.8±0.4 | 71.3±2.1 | 83.5±0.4 | 75 | 82.6 | 111.3 |
| Brood viability, % | 95.8±0.4 | 95.5±0.2 | 88.1±0.5 | 94.3±0.3 | 80 | 93.5 | 117.9 |
| Disease resistance, % | 86.3±0.7 | 92.6±0.6 | 91.6±0.7 | 93.1±0.4 | 60 | 90.9 | 155.2 |
| Honey production, kg | 44.2±0.6 | 34.2±0.4 | 49.9±0.6 | 57.5±0.6 | 45 | 46.4 | 127.8 |
| Rating class, %: | | | | | | | |
| El. record + Elite | 8.0±3.9 | - | 22.0±5.9 | - | | 7.5 | |
| Class I | 36.0±6.8 | - | 10.0±4.3 | 58.0±7.1 | 100 | 26.0 | 58.0 |
| Class II | 38.0±6.9 | 2.0±2.0 | 12.0±4.6 | 38.0±6.9 | | 22.5 | |
| Class III | 18.0±5.5 | 42.0±7.1 | 16.0±5.2 | 4.0±2.8 | | 20.0 | |
| Extraclass | - | 56.0±7.2 | 40.0±7.0 | - | | 24.0 | |

The progressive targeted selection, carried out during seven consecutive years, contributed to the consolidation of the performances of the morpho-productive characters selected at the average level of development above the breed standard.

Thus, the prolificacy of queens in the period 2015 - 2021 was on average 1708 eggs / 24 hours, varying depending on climate change of the year, from a minimum of 1644 ± 17 eggs / 24 hours in 2021 to a maximum of 1795 ± 17 eggs / 24 hours in 2015. It should be noted that, after average prolificacy, queen bee families in the experimental apiary exceed the standard breed level by 108 eggs / 24 hours or 6.8% (td = 4.8; P <0.001).

The strength of bee families has been strengthened to an average of 2.53 kg, varying depending on climate change of the year, from a minimum of 2.34 ± 0.02 kg in 2021 to a maximum of 3.04 ± 0.03 kg in 2015. It should be noted that, according to the average number of bees in the nest (power), the bee families in the experimental apiary exceeded the standard level of *Apis mellifera Carpatica* by 0.2 kg or 10.1% (td = 5, 6; P <0.001).

The winter hardiness of bee families in the selected population has been consolidated at an average level of 82.6%, with variations depending on the climate changes of the year, from a minimum of 71.3 ± 2.1% in 2019, to maximum 88.6 ± 0.4% in 2015. We would like to mention that, after winter hardiness, the bee families in the experimental apiary exceeded the standard level of the *Apis mellifera Carpatica* race by 7.6 absolute units or 10.1% (td = 3.6; P <0.001).

The viability of broods in the bee families of the selected apiary has been strengthened to an average of 93.5%, with variations depending on the climate change of the year, from a minimum of 88.1 ± 0.5% in 2019 to a maximum of 95, 8 ± 0.4% in 2015. It should be noted that, according to the average viability of the brood, the bee families in the experimental apiary exceeded the standard level of the *Apis mellifera Carpatica* race by 13.5 absolute units or 16.9% (td = 25.0; P <0.001).

Disease resistance of bee colonies, subject to progressive targeted selection during this period, was maintained at an average level of 90.9%, showing a consecutive increase from 86.3 ± 0.7% in 2015 to 93.1 ± 0.4% in 2021. We note that, according to disease resistance, bee families in the experimental apiary exceeded the standard level of the race *Apis mellifera Carpatica* by 30.9 absolute units or 51.5% (td = 38.1; P <0.001).

The strengthening of the morpho-productive capacities of the bee families in the selected population at a level above the race standard resulted in the increase, during this period, of the honey production accumulated in the nest from 44.2 ± 0.6 kg in 2015, until 57.5 ± 0.6 kg in 2021.

We mention that climate change has caused, in some years (2017), considerable decreases in honey production below the standard of the race. Despite these challenges, after the average honey production accumulated in the nest during this period, the bee families in the experimental apiary had an obvious tendency to exceed the standard level of the *Apis mellifera Carpatica* race by 1.4 kg or 3.1% (td = 1.94; P <0.1).

Annually, from the population of bee families, evaluated according to the complex of characters, were selected batches of descendants with the most valuable families, which according to the morphological features of the outside corresponded to the requirements of the race standard, and morpho-productive characters significantly exceeded these requirements (Table 2).

Table 2. Dynamics of the development level of the morpho-productive characters of the bee families from the breeding batches in the period 2015-2021

| Name of morpho-productive characters | Level of development of morpho-productive characters, M ± m | | | | Standard of the race | Mediate 2015-2021 | 2021, % compared to the standard |
|---|---|---|---|---|---|---|---|
| | 2015 N=13 | 2017 N=12 | 2019 N=8 | 2021 N=11 | | | |
| Prolificity of queens, eggs / 24 hours | 1846±20 | 1757±20 | 1721±44 | 1727±23 | 1600 | 1763 | 107.9 |
| Family power, kg | 3.20±0.04 | 2.56±0.02 | 2.36±0.08 | 2.48±0.02 | 2.33 | 2.65 | 106.4 |
| Winter hardiness, % | 89.9±0.7 | 88.5±0.7 | 83.0±0.9 | 84.8±1.0 | 75 | 86.6 | 113.1 |
| Brood viability, % | 96.4±0.6 | 96.1±0.4 | 87.8±1.1 | 94.4±0.6 | 80 | 93.7 | 118.0 |
| Disease resistance, % | 85.9±1.0 | 92.0±1.1 | 92.9±0.8 | 93.4±1.0 | 60 | 91.1 | 155.7 |
| Honey production, kg | 49.6±0.5 | 38.2±0.3 | 54.3±1.5 | 62.3±0.6 | 45 | 51.1 | 138.4 |
| Rating class, %: | | | | | | | |
| Elite record | - | - | 37.5±18.3 | - | | 9.4 | - |
| Elite | 30.8±13.3 | - | 62.5±18.3 | - | | 23.3 | - |
| Class I | 69.2±13.3 | - | - | 100±0.0 | 100 | 42.3 | 100 |
| Class II | - | 8.3±8.3 | - | - | | 2.1 | |
| Class III | - | 91.7±8.3 | - | - | | 22.9 | |

According to the average level of development of the morpho-productive characters, the bee families in the breeding batches in the experimental apiary substantially exceeded the races standard: the queen's prolificacy - by 10.2%, the family strength - by 13.7%, the winter hardiness - by 15.5%, at the viability of the brood - by 17.1%, after the resistance to diseases - by 51.8%, at the production of honey - by 13.5%. From the data presented, it can be seen that the genetic value of the bee families in the breeding batches has permanently increased during this period in terms of disease resistance and honey production.

Thus, the disease resistance of bee families increased from 85.9 ± 1.0% in 2015 to 93.4 ± 1.0% in 2021, the increase being 7.5 absolute units or 8.7 % (td = 5.3; P <0.001). The honey production of bee families in the breeding flocks increased from 49.6 ± 0.5 kg in 2015 to 62.3 ± 0.6 kg in 2021, the increase being 12.7 kg or 25, 6% (td = 16.3; P <0.001). With the exception of 2017 (which had unfavorable climatic conditions), the genetic value of beekeepers of bee families in these batches, assessed by the complex of morpho-productive characters, is expressed in the highest classes of bonitas (Elite record, Elite and Class I). The average share of bee families of the upper class of rating - Elite-record is 9.4%, Elite - 23.3% and Class I - 42.3%.

It should be noted that the previous results of guided selection of bee families after natural antivirus resistance (Cebotari et al., 2019c) showed that bee families in the brood group significantly outperformed their contemporaries in the experimental batch after queen prolificacy - by 18.4% ($P < 0.05$), the amount of captive brood - by 18.6% ($P < 0.05$) and honey production by 6.8% ($P < 0.01$). At the same time, they tended to have a lower degree of bee infestation with *Varroa* mites, a higher level of colony power and an increased winter hardiness, ranging from 82.3 to 89.0%.

Therefore, the application of progressive targeted selection of bee families with the reproductive use of high value beekeeping material has contributed to the conservation of the *Apis mellifera Carpatica* bee population with increased morpho-productive capacities, resistant to wintering and disease.

**Protecting bee families from pesticide residues**. This objective is achieved by placing bee families stationary and gathering in areas (sites) that are safe and harmless to bees. The content of harmful residues in honey flowers largely reflects the compliance of the environment for the practice of organic beekeeping and the protection of bee families.

Our scientific research (Cebotari et al., 2019a; Cebotari et al., 2021c) has shown that in the samples of acacia and linden flowers collected from the forest site, in the flowers and bee products collected from the industrial lavender fields and sage, as well as those of sunflower and apple, collected from the gardens of households in the rural locality, no detectable values were detected, or pollutants, of residues of some of the 69 pesticides investigated.

Based on the results obtained, it was concluded that forest sites, industrial lavender and sage fields, as well as rural home gardens, do not contain pesticide residues that could affect the health of pollinating insects and have safe areas for the families of pollinators. bees both stationery and harvesting, for the practice of organic beekeeping with the production of organic bee products.

At the same time, in the same and other similar research of ours (Cebotari et al., 2019a; Cebotari et al., 2020), it was found that in the flowers of honey plants in the industrial orchards of apple and plum, as well as the industrial fields of flower- of the sun, rapeseed, peas and maize, contain residues in detectable concentrations from 8.1 to 37.7% of the 62 pesticides investigated, of which, for some pesticides (Azoxystrobin, Carbendazim-L, Dimethoate, Glyphosate, Tiametoxam ) were recorded residues in concentrations from slightly polluting, exceeding the LMA level by 7-20%, to strongly polluting, exceeding the LMA level by 50-78%. These results show that some sites with orchards and industrial crop fields contain pesticide residues in concentrations that are quite dangerous for the health of bees and the safety of the bee products obtained.

In this context, in order to protect the health of bee families, which are to be located in the harvesting and pollination of apple and plum orchards, as well as industrial crops of sunflower, rapeseed, peas and corn, it is recommended to pre-test the flowers in the respective plantations to the content of the residues of my above-mentioned pesticides. It will also ensure the safety of bee products obtained by harvesting and pollinating these crops.

**Keeping bee families in comfortable hives.**

Our research (Cebotari et al., 2012) has shown that the development of bee families in different times of the year depends significantly on the comfort of maintenance offered in hives of different types. The test results showed that vertical hives have comfort advantages for bees compared to horizontal ones. The maintenance of bee families in vertical hives ensures an increase in the prolificacy of queens - by 3.5% ($td = 2.07$; $P < 0.05$), of family power - by 6.0% ($td = 2.41$; $P < 0.05$) and honey production by 19.1% ($td = 5.33$; $P < 0.001$). The exploitation of bee families in vertical hives ensures an economic efficiency of at least 23.8 euros per bee family. Comfortable hives must also be environmentally friendly. They need to be made of natural wood (preferred fir), according to current standards. For weather protection, the hives can be painted with linseed oil, or with special ecological paints. The frames of the hive must also be made of natural wood (fir, lime), and the wires for fixing the honeycombs must be made of stainless steel.

**Organic feeding of bees during poor harvesting periods in nature**. It is known that at the end of the winter period (February) and the beginning of spring (March-April), bee families face annually the problem of depletion of natural food reserves in the nest. In the body of bees there is a deficiency of bioactive nutrients, especially carbohydrates, proteins, trace elements, vitamins, which play a decisive role in the physiological processes of vital activity of the bee's body, determining the reproductive capacity and further development of the bee family. as a whole (Toderaș et al., 2016a; 2016b; 2016c; 2016d).

To compensate for the lack of nutrients in the diet of bees during poor harvesting periods in nature, most beekeepers feed bee families with sugar syrup, which lacks, with the exception of carbohydrates, a significant number of biologically active substances. In order to strengthen the vital activity capacity of bee families during these periods, we have developed a series of procedures for feeding bee families with carbohydrate nutritional supplements, enriched with biologically active substances of different organic origin.

The essence of these processes consists in feeding the bee families during the deficient periods of harvesting in nature with a nutritious carbohydrate supplement, either 50% sugar syrup, or sugar powder cakes mixed with honey in a ratio of 7: 3, enriched with a solution of 1-2% of the biomass extract of the cyanobacterium *Spirulina platensis* - patents: MD 475 Z 2012.09.30 (Toderaș et al., 2012a), MD 476 Z 2012.09.30.17 (Toderaș et al., 2012b) and MD 477 Z 2012.09.30 ( Toderași et al., 2012c); of the biomass of aquatic microalgae - MD 1061 Y 2016.08.31 (Toderaș et al., 2016a), MD 1062 Y 2016.08.31 (Toderaș et al., 2016b) and MD 1079 Y 2016.10.31 (Toderaș et al., 2016c); or some coordinating organic compounds - MD 850 Z 2015.08.31 (Toderaș et al., 2014) and MD 4438 B1 2016.10.31 (Toderaș et al., 2016d).

Scientific research has shown that the biologically active substances of the new nutritional supplements contribute to increasing the prolificacy of queens and the amount of brood per capita by 7.7-45.9%; family power by 9.3-16.9%; flight intensity of bees by 6.8-7.7%; disease resistance by 5.0-8.4%; winter hardiness by 10.5%; the amount of wax raised in the nest by 36.7-39.3%; the amount of pasture with 23.3-27.6% and the amount of honey accumulated in the nest with 19.6-38.9%.

The result is determined by the presence in the nutritional supplements of biologically active substances, such as: amino acids, essential lipid acids, peptides, vitamins (especially B12 and B6), antioxidant pigments and trace elements in necessary quantities, being catalysts of important regeneration functions. queens' ovarian tissue cells, as well as lactating and ceriferous glands of worker bees, with stimulating, immuno-modulatory and antioxidant properties, being a component part of hormones and enzymes in the hemolymph, which contributes to improving the penetrability of organic tissue cells, participates in the process of regeneration of hemocytes and strengthening the body's immune system the first days, the development of the family and the increase of its productivity.

**Valorisation of the species *Apis mellifera***. In order to increase the economic efficiency of the beekeeping branch, bee families must be exploited not only for obtaining traditional bee products, but also for the directed pollination of entomophilous agricultural crops.

Multiple researches in the field of entomophilic crop pollination (Cîrnu et al., 1973; Coman, 2012; Curennoi, 1973; Falaleev, 1973; Gerster, 2013; Frediani, 1973; Furgala, 1973; Magdici, 2005; Vaissicres, 2013) have shown that, the free (cross-pollination) pollination of fruit crops with the participation of insects, contributes 20-150% to the total production. In addition, the quality of fruits and seeds resulting from entomophilic pollinated flowers is at least 10-20% higher than those produced from pollinated flowers without insects. Trees with flowers that are poorly pollinated by insects produce fruit with an affected shape, less sweet and with few seeds.

Numerous researches in the field of sunflower (Gerster, 2013; Frediani, 1973; Furgala, 1973), carried out in different countries on different varieties and hybrids, have shown that the pollination of this agricultural crop with the help

of bees contributes to the increase of seed production. with 16-105%.
According to information (Magdici, 2007), in the US 33% of food consumed in this country comes from plants pollinated by insects, of which 75-90% bees, and the total value of crops and goods to which bees contribute by pollination is amounts to about $ 19 billion.
In the Republic of Moldova, bee pollination of agricultural crops is not widely applied, and some farmers question the effect of this pollination.
In order to highlight the contribution of honey bees to the pollination of agricultural crops and increase their harvest, we have undertaken a series of scientific research in this field.
As a result of the research, some techniques (methods) for bee-directed pollination of plum and apple fruit crops have been developed and proposed for beekeepers and agricultural growers (Cebotari et al., 2015b), as well as sunflower (Cebotari et al., 2015c; Cebotari et al., 2017).
**When pollinating plum and apple trees in industrial orchards** (Cebotari et al., 2015b).
For saturated pollination of plum and apple orchards, the bee load must be at least 3 families/ha. Each family must have at least 7 ranges of bee frames. The hives with the bee families are placed inside the orchard between the rows of trees, in a row, at a distance of 100 m from each other and over every 7th row of trees. In all areas of the orchard, hives with bee families are placed at the beginning of the flowering period of the trees and kept at least 6 days after the day of placement. In order to speed up the process of accustoming bees to the scent of tree flowers and to increase the intensity of flight, all bee families placed at pollination are fed daily throughout the pollination period, with 50% sugar syrup, mixed with flower infusion, freshly collected from the respective trees, in the amount of 50 g of flowers per 1 liter of syrup. The mixture is administered 50 ml at each interval of bee frames.
It was found that bee-directed pollination of plum and apple orchards by the proposed technique (method) ensures a significant increase, compared to the traditional method of pollination, the frequency of visit of bees to flowers - 2.3-2.4 times, the intensity of bees with pollen clumps - with 23.1-24.5%, of the amount of pollen collected - with 46.2-57.4%, of the degree of fertilization of flowers - of 2.1-2.2 or, as a result, a significant increase in fruit yield of at least 15-30%.
**When pollinating sunflower** (Cebotari et al., 2015c; Cebotari et al., 2017). For directed pollination of sunflower, the load of bees must be at least 4 families/ha. Each family must have at least 7 ranges of bee frames. The hives with the bee families are placed in a row around the chain on all four sides, at a proportional distance from each other. The distance between the hives is calculated by dividing the total length of the perimeter around the hive by the number of hives placed.
Experimental results have shown that bee-directed pollination of sunflower crops by the proposed method ensures an increase in the total mass of seeds (harvest) by 21.3-36.3% - compared to the traditional method and 3.6-8 4 times - compared to isolated pollination.

## CONCLUSIONS

Sustainable selection of purebred bee families *Apis mellifera Carpatica*, with the application of innovative methods of instrumental insemination of queens, contributes to the preservation of the bee population at the highest level of development of morpho-productive characters and their breeding value, ensuring confidence in the superior quality of the beekeeping parent material proposed for reproduction.
The conservation of bee populations requires their protection from the residues of some pesticides, which are more and more often attested in the flowers of some entomophilous agricultural plants. Knowledge of the most dangerous and widespread pesticides, as well as the identification of clean sites and biotopes of pesticide residues, are necessary actions of technology for the protection and conservation of bee populations.
The maintenance of bee families in comfortable and ecological hives is one of the technological methods that ensure the conservation of the species and race of bees *Apis mellifera Carpatica*. The hives of vertical models have comfort advantages for bees, compared to the horizontal ones, and ensure an increase of the prolificacy of the queens - by 3.5% (td = 2.07; P <0.05), of the family power - by 6, 0% (td =

2.41; P <0.05) and honey production by 19.1% (td = 5.33; P <0.001) and an economic efficiency of at least 23.8 euros per family of bees.

Feeding bee families during periods of inadequate harvesting in nature with carbohydrate nutritional supplements, enriched with biologically active substances of various organic origin, contributes to strengthening the vital activity capacity of bee families during these periods, ensuring the increase of queens prolificacy and the number of brood 7.7-45.9%; family power by 9.3-16.9%; flight intensity of bees by 6.8-7.7%; disease resistance by 5.0-8.4%; winter hardiness by 10.5%; the amount of wax raised in the nest by 36.7-39.3%; the amount of pasture with 23.3-27.6% and the amount of honey accumulated in the nest with 19.6-38.9%.

Rational use of *Apis mellifera Carpatica* species and bee race can be achieved by exploiting bee families not only for obtaining bee products, but also for their use in directed pollination, through innovative technologies, of entomophilous agricultural crops that contribute to the growth of fruit crops. orchards with 15-30% and sunflower seeds with 21.3-36.3%.

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# TESTING THE EFFECTIVENESS OF TWO METHODS OF EXTRACTING DNA FROM BLOOD SAMPLES FROM COWS

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***Abstract***

*This research aims to validate the most effective method of extracting DNA from a number of 20 blood samples collected from cows. Two methods were tested, namely: DNA extraction using a manual extraction kit-Promega and automatic DNA extraction, using the Maxwell 16 LEV Blood DNA kit-Promega. Following the quantification of DNA samples, by spectrophotometry technique, the best results were obtained by applying the automatic extraction method (77.37 ng/µl DNA concentration obtained by automatic extraction compared to 14.95 ng/µl DNA concentration obtained by manual extraction). Therefore, the effectiveness of this technique has been demonstrated, representing a first step in genomic analysis protocols. The accuracy of the subsequent results depends to a large extent on the results obtained by extracting the DNA from the samples. Therefore, the automatic DNA isolation method is recommended because it has a number of advantages: accuracy, reduced analysis time, low costs, reduced labor and ease of application. This technique can be successfully applied in the analysis of genetic diversity of different animal species.*

***Key words**: blood samples, cows, DNA isolation, genetic analysis.*

## INTRODUCTION

The most widely used biological samples in the genomic analysis of cattle are whole blood. DNA (deoxyribonucleic acid) is extracted from these samples by various working protocols. Since its inception in 1869, DNA extraction has progressed significantly. It's the first stage in many of the molecular biology's downstream applications (Tan & Yiap, 2009). These techniques range from extremely simple manual processes to more advanced automated DNA extraction strategies (Chacon-Cortes & Griffiths, 2014). Although the molecule DNA was discovered in 1869, it was not until 1943 that its involvement in genetic heredity was proved. James Watson and Francis Crick discovered that DNA is a double-helix polymer, a spiral made up of two DNA strands twisted around each other, in 1953, with the help of biophysicists Rosalind Franklin and Maurice Wilkins. Scientists gained a better grasp of DNA replication and hereditary control of cellular activity as a result of the breakthrough (Travers & Muskhelishvili, 2002; Watson, 1953). As a result, nucleic acid extraction is an important step in the laboratory processes needed to conduct additional molecular research. While studying the chemical makeup of cells, Friedrich Miescher became the first scientist to isolate DNA. In 1869, he undertook research to extract and identify proteins found in leukocytes obtained from samples on fresh surgical bandages.

During his research, he discovered an unique chemical in the nuclei that he named "nuclein." He then devised two techniques for separating the nucleus of cells from their cytoplasm and isolating this unique substance, now known as DNA (Dahm, 2005). DNA extraction techniques have now been modified to extract DNA from a wide range of biological sources.

The analysis carried out in this research focused on testing the effectiveness of two methods of extracting DNA from blood samples, collected from cows, respectively the manual method and the automatic extraction method. Thus, the main purpose of this research is to validate the optimal DNA extracting method from blood samples collected from a number of 20 cows, the first key step in genomic analysis in cattle.

## MATERIALS AND METHODS

In this research, the 20 blood samples were collected by puncturing the jugular vein of cows, using vacutainer with EDTA (ethylene-diamine-tetra-acetic) to prevent clotting. Samples were numbered from C1 to C20 (C-cows). Two methods were used to extract the DNA from blood samples, in order to test their effectiveness, respectively: in the case of the first method, the total genomic DNA was isolated using the Wizard Genomic DNA Purification kit – Promega and in the case of the second method, the isolation of the DNA from the blood samples was done by the automated method, with Maxwell equipment ™ 16 and 16 MDx instruments, using a kit special, 48 Maxwell ™16 MCD LEV-Promega. The purity of the extracted DNA samples was assessed based on the A260/A280 ratio and the concentration of the samples was automatically calculated with the NanoDrop-2000 spectrophotometer software.

## RESULTS AND DISCUSSIONS

In order to isolate the total genomic DNA, in the case of the first method, 3 stages were completed: cell lysis, nucleus lysis and protein precipitation and DNA precipitation and rehydration. Rehydrated DNA was stored at -20°C temperature. The purity of the extracted DNA samples was assessed based on the A260/A280 ratio and the concentration of the samples was automatically calculated with the NanoDrop 2000 spectrophotometer software. This spectrophotometry method is based on the following principle: most substances of nature shows a characteristic absorption rate in the field of ultraviolet radiation (UV). Thus, the absorption rate of 260 nm corresponds to the DNA/RNA nucleic acids, that of 280 nm for proteins and 230 nm for various contaminants (Cojocaru et al., 2009). According to the literature, DNA is considered pure enough if the ratio of the two readings, respectively A260/A280, has values in the range 1.7-2.0. Values lower than 1.7 indicate protein impurities and higher than 2.0 impurities with other contaminants (Kamangu, 2019). According to Beer Lambert's law, there is a linear relationship between concentration of a compound and its absorbance at a certain wavelength (Piskata et al., 2019). It is based on this fact calculating the concentration of DNA, making assessments on its purity in relation with protein. In the case of the first method of DNA extraction, using manual Wizard Genomic DNA Purification kit- Promega, following spectrophotometry for all DNA samples, DNA concentration values between 7.0 and 28.6 ng/µl were obtained (Table 1).

Table 1. Spectrophotometric quantification of total DNA extracted from blood samples of cows (using manual Wizard Genomic DNA Purification kit- Promega-*first method*)

| **Samples** | **Abs260** | **Abs280** | **260/280** | **260/230** | **DNA conc. (ng/µl)** |
|---|---|---|---|---|---|
| C1 | 0.573 | 0.354 | 1.62 | 0.82 | 28.6 |
| C2 | 0.442 | 0.293 | 1.51 | 1.00 | 22.0 |
| C3 | 0.473 | 0.310 | 1.53 | 1.08 | 23.6 |
| C4 | 0.254 | 0.185 | 1.37 | 1.02 | 12.6 |
| C5 | 0.183 | 0.120 | 1.53 | 1.00 | 9.1 |
| C6 | 0.182 | 0.106 | 1.72 | 1.01 | 9.0 |
| C7 | 0.334 | 0.205 | 1.63 | 1.18 | 16.7 |
| C8 | 0.272 | 0.193 | 1.41 | 1.13 | 13.5 |
| C9 | 0.159 | 0.113 | 1.41 | 0.95 | 7.90 |
| C10 | 0.270 | 0.171 | 1.58 | 1.16 | 13.4 |
| C11 | 0.272 | 0.188 | 1.45 | 1.30 | 13.5 |
| C12 | 0.340 | 0.218 | 1.56 | 1.20 | 17.0 |
| C13 | 0.379 | 0.254 | 1.49 | 0.97 | 18.9 |
| C14 | 0.266 | 0.193 | 1.38 | 1.09 | 13.3 |
| **Samples** | **Abs260** | **Abs280** | **260/280** | **260/230** | **DNA conc. (ng/µl)** |
| C15 | 0.243 | 0.150 | 1.62 | 1.11 | 12.1 |
| C16 | 0.217 | 0.152 | 1.43 | 1.08 | 10.8 |
| C17 | 0.244 | 0.154 | 1.58 | 1.30 | 12.2 |
| C18 | 0.146 | 0.096 | 1.52 | 0.98 | 7.3 |
| C19 | 0.141 | 0.108 | 1.31 | 1.06 | 7.0 |
| C20 | 0.251 | 0.213 | 1.18 | 0.94 | 12.5 |

From the table it can be seen that the minimum value of the DNA concentration in the blood samples was 7.0 ng/µl, while the maximum value was 28.6 ng/µl. The ratio of the two absorption rates, respectively A260/A280, presented values between 1.18 and 1.72. Figure 1 shows the values of the DNA concentration in the 20 samples of blood.

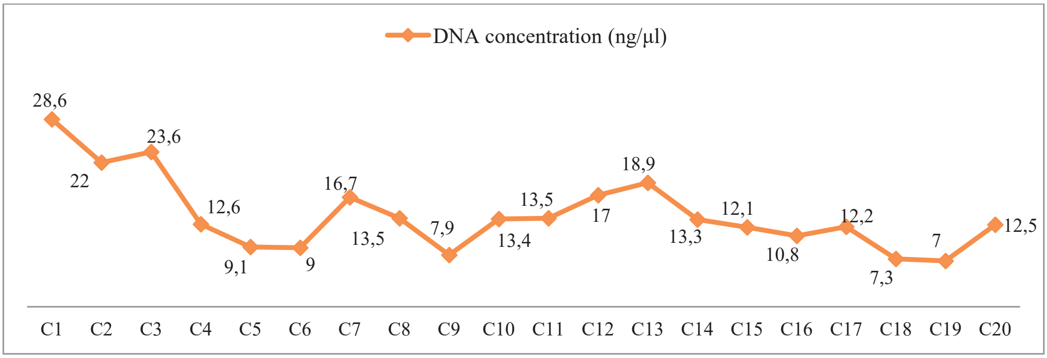


Figure 1. Graphical representation of DNA concentration values, measured with the Nanodrop 2000 (ng/µl) *first method*

Figure 2 shows the difference between the minimum, maximum and average values of the DNA concentration in the blood samples, resulting from the manual extraction method, respectively 7.0/28.6/14.05 ng/µl.

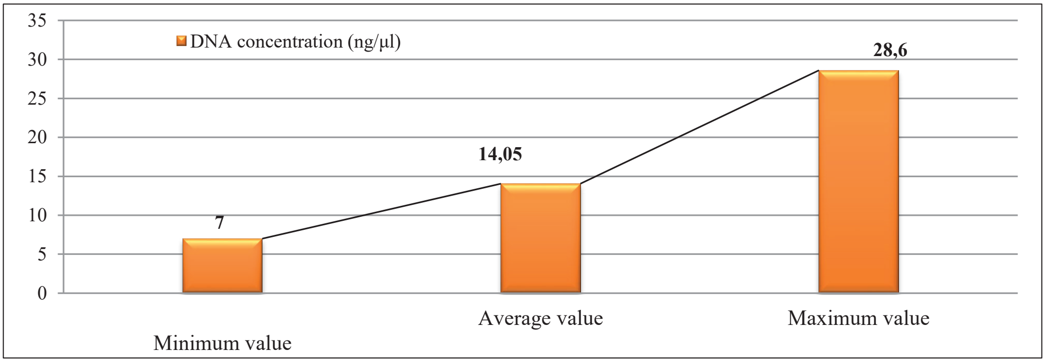


Figure 2. Average value of extracted DNA concentration, relative to minimum / maximum values (ng/µl)- *first method*

Regarding the purity of the extracted DNA, evaluated on the basis of the report of A260/ A280 absorbents, the values obtained can be framed in 3 intervals (<1.7; 1.7-2.0). Therefore, it can be stated that insignificant contamination with protein substances. Proteins have the ability to absorb ultraviolet light with a wavelength λ = 280 nm, which leads to an increase in the absorbance value and at the same time a decrease in the ratio absorbents

A260/A280, the solution in this situation being the repetition of the process of precipitation of proteins. In the case of the second method of DNA extraction, using Maxwell equipment ™ 16 and 16 MDx instruments, and a special kit, 48 Maxwell ™16 MCD LEV-Promega, following spectrophotometry for all DNA samples, DNA concentration values between 33.6 and 161.6 ng/µl were obtained (Table 2).

From the Table 2 it can be seen that the minimum value of the DNA concentration in the blood samples was 33.6 ng/µl, while the maximum value was 161.6 ng/µl. The ratio of the two absorption rates, respectively A260/A280, presented values between 1.22 and 2.53. Figure 3 shows the values of the DNA concentration in the 20 samples of blood.

Table 2. Spectrophotometric quantification of total DNA extracted from blood samples of cows (using Maxwell equipment ™16 and 16 MDx, with 48 Maxwell ™16 MCD LEV-Promega kit-*second method*)

| Samples | $A_{260}$ | $A_{280}$ | $A_{260}/A_{280}$ | $A_{260}/A_{230}$ | DNA conc. (ng/µl) |
|---|---|---|---|---|---|
| C1 | 3.232 | 1.653 | 1.96 | 1.8 | 161.6 |
| C2 | 2.231 | 1.139 | 1.96 | 2.17 | 111.5 |
| C3 | 1.504 | 0.754 | 1.99 | 1.96 | 75.2 |
| C4 | 1.394 | 0.724 | 1.93 | 1.7 | 69.7 |
| C5 | 1.244 | 0.616 | 2.02 | 1.95 | 62.2 |
| C6 | 1.164 | 0.566 | 2.06 | 2.08 | 58.2 |
| C7 | 1.589 | 0.805 | 1.97 | 1.78 | 79.5 |
| C8 | 0.671 | 0.335 | 2.0 | 1.22 | 33.6 |
| C9 | 1.589 | 0.847 | 1.88 | 2.53 | 79.4 |
| C10 | 1.544 | 0.848 | 1.82 | 1.42 | 77.2 |
| C11 | 1.579 | 0.793 | 1.99 | 2.16 | 78.9 |
| C12 | 1.025 | 0.497 | 2.06 | 2.31 | 51.3 |
| C13 | 1.114 | 0.55 | 2.03 | 2.44 | 55.7 |
| C14 | 1.927 | 1.04 | 1.85 | 1.76 | 96.3 |
| C15 | 1.523 | 0.754 | 2.02 | 2.12 | 76.1 |
| C16 | 1.743 | 0.88 | 1.98 | 2.31 | 87.1 |
| C17 | 2.073 | 1.049 | 1.98 | 2.37 | 103.7 |
| C18 | 1.273 | 0.637 | 2.0 | 2.05 | 63.6 |
| C19 | 1.842 | 0.938 | 1.96 | 2.41 | 92.1 |
| C20 | 0.691 | 0.316 | 2.19 | 1.81 | 34.6 |

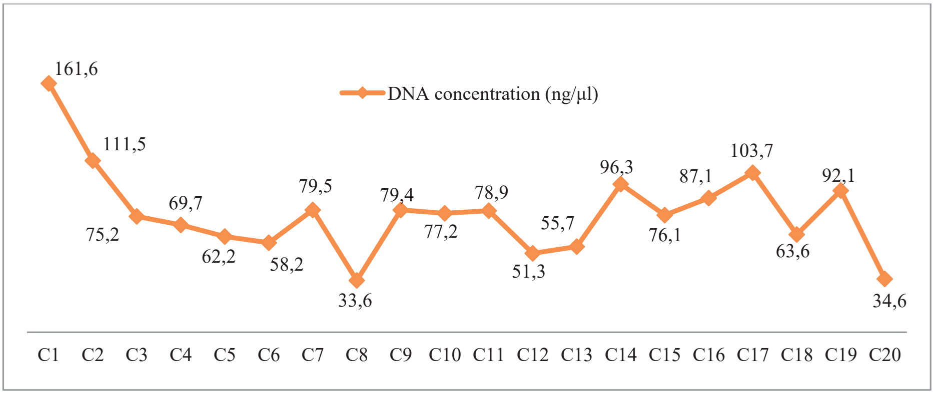


Figure 3. Graphical representation of DNA concentration values, measured with the Nanodrop 2000 (ng/µl)-*second method*

Figure 4 shows the difference between the minimum, maximum and average values of the DNA concentration in the blood samples, resulting from the manual extraction method, respectively 33.6/77.375/161.6 ng/µl.

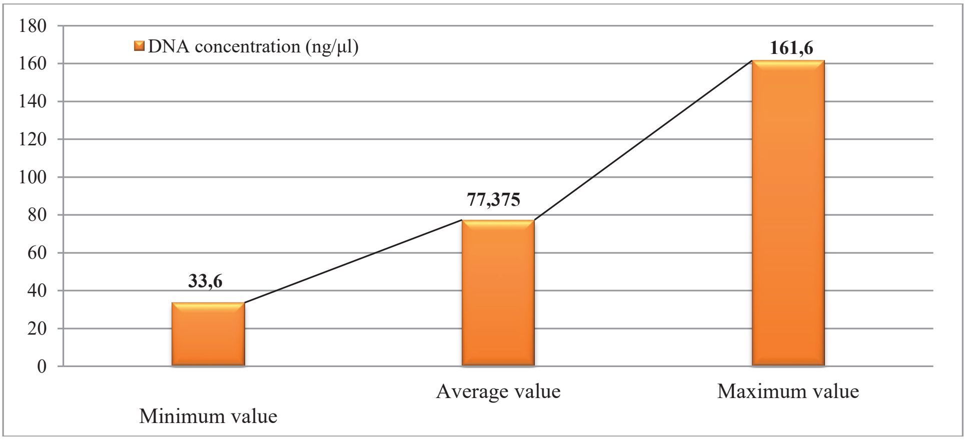


Figure 4. Average value of extracted DNA concentration, relative to minimum/maximum values (ng/µl)- *second method*

Regarding the purity of the extracted DNA, evaluated on the basis of the report of A260/ A280 absorbents, the values obtained can be framed in 3 intervals (1.8-2.0; ≥2).
In this case, the value of the absorbance ratio A260/A280 was in the desired range (1.7-2.0), therefore the presence of other contaminants in the samples is total excluded and thus demonstrates the effectiveness of the automatic method of extracting total genomic DNA from blood samples.
The concentrations of the DNA samples were much lower in the case of the first extraction method compared to the results obtained after the application of the second extraction method (Figure 5).

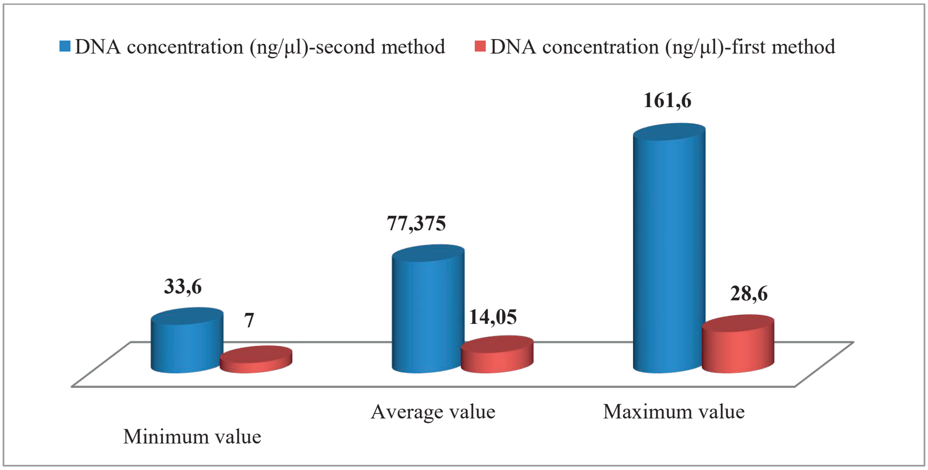


Figure 5. Comparison between the results obtained after performing the two methods of DNA extraction from the 20 blood samples

The major differences between the results are clear. The average values of DNA concentrations resulting from manual kit extraction were only 14.05 ng/µl, while the average values of DNA concentrations resulting from automatic extraction were 77.37 ng/µl. Therefore, a difference of 63.32 ng/µl is observed between the two average values.

## CONCLUSIONS

The results of this research demonstrated the effectiveness of the method of automatic extraction of total genomic DNA (using Maxwell equipment ™ 16 and 16 MDx instruments, using a special kit, 48 Maxwell ™ 16 MCD LEV-Promega) from blood samples collected from cows, the concentration values of the DNA samples obtained by this method, being much higher compared to the results obtained by aliquoting the extraction method using the manual extraction kit (14.05 ng/µl-manual extraction of DNA compared to 77.37 ng/µl-automatic extraction of DNA). Therefore, in performing molecular genetics analysis, it is recommended to use the method of automatic DNA extraction, as a first step to obtain conclusive and highly accurate results.

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# POLYMORPHISM IDENTIFICATION OF *FABP3* GENE IN SHEEP OF BULGARIAN DAIRY SYNTHETIC POPULATION

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***Abstract***

*This experiment was conducted in order to be identified the allelic and genotypic polymorphisms of FABP3 (heart-type fatty acid binding protein) gene in 30 ewes from Bulgarian Dairy Synthetic Population breed reared in Experimental base - Tzarev Brod - part of the Agricultural Institute - Shumen. FABP3 gene is a candidate marker that influences milk fat content and marbling of meat. Thirty blood samples were collected from v. jugularis in vacuum tubes with EDTA. Genomic DNA was extracted manually with commercial kit. By means of PCR-RFLP technique with endonuclease BseDI in exon 2 of FABP3 gene (SNP3) were determined the allele and genotype variants of the investigated animals. In this population were observed two alleles - wild allele A with frequency 0.15 and mutant allele G - with 0.85. Two different genotypes were identified - homozygous GG with frequency 0.67 and heterozygous genotype AG with frequency 0.33. Ho (observed heterozygosity) was 0.330 and He (expected heterozygosity) was 0.255. This herd was found to be in Hardy-Weinberg equilibrium (p>0.05).*

***Key words***: *FABP3 gene, PCR-RFLP method, polymorphism, sheep.*

## INTRODUCTION

In recent years, consumers have paid increasing attention to healthy eating and food quality. In this regard, due to the increased demands of consumers of animal products, the efforts of breeders are aimed at improving the quality of sheep meat and milk through the rapid development of breeding programs in this direction.

The tender and marbled meat is preferable due to its better taste, and the high fat content of milk is a suitable raw material for cheese production. Therefore, researchers seek to identify the main genes affecting economically important traits as initial and decisive steps to develop genetic markers associated with different productive characteristics that will allow the selection of animals producing products of better quality - more tender marble meat, milk with higher fat and protein content, etc. (Deykin et al., 2016; Clark et al., 2017; Xu et al, 2017; Mohammadi et al., 2020).

Many genes affect the milk and meat productivity of sheep. Nowadays, diverse molecular genetic markers are increasingly used in sheep breeding programs in order to improve the quality of milk and meat (Selvaggi et al., 2014; Eer et al., 2020).

Fatty acid binding proteins (FABPs) form a small family of low molecular weight cytoplasmic proteins that have a high binding capacity for long chain fatty acids. FABPs play a crucial role in hormone action and cellular functions in adipocytes and other cells. They are essential mainly in the storage, transport and metabolism of lipids in the cell and are therefore the subject of research. These small proteins coordinate the transport of fatty acids from the plasma membrane to the sites of oxidation, triacylglycerol and phospholipid synthesis (Chmurzynska, 2006; Jurie et al., 2007; Furuhashi & Hotamisligil, 2008; Eer et al., 2020). They accelerate the absorption of long-chain fatty acids and delivering fatty acids to intracellular organelles (Lanier & Corl, 2015). Fatty acid-binding proteins are small intracellular proteins having a molecular size of 14 to 16 kDa with 126 to 134 amino acids (Lang et al., 2017). They are found in all animal species and are involved in their active lipid metabolism (Damcott et al., 2004; Michal et al., 2006; Kulig et al., 2010; Cho et al., 2011; Wang et al., 2015; Wang et al., 2016; El-Mansy et al., 2019).

In sheep, one reliable candidate gene for meat quality and milk fat content, member of this family is FABP3, also known as H-FABP or HFABP, which is associated with cardiac activity and whose molecular weight is 15 kDa (Calvo et al., 2002). According to studies of Lang et al. (2017) in Oula sheep, the H-FABP gene and its expression in muscle tissue are associated with the content of intramuscular fat (IMF) in meat. FABP3 is present in many tissues with a high need for fatty acids such as the heart muscle, skeletal muscle and mammary gland during lactation (Calvo et al., 2002; Jurie et al., 2007). Some studies on the effect of the fatty acid-binding protein 3 gene on different productive traits in sheep have shown the influence of different genotypes on fatty acid metabolism in both muscle and milk and is linked to muscle development, milk fat content and meat marbling (Calvo et al., 2004; Aurora et al., 2014).

The *FABP3* gene was mapped in the distal part of chromosome 2 of *Ovis aries* genome and it consists of four exons according to Calvo et al. (2002) or five according NCBI (2021) which are separated by introns. Thirteen single-nucleotide polymorphisms (SNPs) were identified on sheep *FABP3* gene from Calvo and co-authors (2002) in samples of animals from Manchega breed. Two of SNPs were located in exon 2 and intron 3, respectively named SNP3 (G/A) and SNP13 (G/A) have codominant segregation of the polymorphisms and are suitable to be studied in connection with certain productive qualities. The expression of the *FABP3* gene had a positive effect on the intramuscular fat content in different muscles of sheep (Xu et al., 2020).

In Bulgaria, sheep breeding has a thousand-year tradition and despite the fact that the number of sheep has decreased significantly in the last two decades both in sheep population and the total number of sheep farms, sheep production is essential for the country's economy (Boykovski et al., 2008; Sabkov et al., 2017; Annual Agricultural Reports, 2021). In Bulgaria are bred around 34 breeds of sheep, as 88.4% of the available ewes are dairy, and 70% of them are from the Bulgarian Dairy Synthetic Population. In the case of dairy breeds, the income is generated from the sale of lambs and milk, that is why the breed is a very valuable source for both meat and dairy industries (Slavova et al., 2015; Krastanov et al., 2018; Annual Agricultural Reports, 2021). In this regard, it is interestingly to establish the level of genetic polymorphism in certain productivity-related genes including *FABP3* in farmed sheep breeds. The objective of our study was to determine allele and genotype frequencies of SNP3 of *FABP3* gene in 30 ewes from Bulgarian Dairy Synthetic Population sheep breed.

## MATERIALS AND METHODS

*Animals*

In present investigation were tested 30 animals from Bulgarian Dairy Synthetic Population sheep breed reared in Experimental base in Tzarev Brod - part of the Agricultural Institute - Shumen. The samples of peripheral blood of each individual were collected from the jugular vein into tubes containing EDTA as an anticoagulant factor. The probes were stored at -20°C until DNA extraction process.

*DNA extraction*

The experimental work was carried out in the Laboratory of Genetics, University of Forestry, Sofia, Bulgaria. Genomic DNA was extracted from whole blood using a manual commercial kit for DNA purification according to the manufacturer's instruction (Illustra Blood GenomicPrep DNA Purification Kit, GE Healthcare, US). The DNA concentration of each sample was determined by spectrophotometer Biodrop. The criteria of DNA quality control were DNA concentration must be between 10 to 50 ng/µL. The quality of the obtained DNA was tested also on 1% agarose (Bioline, UK) gel prepared with TAE buffer (Jena Bioscience, Germany).

*PCR amplification*

The polymerase chain reaction amplifications were carried out in total volumes of 10 µl, containing 4 µl DNA template, 0.2 µl sterile water, 0.4 µl of each primer and 5 µl of 2 × (1.5 mM $MgCl_2$) MyTaq TM HS Red Mix 2x (Bioline, UK). The primer set used in this experiment was suggested by Calvo et al., (2004):

F: 5′-GGTTTTGCTACCAGGCAGGT-3′ and
R: 5′-TTCCCTATTCCCCTTCAGGG-3’.
Amplification reactions were accomplished by thermal cycler QB-96 (Quanta Biotech) under the following conditions: primary denaturation at 94ºC for 2 min, followed by 30 cycles of denaturation at 94ºC for 1 min, annealing at 56ºC for 30 s, elongation at 72ºC for 1 min. The reaction was completed by final extension at 72ºC for 10 min.

*RFLP analysis*
The genotypes of all tested animals from Bulgarian Dairy Synthetic Population were determined using restriction fragment length polymorphism analysis (RFLP). All amplifycation products of the *FABP3* gene fragment (exon 2 - 222 bp) were digested separately in 10 µl final volume, containing 6 µl PCR product, 2.5 µl dd $H_2O$, 10 U/µl restriction enzyme *BseDI* (Thermo, US) and 1 µl enzyme buffer. The digestion reactions were carried out at 60˚C for 3 h in thermal block. The fragment sizes were identified using Ready-to-Use DNA Ladder, 50 bp (Thermo, US) on 2.5 % agarose (Bioline) gel and stained by RedGel Nucleic Acid Stain (Bioline, UK). The obtained PCR products and restriction fragments were visualized under UV light.

*Statistical Analysis*
In the present study, the allelic and the genotypic frequencies of *FABP3* gene were estimated using simple gene counting method (Falconer and Mackay, 1996). The expected and the observed genotypic frequencies were compared using $\chi^2$ test. The population was found to be consistent with the Hardy-Weinberg equilibrium so the value of $p$ was > 0.05.

## RESULTS AND DISCUSSIONS

After DNA extraction of received blood samples were obtained 30 specimens of genomic DNA with concentration from 10 ng/µl to 50 ng/µl. All samples were equalized to concentration of 10 mg/µl with TE buffer and tested through 1% agarose gel electrophoresis (Figure 1).
By means of PCR technique were amplified fragments with expected length of 222 bp from exon 2 of *FABP3* locus, included SNP3. The received from amplification products from Bulgarian Dairy Synthetic Population ewes were tested on 2% agarose gel electrophoresis and visualized under UV light (Figure 2).

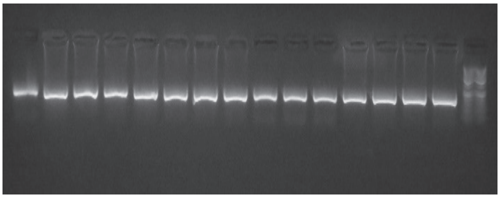

Figure 1. Extracted DNA samples tested on 1% agarose gel electrophoresis

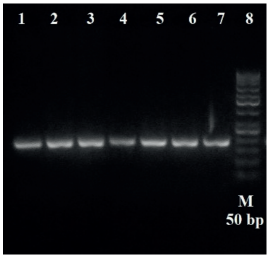


Figure 2. PCR products of *FABP3* gene tested on 2% agarose gel electrophoresis

All samples were digested with *BseDI* restriction enzyme. Two alleles *G* and *A* were detected. The digestion of the PCR products of three different fragments (143, 43 and 36 bp) produced mutant allele *G* and the digestion of the amplification products of two fragments (186 and 36 bp) produced wild allele *A* (Figure 3).

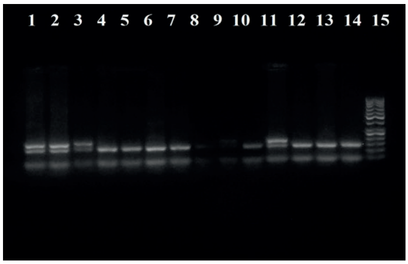


Figure 3. Restriction fragments of *FABP3* gene visualized on 2.5 % agarose gel under UV light. Lane 1, 2, 3, 9, 11 heterozygous genotype *AG*; lane 4, 5, 6, 7, 8, 10, 12, 13, 14 - homozygous genotype *GG*; lane 15 - DNA marker 50 bp

In the investigated Bulgarian Dairy Synthetic Population ewes from herd of the Agricultural Institute - Shumen were identified both possible alleles mutant *G* and wild *A* with frequency 0.85 and 0.15, respectively. Two different genotypes were identified in SNP3 of fatty acid-binding protein 3 gene - homozygous genotype *GG* with frequency 0.67 and heterozygous genotype *AG* with frequency 0.33. Observed heterozygosity ($H_o$) was 0.330 and expected heterozygosity ($H_e$) was 0.255. The variations of alleles and genotypes of *SNP3* of *FABP3* gene were presented in Table 1. This herd was found to be in Hardy-Weinberg equilibrium.

In previous our study of animals from the same breed Bulgarian Dairy Synthetic Population but from herd of Institute of Animal Science - Kostinbrod the allelic frequency was the same. It was obtained also two genotypes - *GG* - with frequency 0.70 and *AG* – with 0.30.

The presence in SNP3 of the fatty acid binding protein 3 gene of the *AG* genotype was associated with increased litter size, while the presence of the *GG* genotype showed a tendency with increased lactation productivity (Dimitrova et al., 2021).

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

| Breed | n | Allele frequencies | | Genotype frequencies | | | Ho | He | Fis | p |
|---|---|---|---|---|---|---|---|---|---|---|
| | | G | A | GG | AG | AA | | | | |
| SPBM | 30 | 0.85 | 0.15 | 0.67 | 0.33 | 0.00 | 0.330 | 0.255 | 0.870 | ns |

The results reported in this experiment were in agreement with other study of the SNP3 of the Fatty acid-binding protein 3 gene in different breeds. It was study five Bulgarian breeds - three fine fleece (Ascanian, Caucasian and Karnobat) and two local (Cooper-Red Shumen and Karakachanian) breeds. The frequency of mutant allele G and homozygous genotype GG was also higher in all breeds, but in all these five breeds all three genotypes have been identified. Research team established that of allele *G* varied between 0.77 to 0.87 and genotype frequency for genotype *GG* - differed from 0.57 to 0.80. The frequency of allele *A* ranges from 0.13 to 0.23, with the lowest frequency observed in the homozygous genotype *AA* - from 0.03 to 0.07, and the heterozygous genotype is in the range from 0.13 to 0.40 (Dimitrova et al., 2020).

The research of Calvo et al. (2002) show that in the predecessor of European sheep breeds - mouflon, only allele A was found in *SNP3* of the fatty acid binding protein 3 gene. The *G* allele was also found in domestic sheep, and it is predominant in the majority of the studied breeds. The frequency of allele *A* in domestic sheep breeds varies between 0.26 in Raza Aragonesa to 0.33 in Awasi, 0.38 in Assaf, 0.39-0.46 in Manchega (Calvo et al., 2002; Calvo et al., 2004).

The results in this study essential differ from the results reported in 100 investigated animals from three populations of Turkish sheep breed Kıvırcık (Oner et al., 2014). It was established genetic diversity in the same region of the *FABP3* gene - SNP3, with frequency of allele *A* was 0.42 and of the allele *G* - 0.58. In contrast to the present study in this Turkish breed was observed homozygous genotype *AA* with frequency 0.30, while the other two genotypes - *GG* (0.46) and *AG* (0.24) were lower in frequency than we found.

Other research team studied 10 different SNPs in *FABP3* gene in 250 Tan sheep and 174 Hu sheep. All of them are located in sequences related to the qualities of meat as H-FABP is considered one of the main genes that affect the content of intramuscular fat and is an important gene that affects slaughter traits and controls meat quality (Huang et al., 2006; Eer et al., 2020).

PCR-RFLP method was used also to detected polymorphism in two regions of fatty acid binding protein 3 gene - *SNP3* and *SNP13* in 50 sheep of the Slovak breed Zošľachtená Valaška (Kowalewska-Łuczak et al., 2017). They found only heterozygous genotypes in *SNP3*, while polymorphism was found in *SNP13*. Study was aimed to determine the prevalence of alleles and genotypes in relation to the SNP polymorphisms in *FABP3* locus and to determine possible relationships between genotypes and qualitative characteristics of sheep's milk. The scientific team reported that animals with the homozygous *AA* genotype had the highest content of fat, protein and solids in the milk of tested sheep. The sheep with the heterozygous genotype *AG* demonstrated the highest content of solids and urea in milk.

The establishment and inclusion of molecular genetic markers in breeding programs in order to improve the quality of meat and milk, as well as their composition is gradually becoming a future goal in sheep breeding (Selvaggi et al. 2014; Kowalewska-Łuczak et al., 2017). The established polymorphism in *SNP3* of the fatty acid-binding protein 3 gene indicated that the study of this locus in Bulgarian Dairy Synthetic Population sheep should be studied more detailed. It is necessary to extend both the number of the tested animals and the studied area of the gene to determine the relationship to specific productive characteristics before its implementation in sheep breeding programs.

## CONCLUSIONS

The results obtained in this study show that sheep fatty acid-binding protein 3 is a polymorphic gene. Genetic diversity was detected with presence of two alleles (wild *A* and mutant *G*) and only two genotypes (*GG* and *AG*). Higher frequency was observed in the homozygous genotype *GG*.

There is no difference in allelic and genotypic frequencies between the animals of the two herds of the breed Bulgarian Dairy Synthetic Population in *SNP3* of the *FABP3* gene.

The established genetic diversity in *SNP3* of *FABP3* gene in 30 animals from Bulgarian Dairy Synthetic Population sheep breed indicated that after additional research this gene could be suitable for implementing in breeding programs for improving milk productivity in sheep.


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# EVALUATION OF THE EXTERIOR AND PRODUCTIVE QUALITIES OF FIRST-HEIFER COWS OF THE JERSEY

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***Abstract***

*The article presents the results of the study of productive indicators and features of the exterior of the trunk and morphological properties of the udder of first- heifer cows of Jersey breed. The research was carried out in the herd of the breeding farm Society of limited liability "Topal-Bereket", town Comrat on first-heifer cows of Jersey breed. Analysis of milk productivity of the pedigrees of female ancestors of first-calf heifers showed that the highest milk yield and fat content were at the mothers of fathers - 8772 kg of milk and 6.02% of fat. On average, the milk yield of first-calf cows for 305 days of the first complete lactation was 4660 kg of milk with a fat content of 5.96%, the amount of milk fat - 276 kg. Exterior assessment showed that the height at the withers of the first Jersey heifers averaged 126.0 cm, and the height at the croup was 131.1 cm. The chest is deep and wide and averaged 63.7 cm and 40.5 cm, respectively. The bone is thin - the girth of the pastern is 16.6 cm. First heifers of the Jersey breed are characterized by a compact physique with an undercut index of 115.7%, which is characteristic to them during the studied period of development. The relationship between girth and width of the udder and milk yield per lactation of Jersey first-calf cows is weak and positive and amounts to 0.092 and 0.102, respectively. The correlation between the length, width and girth of the udder and the fat content in milk is weak and amounts to +0.123, +0.053 and +0.158, respectively.*

***Key words****: correlation, exterior, first-heifer cow, Jersey breed, milk yield.*

## INTRODUCTION

Increasing the production of livestock products, including milk, is the primary task of workers in the agricultural sector of the Republic of Moldova. One of the ways to solve it is the use of highly productive animals.

At present, the most widespread in our republic are the Moldavian type of black-motley cattle, the Holstein breed, the delivery of Jersey heifers has begun. The Jersey cattle breed was bred on the island of Jersey, which is located off the coast of England. This area is characterized by a mild climate that allows animals to be kept in very good pastures for most of the year.

A small area of the island, on which the animals were kept, was isolated, which contributed to the consolidation of the breed characteristics, as a result of which the animals of this breed have a conservative heredity and pass such a quality as fatty milk well to descendants (Dankvert, 2004). In 1789, it was organized a special agricultural society, the purpose of which was to improve the Jersey cattle breed. The first Jersey cattle exhibition took place in 1834, and the first volume of the herd book was published in 1872. The breeding of valuable families and lines was carried out by the scientists- cattle breeders Duncan and Daunan (Ruban, 1960). After the breeding of valuable lines of the Jersey cattle breed, its active spread to other countries of the world began, which continued to engage in the selection of this breed. The Jersey breed is predominantly bred in their homeland of Britain. Currently, the Jersey breed is being bred in Denmark, Hungary, France, Italy, and the USA. According to research conducted by the Ministry of Agriculture of the USA in 2006, Jersey is the main breed in 4% of American dairy farms (Drackley et al., 1996; Drackley, 2008). Due to strong demand for Jersey cattle in the United States, Jersey has become the fastest growing breed. More than 235,000 Jersey cows are enrolled on production testing programs in the United States. Actual yield per cow for 2012: 7,782 kg milk, fat - 371 kg, protein - 283 kg, Cheddar cheese yield - 979 kg. U.S. Jerseys produce, on average, more than 17 times their body weight in milk each lactation (www.usjersey.com/).

To important qualities of this breed refer high genetic potential, early maturity, the presence of a uniformly developed udder with a high milk flow rate, strong limbs and hooves. In 1992, the productivity of Jerseys (standardized milk) was 6779 kg, in 2010 it reached 8673 kg per livestock of 59 604 cows with a fat content of 4.7%, protein - 3.5% (Goncharenko & Vinnichuk, 2014). In terms of dry matter, Jersey milk is significantly superior to that of US Holsteins (Capper & Cady, 2012).

The delivery of Jersey cattle to Denmark began in 1896, then active work began to improve the milk yield and fat milk yield of the breed. Purebred and crossbred animals of this breed make up 18% of the total population. As a result of active breeding work in individual herds, were achieved the highest results in terms of milk yield for 305 days of lactation - 9175 kg of milk with a mass fraction of fat of 5.67% (Barendese et al., 2001, Agasiev, 2005). Jersey is the second-most-common dairy breed in Denmark, making up 13% of the dairy stock in Denmark, whereas Holstein-Friesian is the most dominant breed at 70% of all dairy stock (RYK, 2013; Kristensen et al., 2015). This could be part of the reason why Prendiville et al. (2009) found that Jerseys have higher gross energy efficiency (milk solids/DMI) than Holsteins, despite a lower milk-solid production. Milk yield across systems and breeds has been increasing, from 7,900 kg of ECM in 2004 (Kristensen & Kjærgaard (2004) to 9,500 kg in 2010 (RYK, 2013).

On the exterior, jersey belongs to the pronounced milky type. The constitution of animals of this breed is characterized by a light and thin bone (pastern girth 15-16.5 cm), flat and elongated body, dense and dry muscles, angular shape, small and light head with a shortened facial part, forehead with a narrow and concave profile. The height at the withers is 120-123 cm. The most valuable qualities of the Jersey breed are: uniformly developed udder, early maturity, strong limbs and hooves. The average live weight of full-aged animals varies in the range of 360-400 kg for cows, 650-700 kg for bulls; the birth weight of calves is 18–22 kg (Aleshkina, 2008; Dankvert, 2011; Ruban, 2011).

In countries with developed livestock breeding, animal conformation continues to be a priority in breeding programs, since there is its relationship with productivity, in addition, harmoniously developed individuals are more adapted to progressive technologies for the production of livestock products (Foksha & Konstandoglo, 2012). The exterior is one of the main breeding characteristics of dairy cattle (Loboda, 2012; Stavetska et al., 2013; Khmelnichy, 2007). The entire history of the creation and improvement of cattle breeds in the twentieth century was based on the development of the idea of the desired exterior type of animals Khmelnichy, (2010). In recent years, Jersey heifers have been imported into the Republic of Moldova. The first batch of heifers of this breed was purchased from Denmark in 2019 by SLL "Topal-Bereket".

The purpose of this work was to study the productive indicators and features of the exterior of the trunk and morphological properties of the udder of first-calf cows of Jersey breed.

## MATERIALS AND METHODS

The material for the research was first-heifers Jersey cows (n = 45) on the breeding farm of SLL “Topal-Bereket” (Society of limited liability), Comrat, which were imported from Denmark. Evaluation and analysis of animals for milk productivity was carried out according to generally accepted methods, taking into account: milk yield for 305 days of lactation, fat content in milk (%), amount of milk fat (kg). The genetic productivity potential of first-heifers was determined on the basis of the parental index of cows (PIC) Beauty, (1999) according to the formula: PIC = (2M + MM + MO): 4, where: M - mother's productivity; MO is the productivity of the father's mother; MM is the mother's productivity. The realization of the genetic potential (RGP) was determined by the formula: RGP = actual productivity / expected productivity according to PIC x100%, of the parental index of cows (PIC).

Exterior and constitutional features of first-heifers cows were studied by taking measurements and calculating their constitution indices. The measurements of the animals were carried out 2-3 months after calving (Basovsky, 1983; Belozertsova, 2011). Physique indices were calculated according to the generally accepted method (Kostomakhin et al., 2007). The assessment of the morphological properties of the udder was carried out on 2-4 months of lactation according to the method of Karelin & Starkov (1968). For the studied features were determined, the arithmetic mean (M), the error of the mean (± m), the coefficient of variation (Cv), the reliability of the difference according to the Student's criterion (P). Statistical data processing and correlation analysis were performed according to Merkuryeva & Shangin-Berezovsky (1983) using the Microsoft Excel 2010 software package.

## RESULTS AND DISCUSSIONS

Analysis of the productivity of the pedigrees of female ancestors of first-heifers Jersey breed found that the highest milk yield and fat content were found in mothers of fathers, that, due to the fact that all first- heifers are the daughters of high-value breeding bulls of the Jersey breed, tested for the quality of offspring (Table 1).

Table 1. Productivity of female ancestors of the Jersey breeds population, SLL "Topal-Bereket" (X ± Sx

| Productivity for the highest lactation | Indices | | | | | |
|---|---|---|---|---|---|---|
| | Milk, kg | Cv, % | Fat, % | Cv, % | Fat, kg | Cv, % |
| Mothers | 7459±186.9 | 16.6 | 5.73±0.07 | 8.5 | 425±9.6 | 14.9 |
| Father's mothers | 8772±234 | 16.7 | 6.02±0.08 | 7.8 | 525±11.7 | 14.0 |
| Mother's mothers | 7835±250.5 | 21.2 | 5.81±0.08 | 9.4 | 449±10.5 | 15.5 |

The milk yield of the fathers' mothers in the highest lactation was 8772 kg of milk with a fat content of 6.02%, the amount of milk fat - 525 kg. The milk yield of the highest lactation of mothers averaged 7459 kg of milk, which is by 1313 kg less than that of the father's mothers, the difference is significant (P <0.001).
The milk productivity of mother's mothers for the highest lactation averaged 7835 kg of milk with a fat content of 5.81%, the amount of milk fat - 449 kg. The smallest coefficient of variability for all analysed groups of animals was found for the amount of milk fat 14.0-15.5% (limit 18-32%). In terms of milk yield (16.6-21.2%) and milk fat content (7.8-9.4%), the coefficient of variability was slightly higher than the literature data on average by 1.6-6.0% and 2.8-4.4% respectively.
This confirms the long-term intensive selection according to the analysed economically useful traits of the female ancestors of this population of Jersey breed.
It was carried out the final analysis of milk productivity of first-heifer cows for 305 days of first lactation (Table 2).
It should be noted that the average milk yield of first-heifer cows amounted to 4660 kg of milk with a fat content of 5.96%, the amount of milk fat - 276 kg. The coefficient of variability for all analysed characteristics was below the norm.

Table 2. Characteristics of first-heifer cows of Jersey breed in terms of milk production for 305 days of lactation

| Indices | Milk, kg | Cv, % | Fat content, % | Cv, % | Fat amount, kg | Cv, % |
|---|---|---|---|---|---|---|
| M±m | 4660±30.6 | 4.2 | 5.96±0.03 | 3.4 | 276±3.5 | 8.2 |

For a more complete assessment of the potential capabilities of first-heifer cows according to the analysed indicators of female ancestors, we calculated the parental index of cows (PIC), which shows the genetic capabilities of the animal and the degree of transmission of productive qualities to the offspring (Lapina, 2009) (Table 3).
As it can be seen from the data in table 3, the parental index of cows (PIC) for the milk yield of first- heifer cows of the Jersey breed was 7881 kg of milk, for the fat content - 5.82%.

Table 3. Realization of the genetic potential of first-heifer cows

| Indicii | | X±Sx |
|---|---|---|
| Parental index of cows (PIC) | milk yield, kg | 7881±214.6 |
| | fat, % | 5.82±0.07 |
| Own productivity of first-heifer cows | milk yield, kg | 4660±30.6 |
| | fat, % | 5.96±0.03 |
| Realization of genetic potential (RGP), % | milk yield, kg | 59.1 |
| | fat | 102.4 |

The realization of the genetic potential (RGP) for milk yield in 305 days of lactation was 59.1%.

The realization of the genetic potential in terms of fat content was high and amounted to 102.4%.

**Exterior** is part of the assessment and an important aspect in the breeding of dairy cattle, characterizes the breed, and gives it certain characteristics that distinguish it from other breeds.

The desired type influences not only the level of development of individual signs of the exterior, but also characterizes their most appropriate ratio, which is achieved by directed choice and selection of animals.

Exterior assessment was carried out visually and by taking the main body measurements (Table 4, Figure 1).

As it can be seen from the data presented, all major measurements for Jersey first- heifer cows are within the limits established by the target standards. So, the height at the withers averaged 126.0 cm, the height at the sacrum - 131.1 cm.

Table 4. Indicators of linear measurements of body figure of first-heifer cows, cm (X ± Sx)

| Measurements | Indices | | |
|---|---|---|---|
| | X±Sx | σ | Cv |
| Height at withers | 126.0±0.4 | 2.75 | 2.18 |
| Height at the sacrum | 131.1±0.5 | 3.27 | 2.44 |
| Chest depth | 63.7±0.5 | 3.11 | 4.88 |
| Chest width behind shoulder blades | 40.5±0.4 | 2.44 | 6.02 |
| Width in hook bone | 45.0±0.3 | 1.97 | 4.37 |
| Width at the hip joints | 27.8±0.3 | 1.87 | 6.72 |
| Oblique body length | 150.8±0.5 | 3.28 | 2.17 |
| Chest girth behind the shoulder blades | 174.5±1.0 | 6.41 | 3.67 |
| Pastern girth | 16.6±0.1 | 0.51 | 3.06 |

Figure 1. Exterior of the first- heifer cows of the Jersey breed of the SLL "Topal-Bereket"

The chest is deep and wide and averaged 63.7 cm and 40.5 cm, respectively. The bone is thin - the pastern girth is 16.6 cm.

Analysing the results of the exterior assessment of first-heifer cows, it should be noted that they have a pronounced type of dairy cattle, which is confirmed by a proportional body shape.

The head is small, light, with a concave profile and a wide forehead. The neck is thin, with many small folds of skin. The back with slack, the dewlap is small, the body is somewhat stretched, with angular forms of constitution and a raised tail root, the muscles are poorly developed. The limbs are well developed, strong, the hoof is short, well rounded with a deep back wall and an even sole, the pasterns are strong, flexible, the hocks are clearly defined, good shaped, not rough, dry.

All evaluated animals showed the type characteristic to the Jersey breed, which is also confirmed by the index score (Table 5).

The index of high legs in first-heifers of the Jersey breed of the SLL "Topal-Bereket" herd averaged 49.4%, which characterizes the good development of the organism in the postnatal ontogenesis of animals. The index of high legs in first-heifers of the Jersey breed of the SLL "Topal-Bereket" herd averaged 49.4%, which characterizes the good development of the organism in the postnatal ontogenesis animals.

Table 5. Physique indices of first-heifer cows of Jersey breed, %

| Indexes | Indices | Standard for breeds of different directions of productivity | | |
|---|---|---|---|---|
| | | Dairy | Meat | Dairy and Meat |
| Highlegs | 49.4 | 45.7 | 42.2 | 48.2 |
| Lengthiness | 119.7 | 120 | 122 | 118.4 |
| Pelvic | 90 | 80.2 | 83.5 | 85.5 |
| Chest | 63.6 | 61.8 | 79.6 | 68.8 |
| Consistency | 115.7 | 118.0 | 132.5 | 121.3 |
| Overgrown | 104.0 | 100.9 | 103.2 | 102.5 |
| Osseous | 13.2 | 14.6 | 13.9 | 15.4 |

The stretch index, or format, is inherent to dairy cattle with the best exterior quality characteristics. As evidenced by the values of the indicators of our research, at first- heifers of the Jersey breed, the lengthiness index is 119.7%, which is by 0.3% less than the standard for dairy breeds.

The pelvic and chest indices are by 9.8 and 1.8 units higher than the standard for breeds of dairy productivity.

The general development of the body and body weight can be judged by the consistency or compact index. It should be noted that the first-heifers of Jersey breed is characterized by a compact physique with a consistency index of 115.7%, which is peculiar to them in the studied period of development.

The ratio of the height at the sacrum to the height at the withers is characterized by the overgrowth index, which is a good indicator of the growth and development of the organism in the postembryonic period. The average indicator of our studies of this index (104.0%) testifies to the good development of the physique of the evaluated animals. The bone index was lower than the standard for dairy breeds (1.4%), while the proportions of the physique of the animals of the evaluated Jersey heifers were preserved.

Thus, the results of the visual and index assessment showed that the first Jersey heifers had a pronounced milk type. They are characterized by a good physique and a strong constitution, on which the level of milk production, health status and the duration of productive exploitation largely depend.

**Assessment of the udder** of cows is one of the most important measures of technological selection of cows and is carried out in order to determine the suitability of animals for machine milking, its development is characterized by measurements of the udder and teats of first-heifers.

Measurement indicators, which are given in Table 6, characterize the development of morphological features of the udder in first- heifers of the Jersey breed of the SLL “Topal-Bereket” herd.

Table 6. Morphological properties of the udder of first-heifer cows of Jersey breed

| Signs | Indices | | Points |
|---|---|---|---|
| | X±Sx | Cv | |
| *Udder size, measurements, cm* | | | |
| Girth | 125.8±0.5 | 2.8 | 5 |
| Length | 32.0±0.3 | 6.6 | 5 |
| Width | 37.9±0.6 | 9.8 | 5 |
| Depth | 23.7±0.3 | 7.3 | 4 |
| Distance from the bottom of the udder to the ground | 60.4±0.2 | 6.1 | - |
| *Size of nipples, cm* | | | |
| Front nipple length | 5.30±0.06 | 7.9 | 4 |
| Back nipple length | 4.4±0.03 | 4.1 | - |
| Front nipple diameter | 2.6±0.04 | 10.3 | 5 |
| Back nipple diameter | 2.3±0.02 | 6.6 | - |
| *Distance between nipples, cm* | | | |
| front | 9.0±0.03 | 20.5 | |
| back | 6.2±0.1 | 15.6 | |
| front and back | 10.8±0.2 | 12.9 | |
| *Udder shape:* | | | |
| tub-shaped – 67.4 %; cup-shaped – 32.6 % | | | |

From the given data it follows that the first-calf heifers of SLL "Topal-Bereket" in terms of measurements, the girth, length and width of the udder correspond to the permissible norm and the requirements of the standard for the Jersey breed. The udder girth is large; the depth is medium. The length of the front nipples was within 5.3 ± 0.06 cm, the back - 4.4 ± 0.03 cm. The diameter of the nipples both front (2.6 ± 0.04 cm) and back (2.3 ± 0.02 cm) and the teat spacing was in a standard milking position. The distance from the bottom of the udder to the ground was within the permissible norm - 60.4 ± 0.2 cm. All evaluated first-heifers of the Jersey breed had the desired udder shape (tub-shaped - 67.4% and cup-shaped - 32.6%), the development of the udder quarters was symmetrical, uniform, tight attachment to the body, the bottom of the udder is horizontal, the shape of the nipples is cylindrical (Figure 2).

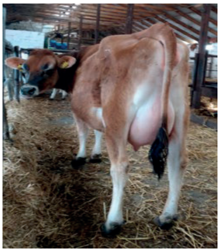

Figure 2. The shape of the udder and teats of first-heifer cows of Jersey breed

Characterizing the variation of the udder measurement indices, it can be noted that to the least variability are subjected measurements of the udder of first-heifer cows by girth - 2.8%; by distance from the bottom of the udder to the ground - 6.1%; along the length of the nipples - 7.9% (front) - 4.1% (back); on the diameter of the nipples - 10.3% (front) - 6.6% (back).

Low coefficients of variation indicate a targeted selection of cows with excellent udder quality. On average for the herd, the coefficients of variation show that this herd of first-heifer cows is the most variable in terms of the distance between the teats - the front teat is 20.5%, the back teat is 15.6%.

When selecting for any complex physiological indicators, it is necessary to establish the degree and direction of the relationship with other indicators. If there is a positive correlation between the sectioned indicators, then the selection of animals for one indicator automatically leads to an improvement of another indicator.

The relationship of morphological signs of the udder and milk yield for 305 days of lactation of first-heifer cows can be judged by studying the correlations, the results of which are given in Table 7.

Between the girth and width of the udder and milk yield per lactation of Jersey first-heifer cows, the relationship is weak positive and is 0.092 and 0.102, respectively. The correlation between the length, width and girth of the udder and the fat content in milk is weak and amounts to +0.123, +0.053 and +0.158, respectively. The weak link between udder measurements and signs of milk production is a consequence of drought, which affected cow feeding levels in 2020 and affected milk yield per lactation.

Table 7. Correlations between udder measurements, milk productivity of first-heifer cows of Jersey breed, r ± mr

| Udder measurements | Correlation coefficient | | |
|---|---|---|---|
| | milk yield for 305 days of lactation | fat content | amount of fat |
| Length | -0.064 | +0.123 | -0.004 |
| Width | +0.092 | +0.053 | -0.062 |
| Girth | +0.102 | +0.158 | +0.213 |
| Front lobe depth | -0.071 | -0.005 | -0.003 |

## CONCLUSIONS

The milk yield of first-heifer cows for the first lactation averaged 4660 kg of milk with a fat content of 5.96%, the amount of milk fat - 276 kg.

All basic measurements of first- heifers of Jersey breed are within the limits established by the target standards: height at the withers averaged 126.0 cm, height at the rump - 131.1 cm, deep and wide chest - 63.7 cm and 40.5 cm, respectively, the skeleton is thin – the pastern girth is 16.6 cm.

It has been established that first-heifers of Jersey breed are characterized by a compact physique with a consistency index of 115.7%, which is characteristic of them in the studied period of development.

All evaluated Jersey heifers had the desired udder shape (tub-shaped - 67.4% and cup-shaped - 32.6%).

The correlation between the length, width and girth of the udder and the fat content in milk is weak and amounts to +0.123, +0.053 and +0.158, respectively.

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# PATTERNS OF LACTATION CURVE IN BULGARIAN MURRAH BUFFALOES FROM TWO FARMS

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***Abstract***

*To assess the different patterns of lactation curve and their parameters, were assigned buffaloes from intensive and pasture farming with respectively 466 and 335 normal lactations. The effects of peak month on overall persistency (PI1), post-peak persistency (PIP) and peak yield (PMY) were tested via LSMLMW and MIXMDL. The curves of lactations with first (LC1), second (LC2), third (LC3) and fourth-plus (LC4) peak month were shaped through conventional statistics. The results show mass deviation from the typical curve, the LC1 lactations being 60%, while LC2 are 1/4. Delaying the peak from 1st to 3rd month, PIP decreases from 88.2 to 86.4% (P< 0.05), but with highest value (90.4%) is LC4 (P< 0.01). Most productive are the lactations with typical pattern (LC2), while LC1 have lower milk yield, despite the higher peak yield, but because of the lower productivity and overall persistency (PI1) after it. It was demonstrated that for the economics of buffalo farming persistency by itself is not the only important parameter, but actually its combination with peak yield and the positioning of the peak.*

***Key words:*** *buffaloes, lactation curve, peak month, persistency.*

## INTRODUCTION

Lactation curve of buffalo cows is to a great extent dependant on non-genetic factors related to management, climate and fodder resources, as it has been reported on global (Chaudhry et al., 2000; Amin, 2003; Macciotta et al., 2006; Anwar et al., 2009) and national (Penchev et al., 2011) scale. The ideal shape of lactation dynamics is a curve with a peak as high as possible and, more importantly, with a gradual decline afterwards (Pryce et al., 1997; Dekkers et al., 1996; Grossman et al., 1999). Though not as persistent as in bovine cows, it was demonstrated by Borghese et al. (2013) on global scale that in the water buffalo species its pattern is similar, the peak yield being normally between 40-th and 50-th day of lactation.

More importantly, such lactation shape was observed in the Bulgarian Murrah in particular - both in earlier grading stage of the population (Polihronov et al., 1977) and at a more recent status of the developed breed (Penchev et al., 2011). Nevertheless, in our previous study in association with days-in-milk (Penchev et al., in press) was established deviation from the principal pattern of lactation curve for the last decade. Hence, the purpose of the present study was to assess the different patterns of lactation curve and their parameters in Bulgarian Murrah buffaloes from two farms.

## MATERIALS AND METHODS

The study assigned milk yield test-day data from the record books of two farms for the period from 2003 to 2018. On one of the farms (Fm1) the buffaloes are housed in a tie-stall barn with an exercise yard, while on the other (Fm2) they are also in a tie-stall barn in the night but on pasture all through the day. From Fm1 was used the information about 466 normal and 115 short (minimum 90 days) lactations, from Fm2 - 335 normal and 58 short.

Three important parameters of lactation were studied to describe the dynamics of milk release throughout it: lactation curve, persistency of lactation, and peak milk yield.

***Lactation curve***. Because of the unequal number of lactation days from parturition to first test day, the pattern of the lactation curve was established via conventional statistical procedure after transformation of the test-day milk into actual lactation months. For this purpose, test-day records were initially divided into ten-day periods and then rearranged so the first test day, in particular, to be transformed into one, two or three ten-day periods. In this way, all lactations could be aligned by their first ten-day period, and then every three ten-day periods grouped back to obtain on monthly milk yield. On this transformed monthly basis daily milk yield was subjected to data processing.

Analysis of variance of milk yield were carried out under the following model (MDL-1):

$Y_{fk} = \mu + H_f + PA_g + MO_i + YR_j + SE_k + R[DIM] + e_{fk}$,

where: $\mu$ is the mean value of the trait;

$H_f$ - the fixed effect of herd/farm ($f$ = 1…2);

$PA_g$ - the fixed effect of parity ($g$= 1…11);

$MO_i$ - the fixed effect of lactation month order ($i$= 1…7);

$YR_j$ - the fixed effect of period of year of calving ($j$ = 1…4): 2003-2006, 2007-2010, 2011-2014, and 2015-2018;

$SE_k$ - the fixed effect of season of calving ($k$= 1…4);

$R[DIM]$ – the regression of days-in-milk;

and $e_{fk}$ - the residual effect.

For that purpose, were used the software products LSMLMW and MIXMDL (Harvey, 1990). The LSM-estimates by the levels of the factor lactation month were used for the shape of the overall lactation curve, while conventional statistical procedure (CSP) was used for the patterns of lactation curve in the cases of peak yield in the first (LC1), second (LC2), third (LC3), and fourth-plus (LC4) month postpartum.

***Persistency of lactation***. Two indices were computed also on monthly basis:

- Overall persistency (PI1) - from 1st to 7th month, as the average ratio between the milk yield of each month (from second on) and of the previous month.
- Post-peak persistency (PIP) - as the average decline after established peak month down to 7th month, which includes milk yield for 7 months (6 ratios) when the peak is in the first month (i.e., PIP= PI1), 6 months when second month is peak, 5 months when third month, and 4 months when the peak is in the fourth month and later.

Analyses of variance of PI1 and PIP were carried out under the following model (MDL-2):

$Y_{fq} = \mu + H_f + PA_g + YR_j + SE_k + DIM_l + DO_m + PM_q + e_{fq}$,

where: $\mu$, $H_f$, $PA_g$, $YR_j$, and $SE_k$ are same as in MDL-1, while $DIM_l$ here is the fixed effect of days-in-milk ($l$ = 1…4) with classes 210-260, 261-305, 306-365, and >365 days; $DO_m$ is the fixed effect of days open ($m$ = 1…5) with classes 0, 1-40, 41-80, 80-120, and >120 days; and $PM_q$ is the fixed effect of peak month ($q$ = 1…4) - 1st, 2nd, 3rd, and 4th-plus.

***Daily milk yield in the peak month (PMY)***. The order of the peak month was established for each lactation (including short lactations) as the maximal monthly milk yield among all available lactation months; in case of two or more consecutive months with equally highest yield, for peak month was taken the earliest. PMY was also subjected to Model-2.

Two relevant traits were studied additionally. Lactation milk yield (LMY) was analyzed via MDL-3, involving the same sources of variance, except for DIM which was included as a regression. DIM was analyzed as a trait via MDL-4, excluding DIM as a source of variance.

For the ease of reading, the abbreviations used in this work are as follows (in alphabetical order): CSP - conventional statistical procedure; DIM - days in milk; LC1, LC2, LC3, and LC4 - lactation curve in the cases of peak yield in the first, second, third, and fourth-plus month respectively; LMY - lactation milk yield; MDL-1 to MDL-4 - linear models; PI1 - overall persistency; PIP - post-peak persistency; PMY - daily milk yield in the peak month.

## RESULTS AND DISCUSSIONS

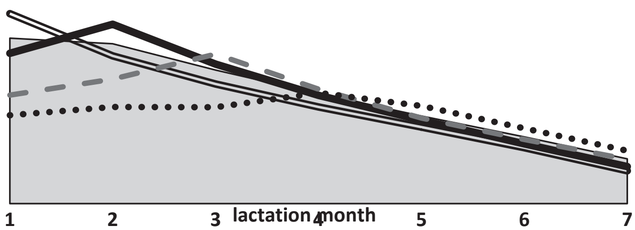
The results of the analyses of variance of the studied traits are presented in Table 1. Concerning daily milk yield per month, the data indicate that all sources of variance have highly significant effect. Most importantly, the factor with best expressed effect is lactation month ($F$= 511.3, $P$< 0.001). This significantly considerable effect and the td-values on Figure 1 render the established overall lactation curve highly reliable. The figure shows that practically the first two months are on the par for peak productivity, first month having 8.36 kg and second by only 2 percent lower - the difference being non-significant. The further differences, however, are all proved at $P$< 0.001. They show gradually increasing relative differences from 2nd-3rd month (10.4 percent) to 6th-7th (13.8 percent).

Table 1. F-values with levels of significance of P-value from the ANOVAs of monthly yield, persistency (PI1, PIP), peak yield (PMY), lactation yield (LMY) and DIM

| Sources of variance | df | Milk yield MDL-1 | PI1 MDL-2 | PIP MDL-2 | PMY MDL-2 | LMY MDL-3 | DIM MDL-4 |
|---|---|---|---|---|---|---|---|
| Factors: | | | | | | | |
| Farm | 1 | 20.4*** | 1.6 NS | 13.8*** | 2.5 NS | 13.6*** | 6.1 * |
| Parity | 10 | 39.8*** | 3.2*** | 5.3*** | 11.3*** | 8.2*** | 5.0*** |
| Period/Year | 3 | 22.9*** | 5.4 ** | 7.9*** | 6.0*** | 5.7*** | 11.2*** |
| Season | 3 | 19.8*** | 4.8 ** | 18.1*** | 7.4*** | 3.6 * | 0.9 NS |
| Lactation month | 6 | 511.3*** | - | - | - | - | - |
| Days-in-milk | 3 | - | 3.4 * | 30.2*** | 5.8*** | - | - |
| Days open | 4 | - | 0.6 NS | 0.8 NS | 2.6 NS | 3.7 ** | 58.3*** |
| Peak month | 3 | - | 27.5*** | 7.3*** | 14.8*** | 3.7 * | 1.9 NS |
| Regressions: | | | | | | | |
| Days-in-milk | 1 | 222.6*** | - | - | - | 264.0*** | - |

Significance of P-value: *** - P≤ 0.001, ** - P≤ 0.01, * - P≤ 0.05, NS - P> 0.05

Figure 1 also represents the differences among the lactations with different order of the peak month. It is seen that these are four different patterns with dynamics that are similar only after fourth lactation month. The LC1 lactations have the highest peak yield but also fast decline to the second month of 15 percent. In this way, the LC1 lactations have by 1.3 kg higher milk yield in the first month while the overall superiority of the LC2 lactations after this is 2.5 kg. The great difference at the second month between LC1 (7.78 kg) and LC2 (8.81 kg) on the basis of the similar productivity at fifth month suggests significant difference in post-peak persistency to be expected. The relative difference from month to month in the case of LC1 (Figure 1) is averagely 11 percent while in the case of LC2 it is 13.5 percent. This is better demonstrated later on by the persistency indices in Table 2.




| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Σ | 8,36 | 8,18 | 7,33 | 6,56 | 5,84 | 5,16 | 4,45 |
| LC1 | 9,15 | 7,78 | 6,87 | 6,13 | 5,48 | 4,81 | 4,06 |
| LC2 | 7,87 | 8,81 | 7,51 | 6,51 | 5,71 | 4,94 | 4,20 |
| LC3 | 6,51 | 7,04 | 7,84 | 6,70 | 5,77 | 5,08 | 4,44 |
| LC4 | 5,86 | 6,13 | 6,12 | 6,59 | 6,17 | 5,44 | 4,71 |

Figure 1. Lactation curves (daily yield per lactation month): from overall data set (Σ) in LSM (MDL-1) and in dependence on peak month (CSP); td (MDL-1): 1-2 - P> 0.05, for all other differences P< 0.001

The overall lactation curve in this study deviates from the ideal pattern described earlier in cattle (Pryce et al., 1997; Dekkers et al., 1996; Grossman et al., 1999). It is principally different from the curves established in the breeds Nili-Ravi (Khan & Chaudhry, 2001), Murrah (Aspilcueta-Borquis et al., 2010; Singh M. et al, 2015*)*, Mediterranean Italian (Catillo et al., 2002), and Anatolian breed (Şahin et al., 2015; Soysal et al., 2016) with peak yield in the second month. Furthermore, it is also different from the lactation curve observed in our previous studies on the Bulgarian Murrah - established peak in the fourth ten-day period (Polihronov et al., 1977) and in the second month in primiparous buffaloes (Penchev et al., 2011).

The distribution of patterns of lactation curves in dependence on their peak can be judged from

the number of observations (n) in Table 2. It is seen that nearly 60% of the LC1 lactations, which is a deviation from the typical curve, while the LC2 lactations are less than one quarter. This observation is unprecedented in the literature on the water buffalo species, the percentage of atypical lactations being below 10%, as reported by Mansour et al. (1992), Khan & Gondal (1996) and Khan & Chaudhry (2001).
As Table 1 shows, post-peak persistency (PIP) is strongly affected by all studied sources of variance (P< 0.001), except for days open (P> 0.05). The effect of peak month on PIP is expressed in F= 7.3 (P< 0.001), while on overall persistency it is even more determinative (F= 27.5, P< 0.001) at the expense of the non-significant farm/herd effect and the weaker influence of the environmental factors (P< 0.01) and of days-in-milk (P< 0.05).

As the results from Table 2 show, delaying the peak from first to third lactation month the post-peak persistency decreases from 88.2 to 86.4% (P <0.05), but the highest PIP = 90.4% belongs to the LC4 lactations (P <0.01). The LC4 lactations are with highest overall persistency, as well - by 13 percent relatively greater value as compared to LC1 – but with also significantly lowest milk yield. This lowest productivity is due to the low productivity of the initial 3 months, where the cumulative milk productivity is by 25% lower than the LC2 lactations, according to the data in Figure 1. The table shows that, judging by lactation milk yield, most profitable should be the LC2 lactations. The LC1 lactations have relatively low milk yield, despite the high peak yield, but because of the low persistency – post-peak persistency that should practically be viewed as overall persistency with significantly lowest index.

Table 2. Effect of peak month (LSM ± SE)

| Curve type | n | PI1 MDL-2 | PIP MDL-2 | PMY, kg MDL-2 | LMY, kg MDL-3 | DIM, days MDL-4 |
|---|---|---|---|---|---|---|
| μ | 801 | 0.929 ± 0.009 | 0.880 ± 0.005 | 8.55 ± 0.16 | 1525.1 ± 25.7 | 285.4 ± 4.94 |
| LC1 | 478 | 0.871 ± 0.009 | 0.882 ± 0.005 | 9.19 ± 0.19 | 1507.8 ± 27.1 | 274.7 ± 5.19 |
| LC2 | 193 | 0.910 ± 0.011 | 0.870 ± 0.006 | 9.03 ± 0.20 | 1578.9 ± 29.2 | 284.0 ± 5.60 |
| LC3 | 74 | 0.947 ± 0.015 | 0.864 ± 0.008 | 8.30 ± 0.28 | 1553.0 ± 40.8 | 275.7 ± 7.83 |
| LC4 | 56 | 0.989 ± 0.016 | 0.904 ± 0.009 | 7.66 ± 0.29 | 1437.5 ± 42.0 | 288.7 ± 8.10 |
| td | | 1-2**, 3-(2,4)* 1-(3,4)***, 2-4*** | 1-(2,3)*, 4-(2,3)** | 1-3**, 2-3*, 4-(1,2)*** | 2-(1,4)* | NS |

Significance of differences: *** - P< 0.001; ** - P< 0.01; * - P< 0.05; NS - non-significant

As most widely treated measure of persistency, the index of post-peak decline established in this study is higher compared to that reported for the Anatolian buffalo (Tekerli et al., 2001) and especially to the Egyptian buffalo (Elmaghraby, 2009), but lower than Nili-Ravi (Zakariyya et al., 1995) and the Mediterranean Italian (Catillo et al., 2002). It is lower also compared to our previous study (Penchev & Peeva, 2013), in view of the included data not only about 305-day lactations but also such with length of down to 210 days.
It is demonstrated herein that persistency (in the worldwide sense, post-peak persistency) by itself is not the only important parameter for the economics of dairy buffalo farming. Actually, its combination with peak yield and especially the positioning of the peak are more essential. In this context, the study also demonstrated the usefulness of the alternative measures of persistency, namely the role of overall persistency index, including the possible initial milk yield increase and a second-month peak that result in higher milk productivity throughout lactation. As they are associated with an economic loss, the dependencies and causes for the established skewed pattern of lactation dynamics are further to be studied.

## CONCLUSIONS

The highly significant effect of lactation month (P<0.001) in this study proves the established overall lactation curve with first month peak productivity. The mass deviation from the typical curve (for the bubaline species and for the Bulgarian Murrah in particular) is expressed in a portion of nearly 60% of the lactations with first month peak, while those peaking in the second month are less than one quarter.

Peak month has highly significant effect on post-peak persistency (P<0.001). Delaying the peak from first to third month, PIP decreases from 88.2 to 86.4% (P <0.05). With highest PIP (90.4%) is the curve with the most delayed (fourth) peak month (P <0.01), but the lactation milk yield is lowest.

Judging by milk yield, most profitable should be considered the lactations with typical pattern (second month peak). The lactations with a peak in the first month have relatively low milk yield, despite the high peak-month yield, but because of their low persistency.

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# PRODUCTIVITY OF FIRST-HEIFER COWS OF LOCAL BREEDING OF THE HOLSTEIN BREED

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***Abstract***

*There are presented the results of a comparative assessment of milk productivity for 305 days of the first lactation of daughters of the local generation of the Holstein breed of various origins and their mothers, as well as a study of the variability and heritability of milk productivity traits. The studies were carried out in 2020-2021 in the herd of Joint-Stock Company "Aydın", Comrat, Administrative and Territorial Unit Gagauzia, Republic of Moldova. There was established a significant advantage in milk yield of daughters on average for the first lactation over mothers by 530 and 1240 kg of milk, respectively, of Dutch and German breeding, with P <0.001. A weak positive relationship was established between the milk yield and the fat content in milk at the offspring of the local generation of various origins (r = +0.069) at first-calf heifers of German breeding also have a weak negative relationship (r = -0.186) - first-calf heifers of Dutch breeding. The relationship between milk yield and the amount of milk fat at the descendants of German breeding is positive, the tightness of the relationship is high (r = +0.768), which is significantly greater at P <0.001 than at the descendants of the Dutch breeding - the tightness of the relationship is moderate (r = +0.366). It should be noted the high degree of heritability in milk yield for the first lactation (mother-daughter) of German breeding - 93.0%, Dutch breeding - this indicator is two times lower - 44.8%.*

***Key words:*** *correlation, daughters of local generation, heritability, productivity, variability.*

## INTRODUCTION

Modern cattle breeding in the leading countries of the world is characterized by dynamic development, assimilation of intensive technologies, an increase in the productivity of animals, which ensures a steady increase in production. Dairy farming in these countries is developing through the accelerated increase of the genetic potential of livestock, intensive rearing of reproductive heifers, reproduction of the herd with first- heifers tested for their own productivity and intensive use of improvers in the selection of bulls, as well as balanced feeding of cows and reproductive calves.

The increase in milk yield and other indicators of milk productivity depends on the correct balanced feeding, climatic conditions of the environment, features of the exterior of cows, etc. The use in breeding work of highly productive cows contributes to the accumulation of the most valuable genetic potential of animals, increases the possibility of obtaining more highly productive breeding herds (Chechenkhina et al., 2018; Zernina, 2019; Zyryanova, 2108; Nemtseva, 2019; Stepanov et al., 2019; Fedoseeva et al., 2018).

Economically useful traits, to a certain extent, are genetically related to each other, that is, they are correlated. The study of correlations between economically useful traits in breeding work with dairy cattle is of particular importance. One-sided breeding for any one trait is impossible without considering the indirect effect that can be obtained from other traits (Dechow et al., 2002; Perez-Cabal & Alenda, 2002; Veerkamp et al., 2001).

Analysis of numerous materials on some breeds of dairy cattle showed that in the selection process, the variability of one of the phenotypic indicators depends on the variability of other economically useful traits. Thus, the variability of the fat content in milk depends on the level of milk yield of cows and, to a large extent, on their live weight. An increase in live weight to an optimal value is accompanied by an increase in milk yield. However, the correlation between these features is curvilinear. Analysis of the relationship between milk yield and live weight of black-motley cattle, carried out on a large livestock (Fenchenko & Nazarchenko, 2003), shows that the highest milk yield is observed at cows with a live weight of 550-600 kg. Further increase of body weight did not affect the increase in milk yield and reduced the value of the milk yield coefficient.

The modern use of the Holstein breed requires additional study of the breeding and genetic parameters of the population, with the help of which it is possible to establish the degree of constancy of economic traits, correlations between traits and their heritability (Adzhibekov, 1995; Nazarchenko, 2007; Nazarchenko, 2011; Ovchinnikova & Rumyantseva, 2012; Fenchenko & Nazarchenko, 2003; Fenchenko et al., 2009; Ernst & Zinovieva, 2008). When assessing the effectiveness of breeding by traits, there are many opinions about the role of the coefficient of heritability. So, the researchers Beguchev et al. (1969) noted that the study of the degree of influence of the coefficient of heritability has a certain value for predicting selection and breeding work for relatively large populations of livestock.

Assessment of the influence of mothers 'cows on milk yield and the qualitative composition of daughters' milk is one of the fragments in the breeding of cattle, which meets the modern requirements of intensive dairy farming (Damarov & Shishin, 2018; Kozlov, 2019; Kulikova & Eremin, 2016; Piotrovskaya & Damarov, 2018; Titova, 2019). In the literature, there are completely different data on the heritability of productivity indicators. As a result of the research by Sklyarenko et al., (2017), it was established the dependence of the milk production of cows on the productivity of their female ancestors in a herd of dairy cows. According to Novak (2012) and Piddubna (2014), the heritability coefficients ($h^2$) between milk yield and the amount of milk fat of daughters and their mothers ranged from 0.264 to 0.356. At the same time, the authors found that the share of the influence of the milk yield of mothers on the milk yield of daughters is in the range of 19.3-46.8%.

In the zootechnical literature there are many reports on the range of variation in the coefficient of heritability of the traits of milk production. So, in terms of milk yield, the heritability coefficient varies from 0.10 to 0.60, in terms of the fat content in milk - from 0.01 to 0.78 (Adzhibekov, 1995; Katmakov and Anfimova, 2013; Kakhikalo et al., 2007; Kuznetsov, 2002; Lyubimov & Martynova, 1995; Mymrin, 1998). An important conclusion follows from the results of the work of these researchers: if a single lactation, for which it is known that it has a relatively small coefficient of heritability, then information about its milk yield, generalized for five lactations, already gives a sufficiently complete picture for judging its breeding value. When calculating the coefficient of heritability of milk production, it is important that there are no sharp differences in the conditions of keeping and feeding mothers and daughters. Therefore, the coefficients may be different not only in different farms, but in the same farm in different feeding years. In good housing conditions and adequate feeding, in most cases they are higher, and lower in bad conditions. At the same time, the higher is the coefficient of heritability of a trait, the higher is the effect of selection of animals by phenotype. A higher value of the heritability coefficient indicates that mass selection for this trait will be effective, and its low indicators indicate a large influence of environmental factors that reduce the selection effect (Rokitsky, 1964; Ruzsky, 1972; Strekozov & Krylova, 1987; Eisner, 1986).

The aim of the study was to assess the milk productivity of first-heifer cows of the local generation and their mothers - Holstein cows of various breeds, to study the correlation of milk productivity traits and their heritability.

## MATERIALS AND METHODS

The material for research was Holstein cattle of various origins in the herd of J-SC "Aydın" (Joint-Stock Company), t. Komrat in 2020-2021. The milk productivity of first- heifers of local generation was assessed at the end of their first lactation. Holstein cows with family ties (mother and daughter) were selected to study the variability and heritability of milk productivity traits for the first lactation. Each sample consists of 20 cows and 20 offspring of local breeding (Dutch breeding) and 23 cows - 23 offspring of local breeding (German breeding). The milk yield for 305 days of lactation, the fat content in milk and the amount of milk fat were studied, and the variability of these parameters (Cv) was determined. It was done a study of the correlative links of cows and primiparous local organic generation with milk production, fat content and overall amount of fat, body mass. The correlation coefficient (r) was calculated on a computer using the CORREL function in the Excel program environment. The heritability coefficient was found to be equal to twice the correlation coefficient between mothers and daughters (h2 = 2rM/F). To determine the significant difference in the data, the Student's test (t-test) was used. Statistical data processing was carried out in a computer application Microsoft Office Excel 2010.

## RESULTS AND DISCUSSIONS

The results of assessing milk productivity and quality indicators of milk of mothers and their daughters - first-heifer cows of local breeding, depending on their origin, are shown in Table 1.

Table 1. Comparative assessment of milk productivity for 305 days of the 1st lactation of daughters and their mothers, depending on the origin, (X ± Sx)

| Mothers | | | Daughters | | | ± in% to mothers | | |
|---|---|---|---|---|---|---|---|---|
| Milk yield kg | Fat | | Milk yield, kg | Fat | | Milk yield, kg | Fat | |
| | % | kg | | % | kg | | % | kg |
| German breeding | | | | | | | | |
| 7239±195.5 | 3.73±0.04 | 273±6.7 | 8479±98.8*** | 3.84±0.03 | 323±5.5*** | +1240 | +0.11 | +50 |
| Dutch breeding | | | | | | | | |
| 7842±212.7 | 3.62±0.05 | 284±8.2 | 8372±69.5* | 3.82±0.02 | 314±3.6** | +530 | +0.2 | +30 |

Note: * - P <0.005; ** - P <0.01; *** - P <0.001

The analysis of the obtained results showed a significant advantage in milk yield of daughters on average for the first lactation over mothers. The milk yield of daughters, on average, exceeded the milk yield of mothers for 305 days of lactation by 530 and 1240 kg of milk of Dutch and German breeding respectively, with P <0.001. The data obtained by us are confirmed in the studies of Bakai & Lepekhina (2016), Latysheva (2018), Tekeev (2014), where cows-daughters exceeded cows-mothers in milk yield for the first lactation. The results of the studies by Poslavskaya (2016) showed a significant superiority of daughters over mothers in terms of the amount of milk produced and the amount of milk fat.

Regarding the fat content of the compared animals, it should be noted that all daughters in terms of fat content in milk exceeded the requirements of the standard by + 0.24% (German breeding) and + 0.22% (Dutch breeding). In terms of the amount of milk fat, the daughters of German breeding exceeded mothers by 50 kg (P <0.001), daughters of the Dutch breeding by - 30 kg (P <0.01).

An analysis of the selection and genetic parameters of economically useful traits of mothers and daughters of various origins showed that the highest coefficient of variability in milk yield for the first lactation was at mothers and daughters of German breeding, which is by 0.3 and 1.7 percent more, respectively, than at mothers and daughters of Dutch breeding (Table 2).

Table 2. Comparative assessment of the coefficient of variability of indicators of milk production mother-daughter (%)

| Indicators | | Milk yield | Fat content | Fat amount |
|---|---|---|---|---|
| German breeding | mothers | 13.0 | 4.7 | 11.7 |
| | daughters | 5.6 | 3.5 | 8.2 |
| Dutch breeding | mothers | 12.7 | 5.7 | 12.9 |
| | daughters | 3.9 | 2.4 | 5.3 |

The coefficient of variability in terms of the content and amount of fat was higher at the daughters of German breeding, respectively, by 1.1 and 2.9 percent.

On average for the sample, the coefficients of variability for all analyzed indicators are lower compared to the literature data, with the exception of the coefficient of variability of mothers of Dutch breeding in terms of fat content (5.7%).

Thus, the low value of the coefficient of variability for all analysed characteristics of mothers and daughters of different origins indicates a small range of variability and a decrease in the genetic diversity of this livestock population. The results of the realization of the genetic potential in terms of milk production indicators by first- heifers - daughters of local breeding are shown in Table 3, for which the parental index of mothers cows (PIC) was calculated.

Table 3. Realization of genetic potential by daughters of local breeding of various origins

| Indicators | | Dutch breeding | German breeding |
|---|---|---|---|
| Parental index of mothers cows (PIC) | Milk yield, kg | 9154±121.3 | 9203±116.4 |
| | fat, % | 4.06±0.03 | 3.92±0.04 |
| Daughters Own productivity | Milk yield, kg | 8372±69.5 | 8479±98.8 |
| | fat, % | 3.82±0.02 | 3.84±0.03 |
| Realization of genetic potential (RGP), % | Milk yield | 91.4 | 92.1 |
| | fat | 94.1 | 97.9 |

As it can be seen from the data in Table 3, the parental index of mothers (PIC) in terms of milk yield was the highest at German-bred mothers - 9203 kg, which is by 49 kg of milk more than the parental index of Dutch-bred mothers, the difference is insignificant.

Realization of the genetic potential (RGP) of first- heifers - daughters of both breeding was quite high - 91.4% (Dutch breeding) and 92.1% (German breeding), in general, according to the analysed traits, it is observed some superiority of the daughters of German breeding over their peers of the Dutch breeding.

The quality of animal evaluation and selection is influenced by the relationship between traits of milk production. It is known that the correlation between the milk yield and the fat content in milk depends on many factors, including the breed or belonging of animals to a particular breeding. As a result of studying the relationship between milk yield and fat content in milk at the offspring of local generation of various origins, a weak positive relationship (r = +0.069) was found at first- heifers of German breeding and a weak negative relationship (r = -0.186) at first- heifers of Dutch breeding (Table 4).

Table 4. Correlations between the main characteristics of the productivity of daughters - descendants of the local generation of various origins, r ± mr

| Origin of first-heifers | Indicators | | | | |
|---|---|---|---|---|---|
| | Milk yield - Fat content | Milk yield - the amount of fat | Milk yield - Live weight | Live weight - Fat content | Live weight - amount of Fat |
| German breeding | +0.069±0.22 | +0.768±0.14*** | -0.161±0.21 | -0.437±0.20 | -0.396±0.20 |
| Dutch breeding | -0.186±0.22 | +0.366±0.21 | +0.043±0.22 | -0.267±0.21 | -0.108±0.22 |

Note *** - P <0.001

The relationship between milk yield and the amount of milk fat at the descendants of German breeding is positive, the tightness of the relationship is high (r = +0.768), which is significantly greater at P <0.001 than at the descendants of the Dutch breeding - the tightness of the relationship is moderate (r = +0.366).

On the high indices of correlation between milk yield and the amount of fat in milk (r = + 0.94) and (r = + 0.91), when studying the milk productivity of the Holstein breed, was also indicated in the work of Carabano et al., (1989). With a high live weight of the offspring of both German breeding (606 kg) and Dutch breeding (611 kg), a weak negative relationship (r = –0.161) and a weak positive (r = +0.043), respectively, was established. This indicates that a further increase in the live weight of cows can lead to a decrease in their milk yield. Therefore, the main attention in the selection should be paid to the amount of milk yield while maintaining sufficient parameters for live weight.

Correlation relationships between live weight and fat content in milk and live weight and milk fat are negative at the entire analysed livestock of first- heifers of both breeds, however, the closeness of the relationship for animals of Dutch breeding is weak, for animals of German breeding - moderate.

Assessment of the genotype for productive qualities is inextricably linked with the determination of the heritability of traits. Therefore, to assess the indicators of heritability of traits, it was used the method of heritability of traits, it was used the method of correlating traits of daughters (descendants of local breeding) with their mothers (Table 5).

Table 5. Correlations between the main features of productivity of daughters and mothers of different origins, r ± mr

| Indicators | Number of animals: mothers - daughters, n | Milk yield, kg | Fat content, % | Fat amount, kg |
|---|---|---|---|---|
| Mothers- daughters (NL) | 20-22 | +0.224±0.15 | +0.066±0.15 | -0.036±0.15 |
| Mothers- daughters (DE) | 23-23 | -0.465±0.14*** | +0.057±0.16 | -0.087±0.16 |

Note: *** - P <0.001

A positive correlation coefficient of milk yield for 305 days of lactation was revealed between mothers and daughters of the Dutch breeding, the tightness of the relationship is weak (+0.224). A negative correlation in milk yield for 305 days of lactation (-0.465) was found between mothers and daughters of German breeding, the closeness of the relationship is moderate, which confirms the superiority of daughters in milk yield for the first lactation. A weak positive correlation was found for the fat content in milk between mothers and daughters of both breeds. It should be noted that there is a negative correlation in the amount of milk fat at mothers-daughters of both breeds, the tightness of the relationship is weak.

As a comparative analysis between mothers and daughters of Dutch and German breeding in terms of the correlation coefficient (milk yield per 305 days of lactation) is revealed a significant difference (-0.241) at P <0.001.

Heritability indicator was studied by us by the double correlation method (mother-daughter), that is, the degree of inheritance of traits from the mother (Table 6).

Table 6. Heritability ($h^2$) of productivity traits (mother-daughter) of the Holstein breed of different breeding for 305 days of lactation, %

| Indicators | Dutch breeding | | German breeding | |
|---|---|---|---|---|
| | Number of animals: mothers - daughter, n | $h^2$=2$r_{m/d}$ | Number of animals: mothers - daughter, n | $h^2$=2$r_{m/d}$ |
| By milk yield | 42 | 44.8 | 46 | 93.0 |
| By fat content in milk | 42 | 13.2 | 46 | 11.4 |
| By the amount of milk fat | 42 | 7.2 | 46 | 17.4 |

It should be noted that a high degree of heritability was revealed in terms of milk yield for the first lactation (mother-daughter) of German breeding, which amounted to 93.0%. To our opinion this is due to the fact that over the past few decades in Germany, breeding work has been carried out to combine high milk yield and high fat and protein content in milk.

As for the Dutch breeding cows, this index is two times lower - within 44.8%. Heritability coefficients (h2) of fat in milk and the amount of milk fat are lower than the literature data. According to the literature (Ruzsky, 1977), in different populations, the coefficient of heritability (h2) of the fat content in milk and the amount of milk fat are higher and vary between 17-70 and 45-70.

Thus, the results obtained testify to the observance of the technology of growing, feeding and keeping cattle on the JSC farm "Aydın" in all age periods of animals. Further study of the degree of influence of the heritability coefficient will be continued after the daughters finish the second and subsequent lactations, which is of certain importance for predicting selection and breeding work for a relatively large population of livestock.

## CONCLUSIONS

The established negative relationship between the fat content in milk and the milk yield of mothers and daughters (descendants of the first local generation) indicates that further selection for milk yield in the J-SC "Aydın" must be carried out taking into account the fat content of milk.

Low correlation coefficients between milk yield and live weight (positive - German breeding and offspring of the first generation) and (negative -

Dutch breeding) indicate a non-linear nature of the relationship between them, and characterize the homogeneity of the herd of J-SC "Aydın" by live weight.

The study of the relationship between milk productivity, fat content in milk, amount of milk fat, milk yield and live weight at first- heifers of local generation made it possible to establish correlation coefficients of positive and negative directions of different strength.

The obtained results of studies of the coefficients of correlation and heritability of the main breeding traits, the correlation between the milk production of daughters and their mothers of the Holstein breed of various origins, should be used in the selection and assortment of animals and in the long-term planning of breeding programs of the herd of J-SC "Aydın".

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# GENETIC CHARACTERISTICS OF WERIS (*GALLIRALLUS PHILIPPENSIS*) FROM MINAHASA BASED ON MITOCHONDRIAL-DNA *CYTOCHROME-B* GENES

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***Abstract***

*Utilization of animal genetic resources that live wildly needs to be maintained as well as one of the conservation efforts in a sustainable manner. This study aims to obtain information on genetic characteristics and kinship of Weris (local name) Gallirallus philippensis in several locations in Minahasa through molecular analysis using the Cyt-b gene. Gallirallus philippensis in Minahasa seems to have considerable genetic differences with their relatives from the Philippines and Australia where the results of the analysis show that the species found in Minahasa (Papontolen, Ranoyapo, Tondano, and Wusa) although still have a high genetic diversity based on the existence of 7 different haplotypes and form several branches but still in the same cluster. Alleged Weris in Minahasa may be a separate species (Gallirallus celebensis) need to be considered.*

***Key words****: cytochrome-B, Gallirallus philippensis, genetic, Minahasa birds.*

## INTRODUCTION

The Weris (*Gallirallus philippensis*) known as the "Buff Banded Rail" belongs to the genus *Gallirallus*, the Rallidae family (Allen *et al*. 2004), its widespread distribution includes the Philippines, Indonesia, New Guinea, Australia and New Zealand. In Indonesia it was known by the name of the "Mandar Padi Kalung Kuning" and in Minahada, North Sulawesi was known as the "Weris Bird". In Sulawesi there were three species namely *Gallirallus torquatus*, *Gallirallus striatus* and *Gallirallus philippensis* (GP) which live wild in nature. In addition, Coates & Bishop (1997) stated that *Gallirallus hilippensis* was also found in the regions of Maluku and Nusa Tenggara.

The three species of the genus *Gallirallus* had a diversity that distinguishes one species from another, with several key factors of each species, besides that each species was monomorphic which can be the main marker of differences of each species. As a comparison of the Rallidae family, for example the species *Gallirallus torquatus* had a dark beak and legs, a dark brown back, face, cheeks, and neck that was black and there was a prominent white line on the cheeks under the eyes, while the bottom was a black and white zebra stripe. *Gallirallus striatus* had a yellow horn beak and the base was pink, the legs were gray, the sides of the face and chest were light gray, the back was brown (Taylor 1998; Kennedy et al. 2000). *Gallirallus okinawae* had a red beak and legs, a brown back, face, cheeks and a black neck, with prominent white lines on the cheeks under the eyes and at the bottom were black and white.

The existence status of each of these species varies according to Bird Life International (2004) where *Gallirallus torquatus, Gallirallus striatus*, and *Gallirallus philippensis* were in the "least concern" status, while *Gallirallus okinawae* was in the "endangered" category. Some groups of birds were often difficult to distinguish morphologically despite occupying a variety of habitats and spread over diverse geographies, especially variations in intraspecies. However, molecularly Fain et al. (2007) stated that mitochondrial DNA had many advantages as a molecular marker at the level of vertebrate intraspecies because, according to Avise (1994) the form of the pattern of inheritance of Mitochondrial DNA through the maternal line causes no recombination and high mutation rates. The encoding area of Cytochrome B (*Cyt b*) in the bird group (*Gallus gallus*) had a size of 1143 nucleotides (nt) located between ND5 and tRNA (Desjardins & Morais 1990). *Cyt-b* in each level of species had a relatively high variation so that this encoding can be used as a comparison for phylogenetic analysis at the level of the same species, genus or family (Bretagnole et al. 1998).

The lack of information and data regarding phylogeny and morphology of birds in the genus *Gallirallus* makes the study of phylogeny (kinship) that plays a very important role in animal management, especially to regulate breeding strategies to avoid extinction, needs to be done. The existence of Weris in their habitat serves as a reservoir of germplasm wealth, both as a collection and conservation of biodiversity and for breeding material. Animal genetic resources had an important role in the formation of superior strains, while genetic diversity can only be known through continued characterization and evaluation. In terms of the utilization of existing animal genetic resources, diversity needs to be maintained as well as one of the preservation efforts in a sustainable manner. In addition, this genetic information will be very useful and valuable for ecological, behavioral and physiological studies of Weris and other genus *Gallirallus*.

Obtaining maps and phylogenic relationships and genetic variations of Weris *(Gallirallus philippensis*) in several locations in Minahada (Papontolen, Ranoyapo, Tondano, Wusa) were the main objectives of this study.

## MATERIALS AND METHODS

Research activities carried out in two stages, namely field activities for blood sampling (DNA analysis) and activities in the laboratory. Sampling was done purposively by selecting several locations in Minahada, namely Tondano (1º 17' 31.60 "N 124º 54' 03.94" E 681.5 m asl), Wusa (1º 34 '01, 24 "N 124º 55 '37.97" E 81.82 m asl), Papontolen (1º 16' 22.17 "N 124º 37 '27.73" E 13.3 m asl) and Tompaso Baru (0º 54 '47.95 "N 124º 28' 22.25" E 346.7 m asl) (Figure 1). While the molecular analysis was conducted at the Laboratory of Molecular Biology Research Center for Biological Resources and Biotechnology, IPB University.

The equipment used in sampling was to use 40 m x 2 m green trawlers, GPS, digital hygrometer thermometers and DSLR cameras. Trawl was installed in accordance with the area of rice fields Trapped birds were usually adults. A total of 150 birds were obtained from the catcher, and 30 blood Weris were taken for DNA samples. A total of 25 samples were sequenced, and those successfully dialeded were 16 samples (Four samples in each at Papontolen, Tondano, Wusa and Ranoyapo sites.

Blood samples were taken by using a syringe on a wing vein (jugular vein) of 0.5 mL and put in a micro tube (1.5 mL) that had been partially filled with absolute alcohol and shaken until homogeneous. Then the absolute alcohol sample was added again until the eppendorf tube was full, closed and stored at room temperature.

DNA extraction was carried out by modification of the phenol purification method developed by Sambrook et al. (1989).

Total isolated DNA was then electrophoresed on 1.2% agarose gel using 1 X TBE buffer solution (89 mM Tris, 89 mM Boric Acid and 2 mM EDTA, pH 8.0) in a submarine electrophoresis (Hoefer) device. DNA was visualized using UV Transluminator ($\lambda$ = 260 nm).

The primary was used to amplify the Cyt b region, which was based on Siahaan (2006), the results of extraction in the form of total DNA are used as a template to amplify the Cyt b region. A pair of primers are used to amplify the Cyt b region, namely the forward primer M101 5'-CAA ATC CTC ACA GGC CTA TTC CTA GC-3 ', and reverse primer M102 5'-TAG GCG AAT AGG AAA TAT CAT TCG GGT TGA T-3' . The PCR was performed using the AB system system 9700. The composition for each PCR reaction consisted of 2 times the Gotaq green (promega) 12.5 μl master mix, total DNA 3 μL (10-100 ng), Enhancer 2 μL, 50 mM MgCl¬2 1.5 μL, each as much as 1 μL of 20 μm primary M101 and M102 and dw (ddH 2 O) sterile water as much as 4 μL to a total volume of 25 μL. DNA Cyt b PCR products were traced using the automatic DNA machine ABI Prism version 3.4.1. (USA). The tracking process was carried out at PT. Genetic Science, Singapore. Traceability data obtained were then aligned using MEGA software version 4.0 (Tamura et al., 2007). As a comparison of the ingroup in this study the complete nucleotide of Buff Banded

Rail (*Gallirallus philippensis*, from Australia with access number Gen Bank DQ485907), and from the Rallidae family out group namely Rallus longirostris (Access number Gen bank DQ485908) (Fain et al., 2007). Analysis of phylogenetic reconstruction using MEGA software version 4.0 (Tamura et al., 2007). The results of the tracing were seen the close relationship with each other based on the genetic distance of Kimura 2 parameters with the phylogeny tree construction using the Bootstrap Neighboor Joining method 1000 repetitions.

## RESULTS AND DISCUSSIONS

### Habitat Description of Weris (*Gallirallus philippensis*)

The Minahasa region in general had mountainous topography and there were stretches of rivers, lakes and rice field which were widely available in Indonesia.

Rice fields can be divided into tidal paddy fields (influences to obtain water), non-tidal paddy fields (water from rivers or from irrigation channels) and rain-fed (only utilize rainwater for processing), while lebak type were made on the edge of a swamp or lake at low tide (Davies et al., 1996).

The type of rice field Minahada was non-tidal rice fields which obtain water from rivers or from irrigation channels, generally had 2 growing seasons. In this study, other bird species occupying habitat (based on morphological identification) that had been caught by trawlers were *Gallirallus torquatus, Gallinulla chloropus*, and *Porphyrio porphyrio*.

### DNA Isolation and PCR *Cyt b* Gene

From 20 blood samples analyzed, 16 total DNA results were obtained with a good enough yield. From all analyzed samples successfully amplified by M101 forward primer and M 102 revers reversal DNA fragment at 695 bp. At locations W (Wusa), R (Ranoyapo), P (Papontolen), and T (Tondano), only 16 samples successfully amplified the complete *Cyt b* partial gene.

### Genetic Variation *Cyt b* Gene

The DNA sequencing of the Polytase Chain Reaction (PCR) product of the *Cyt-b* gene of the *Gallirallus philippensis* bird sample from 4 locations in Minahada produces an alignment of DNA along 695-nt (nucleotides). Of the 695 nucleotides of Weris that were aligned, there was the same nucleotide (conserve) of 685 while there were 10 different nucleotides (10) with 3 parsimony sites and 7 singleton sites. There were 685 different sites, showing differences in intraspecies. Different nucleotides or (variable) had parsimony properties (Parsimony informative sites). This means that the results of the nucleotide sequence (at least two sequences) were observed and compared with other sequential data shows the difference from the other two sequential data. Parsimony occurs at sites 55, 180, and 507. However, the singleton site occurs at sites 107, 141, 156, 161, 290, 320, and 661. Nucleotide changes that cause transition substitution (pyrimidine and pyrimidine) namely Cytosine (C) to Thymine (T) in the amount of 12.03% or vice versa Thymine (T) to Cytosine (C) in the amount of 14.58%, (purines and purines) Adenine (A) to Guanin (G) a total of 5.73%, or conversely Guanin (G) to Adenine (A) a total of 12.56%. Nucleotide changes that cause subversion (purine and pyrimidine) substitution, namely Adenine (A) to Cytosine (C) of 8.79% or conversely Cytosine (C) to Adenine (A) 7.9%, Adenine (A) to Thymine (T) of 7.25% % or vice versa Thymine (T) to Adenine (A) of 7.9%. Guanin (G) to Cytosine (C) was 8.79%, or conversely Cytosine to Guanin was 3.6%. The difference in nucleotides that occur was the transition substitution was greater than the transversion substitution. Transition mutations generally occur during DNA replication whereas transversion was more related to DNA repair systems that are prone to errors (Burn & Bottino 1988; Sofro 1994). The results of the alignment along the 695 nucleotides were the most common nucleotide fragments C (31.9%), followed by A (28.7%), T (26.3%) and the least was G (13.1%). This proportion was in accordance with the opinion of Kocher et al. (1989) that for the group of birds and fish, the most nucleotides were C followed by A, T, and G (Table 1).

The data in Table 1 shows the different nucleotide sequences of all *G. philippensis* bird samples. Of the four locations, one location consisting of four samples had the same nucleotide. This similarity of nucleotides was also found in every other location was represented by 1 sample.

Table 1. Difference between nucleotide and haplotype results from 16 samples (4 locations) *G. philippensis*

| Samples | 1 1 | 1 1 1 | 2 3 5 | 6 | Haplotip | Location | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 5 0 4 | 5 6 8 | 9 2 0 | 6 | | | | | |
| | 5 7 1 | 6 1 0 | 0 0 7 | 1 | | P | T | W | R |
| P3 | A C C | C G A | C T T | A | 1 | 1 | | | |
| P4 | . . . | . . C | . C . | . | 2 | 1 | 2 | | |
| P5 | G . . | . . C | . C . | C | 3 | 1 | | | |
| P7 | G . . | . . C | . C . | . | 4 | 1 | 1 | 4 | 2 |
| T1 | . . . | . . C | . C . | . | 2 | | | | |
| T3 | . . . | . . C | . C . | . | 2 | | | | |
| T4 | G . . | . A C | . C C | . | 5 | | 1 | | |
| T8 | G . . | . . C | . C . | . | 4 | | | | |
| W3 | G . . | . . C | . C . | . | 4 | | | | |
| W9 | G . . | . . C | . C . | . | 4 | | | | |
| W7 | G . . | . . C | . C . | . | 4 | | | | |
| W8 | G . . | . . C | . C . | . | 4 | | | | |
| R4 | G . . | . . C | . C . | . | 4 | | | | |
| R6 | G . . | . . C | . C . | . | 4 | | | | |
| R8 | G G G | G . C | G C . | . | 6 | | | | 1 |
| R5 | G . . | . . . | . C C | . | 7 | | | | 1 |
| Sum of haplotip | | | | | 7 | 4 | 3 | 1 | 3 |

Remarks: P (Papontolen), T (Tondano), W (Wusa), R (Ranoyapo), G (Guanin), A (Adenine), T (Thymine), C (Cytosine).

Changes in nucleotides that cause transition substitution (purine bases to other purines), namely from Adenine (A) to Guanin (G) amounting to one site, namely the 55$^{th}$ nucleotide and vice versa from Guanin (G) to Adenine (A) amounting to one site namely the 161$^{st}$ site, and the transition substitution (pyrimidine base to another pyrimidine) from Thymine (T) to Cytosine (C) amounted to two sites namely 320th and 507 nucleotides. Transitional substitution (pyrimidine base to purine) from Cytosine (C) to Guanin (G) consists of four sites, namely the 107$^{th}$, 141$^{st}$, 156$^{th}$, and 290$^{th}$ nucleotides or from the purine base to pyrimidine, namely from Adenine (A) to Cytosine (C) totaling two sites, namely the 180$^{th}$ site and 661$^{st}$. The number of haplotypes produced from 16 samples of Cyt-b sequences along 695-nt from four locations amounted to 7 haplotypes. The Papontolen had 4 haplotypes, the Tondano had 3 haplotypes, the Wusa had 1 haplotype and the Ranoyapo had 3 haplotypes. These results indicate that Papontolen locations had the most diverse haplotype diversity compared to Tondano, Wusa and Ranoyapo populations. From the data shows that the Wusa location only had one haplotype (uniform).

The genetic distance was used to see the close genetic relationship between birds of Werist through the use of pairwise distance calculation analysis. With the Kimura 2 Parameter model, it can be shown a genetic difference matrix in paired nucleotides that take into account the degree of substitution of transitions and transversions. The kimura 2 parameter genetic distance value from the smallest (null) to the largest 0.009.

This shows that that *G. philippensis* cannot be distinguished between locations. A very close relationship occurs in several birds in four different locations (inter-location) because it had a genetic distance of null, but also in the intra-species relationship had a genetic distance of null or had a nucleotide similarity. Samples P4, T1, and T3 had the same nucleotide sequence. The main differences with samples P3, P5, P7, T4, T8, W3, W9, W7, W8, R4, R6, R8, and R5 were shown by the 55$^{th}$ nucleotide sequence (A-G). In other groups that had the same nucleotide sequence were samples of Weris, P7 T8, W3, W9, W7, W8, R4, and R6. The main difference from the T4 sample was the 161$^{th}$ nucleotide sequence (G-A). The R8 sample differs from the other 15 samples in the nucleotide sequence 107, 131, 147, and 290 (C-G). A close association arises in intra-species that was the species in W3, W8, W7, W9 and aware in one branch with the sample R4, R6, T8, and P7.

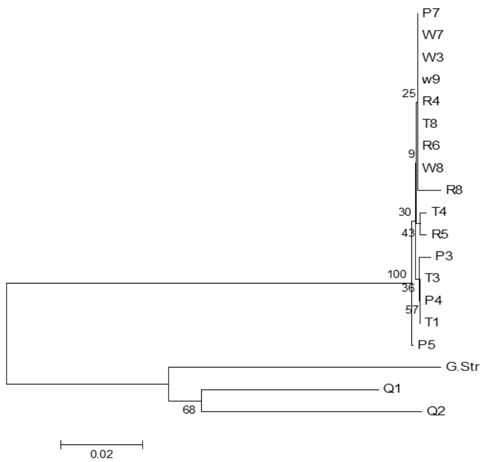


Figure 1. The phylogeny of *G. philippensis* between locations in Minahasa (P, R, T, W) and *G. philippensis* from Australia (Q1), compared to the *R. longirostris* (Q2) outgroup, *G. striatus*

The phylogeny tree construction of *G. philippensis* uses the neighbor joining method with a genetic distance of 2 kimura bootstrap parameters 1000x delivered in Figure 1, which was divided into two large clusters. Cluster A consists of 16 samples and cluster B only represented by

2 samples based on differences in nucleotides and genetic distance from each sample from four different locations. Even more clearly there were differences in interpopulation and intrapopulation, as well as similarities in intraspecies and interspecies. Samples that form clusters had slight differences (no by location). This can be explained by the fact that the four habitat sampling sites were the same even though the height of the sea level and the average location were different. The difference was only because the elders were too far away. The four sampling locations were quite far apart, but it was still possible for mating to occur between birds from one location, with another location as a population in one island typically. Another possibility that can occur was the accidental introduction by farmers or catchers. This can result in mating between birds in one location with another location. The phylogeny tree construction of *G. philippensis* originating from four populations in Minahasa compared to *G. philippensis* from Australia. and other groups of *R. longirostris*, were listed in Figure 1.

Based on the phylogeny tree above it appears that there were two main clusters, namely *G. philippensis* from Minahasa was in one cluster. The other cluster was *G. philippensis* from Australia, *R longirostris* and *Gallirallus striatus* in same branch.

A quite distant relationship between cluster Minahasa and Australia were limited distribution due to the flight range was close so that migration from Minahasa to Australia was not possible. This was supported by the theory of tectonic plate movements, namely Austaralia and Celebes (Minahasa) that were separated several thousand years ago, so that the Minahasa and Australian bird even though one species, but had different nucleotides caused by mutations resulting in evolution that can form new subspecies. The high variety of Weris haplotypes indicated cross-breeding between locations that had different haplotypes. Information about genetic diversity based on the variety of haplotypes makes Papontolen site was the most appropriate location for in-situ conservation. Conversely, by looking at a uniform haplotype, the ex-situ domestication of birds should take at the Wusa site. The breeding program through the domestication process was not only aimed at economic interests, but also to yield Weris bird that had phenotypes in accordance with the purpose of maintenance, for example producing birds for the production of meat, resistance to diseases, and so on. However, the results of captivity process was not returned to nature because it will affect the genetic purity of Weris in Minahasa. A uniform haplotype at also indicates that the ancestors did not originate from the Wusa due to this haplotype exists at the other three locations. Likewise be said that area had the most diverse haplotypes was the area of origin of the bird, in this case it should be assumed that the area was Papontolen.

If the alignment includes other species that were more closely related to *G. striatus* (Gen Bank JQ342144), *G. philippensis philippensis* (Gen Bank GJQ348003), *G. torquatus celebensis* (JQ347982), the number of nucleotides which was shorter is only 272 bp.

It can be seen (Figure 2) that *G. philippensis* from Minahasa formed a separate cluster different from *G. philippensis* from Australia and *G. philippensis philippensis*. This was likely a species of *G. celebensis* that was different from the two nearby species, based on large genetic distances. This was shown by cluster A, namely *G. philippensis* from Minahasa (1-16) with a genetic distance of 0.107, different from cluster B (17-21).

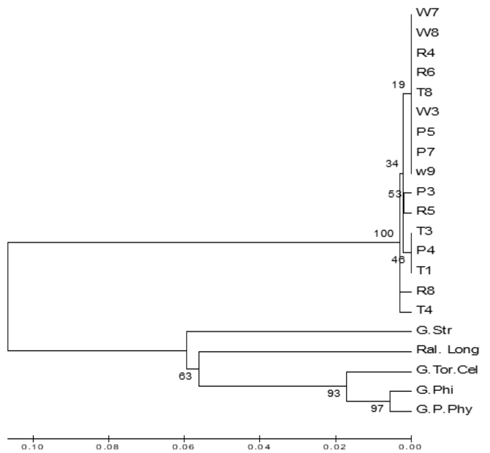


Figure 2. Phylogeny tree of Indonesia (Minahasa) *Gallirallus* sp. compared to 272 NT of Gen Bank Data

## CONCLUSIONS

*G. philippensis* in four locations in Minahasa, namely Papontolen, Ranoyapo, Tondano and

Wusa, were still closely related but had genetic differences with *G. philippensis* from *Australia* and *G. philippensis philippensis* therefore reinforcing the notion that Minahasa's Weris bird possibly will be a separate species *(Gallirallus celebensis)* from two species from Australia and the Philippines

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# RESEARCH OF MORPHOPRODUCTIVE PERFORMANCE OF THE MEAT GOAT POPULATION COMPARED TO CARPATINA GOAT BREED

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***Abstract***

*The ever-increasing requirements for goat meat production have led to create and consolidate a specialized goat population for meat production, well adapted to local environmental conditions, within the Research and Development Institute for Sheep and Goat Breeding - Palas. The new R1 population (75% Boer and 25% Carpatina) showed superior attributes compared to Carpatina breed. During the period of intensive fattening of the kids, the R1 males achieved an average daily gain of 152 grams compared to the group of males from the Carpatina breed, in which the increase was 119 grams/day. The R1 hybrids had 2.75 percentage points more muscle tissue in the carcass and 3.11 percentage points less bones than the Carpatina kids. The adult goats in the newly created population had a body compactness index with values between 84.26 and 94.31 and the muscularity index of the gigot had values of 245.58 - 249.01. In regards to our research was observed the superiority of R1 Boer x Carpatina goats compared to the Carpatina breed regarding to the meat production and the quality of carcass.*

***Key words**: carcass, goat, meat, Romania, yield.*

## INTRODUCTION

The orientation and development of goat breeding in the direction of meat production is determined by the market demand for lean meat, while there are trends to implement a diet, limited in fats and especially in saturated lipids. In recent years there has been an increased interest for goat meat, which provides high biological value proteins and healthy fats due to a high ratio of unsaturated and saturated fatty acids to a low in cholesterol contents (Van Niekerk et al., 1988). It was found that this meat has a lower fat content by 50-65% compared to beef, by 42-59% compared to lamb and 25% lower compared to veal; also, the quality of the fat is better, respectively the content in saturated fatty acids is lower by 40% compared to chicken meat (without skin) and comparisons made with beef, pork or lamb meat emphasize that it has 85%, 100% and 90% less saturated fatty acids (Colomer-Rocher et al., 1987).

The objective of the research consisted in creating and consolidating a meat goat population well adapted to the environmental conditions in Romania, through crossing Boer and Carpatina breed, obtaining R1 goat population (75% Boer x 25% Carpatina) and selecting in the direction of increasing and improving meat production.

## MATERIALS AND METHODS

The research was carried out on the new meat goat population and on the kids obtained at Research and Development Institute for Sheep and Goat Breeding Palas.

The main body dimensions were determined using the zoometer, compass and ribbon, calculating two conformation indices for goats and kids in the new population (Colomer-Rocher et al., 1987).

Breeding indices in mother goats (fertility, prolificacy, birth rate and survival of the kids until weaning) were determined compared to the values recorded by the goats Boer from South Africa, taken from the literature (Amoah et al., 1996; Crepaldi et al., 1999; Galina et al., 1995; Mellado et al., 2000).

Feed consumption was determined for weight gain in goats (subjected to intensive fattening for 120 days) $R_1$ Boer x Carpatina compared to Carpatina contemporaries, in which the growth performances were tested determining the weight increase, the nutrient consumption and the quality of the resulting carcasses.

The combined feed used had an energy content of 2570 kcal / kg, 16% raw protein, 3.50% raw fat and 8.50% raw cellulose.

At the end of the fattening period, the control slaughters were performed (3 animals from each batch), the slaughter yield was calculated (yield 1 and yield 2), and the carcasses were assessed.

The yield at slaughter and the existing differences (with statistical significance) between the $R_1$ Boer x Carpatina and Carpatina kids were determined, subsequently determining the section areas of the muscle *Longissimus dorsi* and the thigh section in the middle of the femur, perpendicular to its longitudinal axis. Section areas were determined using the autoCAD program (Van Niekerk et al., 1988).

Carcass conformation indices, genotype differences and statistical significance were determined (Pascal, 2015; Taftă, 1996).

The slaughter yield was calculated as follows:

$$\text{Yield 1} = \frac{\text{Weight of cooled carcass (kg)}}{\text{Living weight (kg)}} \text{ x } 100$$

$$\text{Yield 2} = \frac{\text{Weight of cooled carcass (kg)}}{\text{Empty living weight (kg)*}} \text{ x } 100$$

* Empty living weight = live weight from which the contents of the digestive tract have been subtracted.

Each carcass was cooled for 24 hours at +2 - +4°C, after weighing, it was sectioned into 2 half carcasses. All determinations were made on the right half of the carcass, the division into different commercial regions was made according to the French cutting system.

The gigot was separated from the carcass by sectioning between the sacrum joint and the 6th lumbar vertebra (L6). The shoulder blade was detached from the thoracic muscle insertion.

The rest of the carcass was represented by: neck, with the bone base of the 7 vertebrae; thorax, with the bone base of the 13 ribs and the sternum; lumbar area, with the bone base of the 6 lumbar vertebrae, all dressed in the afferent muscles, in the carcasses was also included abdominal muscles.

After cutting the carcasses into the 3 pieces (gigot, shoulder blade, the rest of the carcass), each piece was dissected, separating the muscles, fat (covering - ribs and intermuscular) and bones. Each tissue was then weighed to the nearest ± 5 grams.

The caliper measured the large diameter and small diameter of the *Longissimus dorsi muscle,* as well as the thickness of the surface fat layer, calculating conformation indices (Compactness Index of the Gigot - C.I.G.***, Muscularity Index of the Gigot - M.I.G.****) and differences between genotypes with statistical significance (Lu et al., 1988; Rău, 1989; Sodiq et al., 2004).

$$\text{*** C.I.G.} = \frac{\text{Width of coxofemural joints}}{\text{Length of gigot}} \text{ x } 100$$

$$\text{**** M.I.G.} = \frac{\text{Perimeter of gigot}}{\text{Length of gigot}} \text{ x } 100$$

The tissue structure of carcasses was established in $R_1$ Boer x Carpatina kids compared to Carpatina kids and differences between genotypes, with statistical significance (Fisher Test).

Statistical data processing was performed by classical methods (Sandu, 1995).

## RESULTS AND DISCUSSIONS

The main dimensions of goats were: a body length of 85.17 cm, the height at the withers and at the croup was 79.42 cm, the width at the shoulders 26.17 cm, the hip joint width 25.5 cm, rib width was 29.92 cm, chest depth 40.25 cm, the chest girth was 104.58 cm, the perimeter of the whistle was 12.25 cm, the perimeter of the hind leg was 73.42 cm and the length of the hind leg had an average value of 30.0 cm.

The goats had a trunk length of 70.67 cm, the height at the withers was equal to the height at the croup with a value of 68.67 cm, the width at the shoulders was 21.75 cm and at the hip joints it had an average value of 23.44 cm; the width at the ribs was 28.97 cm, the depth of the chest had an average value of 29.89 cm, the chest girth was 95.28 cm, the perimeter of the whistle was 10.69 cm, the perimeter of the hind leg was 61.33 cm and the length of the hind leg had an average value of 25.22 cm (Table 1).

Table 1. The main body dimensions of goats $R_1$ Boer x Carpatina by sex (cm)

| No. | Category | Trunk length | Withers height | Croup height | Shoulder width | Hip joint width | Rib width | Chest depth | Chest girth | Whistle perimeter | Hind leg perimeter | Hind leg lenght |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $x \pm s_x$ | $x \pm s_x$ | $x \pm s_x$ | $x \pm s_x$ | $x \pm s_x$ | $x \pm s_x$ | $x \pm s_x$ | $x \pm s_x$ | $x \pm s_x$ | $x \pm s_x$ | $x \pm s_x$ |
| 1. | Bucks | 85.17 ± 0.6945 | 79.42 ± 0.6793 | 79.42 ± 0.6793 | 26.17 ± 0.4410 | 25.50 ± 0.4523 | 29.92 ± 0.7732 | 40.25 ± 0.2500 | 104.58 ± 0.4840 | 12.25 ± 0.1306 | 73.42 ± 0.8390 | 30.0 ± 0.5222 |
| 2. | Goats | 70.67 ± 0.5941 | 68.67 ± 0.6812 | 68.67 ± 0.6812 | 21.75 ± 0.4358 | 23.44 ± 0.3636 | 28.97 ± 0.5920 | 29.89 ± 0.3223 | 95.28 ± 1.3499 | 10.69 ± 0.2463 | 61.33 ± 1.3431 | 25.22 ± 0.6981 |

In goats, the compactness index of the gigot had the value of 84.26 and the muscularity index of the gigot was 245.58, the value being lower than goats by 10.65% in the first index and by 1.38% in the second index (Table 2).

The number of goats $R_1$ Boer x Carpatina at breeding was 102 heads, of which they were mated 99 and gave birth 98, obtaining 152 kids, from which 135 were weaned (88.81%) resulting 1.38 kids weaned/goat (Table 3).

Table 2. Conformity indices on live animal in goats $R_1$ Boer x Carpatina

| No. | Category | Compactness index of the gigot (C.I.G.) | The muscularity index of the gigot (M.I.G.) | ± Differences between goats and goats | | | |
|---|---|---|---|---|---|---|---|
| | | | | C.I.G. | | M.I.G. | |
| | | $x \pm s_x$ | $x \pm s_x$ | MU | ( % ) | MU | ( % ) |
| 1. | Bucks | 84.26 ± 2.0343 | 245.58 ± 5.2409 | - 10.05 | - 10.65 | - 3.43 | - 1.38 |
| 2. | Goats | 94.31 ± 3.1712 | 249.01 ± 1.0019 | | | | |

Table 3. The result of mating in goats $R_1$ Boer x Carpatina, 2021 season

| No. | The goat stud (head) | Goats mounted (head) | Giving birth goats (head) | Born kids (head) | | | | Weaned kids | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Total | Alive | Dead | Aborted | Total | % | On giving birth goat |
| 1. | 102 | 99 | 98 | 152 | 135 | 16 | 1 | 135 | 88.81 | 1.38 |

In the giving birth 2021 season, the $R_1$ Boer x Carpatina goats had a fecundity of 98.99%, a prolificity of 155.10% and a birth rate of 137.76% (Table 4).

The weight of the male $R_1$ kids (75% Boer, 25% Carpatina) at birth was 2.87 ± 0.1677 kg and of the kids 2.51 ± 0.0900 kg. At the time of weaning, the weight of the kids of both sexes was similar.

The average daily gain made by male kids during this period was 125.9 g, 7.9% higher than the female kids - 116.60 (Table 5).

Table 4. Breeding indices in goats $R_1$ Boer x Carpatina in giving birth 2021 season

| No. | Fertility (%) | Prolificity (%) | Birth rate (%) |
|---|---|---|---|
| 1. | 98.99 | 155.10 | 137.76 |

Table 5. Body weight dynamics in kids from birth to weaning

| No. | Sex | Body weight (kg/head) | | Age at weaning (days) | Average daily gain (g/head) |
|---|---|---|---|---|---|
| | | At birth | At weaning | | |
| | | $x \pm s_x$ | $x \pm s_x$ | $x \pm s_x$ | $x \pm s_x$ |
| 1. | Males | 2.87 ± 0.1967 | 16.91 ± 1.1843 | 113.80 ± 0.9638 | 125.90 ± 11.9819 |
| 2. | Females | 2.51 ± 0.0900 | 16.14 ± 0.9599 | 116.40 ± 0.8327 | 116.60 ± 9.1119 |

In $R_1$ Boer x Carpatina kids, the average daily weight gain was 152 g compared to 119 g in the Carpatina, the difference of approximately 28% being statistically significant.

It is also observed that the $R_1$ Boer x Carpatina kids consumed to achieve 1 kg weight gain: 18095 Kcal, 1053 g digestible protein (DP) and 5790 g dry matter (DM) compared to contemporary Carpatina, who consumed 24138 Kcal, 1430.4 g DP and 7867.2 g DM, the differences between genotypes being 25%, 26.38% and 6.40% higher in the Carpatina than the $R_1$ Boer x Carpatina kids, being very statistically significant (Table 6).

Table 6. Consumption per kg gain in $R_1$ Boer x Carpatina kids compared to Carpatina

| No. | Genotype | Average daily gain (g/head) | Consumption per kg gain | | |
|---|---|---|---|---|---|
| | | $x \pm s_x$ | Metabolizable energy (Kcal) | Digestible protein (g) | Dry matter (g) |
| 1. | $R_1$ Boer x Carpatina | 152 ± 6.52 | 18095 | 1053 | 5790 |
| 2. | Carpatina breed | 119 ± 6.05 | 24138 | 1430.4 | 7867.2 |

R1 Boer x Carpatina kids had a slaughter yield of $Y_1$ with a value of 50.46% compared to 44.48% in the Carpatina and the $Y_2$ yield was for Boer x Carpatina 57.67% compared to 51.38% in the Carpatina (Table 7).

Table 7. Slaughter yield in $R_1$ Boer x Carpatina goats compared to the Carpatina

| No. | Genotype | Body weight (kg/head) | Cooled carcass weight (kg/head) | Empty lived weight carcass (kg/head) | Yield at slaughter $Y_1$ | $Y_2$ |
|---|---|---|---|---|---|---|
| | | $x \pm s_x$ | $x \pm s_x$ | $x \pm s_x$ | $x \pm s_x$ | $x \pm s_x$ |
| 1. | $R_1$ Boer x Carpatina | 39.40 ± 2.7221 | 18.19 ± 0.4130 | 34.88 ± 2.9127 | 50.46 ± 1.3262 | 57.67 ± 1.1159 |
| 2. | Carpatina breed | 34.67 ± 2.4340 | 15.49 ± 1.7555 | 30.07 ± 2.8085 | 44.48 ± 2.8787 | 51.38 ± 2.1068 |

It should be remarked that in terms of $Y_1$, the difference between $R_1$ Boer x Carpatina and Carpatina breed was 5.98 percentage points and in $Y_2$ the difference between $R_1$ Boer x Carpatina and Carpatina breed was 6.29 percentage points, both differences being very statistically significant (Table 8).

Table 8. Differentiation of slaughter yield and significance of differences

| No. | Genotype | Yield | | ± percentage points between $R_1$ Boer and Carpatina breed | | The meaning | |
|---|---|---|---|---|---|---|---|
| | | $Y_1$ | $Y_2$ | $Y_1$ | $Y_2$ | $Y_1$ | $Y_2$ |
| 1. | $R_1$ Boer x Carpatina | 50.46 | 57.67 | + 5.98 | + 6.29 | P <0.001 Very significant | P <0.001 Very significant |
| 2. | Carpatina breed | 44.48 | 51.38 | | | | |

In the $R_1$ Boer x Carpatina kids, the area of the *Longissimus dorsi* muscle section was 12.06 $cm^2$ compared to 8.38 $cm^2$ in the Carpatina, the difference of 3.68 $cm^2$ (44.0%) being very statistically significant (Table 9).

Table 9. Area of the *Longissimus dorsi* muscle section in goats in the meat goat population compared to Carpatina contemporaries

| No. | Genotype | The area of *Longissimus dorsi* muscle section ($cm^2$) | ± Differences between $R_1$ Boer and Carpatina breed | | |
|---|---|---|---|---|---|
| | | $x \pm s_x$ | $cm^2$ | % | The meaning |
| 1. | Meat goat population ($R_1$ Boer x Carpatina) | 12.06 ± 0.7419 | + 3.68 | + 44.00 | P <0.001 Very significant |
| 2. | Carpatina breed | 8.38 ± 1.0366 | | | |

The section area of thigh in the $R_1$ Boer x Carpatina kids had an average value of 103.95 $cm^2$ compared to 88.11 $cm^2$ in the Carpatina breed, the difference of 15.84 $cm^2$ (7.98%) in favor of the $R_1$ Boer x Carpatina kids, being very statistically significant (Table 10).

Table 10. Thigh section area (half of the femur perpendicular to its axis) in the new goat population compared to the Carpatina breed

| No. | Genotype | Thigh section area ($cm^2$) | ± Differences between $R_1$ Boer and Carpatina | | |
|---|---|---|---|---|---|
| | | $x \pm s_x$ | $cm^2$ | % | The meaning |
| 1. | Population $R_1$ Boer x Carpatina | 103.95 ± 5.5643 | +15.84 | +17.98 | P <0.001 Very significant |
| 2. | Carpatina breed | 88.11 ± 1.4580 | | | |

Research has shown that in kids $R_1$ Boer x Carpatina, compactness index of the gigot (C.I.G.) had an average value of 85.47 compared to 53.60 in the Carpatina contemporaries and the muscularity index of the gigot (M.I.G.) had a value of 211.97 in the $R_1$ Boer x Carpatina and 131.16 in the Carpatina, the differences between $R_1$ Boer and Carpatina kids of 31.87 MU in the first index and 80.81 MU in the second being very statistically significant (P <0.001) - according to Table 11.

Table 11. Live animal conformation indices in $R_1$ Boer x Carpatina males compared to Carpatina contemporaries

| No. | Genotype | Compactness index of the gigot (C.I.G.) * | The muscularity index of the gigot (M.I.G.) ** | ± Differences between genotypes and significance | |
|---|---|---|---|---|---|
| | | $x \pm s_x$ | $x \pm s_x$ | C.I.G. (MU) | M.I.G. (MU) |
| 1. | $R_1$ Boer x Carpatina | 85.47 ± 1.5408 | 211.9 7 ± 6.0285 | + 31.87 P <0.001 | + 80.81 P <0.001 |
| 2. | Carpatina breed | 56.60 ± 6.1748 | 131.16 ± 4.3824 | | |

The compactness index of the gigot (C.I.G.) for $R_1$ Boer x Carpatina male kids had an average value of 83.43 compared to 50.07 for Carpatina male kids and the gigot muscularity index (M.I.G.) had a value of 201.79l for $R_1$ Boer x Carpatina kids and 106.16 in the Carpatina breed. The differences between the $R_1$ Boer x Carpatina and Carpatina breed genotypes of 33.36 MU in the first index and 95.63 MU in the second, being very statistically significant (Table 12).

Table 12. Male kids carcass conformation indices $R_1$ Boer x Carpatina compared to Carpatina contemporaries

| No. | Genotype | Compactness index of the gigot (C.I.G.) | The muscularity index of the gigot (M.I.G.) | ± Differences between genotypes and significance | |
|---|---|---|---|---|---|
| | | $x \pm s_x$ | $x \pm s_x$ | C.I.G. (MU) | M.I.G. (MU) |
| 1. | $R_1$ Boer x Carpatina | 83.43 ± 2,0009 | 201.79 ± 1.1134 | 33.36 P <0.001 | 95.63 P <0.001 |
| 2. | Carpatina breed | 50.07 ± 3.66668 | 106.16 ± 6.6836 | | |

In the kids group of $R_1$ Boer x Carpatina, the half-carcass weighed 8.87 kg of which 5.57 kg muscle, 2.15 kg bone and 1.15 kg fat, while in the Carpatina kids carcass weighed an average of 7.46 kg of which 4.48 kg muscle, 2.04 kg bones and 0.94 kg fat (Table 13).

Table 13. The tissue structure of the carcass in according to genotype

| No. | Genotype | Carcass weight (kg) of which: | | | |
|---|---|---|---|---|---|
| | | Total | Muscle | Bones | Fat |
| | | $x \pm s_x$ | $x \pm s_x$ | $x \pm s_x$ | $x \pm s_x$ |
| 1. | $R_1$ Boer x Carpatina | 8.87 ± 0.1660 | 5.57 ± 0.2142 | 2.15 ± 0.0115 | 1.15 ± 0.0573 |
| 2. | Carpatina breed | 7.46 ± 0.7449 | 4.48 ± 0.5179 | 2.04 ± 0.1884 | 0.94 ± 0.0755 |

$R_1$ Boer x Carpatina kids have 2.75 percentage points more muscle in the carcass and 3.11 percentage points less bones compared to Carpatina kids, the differences being statistically significant ($p < 0.05$) (Table 14).

Table 14. Percentage tissue structure in carcasses

| No. | Genotype | Carcass weight of which (%) | | | | ± Differences between genotypes (percentage points) | | |
|---|---|---|---|---|---|---|---|---|
| | | Total | Muscle | Bones | Fat | Muscle | Bones | Fat |
| 1. | $R_1$ Boer x Carpatina | 100.0 | 62.80 | 24.23 | 12.97 | + 2.75 P <0.05 | - 3.11 P <0.05 | + 0.37 P <0.05 |
| 2. | Carpatina breed | 100.0 | 60.05 | 27.34 | 12.60 | | | |

## CONCLUSIONS

From research regarding the morpho-productive performances of the R1 goat population (75% Boer x 25% Carpatina) compared to the Carpatina breed, the following conclusions can be drawn:

The main body dimensions performed on the live animal showed higher values for bucks by 3.23 - 34.66% compared to goats.

Compactness index of the gigot and the muscularity index of the gigot had values of 84.26-94.31, respectively of 245.58-249.01, being lower for bucks by 10.65% and respectively by 1.38% compared to the goats.

Breeding index for $R_1$ goats had the following values: fecundity 98.99%, prolificacy 155.10%, birth rate 137.76%.

The average daily gain made by kids during the lactation period was 116.60-125.90 g, being 7.9% higher in males compared to females.

During the fattening period (120 days), the $R_1$ Boer x Carpatina kids made an average daily gain of 152 g, being by 28% higher compared to the Carpatina kids, in which the increase achieved was 119 g/day.

For one kg of live weight gain, the $R_1$ kids achieved a lower specific consumption by 25% Kcal, 26.38% DP and by 26% DS compared to the Carpatina contemporaries. Also, the efficiency of feed conversion in growth increase was higher by 15.2% in Boer x Carpatina.

The slaughter yield was 44.48 - 50.46 %, being 5.98 percentage points higher in the group of $R_1$ compared to the Carpatina goats, the differences being very significant ($P < 0.001$).

The area of the *Longissimus dorsi* muscle section was content between 8.38 $cm^2$ and 12.06 $cm^2$, being 44% higher in the $R_1$ kids (75% Boer x 25% Carpatina).

The area of the thigh section was 103.95 $cm^2$ for the group of $R_1$ and 88.11 $cm^2$ for the Carpatina breed, being larger for cross breeds by about 17.98%.

The conformation indices on the live animal, respectively the compactness index of the gigot and the muscularity index of the gigot, as well as the conformation indices of the carcass (the compactness and muscular indices of the gigot) presented superior values to the batch of $R_1$ compared to the group of Carpatina goats, the differences being very significant ($P < 0.001$).

The tissue composition of the carcass was defined by the following relative values: muscles 60.05 - 62.80%, bones 24.23 - 27.34%, fat 12.60 - 12.97%, $R_1$ Boer x Carpatina products with 2.75 percentage points more muscle tissue in the carcass and 3.11 percentage points less bone compared to Carpatina kids, the differences being significant ($P < 0.05$).

The data obtained reveal the superiority of the population of $R_1$ 75% Boer x 25% Carpatina compared to the Carpatina breed in all morpho-productive traits, reproduction indices, slaughter yield and carcass quality indices.

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# THE USE OF DAIRY COWS OF FRENCH BREEDING IN THE CONDITIONS OF UKRAINE

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***Abstract***

*The study of milk productivity of French Holstein and Montbéliard breeds is regarded in the article, the uniformity of lactation is investigated, the lactation activity is assessed, as well as the cows are evaluated by the shape of their udder. The obtained results make it possible to affirm that the breed affects the production efficiency and the quality indicators of milk from cows of different French breeds, which are used in Ukraine. The cows of Holstein breed have a uniform course of lactation, high and constant productivity in terms of lactations and the better milk yield, but their milk quality indicators are inferior to those in the cows of Montbéliard breed. The cows of Montbéliard breed have a constant productivity and a uniform flow of lactation, they give high milk yield immediately after calving, but later they rapidly decrease productivity.*

***Key words***: *breed, fat, Holstein breed, lactation, milk yield, Montbéliard breed, productivity, protein.*

## INTRODUCTION

The factors determining milk productivity include breed, type of livestock, feeding, keeping, milking technique, age, body weight, dry period and calving interval (Basovsky et al., 2001; Piddubna & Pelekhaty, 2012; Marin et al., 2020).
The cattle breed is the main factor affecting milk yield, its composition, physicochemical and technical properties of milk. Changes in the milk composition of the same breed cows are explained by hereditary factors, as well as by different housing conditions (Grymak et al., 2020). Since only the ability to produce a certain amount of milk with an approximately constant composition (milk yield) is inherited, the conditions for keeping cows are of great importance for its realization (Mashkin & Parish, 2006).
One of the main characteristics in the breeding of dairy cows is their dairy productivity, exterior constitutional features and reproductive qualities. The breeding work should be carried out on the selection of cows of a strong type of constitution, which, according to the main characteristics, is the most consistent with the desired. This will increase the milk productivity of livestock and will not lead to a decrease in their reproductive ability (Mashkin & Parish, 2006; Oltenacu & Broom, 2010).
Modern breeding programs should be aimed at the genetic improvement of production characteristics such as milk yield, growth rate, live weight and reproductive function. At the same time, an increase in milk yield should not be accompanied by a decrease in fertility, an increase in limb diseases, metabolism and a decrease in life expectancy. Therefore, these programs are often developed on the basis of the breeds of the world's best gene pool: Holstein, Simmental, Montbéliard, Charolais and others (Oltenacu & Broom, 2010; VanRaden & Cole, 2000; Uzun & Koç, 2019).
Tellah et al. (2019) investigated the influence of the genotype of breeding bulls of Holstein, Montbeliard and Jersey (Holstein, Montbéliard and Jersey) breeds on improving the milk productivity of local breeds (Kuri & Bokolodji). The analysis of productivity of the dairy herd showed that the amount of produced milk varied depending on the genotype of the crossbred cows. Crossbreeds of Holstein, Montbéliard and Jersey bulls with local breeds (Kuri and Bokolodji) are more favorable for increasing milk yield, but reproductive indicators (age of first calving and calving interval) must be taken into account.

Getu et al. (2016) dealt with the problems of crossing and its impact on the productivity of native dairy cattle. The studies have shown that when using bulls of the Holstein breed, the indicators of milk productivity (both in quantity and quality), as well as functional conformational signs of the exterior have been significantly improved; the type of body structure has become more consistent with the dairy type.

Holstein, Dutch, Montbéliard cattle, Jersey, Charolais and other breeds are promising for improving milk productivity, body structure, lactation characteristics, milk quality, morphological and functional characteristics of the udder, milk yield depending on the cow's level of development and live weight, as well as the age of its first insemination and calving (Mirulugovna, 2020; Bensaha & Arbouche, 2014; Vallée-Dassonneville, 2017; Vitorino et al., 2017; Tawah et al., 1999).

The Holstein-Friesian breed dominates in the dairy sector, although there is a general concern that these cows may not be well adapted to certain climatic conditions and livestock farming technologies. Rodríguez-Bermúdez et al. (2019) compared the reproductive ability of the Holstein-Friesian breed in different livestock management systems, as well as with other breeds and crosses having been used. The obtained results give grounds to assert that the reproductive ability of the Holstein-Friesian breed is limited not by the technology of management, but by the manifestation of estrus, which is less pronounced in Holstein than in other breeds. Therefore, the reproductive breeding and proper management of estrus detection will improve the reproductive indicators in the cows of Holstein-Friesian breed.

The Montbéliard breed is a part of the Simmental breed and is very widely used to improve the productive, reproductive and technological qualities, as well as the harmonious body structure of cows. The Montbéliard breed spread from France to other European countries, especially Switzerland, Romania, Bulgaria, Moldova and Poland (Vidu et al., 2011; Ponsart et al., 2014).

Over the past 40 years, the milk productivity of cows has significantly increased due to the use of breeds of world leaders in the milk production. Different foreign breeds are also widely used for intensive livestock farming in Ukraine. Therefore, the aim of our study is to analyze the main indicators of milk productivity in the cows of different French breeds, which are used in Ukraine.

## MATERIALS AND METHODS

The production facilities of the enterprise are located in the village of Mukhovtsy of Nemirov district, Vinnytsia region. The enterprise has a fully completed production cycle of dairy products: its own arable land for growing feed, the specialized equipment for harvesting a forage base, modern premises for keeping cows, a milking parlor equipped with a milking complex, as well as the modern equipment for storing, cooling and processing milk.

All equipment for the farm and dairy plant was purchased in Germany and Israel; the genetic material for breeding dairy cows was imported from France. The cows on the farm are kept unleashed, with free access to feed, water, and walking areas.

In order to conduct the research, experimental groups from Holstein cows (n=40) and Montbéliard cows (n=34) were formed.

The data on milk productivity of cows, including milk yield per lactation, the average daily milk yield, the fat content in milk, the amount of milk fat, the protein content, the amount of milk protein and live weight were used as the material for the study.

The research used methods generally accepted in zootechnics, while the methods of variation statistics were used for biometric data processing (Basovsky et al., 2001; Lakin, 1990).

In order to assess the lactation curve, the following indices were determined: (Orikhivskyi et al., 2019)

The first way - according to the formula of Turner:

$$ILC_1 = \frac{C}{D},$$

where: $ILC_1$ is the index of lactation constancy;

C - milk yield per lactation, kg;

D - the highest milk yield per month, kg.

The second way - according to the formula of Johansen and Hansson:

$$ILC_2 = \frac{a \times 100}{в},$$

where: $ILC_2$ is the index of lactation constancy;
a - milk yield from the 101st to the 200th day of lactation, kg;
c - milk yield from the 1st to the 100th day of lactation, kg.
The third way is the index of milk yield decline up to 7 months:

$$IYD = \frac{a}{B} \times 100,$$

where: IYD is the index of milk yield decline up to 7 months;
a - actual milk yield for the first 7 months, kg;
B - total milk yield per lactation.

## RESULTS AND DISCUSSIONS

In an unstable economic environment, when restructuring dairy farms, the choice of cattle for breeding can determine the economic effect of running the industry and become a big problem. Since each breed reacts in its own way to the process of adaptation to certain environmental conditions, fodder resources and the technology of animal husbandry.

The Holstein cows of various breeding occupy the main segment for milk production in Ukraine, but French breeding cows have been studied rather poorly. In recent years, other foreign breeds of dairy production have also begun to be used. There is practically no data on the use of Montbéliard breed in Ukraine, which aroused our interest in this study.

**Milk productivity in the cows of different French breeds.** Studies have determined that the average milk yield per lactation in the herd of Holstein cows was 7662 kg, which significantly (P<0.001) exceeded the milk yield of Montbéliard cows by 13.2% (Table 1).

Table 1. Milk productivity in the cows of French breeding, $\overline{X} \pm S\overline{x}$

| Indicators | Lactation | | | |
|---|---|---|---|---|
| | I | II | III and older | $\overline{X}$ for herd |
| Holstein breed | | | | |
| n | 9 | 12 | 29 | 40 |
| Milk yield per lactation, kg | 6948±185.4 | 7689±190.5 | 8350±124.7 | 7662±163.9 |
| Average daily milk yield, kg | 27.8±0.55 | 39.4±0.89 | 48.0±0.44 | 38.3±0.83 |
| Fat content, % | 3.69±0.01 | 3.72±0.01 | 3.78±0.02 | 3.73±0.02 |
| Amount of milk fat, kg | 265.2±5.45 | 2.0,8±6.78 | 325.1±7.65 | 293.7±7.84 |
| Protein content, % | 3.14±0.02 | 3.24±0.01 | 3.28±0.01 | 3.22±0.01 |
| Amount of milk protein, kg | 213.1±5.74 | 229.1±5.99 | 254.0±6.32 | 232.1±5.24 |
| Live weight, kg | 536±6.78 | 585±5.90 | 612±7.23 | 577.7±6.89 |
| Montbéliard breed | | | | |
| N | 7 | 6 | 21 | 34 |
| Milk yield per lactation, kg | 5840±145.8*** | 6641±173.6*** | 7479±128.1*** | 6653±154.7*** |
| Average daily milk yield, kg | 25.5±0.63* | 32.9±0.78*** | 39.0±0,80*** | 32.5±0.78*** |
| Fat content, % | 4.01±0.01*** | 4.13±0.01*** | 4.16±0.03*** | 4.10±0.02*** |
| Amount of milk fat, kg | 289.1±6.52* | 354.0±7.30*** | 382.0±7.42*** | 341.7±6.94*** |
| Protein content, % | 3.20±0.01* | 3.35±0.01*** | 3.43±0.02*** | 3.32±0.01*** |
| Amount of milk protein, kg | 255.6±6.60*** | 267.4±6.32*** | 295.3±5.45*** | 273.5±6.34*** |
| Live weight, kg | 545±5.80 | 588±4.09 | 634±6.14* | 589±5.35 |

*Note:* * P≤0.05, ** P<0.01, *** P<0.001 compared to Holstein breed

The cows of Holstein breed also reliably (P<0.05–0.001) dominated the animals of Montbéliard breed in terms of milk yield for the first, second, third and older lactations by 10.4-15.9%; in terms of the average daily milk yield - by 8.2-23.0%.

The quality indicators of milk in the cows of Montbéliard breed reliably (P<0.05-0.001) dominated those of Holstein breed in terms of the fat content in milk by 0.4% (P<0.001), of the amount of milk fat - by 16.3% (P<0.05-0.001), of the protein content - by 0.1% (P <0.05-0.001) and the milk protein content - by 17.8% (P <0.001).

It was also determined a significant difference between the qualitative indicators of Holstein and Montbéliard cows (P<0.05-0.001) in the first, second, third and older lactations. The difference varied in the fat content within the range of 0.32-0.41%, in the amount of milk fat -

9.0-21.7%, in the protein content - 0.06-0.15% and the amount of milk protein - 16.3-19.9%.

There was no significant difference between the live weight of cows in terms of the average indicator by the herd, except for the indicator of cows of the third lactation and older.

Balandraud et al. (2018) studied the phenotypic differences between Montbéliard and Holstein breeds. In the similar production environment, the Montbéliard cows were found to produce by about 12% less milk than the Holstein cows, but the fat and protein content in their milk was higher.

The age of the first calving in Holstein cows was, on average, by 3 months earlier than in Montbéliard cows, but with a lower reproductive function (the interval between calving was by 25 days longer) and more frequent mastitis.

When analyzing the indicators of characteristics variability (Table 2), it was found that the variation coefficient of milk yield in Holstein cows was 15.8% (high characteristics variability); the coefficient of the average daily milk yield was 16.8% (high characteristics variability); the coefficient of fat content in milk was 3.9% (low characteristics variability); the coefficient of the amount of milk fat was 15.5% (high characteristics variability); the coefficient of protein content was 2.1% (low characteristics variability); the coefficient of the amount of milk protein was 18.9% (high characteristics variability); the coefficient of live weight was 5.2% (medium characteristics variability).

Table 2. Indicators of characteristics variability in the dairy cows of French breeding ($C_v$), %

| Indicators | Lactation | | | |
|---|---|---|---|---|
| | I | II | III and older | $\overline{X}$ for herd |
| Holstein breed | | | | |
| n | 9 | 12 | 29 | 40 |
| Milk yield per lactation, kg | 12.8 | 16.4 | 18.3 | 17.8 |
| Average daily milk yield, kg | 8.2 | 90.1 | 12.1 | 9.6 |
| Fat content, % | 2.4 | 5.5 | 3.8 | 3.9 |
| Amount of milk fat, kg | 15.0 | 14.7 | 16.9 | 15.5 |
| Protein content, % | 1.9 | 2.8 | 1,6 | 2.1 |
| Amount of milk protein, kg | 17.3 | 20.4 | 18.9 | 18.9 |
| Live weight, kg | 6.0 | 4.5 | 5.2 | 5.2 |
| Montbéliard breed | | | | |
| n | 7 | 6 | 21 | 34 |
| Milk yield per lactation, kg | 16,.2 | 18.6 | 13.8 | 16.2 |
| Average daily milk yield, kg | 6.6 | 9.7 | 8.9 | 8.4 |
| Fat content, % | 1.9 | 3.4 | 5.9 | 3.7. |
| Amount of milk fat, kg | 18.1 | 12.7 | 10.1 | 13.6 |
| Protein content, % | 1.8 | 2.6 | 4.0 | 2.8 |
| Amount of milk protein, kg | 16.6 | 17.9 | 20.5 | 18.3 |
| Live weight, kg | 3.2 | 5.9 | 8.0 | 5.7 |

The indicator of the variation coefficient of milk yield in the cows of Montbéliard breed was 16.2% (high characteristics variability); the coefficient of the average daily milk yield was 8.4% (medium characteristics variability); the coefficient of fat content in milk was 3.7% (low characteristics variability); the coefficient of the amount of milk fat was 13.6% (medium characteristics variability), the coefficient of protein content was 2.8% (low characteristics variability); the coefficient of the amount of milk protein was 18.3% (high characteristics variability); the coefficient of live weight was 5.7% (medium characteristics variability).

Consequently, the coefficient of variability of milk yield, fat and protein content in milk is not constant and is corrected by the influence of environmental factors, the intensity and type of breeding, as well as by the genetic structure of the herd.

It has been found that the indicators of milk productivity of Holstein cows versus those of Montbéliard cows are characterized by a high degree of phenotypic variability.

**The uniformity of lactation in the cows of French breeding.** Milk productivity largely depends on the nature of the formation of cows' lactation, the value of the maximum milk yield

and the ability to maintain milk yield at a certain level for a long time. The uniformity of lactation is characterized by a coefficient of constancy, which can be determined in various ways (Karateeva & Polishchuk, 2018).
Under the optimal conditions of feeding and keeping cows, daily milk yield in the first months of lactation, as a rule, increases and reaches a maximum in the second month of lactation. Subsequently, milk yield for lactation decreases and the lactation curve falls. But our studies have defined a different intensity of lactation decline and the level of milk yield depends on the breed characteristics of dairy cows (Orikhivskyi et al., 2019).
The studies have determined that the milk yield of Holstein cows was significantly higher (Table 3).

Table 3. Indicators of lactation constancy in the dairy cows of French breeding, $\overline{X} \pm S_{\overline{x}}$

| Indicators | Lactation | | | |
|---|---|---|---|---|
| | I | II | III and older | $\overline{X}$ for herd |
| Holstein breed | | | | |
| n | 9 | 12 | 29 | 40 |
| Milk yield per lactation, kg | 6948±185.4 | 7689±190.5 | 8350±124.7 | 7662±163.9 |
| Index of lactation constancy according to Johansen and Hansson, % | 91.9±0.95 | 90.4±1.54 | 96.3±0.83 | 90.1±1.84 |
| Index of lactation constancy according to Turner, % | 7.7±0.25 | 7.9±0.41 | 8.2±1.10 | 7.9±0.83 |
| Index of milk yield decline up to 7 months, % | 80.5±1.67 | 83.4±2.14 | 88.1±1.85 | 84.0±1.94 |
| Montbéliard breed | | | | |
| n | 7 | 6 | 21 | 34 |
| Milk yield per lactation, kg | 5840±145.8*** | 6641±173.6*** | 7479±128.1*** | 6653±154.7*** |
| Index of lactation constancy according to Johansen and Hansson, % | 92.4±1.12 | 95.6±1.39* | 94.3±1.84 | 94.1±2.14 |
| Index of lactation constancy according to Turner, % | 7.5±1.10 | 7.6±1.82 | 7.9±0.93 | 7.7±1.64 |
| Index of milk yield decline up to 7 months, % | 82.8±2.51 | 84.9±1.74 | 85.9±1.65 | 84.5±2.10 |

*Note:* * P≤0.05, ** P<0.01, *** P<0.001 compared to Holstein breed

There was no significant difference in the groups between the indices of lactation constancy and the decline in milk yield, except for the indicator of the second lactation of Holstein cows.
The data in the Table show that lactation of experimental cows of both breeds is characterized by a high indicator of lactation constancy according to Johansen and Hanson and Turner (90.1 and 94.1%; 7.7 and 7.9%). The index of milk yield decline in Holstein and Montbéliard cows was at the same level.
So, the main indicator characterizing the lactation activity of dairy cattle is the amount of milk received during lactation, and the latter one is due to high milk yields per month and the stability of lactation. The lactation in the cows of Holstein and Montbéliard breeds is characterized by constancy, since the higher are the indices, the more stable is the lactation curve of the cows under study.

**Assessment of the cows' lactation activity.**
Milk productivity of a dairy herd is one of the main indicators characterizing the efficiency of dairy cattle breeding. The optimal internal and external factors of cow productivity directly depend on the dynamics of milk yield during lactation, displayed by the lactation curve. In the production conditions, preference is given to the cows, the yield curve of which gradually increases and decreases evenly, which means that such animals have a high lactation activity. The high and stable lactation curve indicates the cow's ability to withstand a prolonged physiological stress for a long time (Orikhivskyi et al., 2019; Karateeva & Polishchuk, 2018).
But even if these conditions are optimal, the productivity is uneven during lactation. The

highest milk yield occurs in the first 2-3 months after the calving of cows, and then begins to gradually decrease until the end of lactation. The period of calving has a significant effect on reducing milk yield.
There are several ways to assess the course of lactation activity, among which the simplest is to graphically depict changes in daily or monthly milk yield.
The study of the lactation curves of Holstein cows shows that the animals had the maximum productivity during the $2^{nd}$ month of lactation, and then the lactation curve passed with different intensities (Figure 1).

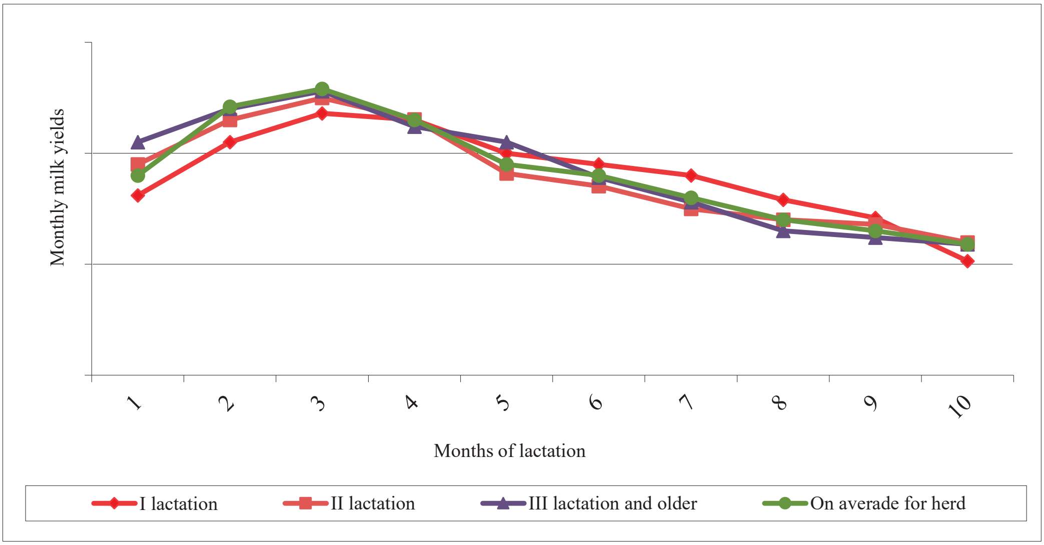


Figure 1. Lactation curves of monthly milk yield in the cows of Holstein breed

At the same time, the cows of the third and older lactations had the highest monthly milk yields; subsequently, they gradually decreased monthly with a rapid fall after the fifth month of lactation. In this case, the animals first increase milk secretion at the expense of a physiological maximum. The lactation curve is characterized by a high index of milk yield decline, which indicates that the conditions of keeping, feeding and using animals of this breed provide the highest productivity. However, in such conditions, the cows of the second lactation reduce productivity with a slightly more intense decline from the third to the fifth month of lactation. On average for a herd of Holstein cows, the curve is characterized by a similar decline from the third to the fifth month of lactation.
The lactation curve of the first lactation cows showed a high milk yield after calving in the second or third month of lactation and was characterized by a stable decrease in milk yield in the following months until the end of lactation.
The milk yield curves in Montbéliard cows were characterized by rapid growth to the peak of lactation with a decrease by certain months and a subsequent gradual decrease until its end (Figure 2).
The lactation curve in the cows of the first lactation increased until the second month of lactation and gradually decreased until its end. The curve in the cows of the third and older lactations increased until the second month and fell rapidly until the fifth; then a gradual decrease was recorded.
The second lactation of experimental cows is characterized by a rapid increase in milk yield until the second month of lactation, decreasing to the fourth month; after that a stable decrease in milk yield was observed in the following months until the end of lactation.
On average, the herd of Montbéliard cows showed a rapid increase by the second month, a stable decrease until the fourth month of lactation and a gradual decrease until its end.

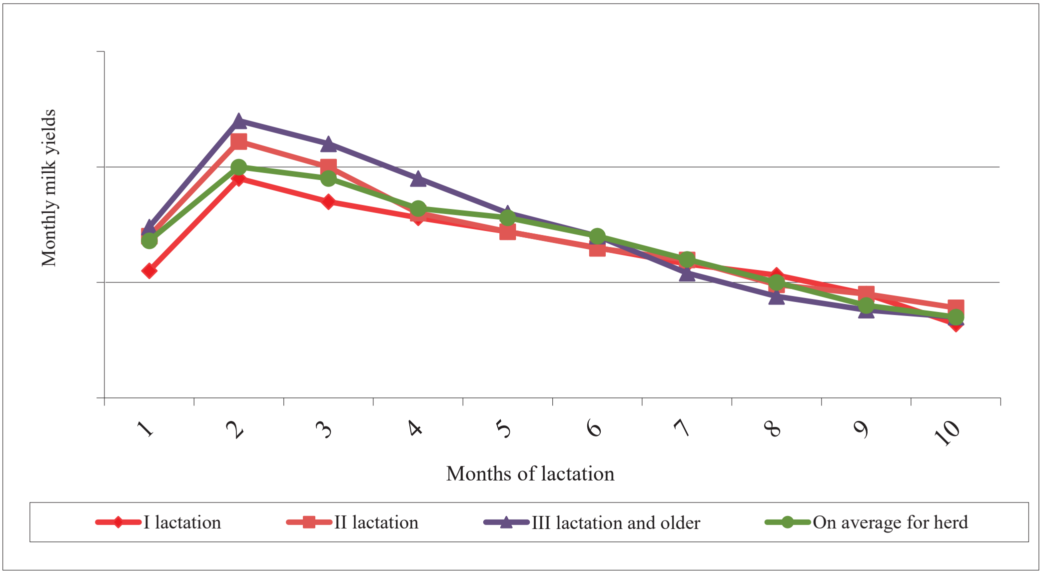


Figure 2. Lactation curves of monthly milk yield in the cows of Montbéliard breed

Consequently, the formation of lactation curves makes it possible to determine a positive or negative effect on the productivity of the breed characteristics of cows. By the course of lactation, the Holstein cows can be attributed to the type of cows with high and constant productivity and a uniform course of lactation, while the Montbéliard breed to those that immediately after calving show high productivity, which subsequently sharply decreases; the lactation curve after a short upward movement quickly decreases.

A number of foreign scientists have also been involved in identifying different forms of the lactation curve for dairy cattle using empirical mathematical models (Macciotta et al., 2019; Congleton & Everett, 1980; Landete-Castillejos & Gallego, 2000).

The correlation between mathematical characteristics and lactation curve shapes were analyzed by Macciotta et al. (2005) by selecting several common functions (Wood incomplete gamma, Wilmink's exponential, Ali and Schaeffer's polynomial regression, and fifth-order Legendre polynomials). Among the studied models, the three-parameter models (Wood and Wilmink) corrected the lactation activity better and were able to identify 2 main groups of the curve shape: standard and atypical. The five-parameter models (Ali and Schaeffer's function and Legendre polynomials) were able to recognize more curve shapes. However, each group of curves can be considered as a result of a certain deformation of two main forms, standard or atypical, which become more variable due to the presence of waves in the second half of lactation.

The other authors state that the appearance of curves without peak lactation is mainly the result of a lack of recordings in the early days of lactation (Congleton & Everett, 1980).

Landete-Castillejos & Gallego (2000) also reported about differences in the ability of models to describe different forms of the lactation curve.

**Assessment of dairy cows of French breeding according to the udder shape.** The assessment of shape, size and health of the udder in the lactating cows during breeding is of great importance since the minimum body weight is reached earlier than the peak milk yield, and much earlier than the peak feed intake. The genetic breeding for a higher milk yield will result in the cows with lower body weight gain during lactation and therefore the cows that are less well prepared to meet the energy requirements of subsequent lactation. The breeding of cows based on milk production alone will increase the physiological load on the udder and therefore increase the incidence of mastitis (Søndergaard et al., 2002).

A deep and well-attached udder is closely associated with high productivity. The udder must be well attached to support more milk without functional problems. The placement of the teats for mechanical milking should be vertical and their size should be the same to fit the teat cup. Therefore, we were tasked with assessing the shape and size of the udder in the dairy cows of studied groups, as well as their suitability for machine milking technologies.

It was found that among the Holstein cows the majority of animals (70%) had a bath-like udder shape, while the percentage among the Montbéliard breed was 74% (Table 4).

Table 4. Assessment of dairy cows of French breeding according to the udder shape

| Indicators | Parameters including the udder shape | | | | | | |
|---|---|---|---|---|---|---|---|
| | bath-like | | | cupped | | | $\overline{X}$ by herd |
| | *n* | % | $\overline{X} \pm S\overline{x}$ | *n* | % | $\overline{X} \pm S\overline{x}$ | $\overline{X} \pm S\overline{x}$ |
| Holstein breed, n=40 | | | | | | | |
| One-time milk yield, kg | 28 | 70 | 18.6±0.31 | 12 | 30 | 18.1±0.26 | 18.4±0.28 |
| Time of milking, min | 28 | 70 | 9.62±0.220 | 12 | 30 | 8.33±0.32 | 8.85±0.193 |
| Intensity of milk flow, kg/min | 28 | 70 | 2.08±0.018 | 12 | 30 | 2.09±0.026 | 2.08±0.022 |
| Montbéliard breed, n=34 | | | | | | | |
| One-time milk yield, kg | 25 | 74 | 17,.6±0.29* | 9 | 26 | 17.4±03.8 | 17.5±0.33* |
| Time of milking, min | 25 | 74 | 9.62±0.351 | 9 | 26 | 9,49±0,362* | 9.56±0.356 |
| Intensity of milk flow, kg/min | 25 | 74 | 1.84±0.026*** | 9 | 26 | 1,82±0,032*** | 1.83±0.029*** |

*Note:* * P≤0.05, ** P<0.01, *** P<0.001 compared to Holstein breed

As a result of the studies carried out, it was found that the cupped shape of the udder is characteristic for both Holstein (30%) and Montbéliard cows (26%). The animals of Holstein breed are characterized by high intensity of milk yield (2.08 kg/min) under the conditions of milking on the automated installation “Parallel”, which was by 12.0% higher than the same indicator of Montbéliard breed (1.83 kg/min) (P≤0.001). This is due to their manufacturability and better suitability for machine milking and intensive technology conditions.

In order to assess effectively the udder condition of highly productive cows during their breeding for machine milking at modern dairy complexes, Palii et al. (2020) developed a methodology that provides the classification of the udder into the following categories: Category I (not suitable); II category (suitable); Category III (not suitable). The minimum percentage of cows leaving the main herd according to this method was determined by linear assessment indicators: the udder depth (6 points), the attachment of the front lobes of the udder (5 points) and the placement of the front teats (6 points).

Gussmann et al. (2019) used records from the Danish cattle database to identify factors associated with culling at different stages of lactation, with particular attention to udder health. The authors proved that most of the factors were predominantly motivating factors for culling, however, high average milk yield reduced the possibility of culling. For early lactation cows, the treatment of dry cows in the previous lactation also reduced the risk of culling in most herds.

## CONCLUSIONS

Thus, the obtained results make it possible to assert that the French breeds of cows are well adapted for breeding in Ukraine, and their breed influences the efficiency of milk production. Based on the foregoing, it can be concluded that Holstein cows, in terms of lactations, are characterized by better milk yield, but at the same time they are inferior to Montbéliard cows in terms of quality indicators, namely, the content and amount of milk fat and protein.

It has been proved that lactation in the experimental cows of both breeds is characterized by a high coefficient of lactation constancy and index of milk yield decline, which indicates the stability of their lactation curves. By the course of lactation, the Holstein cows can be attributed to the type of cows with high and constant productivity and a uniform course of lactation, while the Montbéliard breed to those that immediately after calving show high productivity, which subsequently sharply decreases. Their lactation curve after a short upward movement quickly decreases.

It was found that among the Holstein cows the majority of animals (70%) had a bath-like udder shape, while the percentage among the Montbéliard breed was 74%. The animals of Holstein breed are characterized by high intensity of milk yield under the conditions of milking on the automated installation "Parallel", which was higher than the same indicator of Montbéliard breed.

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# PARTIAL RESULTS OF GENETIC ANALYSIS IN ROMANIAN TROTTER HORSE FROM DOR MARUNT STUDFARM - REPRODUCTIVE ISOLATION AND AGE STRUCTURE

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***Abstract***

*The paper aimed to present an important part of genetic analysis in Romanian Trotter horse. The sample extracted from the population is represented by entire reproductive nucleus of Romanian Trotter breed from Dor Marunt studfarm. We analyzed the reproductive isolation and the age structure. The reproductive isolation are the most important criteria for a flock to be accepted as a population. The other three are morphological and physiological differences, environmental requirements and genetic size, but all these three criteria evolving according to reproductive isolation coefficient. The age structure have an important role in animal breeding (horse breeding in this case) and also in exploitation. Both analyzed components have a capital importance in animal breeding because there has a directly influence in animal population evolution. The reproductive isolation situation was quantified using the relation elaborated by S. Wright and the age structure situation is based on the age distribution histogram.*

***Key words***: *age structure, reproductive isolation, Trotter.*

## INTRODUCTION

The reproductive isolation is the most important criteria for a flock to be accepted as a population (Draganescu, 1979; Popescu-Vifor, 1990). In case of sport horses the situation is a little bit different because the stallions with great sportive performances have the wright to be used for reproduction in other breeds than the one it belongs to (Marginean et al., 2005). The explication is very simple: the breeders are focused in principal on performance and not on genetic conservation. But even in this case of sport horses we can talk about infusion and not about absorption. In order to elaborate strategies for inbreeding management, or breeding programme, we must start from genetic analysis (Maftei, 2011). Only in this situation it is possible to obtain great individuals, with a high genetic value, capable to be the parents of a new and valuable generation. Of course, in the same time we will assure about a better genetic and economic efficiency (Maftei et al., 2011; 2022). Regarding the age structure, Regarding the age structure, it has been shown that it directly influences the generation interval and population variability (Popa, 2009). In the same time the age structure is very important in exploitation because influenced the average age directly (Marginean, 2012).

## MATERIALS AND METHODS

The biologic material it is represented by entire reproductive nucleus of Romanian Trotter horse from Dor Marunt stud, 60 individuals, 6 stallions (Table 1) and 54 broodmares (Table 2).

Table 1. The sire stallions active in the reproductive nucleus of Romanian Trotter horse

| No. | Name | Year of birth | Specification |
|---|---|---|---|
| 1 | NUROFEN | 2002 | II |
| 2 | VIS | 2002 | AI |
| 3 | BIZAR | 2004 | AA |
| 4 | OLIMP | 2009 | AA |
| 5 | VARTEJ | 2009 | AA |
| 6 | NELUTU | 2010 | AA |

The reproductive isolation coefficient was calculated using the relation developed by S. Wright:

$$R.I.C. = \frac{AA - (AI + II)}{AA + AI + II}$$

where: AA - number of individuals, from reproductive nucleus, with both autochthonous parents;

- AI - number of individuals, from reproductive nucleus, with one autochthonous and one immigrant parent;
- II - number of individuals, from reproductive nucleus, with both immigrants parents.

Table 2. The broodmares from reproductive nucleus of Romanian Trotter horse

| No | Name | Year of birth | Specification |
|---|---|---|---|
| 1 | KINTA | 1998 | AI |
| 2 | RECEPTIA | 1998 | AI |
| 3 | CAMILA | 1998 | AI |
| 4 | STEMATA | 1998 | AA |
| 5 | VRAJA ZORILOR | 1998 | AI |
| 6 | SIAMEZA | 1999 | AI |
| 7 | BRENDA | 1999 | AI |
| 8 | KATRINA | 2000 | AI |
| 9 | RAZA DE LUNA | 2000 | AA |
| 10 | SIMETRIA | 2000 | AI |
| 11 | SOGUNA | 2000 | AI |
| 12 | IALTA NU | 2001 | AA |
| 13 | SOLOMIA | 2001 | AA |
| 14 | NEVADA | 2003 | AA |
| 15 | VRAJA SOU | 2003 | AI |
| 16 | REGINA ANA | 2003 | AI |
| 17 | KATIUSA | 2004 | AI |
| 18 | ROMANITA | 2004 | AA |
| 19 | DIACONITA | 2005 | AA |
| 20 | OSANDA | 2005 | AA |
| 21 | PAMFILA | 2005 | AA |
| 22 | VRAJA LIREI | 2005 | AI |
| 23 | AMICA III | 2007 | AI |
| 24 | PAMELA | 2007 | AA |
| 25 | NEDORA | 2007 | AA |
| 26 | ONDA | 2007 | AA |
| 27 | SARA | 2007 | AA |
| 28 | VOIAJORA | 2007 | AI |
| 29 | RELAXA | 2008 | AI |
| 30 | REGINA ANTOANETA | 2009 | AA |
| 31 | SULTANA | 2009 | AA |
| 32 | VICTORIA | 2009 | AA |
| 33 | KITTY | 2010 | AA |
| 34 | SORANA | 2011 | AA |
| 35 | KISS ME | 2011 | AA |
| 36 | SERENA | 2011 | AA |
| 37 | IRENA | 2011 | AA |
| 38 | FINUTA | 2012 | AA |
| 39 | VRAJA STANCA | 2012 | AA |
| 40 | SENIORITA | 2013 | AA |
| 41 | PATIMA | 2014 | AA |
| 42 | RASFATATA | 2014 | AA |
| 43 | VENERA | 2014 | AA |
| 44 | PANDORA | 2015 | AA |
| 45 | OPS | 2015 | AA |
| 46 | ASTARTE | 2015 | AA |
| 47 | VIDIA ROSIE | 2016 | AA |
| 48 | VRAJA ZAPEZII | 2016 | AA |
| 49 | KIRRA | 2016 | AA |
| 50 | RAMYA | 2017 | AA |
| 51 | NEMARA | 2017 | AA |
| 52 | KINA | 2017 | AA |
| 53 | SOLEDAD | 2017 | AA |
| 54 | SOPHIA | 2017 | AA |

The weight of different age categories from entire population, expressed in years, is defined as the age structure, and it is based on the age distribution histogram.

## RESULTS AND DISCUSSIONS

The results regarding reproductive isolation coefficient (RIC) are showed in Table 3.
The age structure for Romanian Trotter, from Dor Marunt stud farm is presented in Table 4, and the share of autochthonous and immigrant individuals, for all analyzed generations are graphically represented in Figures 1, 2 and 3 being much more easy to observe the share of native and immigrant individuals in the reproductive nucleus.

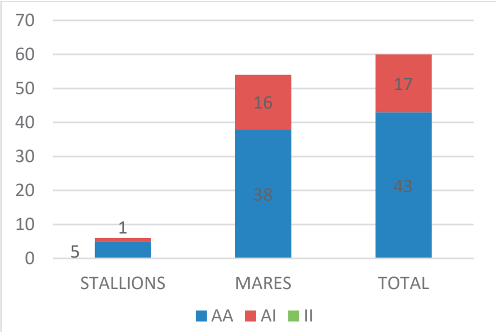


Figure 1. Share of immigrants and autochthonous individuals in the reproductive nucleus

The analyzed data from Table 3 relive the fact that the Romanian Trotter it is a population because the values of reproductive isolation coefficient is between 0 and +1 (Popa, 2009; Maftei et al., 2011) for all three generation.

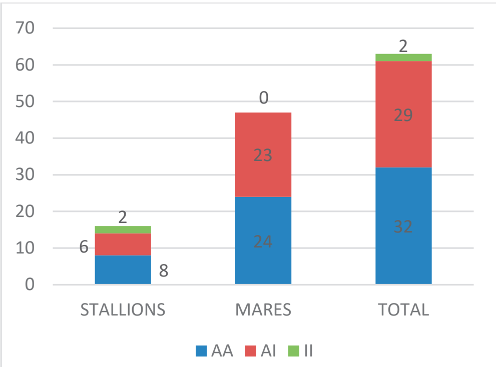


Figure 2. Share of immigrants and autochthonous individuals in case of parents of reproductive nucleus

We are relatively close to the period of the last import, and that is obvious when we look at the R.I.C. value for parents and grandparents of reproductive nucleus. The R.I.C. value for reproductive nucleus (0.4333) reveal the fact that the population is still in the influence area of immigrant populations.

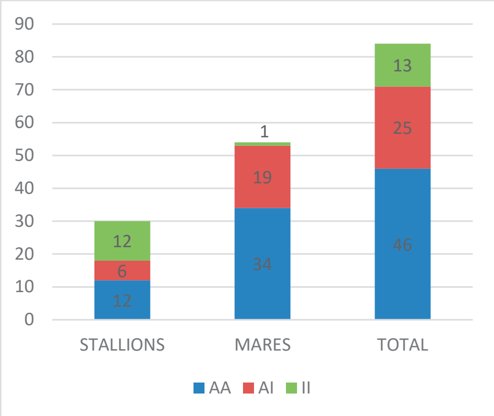


Figure 3. Share of immigrants and autochthonous individuals in case of grandparents of reproductive nucleus

At the grandparents level, in stallion case, R.I.C. value at -0.2 highlights the lack of reproductive isolation. Certainly, at the level of the grandparents of the reproductive nucleus, an infusion cross was made, in order to improve certain characters (especially the speed), using stallions from other trotter populations for reproduction. The value of the same coefficient, but at the level of parents, could not increase significantly due to the overlapping generations. At the same time, the RIC value it was influenced, in this 3 generation, by the promotion to reproduction of a large number of mares with an immigrant parent. All this situation, as mentioned above, is normal if infusion crosses are used, the reproductive isolation being then achieved gradually.

In terms of animal breeding, in order for a herd to be considered a population, respectively to have its own evolutionary path, it must have a value of reproductive isolation coefficient with positive values (to be found in the range 0 ; +1) for a number large enough of generations to allow population differentiation (minimum 3). The situation of perfect reproductive isolation is found at the value of +1 of this coefficient. Wright (cited by Draganescu, 1979 and Popa, 2009) set an optimal value of the coefficient equal to 0.8. This means that populations have an ideal evolution when 20% of the genes are immigrants (a percentage that corresponds to 10% of immigrant individuals, usually males). In this case it is obvious that the Romanian Trotter population is under the effect of infusion crossing, but there is a concern for reproductive isolation and fixation of the desired characters in the population, as can be seen in the figure 4.

The age structure in reproductive nucleus of Romanian Trotter from Dor Marunt stud farm is presented in Table 4 and in Figures 5 and 6. Analyzing presented datas, and also from graphical representation of age distribution in sire stallions and in broodmares livestock, it is easy to observe an unbalanced structure. I such a situation it is difficult, to not say impossible, to maximize the genetic gain and the economic efficiency, even if the age structure in sire stallions case looks a little bit more balanced (2 stallions at 20 years old, 1 stallion at 18 years old, another 2 stallions at 13 years old and one at 12 years old). In the broodmare case the individuals records ages from 5 to 24 years old, from which 24.07% have over 20 years old (almost a quarter from broodmares). This important share of old mares negatively influences the age structure and also the average age, implicitly also the maximization of genetic progress as well as the increasing of economic efficiency. The rest of mares from reproductive nucleus of Romanian Trotter horse is represented by 31.48% mares between 5 and 10 years old (9.27% mares at first parturition), and 44.44% mares between 13 and 19 years old. We suppose that at least 13 mares, born between 1998 and 2001, it will be removed from reproduction due to the old age. This action, associated with the promotion in the reproductive nucleus of a number of mares at least equal to the number of reformed mares can contribute to the optimization of the age structure. At this moment, if we take into account an average gestation time, in horses, of 0.92 years (11 months), at an average age of stallions of 10.7 years, we obtain a generation interval through males at 11.62 years which is not bad. In the broodmares case, the generation interval, calculated in the same style, is 15.23 years (broodmares average age 14.31 years old).

Table 3. Reproductive isolation coefficient values

| Specifications | | No. | Immigrants (I) | Parents | | | R.I.C. |
|---|---|---|---|---|---|---|---|
| | | | | AA | AI | II | |
| Reproductive nucleus (RN) without last imported stallions | ♂ | 6 | - | 5 | 1 | - | +0.6667 |
| | ♀ | 54 | - | 38 | 16 | - | +0.4074 |
| | Total | 60 | - | 43 | 17 | - | +0.4333 |
| Parents of RN | ♂ | 8 | 2 | 8 | 6 | 2 | 0.0000 |
| | ♀ | 47 | - | 24 | 23 | - | +0.0213 |
| | Total | 67 | 2 | 47 | 18 | 2 | +0.0159 |
| Grandparents of RN | ♂ | 30 | 12 | 12 | 6 | 12 | -0.2000 |
| | ♀ | 54 | 2 | 34 | 19 | 1 | +0.2593 |
| | Total | 84 | 8 | 46 | 25 | 13 | +0.0952 |

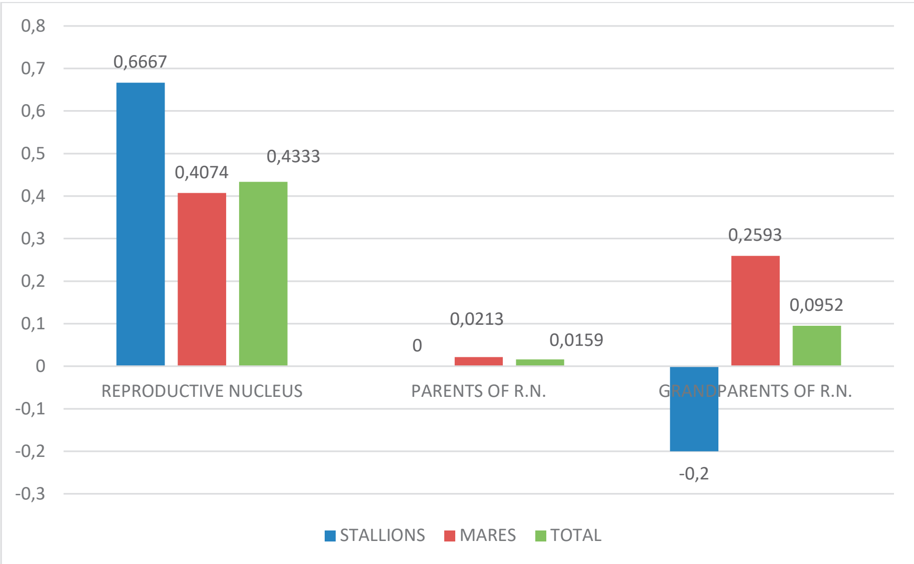


Figure 4. Evolution of R.I.C. values in analyzed population

Table 4. Age structure in the reproductive nucleus of Romanian Trotter horse

| **Sex** | **Total** | **Year of birth** 1998 | | 1999 | | 2000 | | 2001 | | 2002 | | 2003 | | 2004 | | 2005 | | 2007 | | 2008 | | 2009 | | 2010 | | 2011 | | 2012 | | 2013 | | 2014 | | 2015 | | 2016 | | 2017 | | **Average age** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *N* | *%* | *N* | *%* | *N* | *%* | *N* | *%* | *N* | *%* | *N* | *%* | *N* | *%* | *N* | *%* | *N* | *%* | *N* | *%* | *N* | *%* | *N* | *%* | *N* | *%* | *N* | *%* | *N* | *%* | *N* | *%* | *N* | *%* | *N* | *%* | *N* | *%* | |
| **M** | 6 | | | | | | | | | 2 | 33.33 | | | 1 | 16.66 | | | | | | | 2 | 33.33 | 1 | 16.66 | | | | | | | | | | | | | | | 10.7 |
| **F** | 54 | 5 | 9.26 | 2 | 3.71 | 4 | 7.41 | 2 | 3.71 | | | 3 | 5.55 | 2 | 3.71 | 4 | 7.41 | 6 | 11.11 | 1 | 1.85 | 3 | 5.55 | 1 | 1.85 | 4 | 7.41 | 2 | 3.71 | 1 | 1.85 | 3 | 5.55 | 3 | 5.55 | 3 | 5.55 | 5 | 9.26 | 14.31 |

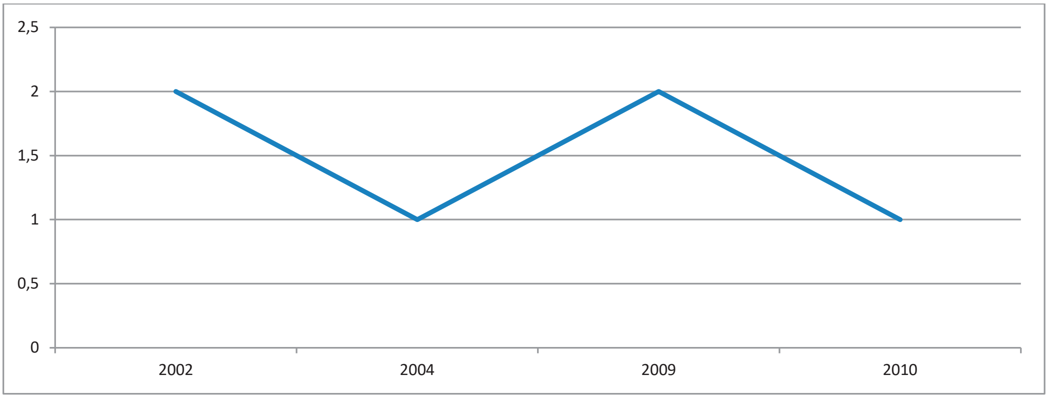


Figure 5. Romanian Trotter - sire stallions age structure

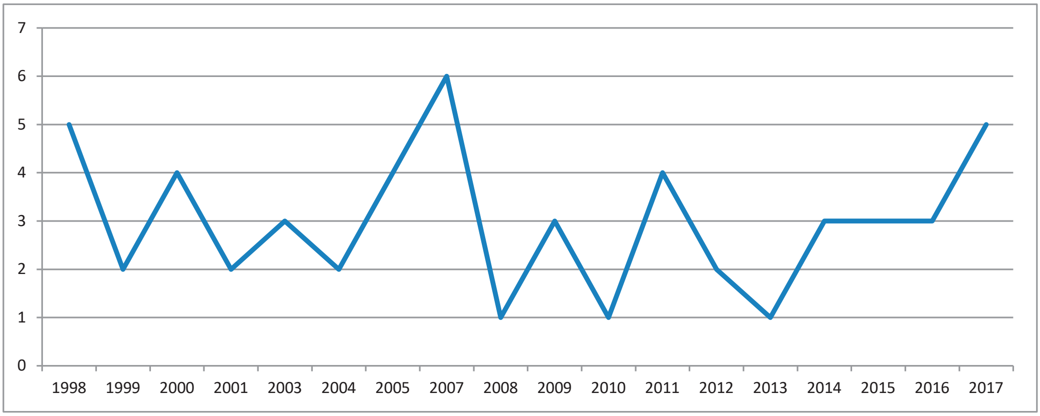


Figure 6. Romanian Trotter - broodmares age structure

Figure 7. View from Ploiesti Hippodrome - Romanian Trotter in action

## CONCLUSIONS

1. ***Reproductive isolation*** - It is obvious that, in case of Romanian Trotter horse from Dor Marunt studfarm, exists concerns regarding the improvement of some characters, especially productive traits as velocity, or even the concerning for increasing the genetic variability in order to create a larger action field for selection. All this situation is highlighted also by the important number of broodmares, from reproductive nucleus, with one immigrant parent. Using the infusion crossing through immigrant stallions can contribute to the improvement of the breed in the desired direction but it is very important to not forget that it is a most to return to the reproductive isolation after the infusion process. Otherwise we will not be able to have a breed with his own evolutionary path. It will be just a herd influenced by the genetic structure of immigrants or it will become just a flock from another population because of genes substitution process. Int this situation, when we will import more immigrants to be used in the reproductive nucleus of Romanian Trotter, in time, will make

from Dor Marunt studfarm only a multiplication farm for immigrants and a good financing point for studfarms from which immigrants became. Unfortunately, the comparison of the productive performances of the Romanian Trotter with those of the French Trotter or Standardbred (American Trotter) is made empirically, by ear, without taking into account the influence of the general and special environment. A brief retrospective look at the Romanian Trotter shows that this is a later breed that shows its maximum productive potential after the age of 4-5 years, and that the performances of individuals of this breed, on other European hippodromes, were clearly superior to the performances obtained in Romania.

2. ***Age structure*** - An unexplained fact is the big numbers of old mares, born between 1998 and 2001, and kept till 2021 as broodmares. All this situation, associated with the very small number of mares born in 2008 (1 mare), 2010 (1 mare), 2012 (1 mare) and 2013 (1 mares) and promoted in the reproductive nucleus of the breed led to the existence of an unbalanced age structure. However, if the trend of the last two years is maintained, and if the old specimens are reformed (born between 1998 and 2001) it will be possible to have a balanced age structure. By simply reforming the old mares (born between 1998 and 2001), the generation interval, calculated by mares, can be reduced from 15.23 to 12.55, taking into account an average gestation period of 11 months (0.92 months). Regarding the sire stallions it is very important to promote in reproductive nucleus young stallions, tested on the hippodromes from Romania but also from other european countries and to not at european level. Let's not focus on the use of only imported stallions for breeding. In this way we will be able to maintain the Romanian Trotter livestock as a breed, but also to improve the much desired productive performance. Great care must be taken when keeping mares in the reproductive nucleus with an immigrant parent in order to avoid inbreeding and to have a judicious matching of the pairs. Only in this way will we obtain young and valuable generations, both genetically and athletically point of view.

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# EFFECT OF DIFFERENT SOURCES OF SPECIFIC VARIANCE ON THE WOOL PRODUCTIVITY OF SHEEP FROM THE NORTH EAST BULGARIAN MERINO BREED

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***Abstract***

*Subject of the study were 678 sheep at 18 months from the North-East Bulgarian Merino breed, born in the period 2013-2019, ownership of the Scientific Agricultural Center - Targovishte. The following traits were analyzed: wool productivity, staple length, clean wool yield, clean fiber and tenderness of the fibers. The influence of the factors - year of birth, type of mating and breeding line was researched. The variance analysis was based on a multifactor linear statistical model. Year of birth had a highly significant influence of the studied traits. The average wool yield was 8.154 kg., staple length was 11.469 cm., average clean wool yield was 61.634 % and clean fiber was 5.101 kg. The genetic factors - selection type and breeding line had no significant effect on the traits for wool productivity. Heritability values for the studied traits were low which indicates a decrease of the genetic diversity and limited abilities for effective selection for increasing wool productivity.*

**Key words:** *clean fiber, clean wool yield, North East Bulgarian Merino sheep, staple length, wool productivity, tenderness of the fibers.*

## INTRODUCTION

Fine fleece sheep breeding has been hit hardest by the reduction in the number of sheep in our country over the last three decades. The population of fine fleece sheep in the late 80's of the last century occupied the largest relative share in this sector. From the Bulgarian breeds, the largest share of the total volume was occupied by the herds of the sheep breeds: North-East Bulgarian Merino breed (NEBM) (52.30%), Danube (DM) (17.92%), Thracian (TM) (17.58%), Karnobat (KM) (9.72%), Caucasian (CM) (1.36%) and Askanian (AM) (1.05%) (Boykovski et al., 2015). The change in the supply and demand of the market at the beginning of the transition changed the economic weight of the individual products produced in sheep farms. Priority was given to meat and milk, which led to the devaluation of wool and, accordingly, to the reduction of fine fleece herds in our country. This process continues today for a number of complex reasons and currently the fine fleece population occupies less than 1% of the total volume of the subsector in the country. Since 2008 the breeding activity with fine fleece sheep has been undertaken by the Association for Breeding of Merino Sheep in Bulgaria (ABMSB). Breeding programs to improve the main productive traits are being replaced by programs for the preservation and maintenance of breeds as a valuable part of the national gene pool. Breeding program of merino and fine fleece sheep in Bulgaria for the period 2011-2020 of ABMSB was adopted in 2011. (Boykowski et al., 2011). In 2013 fine fleece breeds have been declared endangered and farmers have the opportunity to participate in measures and schemes for national and European subsidies. According to ABMSB, in 2021 just over 4,000 fine fleece sheep in the country are covered under selection control. The North East Bulgarian Merino breed still has the largest number of animals - 2896 sheep. Most of them are grown mainly in the institutes and research centers, which are branches of Agricultural Academy - Sofia. The flock in the National Center for Agriculture - Targovishte is the nucleus of the North East Bulgarian Merino breed - Shumen type. Purebred linear breeding is the main method for storing endangered breeds. The flock maintains a genetic structure with 7 breeding lines and produces quality male and female breeding material for the other flocks of the breed. The analyses of the productivity in the nucleus flocks of fine fleece breeds contribute to the formation of a new breeding strategy in the fine fleece sheep breeding, as well as to the economic survival of the flocks. Studies on genetic structures, the main productive traits and factors influencing the phenotypic expression of genetic potential in fine fleece breeds are conducted by Boikovski et al. (2009; 2012); Dimitrov, D. (2001; 2006a; b); Slavov, R. (2007); Slavov et al. (2008); Staikova, G. & N. Stancheva, (2009); Stancheva et al. (2015; 2017; 2020); Stefanova, G. (2000); Tzonev, T. (2014); Tyankov et al., (2000). In recent years, the strategy for unification of the three breeds in the Bulgarian Merino breed, with preserved, differentiated intrabreed types, has been discussed. This vision for the preservation and improvement of the population raises the need for current research and motivates our study.

The aim of the study was to establish the effect of different sources of specific variance on the wool productivity of sheep of the North East Bulgarian Merino breed.

## MATERIALS AND METHODS

Subject of the study were 678 sheep at 18 months of the North-East Bulgarian Merino breed, owned by the National Center for Agriculture - Targovishte. The animals were born in the period 2013-2019. The following breeding traits were included in the study: wool yield, staple length, clean wool yield, clean fiber, and wool fiber tenderness according to the Bradford classification. The information was obtained from the genealogy book of the farm. The data were obtained according to the standard methods and guidelines provided in the Instruction for control of productive traits (2011) of the Association for Breeding of Merino Sheep in Bulgaria. 678 measurements of wool yield, staple length and determination of wool quality were made, 614 samples were tested for clean wool yield and clean fiber. The amount of obtained wool was determined individually and measured to the nearest 0.1 kg. The wool yield, reported at 1.5 years, was achieved with 18 months of wool growth. The staple length was measured with a measuring line. The quality of the wool was determined on samples from a topographic section on the side of the body. Samples of 50 g of wool from the same section were taken to determine clean wool yield and to calculate the clean fiber. The samples were tested in the wool science laboratory in Shumen according to standard methods. The influence of non-genetic and genetic factors - year of birth, type of mating and breeding line has been studied. An analysis of the variance was made on the basis of a multifactor linear-statistical model, which has the following form:

$$\mathbf{Y}_{ijk} = \boldsymbol{\mu} + \mathbf{A}_{i\,(1-7)} + \mathbf{B}_{j\,(1-2)} + \mathbf{C}_{k\,(1-6)} + \mathbf{e}_{ijk}$$

where:

**μ** - total average

$\mathbf{A}_{i\,(1-7)}$ – effect of the factor year of birth (fixed) – 7 levels (2013 – 2019)

$\mathbf{B}_{j\,(1-2)}$ – effect of the factor type of mating (fixed) - 2 levels (intralinear and multi linear);

$\mathbf{C}_{k\,(1-6)}$ - effect of the factor breeding line (fixed) - 6 levels (lines);

$\mathbf{e}_{ijk}$ – residual effects, $\approx N(O, \delta e^2)$

The heritability coefficients of the studied traits were evaluated according to Becker (1968). Differences between the levels of the studied factors were established on the basis of the degree of distribution measured by Studant (Hayter, A. 1984):

$$\mathbf{(yi - yj) / S \sqrt{(1/ni + 1/nj)} /2}$$

where: (yi - yj) - differences between the average values of the levels of the studied factor, S - square deviation, ni and nj - number of observations (individuals) for the respective levels.

## RESULTS AND DISCUSSIONS

The year of birth had a highly significant effect (P <0.001) on all studied traits of wool productivity (Table 1). The values of the F-criterion range from 22,351 for clean wool yield to 82,871 for wool yield. Staikova, G. & N. Stancheva, (2009) also found a significant influence of this complex factor on wool yield and staple length.

Table 1. Analysis of variance of the traits of wool productivity at 1.5 years

| Sources of variance | df | F | P | $R^2$ | CV% |
|---|---|---|---|---|---|
| **Wool yield** | | | | | |
| Year of birth | 6 | 82.871 | *** | 0.664 | 9.87 |
| Type of mating | 1 | 0.196 | **n. s.** | | |
| Breeding line | 5 | 0.608 | **n. s.** | | |
| **Staple lenght** | | | | | |
| Year of birth | 6 | 44.817 | *** | 0.547 | 13.55 |
| Type of mating | 1 | 2.174 | **n. s.** | | |
| Breeding line | 5 | 1.726 | **n. s.** | | |
| **Clean wool yield** | | | | | |
| Year of birth | 6 | 22.351 | *** | 0.412 | 8.44 |
| Type of mating | 1 | 0.455 | **n. s.** | | |
| Breeding line | 5 | 1.216 | **n. s.** | | |
| **Clean fiber** | | | | | |
| Year of birth | 6 | 64.125 | *** | 0.599 | 12.98 |
| Type of mating | 1 | 0.004 | **n. s.** | | |
| Breeding line | 5 | 0.849 | **n. s.** | | |

*** – P< 0,001; ** – P< 0,01; * – P< 0,05

The results of our study indicate that the two genetic factors - type of mating and breeding line do not have a significant effect on the phenotypic manifestation of the studied traits. The coefficients of determination range from $R^2$ = 0.412% to $R^2$ = 0.664%, which shows that much of the variation in traits is due to the sources of variability included in the model. The coefficients of variation were low to medium and range from 8.44% to 13.55%. Stefanova, G. (2000) published data on a significant effect of lineage on the traits of wool productivity in the North East Bulgarian Merino breed. Boykovski et al. (2002) reported a significant effect of the line on staple length, clean wool yield and amount of clean fiber at 18 months (P <0.001, P <0.01). In other studies of the same flock, linear differentiation was found on the basis of wool yield, as well as variation, depending on the type of mating of some of the studied ages (Staikova, G. & N. Stancheva, 2009). The comparison shows that for a period of about 20 years, the genetically determined variance decreases, and the environmental effects dominate in the formation of the phenotype and productivity of sheep.

The results in Table 2 show that the highest wool yield with positive LS-scores was represented by sheep born in 2018, followed by 2016 (P <0.001, P <0.01, P <0.05).

Table 2. LS-estimates (LSC) of the effect of different factors on the wool yield and staple length at 1.5 years

| Trait | Wool yield, kg | | | Staple length, cm | | |
|---|---|---|---|---|---|---|
| **Factor levels** | **n** | **LSC** | **LSM ± SE** | **n** | **LSC** | **LSM ± SE** |
| **Year of birth** | | | | | | |
| 2013 | 66 | -1.847ABCDE | 6.307 ± 0.103 | 66 | 1.153ABCa | 12.621 ± 0.196 |
| 2014 | 80 | 0.191Aa | 8.345 ± 0.094 | 80 | 1.418DEFG | 12.887 ± 0.177 |
| 2015 | 164 | 0.128 BFbGc | 8.282 ± 0.067 | 164 | 0.771HIJK | 12.240 ± 0.128 |
| 2016 | 141 | 0.504CFd | 8.658 ± 0,074 | 141 | - 1.235ADH | 10.234 ± 0.140 |
| 2017 | 118 | 0.435Dble | 8.588 ± 0,079 | 118 | - 0.963BEI | 10.506 ± 0.151 |
| 2018 | 68 | 0.931 EaGlH | 9.085 ± 0.100 | 68 | - 0.644CFJ | 10.824 ± 0.191 |
| 2019 | 41 | - 0.343cdeH | 7.811 ± 0.132 | 41 | - 0.499aGK | 10.969 ± 0.251 |
| **Type of mating** | | | | | | |
| Interlinear | 273 | - 0.016 | 8.138 ± 0.058 | 273 | - 0.099 | 11.369 ± 0.110 |
| Between the lines | 405 | 0.016 | 8.169 ± 0.045 | 405 | 0.099 | 11.568 ± 0.084 |
| **Breeding line** | | | | | | |
| 61 | 60 | 0.038 | 8.191 ± 0,109 | 60 | 0.311 | 11.779 ± 0.206 |
| 239 | 141 | - 0.060 | 8.094 ± 0,071 | 141 | 0.138 | 11.607 ± 0.135 |
| 583 | 145 | 0.001 | 8.155 ± 0,070 | 145 | - 0.276 | 11.192 ± 0.134 |
| 755 | 155 | - 0.049 | 8.105 ± 0,071 | 155 | 0.033 | 11.501 ± 0.133 |
| 777 | 114 | 0.099 | 8.253 ± 0,080 | 114 | - 0.029 | 11.440 ± 0.154 |
| 845 | 63 | - 0.029 | 8.125 ± 0,105 | 63 | - 0.176 | 11.292 ± 0.199 |
| **μ** | **678** | **8.154** | | **678** | **11.469** | |

**μ** – overall LS mean;
Significance of differences within columns – when symbols identical: **A** to **Z** – P< 0.001; **a** to **k** – P< 0.01; **l** to **z** – P< 0.05

The animals born in 2013 and 2019 have the lowest levels of the trait (P <0.001, P <0.01, P <0.05). Sheep, which were a product of intralinear cross, lag behind their peers in terms of the amount of wool quantity. The descendants of lines 777, 61 and 583 were superior to the other groups in wool yield, but without statistical significance for the differences. Sheep born in 2013, 2014 and 2015 had significantly higher staple length (P <0.001, P <0.01). This corresponded to a higher wool yield for those born in the last two years. Animals from intralinear cross again showed below average length for the studied group of animals. Lines 61 and 239 had longer staple length and line 583 - shorter than the average. The interlinear differentiation on this basis had low values and no statistical significance. The average wool yield was 8,154 kg with an average staple length of 11.46 cm. Lower value for the staple length of 10.50 cm was reported by Dimitrov D. (2006 b). Tzonev T. (2014) published similar to our results for wool yield at 1.5 years for sheep from the North East Merino breed - 8.440 kg, with a relatively high variation of the trait of 26.173%. Boikovski et al. (2015) found 8,350 kg average yield of wool from fine fleece sheep population under selective control. Slightly higher average wool yield of 8,989 kg and lower average wool lengths of 10.06 cm for the same breed were reported by Stancheva et al. (2015).

The highest and positive LS-scores for clean wool yield were found in sheep born in 2014 and 2015 ($P < 0.001$, $P < 0.01$) (Table 3). They were also superior to their peers in terms of clean fiber ($P < 0.001$). Those born in 2013 and 2017 lagged behind the average for the study group for the two studied traits ($P < 0.001$, $P < 0.01$, $P < 0.05$). Those born in 2016 and 2018 showed a lower than average percentage of clean wool yield, but gave more clean fiber from the group average. LS-scores in different types of mating were low in value, with different direction and without statistical significance. Lines 845, 777 and 61 had positive LS-scores for clean wool yield and clean fiber, but without significant differences between groups. Animals from lines 239 and 583 deviated in a negative direction from their peers on these traits. Our study found 61.634% average clean wool yield and 5,101 kg of clean fiber at 18 months. Lower values than our results for these traits were published by Stefanova (2000) and Dimitrov (2006 b), respectively 52.98% and 50.59% clean wool yield, also 4.554 kg and 4.223 kg obtained clean fiber of the same age. According to Tzonev (2014) for a period of 5 years the clean wool yield of sheep at 1.5-year varied from 54.75% to 59.98%, an average of 57.64%. Clean fiber ranged from 4.481 kg to 5.902 kg, an average of 5.234 kg and these data were close in value to our results. Boikovski et al. (2015) found 57.91% average clean wool yield and 4,784 kg of clean fiber for the Merino population under selective control.

Table 3. LS-estimates (LSC) of the effect of different factors on the clean wool yield and quantity of clean fiber at 1.5 years

| **Trait** | **Clean wool yield, %** | | | **Clean fiber, kg** | | |
|---|---|---|---|---|---|---|
| **Factor levels** | **n** | **LSC** | **LSM ± SE** | **n** | **LSC** | **LSM ± SE** |
| **Year of birth** | | | | | | |
| 2013 | 65 | - 0.406Aab | 61.228 ± 0.659 | 65 | -1.257ABCDE | 3.844 ± 0.085 |
| 2014 | 78 | 3.491Acd | 65.125 ± 0.602 | 78 | 0.443Al | 5.544 ± 0.077 |
| 2015 | 156 | 1.543aBCD | 63.177 ± 0.443 | 156 | 0.144B | 5.246 ± 0.057 |
| 2016 | 133 | - 0.297cB | 61.336 ± 0.483 | 133 | 0.190Cm | 5.291 ± 0.062 |
| 2017 | 115 | - 3.849bdC | 57.785 ± 0.516 | 115 | - 0.089Dlmn | 5.012 ± 0.067 |
| 2018 | 67 | -0.482D | 61.152 ± 0.645 | 67 | 0.569En | 5.670 ± 0.083 |
| **Type of mating** | | | | | | |
| Interlinear | 263 | 0.155 | 61.789 ± 0.367 | 263 | - 0.002 | 5.099 ± 0.047 |
| Between the lines | 351 | - 0.155 | 61.478 ± 0.308 | 351 | 0.002 | 5.103 ± 0.040 |
| **Breeding line** | | | | | | |
| 61 | 50 | 0.047 | 61.681 ± 0.755 | 50 | 0.001 | 5.102 ± 0.097 |
| 239 | 130 | - 0.876 | 60.767 ± 0.463 | 130 | - 0.103 | 4.998 ± 0.060 |
| 583 | 137 | - 0.489 | 61.145 ± 0.449 | 137 | - 0.006 | 5.095 ± 0.059 |
| 755 | 143 | 0.196 | 61.830 ± 0.458 | 143 | - 0.001 | 5.100 ± 0.059 |
| 777 | 108 | 0.245 | 61.879 ± 0.515 | 108 | 0.068 | 5.169 ± 0.066 |
| 845 | 46 | 0.867 | 62.501 ± 0.775 | 46 | 0.042 | 5.143 ± 0.099 |
| **μ** | **614** | **61.634** | | **614** | **5.101** | |

**μ** – overall LS mean;
Significance of differences within columns – when symbols identical: **A** to **Z** – $P< 0.001$; **a** to **k** – $P< 0.01$; **l** to **z** – $P< 0.05$

The results in Table 4 show low values of the heritability coefficients of the studied traits, which range from 0.092 for clean wool yield to 0.133 for wool yield. Stefanova (2000) found higher values of heritability coefficients for the same selection traits. The heritability for wool yield was 0.713, for wool length - 0.622, for clean wool yield - 0.880 and for clean fiber - 0.714. Staikova & Stancheva (2009) also found higher values of the coefficients, respectively

0.554 for wool yield and 0.603 for the amount of clean fiber at 18 months for the same breed. The data indicated a tendency for a stable decrease in the values, respectively narrowing of the genetic diversity in the population and limited possibilities for achieving progress on the studied productive traits through the methods of selection.

Table 4. Heritability coefficients ($h^2$) of the traits of wool productivity in sheep from the North East Bulgarian Merino breed

| Traits | N | n | $h^2$ |
|---|---|---|---|
| **Wool yield** | 69 | 678 | 0.133 |
| **Staple lenght** | 69 | 678 | 0.106 |
| **Clean wool yield** | 56 | 614 | 0.092 |
| **Clean fiber** | 56 | 614 | 0.121 |

The data in Table 5 provide information on the percentage distribution of fibers of different thickness in the fleece. Nearly 67% of the fibers were 20.6 to 23 microns (μ) thick (Bradford quality 64). About 23% ranged from 23.1 μ to 25 μ (quality 60) and nearly 10 percent ranged from 18.1 μ to 20.5 μ thickness (70 quality), which was normal for fine fleece sheep breeds. Boikovski et al. (2002) published data on 21.96 μ average tenderness of the fibers in the North-East Bulgarian Merino breed, which confirmed our findings that animals of quality 64 predominate. Tzonev T. (2014) found an average wool fiber tenderness of 22.12 μ, which corresponds to 64 Bradford quality. The degree of variation in this indicator was 4.86%, which shows a good evenness of the wool in thickness of the fibers in the staple. Boikovski et al. (2018) obtained similar results when studying the wool tenderness of 467 sheep of the studied breed. The studied samples from two topographic sections gave average values in microns within the range of quality 64 according to the Bradford classification.

Table 5. Fineness of wool fibers in the fleece of sheep from the North East Bulgarian Fine Fleece breed

| Fineness of the wool / Quality of Bradford/ | Amount | Percentage of the total amount |
|---|---|---|
| **60** | 159 | 23.45 |
| **64** | 454 | 66.96 |
| **70** | 65 | 9.59 |
| **Total** | **678** | **100%** |

## CONCLUSIONS

The year of birth has a significant influence on the selection traits - wool yield, staple length, clean wool yield and clean fiber in sheep of the North East Bulgarian Merino breed at 18 months.

The average wool yield was 8.154 kg, 11.469 cm - staple length, 61.634% - average clean wool yield and 5.101 kg - clean fiber.

Genetic factors - type of mating and breeding line did not have a significant effect on the traits for wool productivity of the studied group of animals.

Heritability values for the studied traits were low, which indicated a narrowing of genetic diversity and limited opportunities for effective selection to increase wool productivity.


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# DYNAMIC OF RECTAL TEMPERATURE OF GOAT KIDS OF DIFFERENT TYPE OF BIRTH IN THE FIRST HOUR AFTER BIRTH

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***Abstract***

*Rectal temperature dynamic was studied in newborn kids of different type of birth during the first hour of postnatal life. The study involved 37 goat kids of Bulgarian White Dairy breed and its crossbreeds with Anglo-Nubian and Togenburg - 19 single kids and 36 twins kids. Rectal temperature of the newborns kids was recorded at birth, at 15, 30, 45 and 60 min after delivery. The dynamic of the rectal temperature during the first hour after birth did not differ significantly in single and twins. In both singles and twins, the rectal temperature began to decline, with singles it reached its minimum on the 45th minute and on the 30th in twins. The difference between the highest and the lowest value of the indicator was 0.94ºC in the singles and 0.77ºC in the twins. The established values indicated activation of appropriate thermoregulatory responses responsible for the kid's ability to maintain the body's homeotherm within normal physiological limits during the early postnatal period.*

***Key words**: goat, newborn, singles, thermoregulation, twins.*

## INTRODUCTION

According to Arfuso et al. (2021), the transition from the fetal state, protected and nourished within the uterus, to the free-living neonate is probably the most profound change the newborn have to face.

Along with changes in the immune status, behavior, and physiology (Dwyer et al., 2016), thermoregulatory changes occur. This transition period is of paramount importance, as newborns have limited energy reserves and, in order to maintain their homeothermy and survive, they must be weaned in a timely manner (Piccione et al., 2013).

The homeostasis temperature is based on a complex of physiological and biochemical mechanisms that ensure the variation of body temperature within limits, optimal for the course of biochemical reactions and the functioning of various tissues and organs (Mellor, 1988). The adaptive changes in the thermoregulatory system include morphological and functional changes, manifestation of which requires different periods of time (Brück & Zeisberger, 1987).

The main factor influencing the survival rate of newborn kids in countries with extensive breeding systems is hypothermia accompanied by late weaning, which are often caused by difficult births (Kumar et al., 2010; Chauhan et al., 2019).

Mortality in Merino kids in Australia reaches 20-30%, and in twins 30 to 40% depending on the environmental conditions at birth (Walker et al., 2003).

Melado et al. (2000) report that newborn kids are more sensitive to cold stress, compared to newborn lambs (Muller & McCutcheon, 1991) because of a higher heat production capacity per unit of body weight and a lower surface area to body weight ratio (reviewed by Mellor & Stafford, 2004).

At ambient temperatures lower than 4°C, during the first 5 days after birth, the survival of the kids was significantly lower than that of the kids born at temperatures higher than 4°C (Melado et al., 2000).

According to Binns et al. (2002), neonatal mortality among newborn lambs in the United Kingdom is around 10%, and 50% of pre-weaning mortality occurs on the first day after birth (Singh et al., 2008; Dwyer et al., 2016). This can have a significant negative effect on both the farm's reproductive performance and income (Shiels et al., 2022).

Most animal species require time to activate the processes of protection against cooling, and this largely depends on the accumulated reserves of adipose tissue (Plush et al., 2016).

The ability to rapidly activate adaptive mechanisms related to heat production, heat loss reduction and prevention of hypothermia were indicators that influence early survival of the infant (Morris et al., 2000; Southey et al., 2001).

The generation of endogenous heat was accomplished by non-contractile and contractile thermogenesis, both mechanisms being activated immediately after birth, contributing respecttively to the formation of 46 and 31% of the total heat production at the maximum level of metabolism - the highest possible metabolic activity that can is achieved by cold exposure of the newborn (Eales & Small, 1981).

Műller & McCutcheon (1991), however, found that in newborn kids the metabolism rate per unit of live weight was significantly lower than in newborn lambs. The authors believe that it was precisely the combination of low heat production capacity, lower birth weight and less efficient peripheral insulation that caused the lower cold resistance of the kids compared to the newborn lambs.

The aim of this work was to study the rectal temperature dynamic in newborn kids in different type of birth (singles, twins) during the first hour of postnatal life.

## MATERIALS AND METHODS

The study was conducted in the goat farm of the Research Institute on Mountain Stockbreeding and Agriculture in the town of Troyan, Bulgaria.

The facility is located at an altitude of 380 m, (42° 53' 39" N / 24° 42' 57" E).

The study involved 37 goat kids of Bulgarian White Dairy breed (BWD) and its crossbreeds with Anglo-Nubian (AN) and Togenburg (TG) - 19 single kids and 36 twins kids. All goats were housed and cared for under the same conditions. During the winter period animals were kept in a barn and fed with a ration containing of 2 kg hay, and 0.8 kg concentrated fodder per head.

There was free access to water and salt. In the spring months (May-November) goats were grazing. Goats were vaccinated against enterotoxemia, treated for parasites, and given vitamins A, D, and E (Vialiton, Biovet).

Kidding of goats took place in February and March. Before kidding goats were separated in individual pins and were under surveillance.

The study included term-born, clinically healthy kids. The kids' were weighed right after birth.

Rectal temperature was recorded by digital clinical thermometer Microlife MT 16C2 inserted in a depth of 6 cm after delivery. The first rectal temperature measurement was made within a few minutes of birth, immediately after expulsion of the kid.

The second rectal temperature measurement was taken at 15 min of birth when the newborn were already located in the pen. The next measurements were performed at 30 min, 45 min and 60 min after delivery.

Environmental parameters, including air temperature, relative humidity and air velocity were monitored at various locations in the barn at 07.00 h, 14.00 h, and 21.00 h, using thermometers, whirling psychrometer and katathermometer respectively.

All environmental measurements were conducted within kid height. During the campaign, the room's electric lights were turned on at dusk and turned off in the morning around 7-8 p.m.

One-way ANOVA was used for statistical comparison. The differences were tested by Student t-test.

## RESULTS AND DISCUSSIONS

The dynamics of rectal temperature in the first hour after birth did not differ significantly between singles and twins (Figure 1).

From birth, the temperature dropped, reaching the same values in 15 minutes in singles and twins (P>0.05).

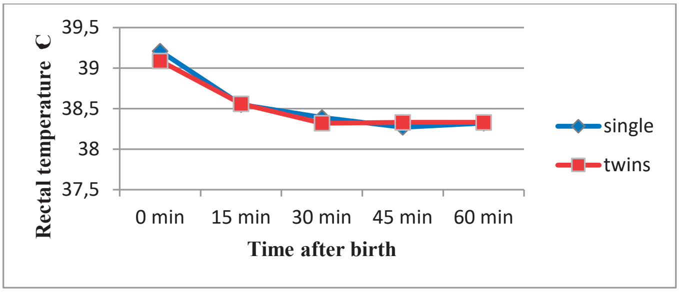


Figure 1. Rectal temperature dynamic in male and femae kids during the first hour of postnatal life

After a drop at around the 30th minute in the twins, the rectal temperature curve remained the same until the end of the first hour

In singles, the rectal temperature dropped until the 45th minute, after which it began to rise and at the end of the first hour the temperature equalized with that of the twins.

The difference between the highest and the lowest value of the indicator was 0.94ºC in the singles and 0.77ºC in the twins .

The increase in rectal temperature up to the 60th minute after birth can be explained by an increase in the metabolism of brown adipose tissue, an increase in heat production and an improvement in peripheral insulation (Cannon & Nedergaard, 2004).

In addition, during this period, the kids exhibited increased locomotor activity when attempting to stand up and seek the udder, whereby the heat generated in the muscles also contributed to the thermal status of the newborn (Aleksiev et al., 2009; Plush et al., 2016).

A similar pattern in the dynamics of rectal temperature has been found in newborn lambs (Aleksiev et al., 2007) and kids (Aleksiev, 2009a; Aleksiev, 2009b) of different breeds, in which the increase in rectal temperature after the initial decrease after birth, occurred, as in our experiments, after 45 minutes of postnatal life. The results obtained in both animal species suggested that genetic control exists over the implementation of mechanisms corresponding to the maintenance of homeothermia in newborns.

The live birth weight of the kids in our study did not differ significantly between singles (3.63 ± 0.12) and twins (3.42 ± 0.10).

In their study, Fazio et al. (2016) found that heavier singles have a lower rectal temperature after birth compared to lighter twin kids. According to the authors, this is probably due to the weaker use of the thermogenesis of shivering, as well as insufficient cold stimuli to induce metabolic over secretion.

In a study of lambs of different birth types, Stafford et al. (2007) found that the difference in rectal temperature measured at birth and at 1, 2, 3 and 6 hours after birth was due to the size of the lamb itself and not to its type of birth. This is confirmed by the findings of Chnite et al. (2013), who reported that regardless of liter size, heavier lambs have a higher rectal temperature at 1-12 hours, 24-36 hours and 48-69 hours after birth compared to lighter lambs.

Taking into account the specificity of the timing of the deliveries, it can be assumed that births occurred at the hours when the ambient temperatures were close to the maximum values for the day, which to some extent facilitates the adaptation of the newborns.

The low intensity of the air currents recorded during the experiment also contributed to a significant reduction in the level of heat loss.

Minimum barn temperature values ranged from -1 to 8ºC. Maximum temperatures ranged from 4 to 16ºC (Figure 2).

The relative humidity varied from 53 to 74% and the intensity of the air circulation in the different areas of the room ranged from 0.04 to 0.12 m/s.

The observed changes in rectal temperature indicated the initiation of thermogenesis due to separation from the placenta, removal of placental inhibitors from circulation, and sympathetic stimulation of brown adipose tissue upon cooling of the skin (Ball et al., 1995; Symonds et al., 1995; Clarke & Symonds, 1998).

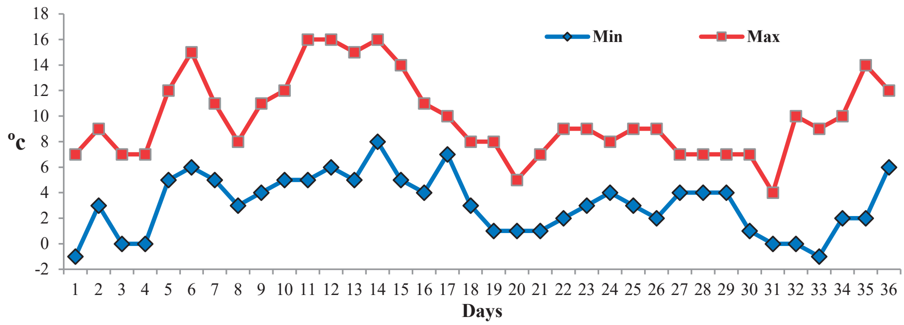


Figure 2. Minimum and maximum values of ambient temperatures during the birth period

Due to thermal inertia, the recorded rectal temperature values did not reflect the rapid changes in temperature of the various tissues and organs, but indicated that activation of non-contractile thermogenesis began within minutes after birth (Aleksiev, 2009a). Therefore, changes in body temperature lagged behind changes in the level of heat production in brown adipose tissue. According to Darwish & El-Bahr (2007), singles lambs and higher weight lambs have higher plasma concentrations of T3 and T4 at birth and higher thermoregulatory capacity (Symonds et al., 1995; Dwyer & Morgan 2006; Sawalha et al., 2007).

Improvement of peripheral isolation resulted from the activation of vasomotor activity and partly to the reduction of moisture content in the hair coat due to maternal care and evaporation (Darcan et al., 2009; Mota-Rojas et al., 2021).

The thermoregulatory mechanisms in lambs are well developed at birth (Faurie et al., 2004), but continue to be refined, reaching maximum efficiency by 4-5 days of age (Mercer et al., 1979).

According to authors (Mercer et al., 1979; Clarke et al., 1994), the process can take up to several weeks, depending on the maturity of the effectors at birth, and during this period, thermoregulatory, cardiovascular, respiratory and metabolic homeostatic mechanisms complete their development.

The dynamics of rectal temperature, especially the recorded increase after 30 minutes after birth in twins, was also influenced by the activation of mechanisms of contractile thermogenesis as an additional source of heat generation.

In a number of cases, in the newborn kids, tremors with different intensity of muscle groups in different parts of the body have been observed.

Likewise, differences in rectal temperature found between singles and twins may indicate variations in the degree of physiological maturation of homeothermic mechanisms at birth and their ability to activate heat production and/or heat conservation mechanisms, which ultimately affect heat retention (Aleksiev, 2009a; Giannetto et al., 2017).

## CONCLUSIONS

No significant differences were observed in the values of rectal temperature recorded at birth of singles and twin goat kids.

The rectal temperature of singles and twins during our study was within normal physiological norms.

The established values indicated activation of the appropriate thermoregulatory responses responsible for the kid's ability to maintain body homeothermia in temperate climates and facilitated adaptation to environmental conditions during the early postnatal period.

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# EFFECT OF POWDER PROBIOTIC ON THE LEUKOCYTE, HETEROPHIL AND LYMPHOCYTE LEVEL ON LAYING HENS

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***Abstract***

*Powder probiotic has a good effect on the digestive tract which improvement of the immune system. This study to know the impact of powder probiotics on the immune system which includes levels of leukocytes, heterophils, lymphocytes, and heterophils to lymphocytes ratio in laying hens age 90 weeks. This study was conducted from February to March 2021 at Laying Hens Farm in Sukarapih Village, Sukasari, Sumedang, Jawa Barat. The object study were 40 laying hens aged 90 weeks. Completely Randomized Design (CRD) was applied which consists of four treatments and five replications. The treatments are basal ration without powder probiotic (T0); basal ration + 2 % powder probiotic (T1); basal ration + 3% powder probiotic(T2); and basal ration + 4% powder probiotic (T3). Statically this study showed no significant difference in levels of leukocyte, heterophil, lymphocyte, and heterophil to lymphocyte ratio.However, the administration of powder probiotic 4 % has improved the levels of, heterophil,lymphocyte, and heterophil to lymphocyte ratio on laying hens aged 90 weeks near rejected.*

**Key words:** *heterophil, laying hen, leukocyte, lymphocyte, probiotic.*

## INTRODUCTION

The productivity of laying hens is influenced by the immune system. A strong immunesystem can increase productivity. In addition, the productivity of laying hens is also influencedby age. In the first year productivity will be optimal, but laying hens with high productivity can produce up to 2-3 years. Laying hens at a productive age of 22-72 weeks can produce as many as 260 eggs/year. Laying hens with the age of 72 weeks will decrease productivity, thus the chickens will be rejected laying hens. According to Salang (2015), the production of laying hens aged 82 weeks is below 50%. Because of the normal rate of egg production decrease in laying hens is 0.4-0.5% per week. One of the efforts to increase productivity uses probiotics as feed additives in the ration, which improve digestibility and increase immunity.

Probiotics are microorganisms that play a role in improving the ecosystem of intestinal flora, which impacts improving health. Some probiotics can reach the colon, which decreases of total pathogenic bacteria in the colon. Giving probiotics from an early period can improvesthe balance of intestinal microflora (Adriani, 2005; Adriani et al., 2019). Administration of probiotics in recent years is beneficial in modulating the immune system by enhancing intestinal barrier function. The gastrointestinal tract is in contact directly with various antigens from the environment, microbes, and pathogens. *Gut-associated lymphoid* tissue includes two main components, respectively intestinal microflora and the local immune system that interact (Kusumo, 2010). The composition of the intestinal tract consists of various defenses. They are physical defense from mucus and epithelial cell layers of the non-specific immune system, innate immunity (macrophages, invariant T cells, and defensins), and specific defense systems(production of antibodies and T cells). The intestine is an organ with the largest immune system in the body. The cells that make up the intestines are coats with a mucous barrier constantly undergoing a process of regeneration. Probiotics can strengthen immunity because of mucus. This mucus provides an advantage for probiotic bacteria, which is a medium for attaching probiotics to the intestinal wall. According to Perdigon et al. (1991), the attachment of probiotics to mucus occurs because of the mucus-binding-protein substances that probioticshave. Mucus-binding-proteins can recognize antibody proteins and several types of probioticsso that they increase specific immune responses as immunomodulators. Resistance to enteric pathogens is influenced by balance interactions between the gut microbiota, epithelium, and the immune system (Patterson & Burkholder, 2003). Lactic Acid Bacteria (LAB) uses common probiotics are *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. However, these two bacteria are reduced in the colon, so they are not reliable probiotics. Meanwhile, bacteria that live along the digestive tract and obtain in large numbers on the small intestine are *Lactobacillus acidophilus* and *Bifidobacterium bifidum*. They are reduced pathogenic bacteria in the colon (Adriani & Lengkey, 2010). Adding beneficial microorganisms to the gut could prevent or delay some diseases by improving the immune response or by producing bioactive metabolites (Lesmana et al, 2021). *Lactobacilli* treatment leads to higher antibody production in chickens. *Lactobacillus acidophilus* will inhibit the growth of pathogenic bacteria, which can damage the permeability of blood cell membranes and will end with leak or damage to blood cell walls (Latipudin et al., 2018). Administration of probiotics in powder form is easier to feed livestock than in liquid form (Adriani et al., 2020).

Leukocytes are divided into two groups, they are granulocytes (heterophils, eosinophils, and basophils) and agranulocytes (monocytes and lymphocytes). Granulocytes and monocytes defense the body from pathogens by phagocytosis, while main function of lymphocytes relates to the immune system. The addition of live microorganisms to the ration has been found to stimulate the immune system (Toms & Powrie, 2001; Koenen et al., 2004)and strengthen non-specific immunity (Placha et al., 2010). Probiotics will stimulate lymphocyte or immunocompetent cells to maintain immunity through the response of lymphoid tissue. Lymphoid tissue is the tissue production and maturation of lymphocytes. Thelymphoid tissue will trigger plasma cells to produce antibodies (Dewi & Herlisa, 2015).

In a previous study, probiotics consisted of *Lactobacillus fermentum, Lactobacillus plantarum,* and *Pediococcus pentosaceus* were given 0%, 1%, 2%, and 3% to Peking ducks. The results of that study showed administration of probiotics as much as 2% was quite effectivein reducing level of basophils, eosinophils, H/L ratio, and increasing lymphocyte level. Leukocyte levels according to treatment (0%, 1%, 2% and 3%) were 23.38 x $10^3$, 20.26 x $10^3$, 21.85 x $10^3$, and 22,34 x $10^3$ cells/mm$^3$, while lymphocyte levels were 35.7%, 46.8%, 51%, and 45.2% (Wulandari, 2014). Another study showed probiotic *L. acidophilus* given as much as 0,1 x $10^9$, 2 x $10^9$; and 3 x $10^9$ CFU/kg, resulted leukocyte levels were 43.96 x $10^3$, 47.76 x$10^3$, 50.78 x $10^3$, and 52.88 x $10^3$. The administration of *L. acidophilus* in this study was not significant effect, but it showed that the higher probiotic level given can increase leukocyte components in the blood turn to increase in lymphocytes, so stimulate the immune response in chickens (Alaqil et al., 2020). Several studies were conducted by Asmara et al. (2019) which showed the administration of probiotics had a very significant effect on the total leukocytes, heterophils, and lymphocytes of broiler chickens. Another study from Februansyah (2018) wasfound probiotic Bacillus plus vitamin and mineral at 0.1%, 0.5%, and 1% increase the immunesystem of broiler chickens as seen from leukocyte level and differential leukocytes levels, especially heterophils. eosinophils, and lymphocytes levels.

In addition, a study by Gunawan & Sundari (2003) used *Lactobacillus acidophilus* as much as 2% and 4% in laying hens rations can increase 5-11% egg production and suppress feed conversion ratio. The study conducted by Lutfiana et al. (2015) stated the administration of probiotics 2% and 3% was able to increase hemoglobin total in laying hens compared to 0% and 1% treatments. Recent study conducted by Kumalasari et al. (2020), the administration of dry probiotic as much as 2% of the total broiler chicken ration were increased growth performance, body weight gain and giblets. In addition, dry probiotic decreased abdominal fat, and lipid profiles of blood and meat.

This research to know effect of powder probiotics on the immune system which includes levels of leukocytes, heterophils, lymphocytes, and heterophils to lymphocytes ratio in laying hens age 90 weeks. The main novelty in this research is using yoghurt based on probiotics as animal feed, the microbiota consortium described above and powdered by spray drying method.

## MATERIALS AND METHODS

Materials: Fresh cow's milk, Lactic Acid Bacteria culture i.e., *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidhophilus*, *Bifidobacterium bifidum,* skimmed milk and maltodextrin DE 10-12 as probiotic encapsulate, Plate Count Agar (PCA).
Bird and Ration: A total of 40 laying hens strain Lohman Brown 90 weeks old with Hen Day Production (HDP) between 50-60 %. The experiment was conducted for 30 days from February to March 2021 at Laying Hens Farm, Sukarapih Village, Sukasari, Sumedang, Jawa Barat. The basal ration used mixture of corn, bran, concentrate, top mix, and macro minerals. The concentrate used consisted of corn gluten, pollard, meat and bone meal, soybean meal, oil, calcium phosphate, calcium carbonate, sodium chloride, amino acids, vitamins, trace minerals, and antioxidants. The basal ration contains a metabolic energy of 2700 kcal/kg and 16.5% crude protein.
Processing of Powder Probiotic: The probiotics were used 5% (v/v) *Streptococcus thermophilus, Lactobacillus bulgaricus, Lactobacillus acidophilus, Bifidobacterium bifidum* inoculated into 250 ml De Man Rogosa and Sharpe (MRS) medium and then incubated at 45°C for 14 hours. Fresh cow's milk was pasteurized and cooled to 45°C before added 5% of the consortium bacteria, then homogenized. The fermentation process was carried out for 14 hoursat room temperature. The process making probiotic powder was liquid fermented milk added with skimmed milk and maltodextrin as encapsulated materials, sterile distilled water (1/2 of the total volume of the solution), and homogenized. After homogenized, the mixture was dried used a spray dryer with an inlet temperature of 160$^0$C and outlet 65-70°C (Juniawati et al., 2019). Administration powder probiotics are 2, 3, and 4 % of total ration. This dose based a reference in the study of Kumalasari et.al. (2020) and Adriani et.al. (2020) by administration 2% probiotics in broiler chicken rations showed significant results on blood biochemistry and broiler chicken production.

### Statistical Analysis

This experiment was used Completely Randomized Design with 4 treatments i.e., T0 = basal ration without powder probiotic; T1 = basal ration + 2 % powder probiotic; T2 = basal ration + 3% powder probiotic; T3 = basal ration + 4% powder probiotic. Under each treatment 5 replicates were considered for each parameter such as leukocyte level, lymphocyte level, heterophil level, and heterophil to lymphocyte ratio. Data analysis were used analysis of variance (ANOVA) and orthogonal polynomials to test significance between treatments. Significance was considered at $P \leq 0.05$ levels.

## RESULTS AND DISCUSSIONS

### Results

### Leukocyte Levels

The effect of ration administration of powder probiotic on leukocyte level of layer hens is presented in Table 1.

Table 1. Immune Response of Laying Hens Supplemented Powder Probiotics

| Variable | Treatments | | | |
|---|---|---|---|---|
| | T0 | T1 | T2 | T3 |
| Leukocyte (cells/mm$^3$) | 43,980 $^a$ | 48,320 $^a$ | 41,060 $^a$ | 43,160 $^a$ |
| Heterophil (%) | 2.8 $^a$ | 3.2 $^a$ | 3.8 $^a$ | 1.8 $^a$ |
| Lymphocyte (%) | 90.8 $^a$ | 90.4 $^a$ | 89.8 $^a$ | 92.2 $^a$ |
| H/L Ratio (%) | 0.031$^a$ | 0.035 $^a$ | 0.042 $^a$ | 0.020 $^a$ |

Note:
T0 = Basal ration without powder probiotic (Control)
T1 = Basal ration + 2 % powder probiotic
T2 = Basal ration + 3% powder probiotic
T3 = Basal ration + 4% powder probiotic

The average leukocyte level was highest at T1 (48,320 cells/mm$^3$) and lowest at T2, which was 41,060 cells/mm$^3$. In T1 was increased in leukocyte levels by 9.87%, while other treatments decreased by 1.86% (T3) and 6.64% (T2). Leukocyte levels were not significantly different (P>0.05).

### Heterophil Levels

The effect of ration administration of powder probiotic on heterophil levels of layer hens is presented in Table 1. The average heterophil level was highest at T2 (3.8%) and lowest at T3, which was 1.8%. In T3 was decreased in heterophil levels by 9.87%, while other treatments increased by 1.86% (T1) and 6,64% (T2). Heterophil levels were not significantly different (P>0.05)

**Lymphocyte Levels**

The effect of ration administration of powder probiotic on lymphocyte level of layer hens is presented in Table 1. The average lymphocyte level was highest at T3 (92.2%) and lowest at T2, which was 89.8%. In T3 was increased in lymphocyte levels by 1.54%, while other treatments decreased by 0.22% (T1) and 1.10% (T2). Lymphocyte levels were not significantly different (P>0.05).

**Heterophil to Lymphocyte Ratio**

The effect of ration administration of powder probiotic on heterophil to lymphocyte ratio (ratio H/L) of layer hens is presented in Table 1. The average ratio H/L was highest at T2 (0.042) and lowest at T3, which was 0.020. In T3 was decreased in ratio H/L by 35.48%, while other treatments increased by 12.9% (T1) and 35.48% (T2). Ratio H/L were not significantly different (P>0.05).

## Discussions

### The Effect of Powder Probiotic on Leukocyte Levels Laying Hens

Leukocytes are the body's immune system (Mushawwir et al., 2020) in cellular and humoral defense against foreign substances at the site of damage (Indah, 2016). Leukocyte observation is used to diagnose the health condition of livestock. Leukocytes consist of heterophils, eosinophils, basophils, lymphocytes, and monocytes.

Normal levels of leukocytes in chickens range from 8-20 x $10^3$ cells/mm$^3$ (Soeharsono et al., 2010). According to Jannah et al. (2017), the range of 225.20 – 487.40 x $10^3$ cells/mm$^3$ is still in normal condition. Leukocytes levels in all treatments showed in the normal range. Leukocytes levels that deviate from normal conditions correlates with the health condition of the animal (Suriansyah et al., 2016). However, the high production of leukocytes cannot be assumed that laying hens are sick. An increase in leukocytes level describes a humoral and cellular response against pathogenic agents that cause disease in the body. This high leukocyte level because laying hens were pathogenic infections or immune system disorders, so that was an increase in the body's defense ability, while the decrease in leukocytes level can also be assumed that there is no infection or disruption of pathogenic bacteria attack the body (Soeharsono et al., 2010). Therefore, it is necessary to measure differential leukocyte levels to determine the health of the laying hens under study.

Factors that affect leukocyte levels include stress, age, environment, biological activity, hormones, and ultraviolet light or radiation. Environmental stress causes physiological processes to become abnormal, which affects the hormonal balance in the chicken body whichcan cause an increase in leukocyte levels. Environmental stress will increase the production of corticosteroids and glucocorticoids, which causes a decrease body's defense system. The increase corticosterone hormone levels in poultry that will trigger cell damage including blood cells due to reduced body oxygen intake. The mechanism of the body's response to the pathogen invasion can be seen from the increase and decrease in total leukocyte (Saputro et al., 2013; Latipudin et al., 2018; Falahudin et al., 2016).

The highest average leukocyte level at T1 with 2% supplemeted powder probiotic was increased by 9.87% compared control treatment. This showed that the administration of 2% probiotic powder of thetotal ration can improve the body's immune system, so that chickens can produce more leukocytes. LAB in probiotic powder can reduce pathogenic microbes in the digestive tract by increase beneficial microbes. High leukocyte levels are caused by a response to disease, both infections and foreign substances. This process will form antibodies. According to Hartoyo et al. (2015), the function of leukocytes is protect the body from pathogens by phagocytosis and produce antibodies. It is also proven by the higher egg productivity on T1 at the time of the study with an average of 80 items for 4 weeks. Probiotics can improve the balance of intestinal microflora, so that can improve immunity and egg productivity (Tang et al., 2017).

The average leukocytes levels were lower at T2 (3% powder probiotic) and T3 (4% powder probiotic) than control, with each decrease at 6.64% (T2) and 1.86% (T3). This decrease in leukocyte levels was related to intestinal microflora balance, which will result in reduced infections in the body. According to Sjofjan et al. (2020), the increase and decrease in the leukocyte levels is a response mechanism against invading pathogens. Based on this, decrease in pathogens in the digestive tract will reduce infection and decrease leukocytes levels.

**The Effect of Powder Probiotic on Heterophil Levels Laying Hens**

Heterophils include the group of granulocytes in leukocytes. They are the forefront (first line) which acts rapidly as a nonspecific immune response and earliest in defense againstpathogens infection (Hewajuli & Dharmayanti, 2015). Heterophils function is the body's line of defense against pathogens, especially bacteria. It is phagocytic with engulfs microflora andremains of dead cells and can enter the infected tissue.

The normal range of heterophil levels in poultry are 20%-40% or 4 – 8 x $10^3$/$mm^3$ (Soeharsono et al., 2010). Meanwhile, the heterophil levels of laying hens in the study were below the normal range. This can be caused age factor of laying hens on the study being old orheading to reject, so they are can not produce heterophil optimally. According to Devi et al. (2019), the factors that influence differential leukocyte levels include environmental conditions, age, and nutritional content of the feed. According to Nasrullah et.al. (2020) and Sukmana (2019), the average heterophil level of broiler chicken aged 40 weeks was 12.8% and the age of 85 weeks was 4.6%. Whereas in this research, 90 weeks of heterophil levels were 2.8%. The average heterophil levels was bigger at T1 (2% powder probiotic) and T2 (3% powder probiotic) compared control, with each increase at 1,8% (T1) and 6,64% (T2). Theincrease in heterophil level occurs due to a physiological stress response. Pathological conditions that cause neutrophilia include acute infection, inflamemation, tissue damage, andmetabolic disorders (Riswanto, 2013). This increase is an effort from leukocytes to fight pathogens by heterophils return the livestock body to normal condition (Mushawwir et al., 2017).

The lowest average leukocyte level at T3 with 4% supplemented powder probiotic was decreased by 1.8% compared other treatments. heterophil can attack pathogen by migrating to attack areas, penetrating vessel walls, and destructing pathogen (Hutasoit, 2010). When there is no infection, it does not affect the increase in heterophil level (Wulandari et al., 2014), because the increase in the heterophils levels is caused by pathogen infection (Sugiharto et al., 2014). The administration of probiotic powder reduces the heterophil levels laying hens in the study. According to Adriani (2010), probiotic plays a role in suppressing the growth of microflora pathogen that cause digestive tract diseases. This is because LAB produces antimicrobials include bacteriocin, hydrogen peroxide, and various natural antibiotics. Antimicrobials produce by probiotic in T3 can help alleviate the work of heterophils in inhibit the growth of pathogenic bacteria, so that can reduce heterophil in phagocytosis and decrease compare to other treatments.

**The Effect of Powder Probiotic on Lymphocyte Levels Laying Hens**

Lymphocytes are a part of leukocytes that do not have agranulocytes or nuclei. Lymphocytes are cells capable of recognizing and destroying antigenic. Lymphocytes consist of B lymphocytes or cells B (naive and active) and T lymphocytes (T cells). When an antigen is detected, T cells and B cells in the bone marrow will enter secondary lymphoid organs such as lymph nodes and spleen to activate these antigens into effector cells and memory cells. Active cells will then migrate to peripheral tissues where infection occurs (Sukmayadi et al., 2014).

The range normal of lymphocyte levels on poultry is 30-70%. However, the lymphocyte levels in this study were all above normal levels. This is caused the old age laying hens. The old age of chicken makes the cortex in the follicles thicker and more lymphocyte cells. According to Nasrullah et al. (2020) and Sukmana (2019), the average lymphocytes levels of broiler chicken aged 40 weeks was 80.2% and the age of 85 weeks was 82.9%. Whereas in this research, 90 weeks of heterophil levels were 90.8%.

The highest average lymphocyte level at T3 with 4% supplemented powder probiotic was increased by 1.54% compared other treatments. This caused powder probiotics can increase LAB in the digestive tract. LAB improves intestinal microbial balance, adheres to the gut, secretes active metabolites, and competes with pathogenic bacteria (Boostani et al., 2013; Trela et al., 2020). According to Saki et.al. (2018), the humoral immune response can be stimulated because of high levels of probiotics so that the leukocyte component in the peripheral blood leads to an increase in the lymphocyte population. The parts of LAB that can stimulate immunity are endotoxic lipopolysaccharide, peptidoglycan, and lipoteichoic acid. Lipoteichoic acid from *Lactobacillus* sp. and *Bifidobacteria* sp. has a high affinity for epithelial membranes, acts as a carrier for other antigens, and binds them to target tissues for an immune reaction to occur. LAB attach to intestinal epithelial cells can activate macrophages. The attachment of microflora to mucosal epithelial cells is the result of a special binding process between surface adhesives on microflora and mucosal receptors on the cell membrane. (Gusils et al., 2002; Surono, 2004).

In addition, probiotics activate dendritic cells in Peyer's patch that stimulates the mucosa circulating pool of T-lymphocytes generated from within the Peyer's patch. In this waythese T-cells might also exert their immune modulation at distant mucosal sites (Clancy, 2003). Probiotic powder modulates the production of cytokines as antibody-producing metabolites from monocyte macrophages, mitogens, and antigens that promote lymphocyte proliferation (Rohyati, 2012). T lymphocytes will release interferon that plays a role in activating macrophages and B cell differentiation. Meanwhile, B lymphocytes will produce antibodies that play a role in humoral-specific immunity (Galdiano et al., 2007).

### The Effect of Powder Probiotic on Heterophil to Lymphocyte Ratio Levels (Ratio H/L) Laying Hens

The ratio heterophil to lymphocyte (ratio H/L) uses as an indicator of stress in chickens, stress conditions will be seen if the value is above the normal range (Sugito & Delima, 2009). Factors that affect ratio H/L are feed, light, age, and environmental temperature (Mashaly et al., 2004).

The level of body resistance on poultry to environment ranges from ratio H/L around 0.2 – 0.8 with a normal value of 0.5 (Emadi & Kermanshahi, 2007). Meanwhile, the percentage ratio H/L in this study showed from 0.020 to 0.042. In this study, laying hens with all treatments supplement powder probiotic did not stress, which showed by ratio H/L below the normal range. According to Kusnadi (2008), the higher stress level makes the ratio heterophil to lymphocyte increase.

## CONCLUSIONS

The administration of powder probiotic overall was not significant. Although not significant, the administration of 4% probiotic can improve total lymphocyte, decrease heterophil and H/L ratio. This research show that probiotic can increase the immunity of laying hens aged 90 weeks/ rejected chicken

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# MORPHOLOGICAL TRAITS AT FIRST CUTTING OF FAST GROWING TREE LEGUME *INDIGOFERA ZOLLINGERIANA* UNDER DIFRENT PLANTING SPACING IN COCONUTS BASED FARMING

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***Abstract***

*The objective of this research was to assess the morphological response of Indigofera zollingeriana to differences in planting spacing at first cutting of three months after grown in the field. This study used a Completely Randomized Design consisting of 6 treatments, namely PS1: 100 cm x 50 cm, PS2: 100 cm x 100 cm, and PS3: 100 cm x 150 cm, each treatment was repeated 6 times. The measured variables were: plant height, stem diameter, the highest number of leaves, and number of branches. Further, we have measured also dry weight yield and leaf/wood ratio. The results showed that plant height and stem diameter in PS2 and PS3 treatments were significantly higher than PS. Number of leaves not effected but branches, leaf/wood ratio and total dry weight were significant effected by treatments. Based on the results of this study it can be concluded that the best morphological response of Indigofera in term of leaf/wood ratio and total dry weight at the three months in the fields was obtained in the 100 cm x 100 cm planting spacing.*

***Key words:*** *coconuts, cutting, indigofera, morphological, planting.*

## INTRODUCTION

Plant growth is increase in length, stem diameter, plant covered area, volume or biomass, wet and dry weight of plants. *I. zollingeriana* is a mainstay plant tree legume alternate to *L. leucocephala* which is susceptible to leucaena psyllid (*Heteropsylla cubana*). This Indigofera is one of important forage legume in Indonesia because agronomical aspect grown well during dry season, produced fertile seeds, branch and leaf developed exponentially up to certain time of defoliation, contains relatively higher crude protein ranging from 22-29% and highly relished by livestock (Figure 1). However, this plant is widely studied in an open environment, while in shade conditions has never been reported. The limited land for forage planting is a common problem in the development of ruminant animals. Along with the increasing of human population, extensive forage fodder is decreasing, due to the development of food agriculture and other infrastructure. Therefore, there needs to be an effort to provide land for growing forage.

Farming systems applied in North Sulawesi eastern part of Indonesia is still an integrated land with industrial plantations include coconut that can be used for the development of forage crops (Anis et al., 2015).

Figure 1. *Indigofera zollingeriana* underneath coconuts plantation

However, this kind of integration is faced with competition for nutrients, water and sunlight. Many research has been done and reported concerning *I. zollingeriana* grown in full sun light but unfortunately limited study assess this plant under natural shade environment especially under coconut plantation.

Therefore, the objectives of this study is to examine the effect of planting spacing configuration patterns and population density of *I. zollingeriana* measured on their morphological response and dry matter yield in the coconut farming area.

## MATERIALS AND METHODS

The study was conducted in the experimental station of Assessment Institute Agricultural Technology (AIAT) of North Sulawesi, located 12 km from Manado City. Experimental site receives an average rainfall of 2700 mm, and the distribution fairly even, except for the period of lower rainfall by 100-150 mm monthly, from July to September every year. The pH of the fertile, sandy loam soil is around 6. Light transmission at 10.00 a.m on a sunny day as PAR underneath mature tall coconuts averaged 73%.

Indigofera seeds sown on land that has been processed as a nursery. Plant seeds that have grown well are then moved into a 2.5 kg plastic bag that has already been filled with soil (one plant / plastic bag). After growing for 2 months in a plastic bag medium, the plant was then transferred to experimental site in a plot size of 3m x4 m that had been processed and divided into 18 plots to accommodate the 3 treatments of planting spacing (PS) namely PS1: 100 cm x 50 cm, PS2: 100 cm x 100 cm, and PS3: 100 cm x 150 cm, corresponding to the population densities of 21 plants/plot (1.75 plant/m$^2$), 12 plants/plot (1 plant/m$^2$), and 9 plants/plot (0.75plant/m$^2$), corresponding 5714 plant/ha, 10,000 plant/ha and 13,333 plant/ha respectively. Each plot of treatment had a size of 3x4 m (12 m$^2$) was then placed individually. Since the distance between plots of treatments were 1 meter apart, caused the space of land utilized of each plot enlarge up to 4x5 m (20m$^2$) in each 10x10 m of square pattern planting of coconuts. There by the number of plots of treatments in each space of coconut of 100 m$^2$ were then 5 plots.

The variables measured were: (1) plant height, (2) stem diameter, (3) leaves number, and (4) number of branches. Ten individual plants has been selected as sample in each treatment. The total number of plants as sample in this experiment were sum of 10 plants x 3 treatment x 6 replication = 180 plants. Morphological traits data were calculated each week along 3 months consecutive observations has been done before harvesting.

The plants that are sampled for counting the number of branches is in the middle of the experimental plot in order to avoid border effect, and to facilitate observation the sample plants are marked with a red ribbon. Harvesting was done by cutting the plant canopy, then the leaves and stems are separated. Samples of 500 g were then dried in an oven at a temperature of 105$^0$C for 24 hours to get dry weight. Crude protein (CP) content of leaf component varied from 33 up to 37% or an average 35%, steam component CP content average 17%, or the whole plant CP was average 26%.

This study used a Completely Randomized Design consisting of 3 treatments of planting spacing and 6 replications. Data were then statistically analyzed by using analysis of variance (ANOVA) by means of MINITAB (Version 16). Honestly Significance Difference (HSD) was applied to determine the difference among treatments. Differences were considered at $P<0.05$.

## RESULTS AND DISCUSSIONS

The influence of planting spacing treatments on plant population and morphological traits variable measured can be seen in Table 1. The highest plant height at the age of 3 months, obtained at the planting spacing 100 cm x 100 cm (PS2) with a height reaching around 86 cm. This treatment (PS2) differs markedly ($P <0.05$) higher than planting spacing at 100 cm x 50 cm (PS1), but there is not difference ($P>0.05$) compared to planting spacing at 100 cm x 150 m (PS3).

Stem diameter and number of branch affected by planting spacing. At treatment PS2 and PS3 gives higher ($P<0.05$) in stem diameter and number of branches compared to those in narrower spacing 100 cm x 50 cm (PS1), but both last treatments were not different ($P>0.05$).

**Table 1. Some morphological traits of *I. zollingeriana* under difference planting spacing in coconuts plantation area**

| Items | Treatments groups | | | SE | p value |
|---|---|---|---|---|---|
| | PS1 | PS2 | PS3 | | |
| Plant height (cm) | $68.73^{b}$ | $86.57^{a}$ | $71.62^{ab}$ | 0.231 | 0.001 |
| Stem Diameter | $0.93^{b}$ | $1.18^{a}$ | $1.04^{ab}$ | 0.061 | 0.034 |
| Leaves number | 15.33 | 16.17 | 15.93 | 0.317 | 0.192 |
| Number of branches | $8.27^{b}$ | $11.60^{a}$ | $11.00^{a}$ | 1.361 | 0.214 |

$^{a,b}$Means in the same row with different letters show differences (P < 0.05).

The highest number of leaves at 3 months after planting obtained at the planting spacing 100 cm x 100 cm with the average number reached around 16 leaf per plant. Analysis of variance showed that treatments has not affects differently (P>0.05), on the number of leaves. The effect of space between plants on the number of branches per plant is presented in Table 1. The highest number of branches at the 3 months, obtained at the planting spacing 100 cm x 100 cm (PS2) reaching at around 11 branch and significantly higher (P<0.01) than treatment PS1 (8.27) but not differ compared treatment PS3 (11.00).

Table 2 below presented data the effects of treatments on biomass dry weight based on population density or number of plants per hectare. Those populations were 5,710 plants, 10,000 plants and 13,333 plants, corresponding to PS1, PS2 and PS3 respectively. The ratio of leaves / wood instead is not affected by planting spacing. The highest dry weight production (24,1 kg/ ha /harvest) resulted from the treatment of planting spacing 100 cm x100 cm (PS2) and 24,9 kg/ha/harvest at planting distance 100 cm x 150 cm (PS3), and both treatments were higher (P<0.05) compared to treatment PS1 (21,2 kg/ha/harvest).

**Table 2. Leaf (L), wood (W), L/W ratio and DW yield of *I. zollingeriana* under difference planting spacing in coconuts plantation area**

| Items | Treatments groups Number plant ($ha^{-1}$) | | | SE | p value |
|---|---|---|---|---|---|
| | PS1 5,710 | PS2 10,000 | PS3 13,333 | | |
| Leaf DW (Kg. $ha^{-1}$) | $13,6^{b}$ | $16,59^{a}$ | $15,75^{a}$ | 0.730 | 0.033 |
| Wood DW (kg.$ha^{-1}$) | $7,54^{b}$ | $9,31^{a}$ | $9,14^{a}$ | 0.231 | 0.001 |
| Leaf/Wood ratio | 1.81 | 1.78 | 1.72 | 0.056 | 0.242 |
| Total DW (kg.$ha^{-1}$) | $21,2^{b}$ | $24,1^{a}$ | $24,9^{a}$ | 0.596 | 0.001 |

$^{a,b}$Means in the same row with different letters show differences (P < 0.05)

Shorter plant height, smaller diameter and lower number of branches in treatment PS1 is in strong relation with the higher plant populations almost double compared to PS2 and PS3. It means this phenomenon occur is probably due to strong competition of nutrient and water, which is markedly arise in crowded plant population (Craine & Dybzinsky, 2013). Contrary the wider spacing of PS2 and PS3 showed plant height, stem diameter and number of branches were significantly superior compared to narrower spacing PS1. The increase in plant height in equidistant spacing (PS2) is probably be due to high rate of stem elongation. Stem elongation is related to the light competition among plants in narrow planting spacing (Widodo et al., 2016), followed with taller plant compared to those in wider spacing (Craine & Dybzinski, 2013). The increasing this plant height in PS2 treatment followed by increasing in stem diameter (1.18) and number of branches (11.60). This founding is in agree with previous statement that narrower row spacing at 1.0 m x 0.5 m (PS1) reduces the number of branches (Kumalasari et al., 2017). It is likely that the greater spacing between adjacent plants within rows enhances the abilities of the plants to convert the intercepted solar radiation to leaf production (Telleng et al., 2016). Nevertheless, leaf number was not affected by all plant spacing treatments. It is mean that this plant could produce same number of leaves at 3 months after planting for all treatments. This probably due to the age of tree legume plant at 3 months still in vegetative development stages which is leave component grown dominantly (Anis et al., 2016).

Plant parts that are preferred by livestock and have higher nutritional quality are leaf fractions (Kaligis et al., 2018) so that the ratio of leaves / stems becomes important. From our finding the highest number of branches of Indigofera at the age of 3 months, obtained at the planting distance 100 cm x 100 cm (PS2) reaching at around 11 branches. The greater number of branches the higher growing point for leave development and will be related to the availability of energy reserves (carbohydrates) sustain re-growth of forages plant (Anis et al., 2016). Previous report stated that leaf and branch of *I. zolingeriana* grown exponentially up to sixth pruning and then decrease gradually (Abdullah, 2014). This research has been done under shading environment in coconut plantations. Even though the number of plant populations increased per hectare but dry weight has not increased linearly. Total dry weight, as well as leaves and wood dry weight increased up to the treatment PS2, and then almost reached plateau at PS3. This phenomenon is probably due to the shortages light in coconuts plantation. Discussion about coconut plantation is still important topic in rural development since this commodity as back bone economy at farmer level (Kaligis et al., 2017). The highest dry weight production of *I. zolingeriana* in this research was found at planting spacing PS2 and PS3. Forages dry matter production is contributed by leaf and stem formation, which was affected by cell division and elongation. Both physiology process was the sites of high metabolic activity, including dry matter accumulation through photosynthetic activity utilizing of CO2 atmospheric (Schaufele & Schneider, 2000).

## CONCLUSIONS

Based on the results of this study it can be concluded:

**1**. The best morphological response of *I. zolingeriana* in term of leaf/wood ratio and total dry weight at the age of 3 months after planting was obtained in the 100 cm x 100 cm planting spacing underneath mature coconuts.

**2**. The more narrowing of planting spacing, results the more reducing the number of branches.

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# ADHESION AND ANTAGONISTS PROPERTIES OF *ENTEROCOCUS* MONOCULTURES AND THE OPPORTUNITY OF THEIR USE AS PROBIOTICS

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***Abstract***

*The adhesion and antagonist capacity of some Enterococcus strains isolated from human and animal intestinal contents was investigated. The obtained results demonstrated the increased adhesive capacity of the studied Enterococcus strains, especially those specific to the human digestive tract. At the same time, the high activity of enterococci monocultures in the control of Escherichia and Salmonella bacteria has been established, which indicates their antagonistic property. Based on the adhesive capacity and high antagonistic activity of enterococci, new microbial associations containing enterococci were investigated. The obtained results revealed a beneficial action of the new microbial preparations, which prevented the appearance and development of diarrheal dysfunctions, which argues the opportunity to include enterococci in the composition of associations or probiotic microbial preparations.*

***Key words:*** *adhesion capacity, antagonistic activity, Enterococcus, probiotics.*

## INTRODUCTION

For the production of probiotics, one of main criterion in the selection of useful strains of microorganisms is the ability to colonize the digestive tube (McNaught & MacFie, 2001; Alp & Kuleasan, 2019).

This process is quite complex and depends on several factors. These factors include the adhesion property of probiotic microorganisms, which is important for the interaction between probiotic strains and the host organism and underlies antagonism to pathogens (Nishiyama et al., 2016; Hanifeh et al., 2021).

The mechanism of adhesion is determined by the interaction between molecular complexes (proteins, oligosaccharides) from the surface of bacteria and intestinal glycoconjugates of epithelial intestinal cells, more precisely their mucus. The peculiarities of the formation of associations between bacterial proteins and components of intestinal mucus establish differential adhesion, which might be employed as a host strategy to possibly select for particular strains or species (Ouwehand & Salminen, 2003; Garcia-Gonzalez et al., 2018; Monteagudo-Mera et al., 2019; Hanifeh et al., 2021).

Some representatives of enterococci, namely specific strains of *Enterococcus faecalis* and *Enterococcus faecium* have been used as probiotics or feed additives (Becquet, 2003; Araújo & de Luces Fortes Ferreira, 2013; Hanchi et al., 2018). Enterococci are a component part of the human and animal intestinal micro-biocenosis, having an important role in the vital activity of the host organism (Sivieri et al., 2008; Araújo & de Luces Fortes Ferreira, 2013).

Some strains of enterococci have been used as triggers in food fermentation and as food preservatives. At the same time, strains of enterococci were tested as probiotics (Araújo & de Luces Fortes Ferreira, 2013; Hanchi et al., 2018). But the development of new enterococcal probiotics requires a more rigorous study in safety aspects in order to select harmless enterococcal strains for safe application (Ben Braïek & Smaoui, 2019).

As it was mentioned, one of the characteristics that apply to the selection of probiotic microorganisms is adhesion to the intestinal mucosa.

Adhesion capacities of *E. faecalis* and *E. faecium* to the intestinal mucosa have been studied in some agricultural and domestic animals.

Some studies confirm the efficacy of selected strains of enterococci as probiotics (Sivieri et al., 2008). Thus, the strain *Enterococcus faecium* WEFA23, isolated from Chinese infant feces, is able to exclude or displace the adhesion of *Escherichia coli* O157:H7, *Salmonella typhimurium* ATCC 13311, *Listeria monocytogenes* CMCC54007, *Staphylococcus aureus* CMCC26003, and *Shigella sonnei* ATCC 25931 to Caco-2 cells (He et al., 2019).
The strain of *Enterococcus faecium* OV3-6 with probiotic properties and its secreted active peptides is able to survive in simulated gastric and small intestinal conditions (Choeisoongnern et al., 2021). It is known that one of the basic characteristics in the selection of probiotics is resistance to gastric juice and bile salts (Araújo & de Luces Fortes Ferreira, 2013) and production of antimicrobial compounds such as enterocin (Franz et al. 1999; Araújo & de Luces Fortes Ferreira, 2013). This strain denotes α-hemolysis and is susceptible to most clinically relevant antibiotics (Choeisoongnern et al., 2021) and reduces the adhesion of *E. coli* and *S. typhimurium* on Caco-2 cells. The strain can prevent the growth of Gram-positive strains belonging to the genera *Bacillus*, *Carnobacterium*, *Listeria* and *Staphylococcus*. (Choeisoongnern et al., 2021).
However, representatives of enterococci are involved in several nosocomial infections due to virulence factors and antibiotic resistance. Thus, the development of new enterococcal probiotics requires strict evaluation in terms of safety aspects for the selection of harmless enterococcus strains (Ben Braïek & Smaoui, 2019).
At the same time, the use of certain strains of microorganisms as probiotics is regulated by the respective decision-making bodies. Thus, the European Food Safety Authority determined that enterococci did not meet „Qualified Presumption of Safety" status (Becquet, 2003; Wang et al., 2020). No enterococcal probiotic has been approved by the United States Food and Drug Administration for the treatment, cure, or amelioration of human disease and enterococcus strains used or proposed for use as probiotics should be carefully screened for efficacy and safety (Wang et al., 2020).
However, *E. faecium* is still used as feed supplements in the USA and China, termed as direct-fed microorganisms (Wang et al., 2020).

The efficacy and safety of microorganisms as probiotics, including enterococci, should be based on rigorous studies that would reveal all aspects of the action of the bacteria investigated. In the paper it was proposed to study the adhesion and antagonist capacities of enterococci, as these properties are considered a crucial step for intestinal bacteria to colonize and further interact with the host epithelium and the immune system and appears to be an important feature for probiotics.

## MATERIALS AND METHODS

The human and animal intestinal contents served as study material. *In vitro* conditions, using classical microbiological methods, the single strains of enterococci with enhanced antagonistic and adhesive properties were isolated, identified and selected.

Table 1. Distributions of *Enterococcus* strains by sources

| Source | The selected strains (number) |
|---|---|
| Human intestinal content | 18; 25; 32; 46; 49; 58; 67; 70; 82; 85; 89; 93; 108; 112; 116 |
| Animal intestinal content | 13; 21; 37; 43; 55; 64; 74; 77; 97; 101; 129 |

The adhesion capacity of enterococci was studied according to the method of Brilis (1986) using erythrocytes as a model of the macroorganism cells. The mixture of human, bovine and porcine erythrocytes was incubated at 30°C for 30 min, being stirred regularly, then the smear was prepared, lyophilized, fixed and colored according to the Romanovschi-Ghimze technique. The adhesion study was performed using a light microscope. The calculation was performed on the basis of 25 erythrocytes, analyzing 5 erythrocytes in the visual field, using the adhesion index of microorganisms. After this index, the adhesion level of the microorganisms is determined.
The antibacterial (antagonistic) activity of the culture supernatant of these strains were tested against *Escherichia* and *Salmonella*. For this purpose, the agar well diffusion method was used to test the antagonistic activity of the isolated strains of enterococci against the selected human pathogens (using nutrient agar media for testing bacteria). Then the plates were incubated for 24 h at 37°C. The zones of inhibition of pathogenic bacteria were measured by a transparent ruler. Three replicates for each

test were done for every evaluated pathogenic species (Bhat & Nalawade, 2016).

Based on the selected enterococcal strains, associations of enterococci with bifido- and lactobacilli were prepared. The newly developed associations were investigated in comparison with other existing microbial preparations in laboratory conditions on laboratory animals (white mice). For this purpose, the laboratory animals were divided into five groups of ten (10) animals each.

Group I received Bifidobacterin (based on bifidobacteria); group II - Lacidophil-WM (containing lactobacilli); group III - Bifi.form (includes bifidobacteria and enterococci); group IV - the new association of bacteria, developed for the first time based on microorganisms of the genus *Enterococcus* (*E. faecium*) and those of the genera *Bifidobacterium* and *Lactobacillus*; group V served as a control.

All preparations were administered orally, on the background of intestinal dysmicrobism, for 6 days, of 1 ml of microbial suspension (1 billion microbial cells) to 1 animal per day (before morning feeding).

Diluted intestinal contents samples were studied at the beginning and end of the experiment (before and after 6 days of administration of the studied microbial preparations). Quantitative indices of microorganisms of the genera: *Bifidobacterium*, *Lactobacillus*, *Escherichia*, *Proteus*, *Enterococcus* were determined.

The samples were subjected to research using classical microbiological methods (Garmasheva & Kovalenko, 2010).

Inoculation was performed on elective agar nutrient media for each genus of bacteria, with subsequent incubation at 37 ± 1ºC, for 24-72 h, under aerobic and anaerobic conditions. The final results are expressed in decimal logarithms (log) (GOST 30518-97, 2000).

## RESULTS AND DISCUSSIONS

The results of investigations regarding the selection of enterococci strains with high adhesion properties and antagonistic activity, revealed that streptococci isolated from human intestinal contents belong mostly to the genera *Streptococcus*, *Lactococcus* and *Enterococcus*, and from the intestinal contents to animals - from the genera *Streptococcus* and *Enterococcus* (data are not presented in this article).

The enterococci strains isolated from human and animal intestinal contents were tested for their adhesion and antagonist capacities.

The results of the research regarding the adhesive capacity of selected *Enterococcus* strains on native human, porcine and bovine erythrocytes (Table 2) show that the adhesion index was it was quite high (within 2.81 - 4.67 c. u.).

Table 2. The adhesive capacity of enterococci specific to the human and animal digestive tract

| Number of isolated and tested enterococcal strains | The number of microbial cells adhering to the surface of 25 native erythrocytes | Adhesion index c. u. |
|---|---|---|
| From the human intestinal content | | |
| 18 | 102.50±3.50 | 3.18 |
| 25 | 106.75±3.25 | 4.27 |
| 32 | 103.75±1.25 | 4.15 |
| 46 | 102.50±2.50 | 4.10 |
| 49 | 103.50±3.50 | 4.14 |
| 58 | 103.00±2.00 | 4.12 |
| 67 | 102.00±3.00 | 4.08 |
| 70 | 104.00±3.00 | 4.16 |
| 82 | 102.75±2.25 | 4.11 |
| 85 | 103.50±2.50 | 4.14 |
| 89 | 99.50±3.50 | 3.98 |
| 93 | 103.00±4.00 | 4.12 |
| 108 | 102.50±3.50 | 4.10 |
| 112 | 104.00±3.00 | 4.16 |
| 116 | 116.75±2.25 | 4.67 |
| From the animal intestinal content | | |
| 13 | 79.50±2.50 | 3.18 |
| 21 | 89.75±2.25 | 3.59 |
| 37 | 86.25±2.75 | 3.45 |
| 43 | 81.00±3.00 | 3.24 |
| 55 | 70.25±1.75 | 2.81 |
| 64 | 86.00±4.00 | 3.44 |
| 74 | 93.00±2.00 | 3.72 |
| 77 | 96.25±1.75 | 3.85 |
| 97 | 89.25±3.75 | 3.57 |
| 101 | 78.00±3.00 | 3.12 |
| 129 | 91.25±3.75 | 3.65 |

A higher adhesion capacity was identified in enterococcus strains from human intestinal contents, ranging from 3.98 to 4.67 c. u. The highest value of the adhesion index was found at strain no. 116, namely 4.67 c. u.

The adhesion index to the selected enterococcal strains from the animal intestinal contents is in the range of 2.81-3.85 c. u. The highest value (3.85 c.u.) was detected at strain no. 77.

Data on antagonistic capacity (Table 3A and B) indicate that high antagonistic activity against pathogens (*Escherichia* and *Salmonella*) shows the selected enterococcal strains from the human intestinal contents, compared to those in the animal digestive tract.

Table 3A. The antagonistic activity of isolated enterococcal strains, specific to the human and animal digestive tract (part I)

| Number of selected entero-coccal strains | Absolute number of microbial cells per 1 ml of microbial suspension in decimal logarithms (lg), inoculated jointly with bacteria of the genera | | | |
|---|---|---|---|---|
| | *Escherichia* | | *Salmonella* | |
| | *at the beginning* | *at the finally* | *at the beginning* | *at the finally* |
| From the human intestinal content | | | | |
| 18 | 3.90±0.09 | 2.74±0.07 | 3.46±0.1 | 2.77±0.07 |
| 25 | 3.82±0.08 | 2.88±0.06 | 4.07±0.11 | 3.13±0.12 |
| 32 | 3.80±0.08 | 2.87±0.06 | 3.77±0.05 | 3.04±0.13 |
| 46 | 3.46±0.05 | 2.70±0.00 | 3.82±0.04 | 3.00±0.19 |
| 49 | 3.70±0.08 | 2.84±0.16 | 3.76±0.17 | 3.04±0.12 |
| 58 | 3.95±0.12 | 3.07±0.13 | 3.70±0.12 | 2.98±0.15 |
| 67 | 4.11±0.11 | 3.23±0.12 | 3.62±0.26 | 2.88±0.13 |
| 70 | 3.69±0.11 | 2.72±0.09 | 3.54±0.14 | 2.64±0.17 |
| 82 | 3.77±0.13 | 2.87±0.13 | 3.46±0.08 | 2.88±0.13 |
| 85 | 4.07±0.12 | 3.04±0.05 | 3.84±0.09 | 2.94±0.04 |
| 89 | 4.20±0.10 | 3.32±0.1 | 3.68±0.11 | 2.92±0.07 |
| 93 | 3.79±0.11 | 2.88±0.04 | 3.62±0.13 | 2.81±0.12 |
| 108 | 4.07±0.13 | 3.14±0.13 | 3.64±0.12 | 2.92±0.09 |
| 112 | 4.23±0.14 | 3.20±0.10 | 3.77±0.15 | 2.90±0.11 |
| 116 | 3.98±0.10 | 2.70±0.08 | 3.72±0.13 | 2.77±0.07 |
| From the animal intestinal content | | | | |
| 13 | 4.25±0.09 | 3.46±0.06 | 3.84±0.09 | 3.13±0.14 |
| 21 | 3.60±0.07 | 2.90±0.08 | 3.41±0.13 | 2.92±0.08 |
| 37 | 3.64±0.11 | 2.98±0.1 | 4.17±0.13 | 3.46±0.09 |
| 43 | 3.50±0.1 | 2.90±0.13 | 3.60±0.07 | 3.07±0.04 |
| 55 | 3.70±0.04 | 3.00±0.12 | 3.65±0.06 | 3.11±0.13 |
| 64 | 3.78±0.13 | 3.11±0.11 | 3.77±0.07 | 3.17±0.13 |
| 74 | 3.75±0.08 | 3.14±0.12 | 3.49±0.08 | 2.96±0.12 |
| 77 | 3.67±0.07 | 2.96±0.14 | 3.50±0.04 | 2.86±0.15 |
| 97 | 4.17±0.09 | 3.43±0.07 | 3.54±0.14 | 2.98±0.14 |
| 101 | 4.04±0.07 | 3.46±0.12 | 3.76±0.08 | 3.25±0.10 |
| 129 | 4.13±0.08 | 3.41±0.11 | 3.80±0.10 | 3.23±0.12 |

Table 3B. The antagonistic activity of isolated enterococcal strains, specific to the human and animal digestive tract (part II)

| Number of selected enterococcal strains | The control coefficient for pathogens, % | |
|---|---|---|
| | *Escherichia* | *Salmonella* |
| From the human intestinal content | | |
| 18 | 29.74 | 19.94 |
| 25 | 24.60 | 23.09 |
| 32 | 24.47 | 19.36 |
| 46 | 21.96 | 21.45 |
| 49 | 23.24 | 19.14 |
| 58 | 22.27 | 19.45 |
| 67 | 21.41 | 20.44 |
| 70 | 26.28 | 25.42 |
| 82 | 23.87 | 19.76 |
| 85 | 25.30 | 23.43 |
| 89 | 20.95 | 25.85 |
| 93 | 24.01 | 22.37 |
| 108 | 22.85 | 19.78 |
| 112 | 24.34 | 23.07 |
| 116 | 32.16 | 25.53 |
| From the animal intestinal content | | |
| 13 | 18.58 | 18.48 |
| 21 | 19.44 | 14.36 |
| 37 | 18.13 | 17.02 |
| 43 | 17.14 | 14.72 |
| 55 | 18.91 | 14.79 |
| 64 | 17.72 | 15.91 |
| 74 | 16.26 | 15.18 |
| 77 | 19.34 | 18.28 |
| 97 | 17.74 | 15.81 |
| 101 | 14.35 | 13.56 |
| 129 | 17.43 | 15.00 |

Thus, the antagonistic activity of enterococcal strains specific to the human digestive tract varies from 20.95% to 32.16% (Table 3B), the most effective in this regard being strain no. 116, for which the coefficient of control of *Escherichia* and *Salmonella* constituted respectively 32.16% and 25.53%.

Among the enterococcal strains specific to the animal digestive tract, the highest activity to combat pathogenic microorganisms was detected in strain no. 77, at which the control coefficient of *Escherichia* and *Salmonella* was 19.34% and 18.28% respectively.

Thus, it is observed a strong correlation between adhesion capacity and antibacterial activity of studied strains of *Enterococcus*. The strain no. 116 from human intestinal content and strain no. 77 from animal digestive tract, which a high adhesion capacity, are also more efficient in fighting against pathogenic bacteria, and capable in this way preventing infectious diseases.

Based on the data obtained, it can be stated that all enterococcal strains, isolated for the first time, highlighted the probiotic potential, expressed by the increased adhesion capacity, high level of antagonistic activity.

The investigations *in vivo* conditions on laboratory animals (white mice) were aimed to elucidate the action of the new developed association based on microorganisms of the genus *Enterococcus* (*E. faecium*) and those of the genera *Bifidobacterium* and *Lactobacilli* compared to other microbial preparations on the quantitative indices of some intestinal microbial representatives (*Bifidobacterium*, *Lactobacillus*, *Escherichia*, *Proteus*, *Enterococcus*) (Table 4).

Analyzing the data from table 4, it was observed that for all the animals (experimental groups I-IV), after 6 days of probiotic preparation administration, the state of the intestinal eubiosis is present, and in those from the control group - of dysmicrobism. The state of dysmicrobism is characterized by high quantitative indices of *Escherichia* and *Proteus* bacteria and the state of intestinal eubiosis - by the low values of these indices respectively and high indices of *Bifidobacterium*, and *Lactobacillus*.

At the same time, all tested preparations had a beneficial effect on the animal body, preventing the occurrence and development of diarrheal dysfunction (in 100% of tested animals), while in the control group such disorders were recorded in 80% of animals and the remaining 20% - intestinal dysmicrobism.

Table 4. Quantitative indices of intestinal microbiocenosis (of experimental animals) at administering of various microbial preparations

| The experimental groups | Types of microorganisms | Number of microbial cells per 1 g of intestinal contents, decimal logarithm (log) | |
|---|---|---|---|
| | | *at the beginning* | *at the finally* |
| I | *Bifidobacterium* | 7.17±0.08 | 9.41±0.07 |
| | *Lactobacillus* | 6.88±0.12 | 7.72+0.09 |
| | *Escherichia* | 8.84±0.13 | 7.46±0.12 |
| | *Proteus* | 3.11±0.09 | 2.07±0.10 |
| | *Enterococcus* | 6.77±0.11 | 7.90±0.14 |
| II | *Bifidobacterium* | 7.53±0.11 | 8.04±0.13 |
| | *Lactobacillus* | 6.32±0.12 | 8.32±0.10 |
| | *Escherichia* | 8.96±0.10 | 7.54±0.14 |
| | *Proteus* | 3.00±0.13 | 1.23±0.12 |
| | *Enterococcus* | 6.84±0.15 | 7.04±0.13 |
| III | *Bifidobacterium* | 7.23±0.13 | 9.20±0.12 |
| | *Lactobacillus* | 6.63±0.11 | 7.70±0.13 |
| | *Escherichia* | 8.68±0.12 | 6.82±0.10 |
| | *Proteus* | 3.07±0.11 | 1.17±0.10 |
| | *Enterococcus* | 6.54±0.14 | 8.62±0.10 |
| IV | *Bifidobacterium* | 7.60±0.10 | 9.47±0.13 |
| | *Lactobacillus* | 6.50±0.11 | 8.65±0.09 |
| | *Escherichia* | 8.46±0.12 | 5.79±0.11 |
| | *Proteus* | 3.17±0.08 | 0 |
| | *Enterococcus* | 6.32±0.13 | 8.88±0.12 |
| V | *Bifidobacterium* | 7.20±0.12 | 7.82±0.10 |
| | *Lactobacillus* | 6.11±0.11 | 6.77±0.11 |
| | *Escherichia* | 8.53±0.11 | 9.49±0.12 |
| | *Proteus* | 3.04±0.10 | 4.14±0.08 |
| | *Enterococcus* | 6.49±0.12 | 7.23±0.13 |

The association developed for the first time based on microorganisms of the genus *Enterococcus* (*E. faecium*) and those of the genera *Bifidobacterium* and *Lactobacilli* has a greater action of inhibiting pathogenic bacteria (*Escherichia* and *Proteus*).

The antagonistic relations between the bacteria are of interest in their use in the fight against pathogenic genes and the infections triggered by them. According to several studies, the antagonistic activity is based on the adhesion capacity of bacteria and the production by microorganisms of substances with antagonistic/antibacterial action (lactic acid, bacteriocins, etc.). Use as a probiotic of lactic acid bacteria - LAB; is recognized by the FAO and the WHO, being recognized as safe status (GRAS) (Zielińska& Kolożyn-Krajewska, 2018; Ben Braïek & Smaoui, 2019).

It has been established that enterococci produce lactic acid and enterocin (a substance with antibacterial action) (Nami et al., 2019), which make some enterococcus strain promising for probiotics, with their application in diarrhea treatment in association with antibiotic medication, viral infection, chemotherapy and diseases originated from food-borne pathogens (Lau & Chamberlain, 2016).

While, probiotic effect of *Enterococcus* is strain dependent. The use of enterococci stains as probiotics must be based on research proving their safety, as well as the lack of virulent factors (Nascimento et al., 2019).

Based on the obtained data, it is argued that the selected strains of enterococci in the composition of associations or microbial preparations with probiotic action, but the investigations on adhesion capacity and antagonistic activity is a first step in the selection of probiotic strains of *Enterococcus*.

## CONCLUSIONS

Enterococci are part of the digestive tract microbiocenosis, having a special role in the normal functioning of the digestive tract.

The strains of enterococci isolated for the first time from the intestinal contents of humans and animals have shown useful properties for the organism, confirmed by increased indications of antagonistic activity and adhesive capacity.

Human digestive tract-specific enterococcal strains showed a higher adhesion and antagonist capacity compared to those specific to the animal digestive tract.

Experimentally, the inclusion of enterococcal strains isolated for the first time in the composition of associations and microbial preparations intended to strengthen the health of the digestive tract (in case of intestinal dysmicrobism and diarrheal dysfunction) has been argued.


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# NATURAL AND INEXPENSIVE NUTRITIONAL HERBAL SOLUTIONS TO ALLEVIATE HEAT STRESS IN POULTRY

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***Abstract***

*Heat stress represents a real menace of the poultry industry all around the world. The World Meteorological Organization (WMO) predicts an increasing temperature by 1.5°C as early as 2024. The normal body temperature of a chicken is about 41°C, and its thermoneutral comfort zone ranges between 18-25°C. To avoid the overheating and to dissipate the excess heat the poultry's body conducts a demanding struggle, therefore it affects production and immune parameters, and also eggs' quality. Reduced appetite and feed intake due to an impaired digestion and metabolism caused by intestinal morphology damage requires different strategies against the negative impacts of heat stress. Dietary herbal supplementation with natural and inexpensive ingredients can be considered an efficient strategy. There are globally available, with scientific demonstrated thermoregulatory effects, and also with antioxidant properties that enhances the production parameters and the health status of poultry. Herbal utilization offers medium and long-term economic and natural potential to minimize the negative effects of heat stress in local and global poultry industry*

***Key words**: diet, heat-stress, herbal, poultry, temperature.*

## INTRODUCTION

The aim of this review is to gather various data about natural, affordable but efficient tested and proved herbal solutions with pharmacological and nutritional values within poultry diets to alleviate the negative effects of heat stress. The heat stress negative effects in poultry industry were already stated on international forums, becoming a real public awareness and concern. It is critical for an optimal poultry production and welfare to understand, find solutions and alternatives to control high environmental temperatures since birds possess no sweat glands and must find other means of dissipating heat. The productive parameters are visible affected: a decreasing body weight, a reduced feed intake and consequently a lower egg production (Mehaisen et al., 2018). Heat stress causes serious physiological, metabolic and genetic changes, such as oxidative stress, acid-base imbalance, and a suppressed immune response, which leads to increased mortality (Wasti et al., 2020) According to several authors, our understanding of basic mechanisms connected to the observed effects, as well as regarding poultry behaviour and welfare under heat stress conditions, is limited, therefore a great point of interests must be still accorded since the data of most stress heat experiments had been variable, inconsistent or scarce (Lara & Rostagno, 2013). Modern poultry genotypes are thought to create more body heat due to their increased metabolic activity (Setar et al., 1999; Deeb & Cahaner, 2002). The shell egg quality is affected due to the increase levels of carbon dioxide that obstructs the bioavailability of bicarbonate into blood therefore results a poor egg mineralization (Marder & Arad, 1989). An increased concentrations of reactive oxygen species (ROS) were observed under heat stress condition which causes an increase intestinal permeability (Quinteiro-Filho et al., 2012). The intestinal barrier is weakened and as a consequence the susceptibility to infections is increasing due to a reduced protection against microbial pathogens from the gastrointestinal tract (Gupta et al., 2017). An increased intestinal permeability was noticed in broilers, a decreased villus height and villus height:crypt depth were observed in laying hens (Garriga et al., 2006; Bozkurt et al., 2012). Heat stress affects also food safety allowing those foodborne pathogens as Salmonella and Camphylobacter to disseminate into human food chain (Eisenberg et al., 2012). The circulating cells amount and the heterophil:lymphocyte ratio by reducing the lymphocytes and increasing the heterophils was noticed when stress heat was experienced in poultry (Deng et al., 2012). Excretion of minerals and vitamins concentrations increases under heat stress conditions, therefore decrease their concentration from serum and liver (Sahin et al., 2009). This review aims to bring into readers attention about ordinary herbal ingredients for us but with important properties when using them under stress conditions in poultry diet.

## MATERIALS AND METHODS

To accomplished the review's objective, by consulting and mentioning 56 of bibliographic sources from the literature. Relevant articles were consulted by identifying scientific databases with keywords as heat-stress, poultry, herbal, temperature, diet. Also, we searched through literature specifically herbal ingredients with demonstrated thermoregulatory effects such as fennel, thyme, parsley, dill, rosemary, coriander.

## RESULTS AND DISCUSSION

According to Panossian (2013) herbal ingredients are considered metabolic regulators able to help the poultry organism to adapt and resist when temperature exceeds the thermo-neutral zone (16-25°C). Inexpensive and natural herbal ingredients with thermoregulatory effects such as fennel, thyme, parsley, dill, rosemary, coriander with their pharmacological and nutritional values improved production and performance in poultry under heat stress conditions (Wang et al., 2008).

Fennel (*Foeniculum vulgare* Mill.) is an aromatic wild edible plant, well appreciated flavouring agent, which is also cultivated extensively in Mediterranean region, used in traditional medicine with a wide range of therapeutic properties, that exhibits hepatoprotective, antioxidant (due to its phenolic compounds), antimicrobial and antifungal activities (Renna et al., 2015). The alleviating effects of fennel seeds were tested on broilers exposed to chronic heat stress (32±2°C) for seven hours using a dietary inclusion rate of 1.6, and 3.2%. The study showed that dietary fennel seed powder 3.2% improved the growth rate of broiler between 19-41 days, enhanced breast meat redness and reduced temperature under chronic heat stress. In conclusion, 3.2% of fennel seed powder could be used as an agent for enhancing the broiler's tolerance during chronic heat stress condition from 19 to 41 days of age (Al-Sagan et al., 2020). Other authors declared that an inclusion rate in broilers diet of 2% or even 1% fennel seeds was enough to improve feed intake, meat breast (%) and leukocytes (Ragab et al., 2013). An inclusion rate of 10 and 20 g/kg dietary fennel fruits were tested in White Leghorn laying hens' diets under 34°C. The results showed that fennel due to its antioxidant properties reduced the MDA and carbonyl egg content values, also registered lower cholesterol and triglyceride yolk concentrations (Gharaghani et al., 2015) A concentration of 0.5% fennel seeds can be added to the laying hens' diets with beneficial effects on egg quality, laying performance, and serum biochemical parameters (Abou-Elkhair et al., 2018). The improvement of egg quality when heat stress is experienced can be attributed to the anethole antioxidant compound of fennel (Oktay et al., 2003). Dietary supplementation with 1, 2, and 3 g fennel/kg diet registered significant improvement ($p<0.05$) of body weight, feed efficiency, red blood cell concentration, haemoglobin, and packed cell volumes (PCV), without heat stress factor (Mohammed & Abbas, 2009).

Thyme (*Thymus vulgaris* L.) is an aromatic herb with a distinctive aroma and flavour often used in the food and pharmaceutical industries. Thyme has many therapeutic properties: antimicrobial, hypocholesterolemic, antioxidative, antiviral, antifungal, immunostimulants (Fachini-Queiroz et al., 2012). Thyme essential oil added into broilers diet as 100, 150 and 200 mg TEO/kg of diet, 22 to 42 days of age, under heats-stress exposure (23.9-38°C daily) improved growth performances and immune responses, and lowered the heterophile concentration of birds Rafat et al., 2019). During the Egyptian hot summer season dietary thyme dried leaves supplementation with 1 and 2% did not influenced the productive parameters and the thermoregulatory responses of broilers according to Ragab et al. (2013). Some authors experimented 250 mg/kg thyme essential oil on broilers in thermoneutral conditions and observed a significantly lower plasma uric acid and triglyceride levels (Noruzi et al., 2022). Others researchers noticed no effects on body weight gain, feed conversion ratio, cholesterol concentration heterophils: lymphocytes ratio when added 0.5 and 1% thyme into broilers diet under heat stress at 34°C ± 2 (Behboudi et al., 2016). Dietary thyme oil (1.0 g/kg feed, 1.5 g/kg feed, 2.0 g/kg feed) was experimented on broilers as a natural growth promoter in hot climate conditions and the best obtained results (white blood cells were noticed for 1.0 g/kg diet during 1-28 rearing days. According to Marino et al. (1999), *in vitro* studies demonstrated the thyme is delaying growth properties of *E. coli* and *S. typhimurium* as stated by Aktuğ et al. (1986).

Parsley (*Petroselinum crispum* Mill.) is a powerful antioxidant plant that belongs to family Apiaceae or Umbelliferae, most appreciated as medicinal adjuvant, food and spice with a rich phytonutrients content of the whole plant: leaf, stem and root (Agyare et al., 2017). Its phenolic content is hold responsible for antioxidant and antibacterial activities (Wong & Kitts, 2006). The valuable antioxidant properties of parsley are due to flavonoid, ascorbic acid, tocopherol and essential oils content (Zhang et al., 2006). Parsley essential oils were used in poultry feeding to improve productive performances and health, as heat stress alleviator with a high antioxidant activity (Gopi et al., 2014). Effect of parsley oil (0.3, 0.6, 0.9 ml/kg diet) in Japanese quail males testicular histomorphometric evaluation and semen quality were tested for 14 weeks in heat stress condition and a significant improvement were observed at a dietary inclusion rate of 0.9 ml parsley oil/kg (Razooqi et al., 2019). Researchers stated that a parsley-rich diets supports the antioxidant system at cellular level therefore reducing stress-induced gastric injury (Akıncı et al., 2017). Adding 80 g/d parsley, 160 g/d parsley, 240 g/d parsley to local Iraqi geese diets, a significant increase of haematological parameters (haemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, thrombocytes, white blood cells, lymphocytes increase) was noticed, as well as a decrease in heterophile: lymphocytes ratio and eosinophils concentration (Al-Daraji et al., 2012). As we studied the previous literature concerning parsley utilization in poultry diets we noticed a shortcoming experiments, concerning its dietary supplementation.

Dill (*Anethum graveolens* L.) is an important aromatic herb of Apiaceae family with multiple utilization: medicinal, foods and beverages flavouring. It is used to treat gastrointestinal disorders, to lower blood total cholesterol, glucose and as antioxidant agent (Oh et al., 2022). Supplementation of 15 ml/100 kg dill essential oil within heat stress conditions (12 h of 18-22°C, 3 h of 20-31°C, 5 h of 31°C, 4 h of 31-20°C) improved production performance and antioxidant activity (glutathione peroxidase) in heat-stressed laying hens according to Torki et al. (2018). Dietary supplementation with 1% dill and combination between pennyroyal (0.5%) and dill (0.5%) in broilers' under thermoneutral conditions registered a significant malondialdehyde content decrease and an antioxidant capacity increasing (Mohammadi, 2020). Also, other authors testing 1% of dill plant powder in broiler diets recorded growth performances improvement under normal temperature conditions (Mohasesi et al., 2021). Under normal temperature conditions but different inclusion rate of dill seeds (0.3%) in combination to hemp seed (0.2%) were tested in broilers' diets. This seeds combination led to triglyceride, LDL and total cholesterol concentration significant serum reduction while gut health was significant improved by caecum and jejunum *Lactobacilli* proliferation (Vispute et al., 2019). A lack of literature information about dill utilization in poultry nutrition under heat stress conditions affects the overall understanding of this plant addition effects on physiological and production performances.

Coriander (*Coriandrum sativum* L.) aromatic herb of Apiaceae family indigenous from southern Europe widely distributed and mainly cultivated for its edible seeds and leaves characterized by a high content of vitamins C, A, K, and minerals I, Mn, Zn, and dietary fiber. It is an antibacterial agent for *B. subtilis* and *E. coli.* (Kumar et al., 2016). The plant is rich in petroselinic acid and high in linalool isolated from the seeds and the aerial parts (Mandal & Mandal, 2015). According to Hamodi et al., (2010), testing different inclusion levels of coriander seeds (1%, 2%, 3%) in broilers' feeding under heat stress conditions (32-36°C) a rate of 2% registered significant higher feed consumption and feed conversion and a general improvement of production performances. Other authors added the same inclusion rate of 2% coriander seeds in broilers aged 14 to 42 days old under 34°C and observed a significant improvement in feeding behaviour, productive performances, dressing percentage, concomitant with a decreasing of corticosterone level (El-Shoukary et al., 2014). Other authors tested different inclusion rates of coriander seeds (1.5%, 2.5%, 3.5%) in broiler feed, under normal temperatures, as growth promoter on productive performances and blood profile. Optimum rate inclusion based on dressing percentage without skin, biochemical blood parameters, and immune response results was 1.5% coriander seeds (Khubeiz et al., 2020). Better results on performance and physiological parameters were obtained using 2% coriander seeds inclusion rate on one day-old Arbor Acer broiler chick under high ambient temperature by Al-Jaff, (2011). Coriander oil inclusion at levels of 0.5% and 1% in broilers' diet decreased significantly plasma cholesterol and glucose and improved production performances during summer conditions (Al-Mashhadani et al., 2011).

Rosemary (*Rosmarinus officinalis* L.) a well-known aromatic plant of Lamiaceae family, widely consumed as fresh and dried leaves, as extract or as essential oil in traditional Mediterranean cuisine and in folk medicine Ribeiro-Santos *et al*., 2015). Its major components of polyphenolic profile are carnosic acid, carnosol, rosmarinic acid and hesperidin (Tai et al., 2019). Related to this polyphenolic compounds, rosemary extracts include anti-inflammatory, hepatoprotective, antidiabetic, and antimicrobial activity. According to some authors rosemary extract antimicrobial activity was considerably higher compared to commonly additives used in food industry (Nieto et al., 2018). Due to antioxidant properties of rosemary (isoprenoid quinones) its inclusion in animal feed as extract was beneficial, especially in broilers' where a delayed lipid oxidation was registered in a diet with 200 mg/kg a-TAc, 500 mg/kg rosemary, 500 mg/kg sage combination, as some authors reported (Lopez-Bote et al., 1998). Also, there are contrary results obtained by other authors that stated that dietary rosemary inclusion had no effect on meat or eggs lipid stability (Galobart et al., 2001). The purified rosemary extract was evaluated in vivo on myocardial cell model to assess its effect on heat stress response by analysing broilers' heat shock proteins profile. The results confirmed that its alleviation heat stress effects in broiler chickens (Tang et al., 2018). Different combination of essential oils of rosemary and other plants were tested as 45 ppm of *Lippia origanoides* with 45 ppm of *Rosmarinus officinalis* and 300 ppm of beetroot with 700 g/ton feed dietary inclusion; or 45 ppm *Lippia origanoides* and 45 ppm *Rosmarinus officinalis* and 300 ppm of Natural Betaine with feed 700 g/ton of feed dietary inclusion. A significant improvement of all productive parameters was noticed concomitantly with a significantly improved bone mineralization, which a reduction of negative effects of heat stress (Ruff et al., 2021). Other studies on Japanese quails showed that a supplementation of 125 mg/kg rosemary oil supplementation reduced heat stress induced oxidative stress due to its potent antioxidant activity (Ozcelik et al., 2014). Positive impact on productive performances, and microbiological cecal composition was observed at an inclusion rate of 0.4% rosemary powder in broilers' feeding (Petricevic et al., 2018).

## CONCLUSIONS

These commonly used aromatic herbs, consumed worldwide ameliorate the negative effects of heat stress in poultry without any adverse effects. There are safe and healthy to use them as supplements especially taking into consideration the increasing consumer's preoccupation for natural ingredients addition in animal feed to obtain quality products.

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# HEALTH STATUS, PERFORMANCE AND CARCASS CARACTERISTICS OF BROILER CHICKS SUPPLEMENTED WITH YEASTS BIOPRODUCTS

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***Abstract***

*The current study aimed to evaluate the effects of spent brewer's yeast (SBY, Saccharomyces spp.), with or without the addition of Rhodotorula spp. biomass (Rh), as dietary supplements on broiler health status and growth performance. A total of 320 one-day-old, Ross 308 broiler chicks were randomly divided into eight experimental groups with five replicate pens of eight birds/replicate. A 4 by 2 factorial design study was used, with SBY different inclusion levels (0, 0.6, 1 and 1.3 g/kg feed) and Rh supplementation (0 or 0.3 g/kg of feed) as treatments. There were no significant effects between the main factors SBY x Rh on the hematologic profiles (P>0.05) of the broilers. Blood serum biochemical profile of SBY and Rh groups and the interactions between treatments were evaluated and no significant effects were found (P>0.05), except for the glucose (p = 0.023), which was influenced by the SBY addition. Moreover, the SBY addition (0.6 and 1g/kg) resulted in similar productive performances for weight gain and average daily gain, with the control group (Corn-SBM diet). In conclusion, yeast bioactive, nutritive, and pharmacologic compounds could serve as a suitable low-cost option to conventional supplements used in meat poultry nutrition.*

***Key words****: brewer's spent yeast, broiler, health, performance, Rhodotorula spp. biomass.*

## INTRODUCTION

In the poultry meat production industry, great attention is directed to protein feed resources (Ruiz et al., 2020), considering their essential role in muscular tissue synthesis (Estevez et al., 2020). It is well known that broiler chicks have high protein requirements (Gous et al., 2018), therefore identifying favourable growth promoting additives to support feed efficiency, while boosting the productive performance and muscular synthesis it is required (Alagawany et al., 2021). Natural feeding additives play considerable parts in the broiler rearing industry, serving as growth promoters, with positive health modulative mechanisms (Swaggerty et al., 2022). In order to support early-stage development and avoiding potential pathogenic threats, a common practice was represented by antibiotics administration, as growth promoters in poultry diets (Cuong et al., 2021). However, following the recent banning of using antibiotics as growth promoters in poultry nutrition, several strategies and potential alternatives, such as the use of bio-applications were tested (Al-Baadani et al., 2018; Peralta et al., 2018; dos Santos et al., 2018; Ciurescu et al., 2021).

Utilizing agro-industry by-products as low-cost and available resources (Jaeger et al., 2020) in poultry nutrition is currently of interest for both nutritionists and producers. The nutritive and growth promotion potential of agro-industrial waste, such as spent brewer's yeast (SBY) was recently studied (Ribeirto-Oliveira et al., 2021). With previous studies on the use of SBY showing encouraging results on improving broilers growth performance (Kumar et al., 2019 and Mulatu et al., 2019) and health status (Chuang et al., 2020). SBY has high levels of protein (39-55%, containing all essential amino acids), vitamin B complex, minerals (5-7% of dry biomass), lipids (4.4% of dry biomass), enzymes, β-glucans, mannan-oligosaccharides and selenium (Amoriello & Ciccoritti, 2020; Patel et al., 2018). The SBY is a by-product derived from beer production, through the use of fermentative yeasts (*Saccharomyces cerevisiae*), throughout the production of one hl of beer, it results up to 2-4 kg of SBY (Cimini and Moresi, 2020). Moreover, the SBY waste management and discarding procedures are imply significant time and financial efforts.

Colorant food additives used in meat production, are well known to improve visual characteristics of animal product, throughout modulating the appearance of meat to meet consumers demand (Faustino et al., 2019). To develop healthier alternatives to synthetic colorants, natural resources such as yeasts (e.g., *Rhodotorula* spp.) represent potential candidates (Aman et al., 2021). *Rhodotorula* spp. are known to synthesize large amounts of valuable xanthophylls and carotenoids (Kreusch & Duarte, 2021) in up to 96 h of fermentation, while using low-cost substrates. The genus includes a variety of colours, starting from pale pink and as far as dark red (Shengnan et al., 2017), while creating gram-positive round-shaped colonies (Soliman et al., 2018). The current study aimed to evaluate the effects of spent brewer's yeast (SBY, *Saccharomyces* spp.), with or without the addition of *Rhodotorula* spp. biomass (Rh), as dietary supplements on broiler health status and growth performance.

## MATERIALS AND METHODS

The experimental design and protocol used were in accordance with the EU Directive 2010/63/EU and Romanian Law on Experimental Animal Protection.

***Birds, design, husbandry and experimental diets***

Day-old 'Ross 308' broiler chick (n = 320) of mixed sexes, produced in a commercial hatchery, were randomly distributed at the start of the trial. The chicks (average body weight 43.11 ± 0.7 g/bird/group) were randomly allocated into 8 dietary treatments groups (see Table 1), during 42 days feeding trial. Each treatment was subdivided into 5 replicates per pens (experimental units) of equal size, with pens being arranged in longitudinal lines in the brooder house. The chicks were vaccinated against New Castle disease (NCD), Marek disease and anti-infectious bursal disease, as the conventional veterinary schedule requires. All birds were reared on a permanent deep litter (using wood shavings), with environmentally controlled conditions (air and temperature), according to birds age (Aviagen, 2019). Temperature was maintained at 32°C at placement followed by a 3°C decrease/week, up-to 20-21°C, using thermostatically controlled heaters, fans and adjustable sidewall inlets. Lighting was provided for 23 hours/day from 1day to 7 days, and then from the 8th day, the light decreased by 1 hour/day until 20 hours, according to EU legislation (EU Council Directive 2007/43/EC).

Table 1. Experimental design and dietary treatments

| Group | Dietary treatments |
|---|---|
| 1 | Negative control group - fed with basal diet (Corn-soybean meal); |
| 2 | Basal diet + SBY 0.6 g/kg feed; |
| 3 | Basal diet + SBY 1.0 g/kg feed; |
| 4 | Basal diet + SBY 1.3 g/kg feed; |
| 5 | Positive control group - fed with basal diet (Corn-soybean meal) + Rh (0.3 kg/t feed); |
| 6 | Basal diet + SBY 0.6 g/kg feed + Rh (0.3 kg/t feed); |
| 7 | Basal diet + SBY 1.0 g/kg feed + Rh (0.3 kg/t of feed); |
| 8 | Basal diet + SBY 1.3 g/kg feed + Rh (0.3 kg/t feed); |

The diets were arranged in a bifactorial design, with the variables being SBY supplement at four levels (0, 0.6, 1 and 1.3 g/kg diet, respectively) with (+) or without (−) of *Rhodotorula spp*. (Rh) supplement at two levels (0 and 0.3 g/kg diet). The SBY was procured as by-product from a local brewery and inactivated using successive thermic induced stress, lyophilised (0.39 mBar, -50°C), mashed and stored at -20°C. The SBY was evaluated for viability and vitality. The proximal composition and amino acid profile of the SBY was determined (Tables 2 and 3). In order to improve the nutritional value of the dietary protein, up to the level of digestible protein, in the diets structure, biosynthetic/ synthetic amino acids were added, respectively L-lysine HCl and DL-methionine, in variable proportions, depending on the level of inclusion of this ingredient in the manufacture of feed (Table 2). All the nutrients met or exceed the nutrient requirements according to the broilers age (Aviagen, 2019).

Table 2. Experimental basal diets of broiler chicks, per each growth period

| | Starter | Grower | Finisher |
|---|---|---|---|
| Ingredients (g/kg) | | | |
| Corn | 557.9 | 567.3 | 629.6 |
| Soybean meal | 331.0 | 311.0 | 255.0 |
| Corn gluten | 43.0 | 43.0 | 35.0 |
| Soybean oil | 14.6 | 29.8 | 34.0 |
| Monocalcium phosphate | 16.9 | 16.6 | 14.5 |
| Calcium carbonate | 16.9 | 14.6 | 13.2 |
| Salt | 2.8 | 2.8 | 2.8 |
| L-lysine HCl | 3.3 | 1.8 | 2.7 |
| Dl-methionine | 2.8 | 2.3 | 2.5 |
| Choline-chloride 50% | 0.8 | 0.8 | 0.7 |
| Vitamin - mineral mixture* | 10.0 | 10.0 | 10.0 |
| *Rhodotorula*[1] | +/- | +/- | +/- |
| Calculated composition | | | |
| ME (MJ/kg) | 12.55 | 13.02 | 13.40 |
| CP (%) | 23.0 | 22.0 | 20.0 |
| Lysine, total (%) | 1.41 | 1.24 | 1.05 |
| Lysine, digestible (%) | 1.28 | 1.16 | 0.98 |
| Methionine + cysteine, total (%) | 1.02 | 0.95 | 0.86 |
| Methionine + cysteine, digestible (%) | 0.94 | 0.87 | 0.75 |
| Ca (%) | 1.00 | 0.90 | 0.80 |
| Available P (%) | 0.45 | 0.45 | 0.45 |
| Crude fat (%) | 4.34 | 5.85 | 6.23 |
| Crude fiber (%) | 2.85 | 2.77 | 2.56 |

*Supplied per kg diet: 12000 IU vitamin A, 5000 IU vitamin D3, 75 mg vitamin E, 3 mg vitamin K3, 3 mg vitamin B1, 8 mg vitamin B2, 5 mg vitamin B6, 0.016 mg vitamin B12, 13 mg pantothenic acid, 55 mg nicotinic acid, 2 mg folic acid, 0.2 mg biotin, 120 mg Mn, 100 mg Zn, 40 mg Fe, 16 mg Cu, 1.25 mg I and 0.3 mg Se, 70 mg Monteban G100. [1]*Rhodotorula* spp. – lyophilised biomass 0.3kg/T0 feed. - = not included in the diet; + = included in the diet.

The Rh supplement was isolated and developed in the laboratory of the Biotechnology Faculty from Bucharest, from a wild strain identified as belonging to the *Rhodotorula* genus. Previously, the strain was isolated from waste milk subproducts, by successive passages on PDA (potato dextrose agar) and maintained at 4°C. The reactivation was conducted on potato dextrose broth, incubated at 28°C on an orbital shaker (100 rpm, 48 h). An optimised process for nutritive substrates and optimal fermentative conditions (aeration, time and temperature) were developed. The best productivity stage (orbital shaker) was found at 96 h of fermentation (on potato waste substrate), at 30°C, pH = 5.15 ± 0.198, showing 849.6 mg/$L^{-1}$ wet biomass. Thermic inactivation (five times of freezing-defreezing cycles for each fermented batch, n = 5) and lyophilisation were developed to ensure the health safety requirements and in order to avoid the potential opportunistic expression of the strain. The developed product was further lyophilised and mashed (powdered, having 77.9mg/$L^{-1}$) and added in broiler chicks' diets at 0 and 300g/t of feed.

Feeding program was divided into 3 phases: starter (0 to 10 days), grower (11 to 22 days), and finisher (23 to 42 days). Diets for each feeding phase were formulated to be isocaloric, isonitrogenous, with similar content of total essential amino acids (Table 3). The feed and water were provided *ad libitum* throughout the entire trial.

***Laboratory Analysis, Sampling and Measurements***

Samples of SBY, and feeds were analysed in triplicate, for content in dry matter (ISO 5984:2002), crude protein (SR EN ISO 5983-2:2009), crude fat (SR EN ISO 6492:2001) and fiber content (SR EN ISO 6865:2002). Amino acids were analysed using a HPLC System (Surveyor Plus, Thermo Fisher Scientific Inc., San Jose, CA, USA), according to the method described by Ciurescu and Pana, 2017). Flame atomic absorption spectrometry (SOLAAR M6 Dual Zeeman Comfort; Thermo Electron Corp., Waltham, MA, USA) was used to determine the macro and micro-mineral concentrations according to the method described by Ciurescu et al. (2018). Nitrogen-free extract (NFE) content was calculated as follows: NFE (%) = dry matter % – (crude protein % + crude fat % + crude ash % + crude fibre %). The content of dietary fibre fraction – neutral detergent fiber (NDF), and acid detergent fiber (ADF) – was determined with the classical semi-automatic Fibertec method (FOSS – Tecator AB, Hoganas, Sweden) as previously described by Ciurescu et al., 2018.

***Growth parameters***

The body weights (BW, n = 40/dietary treatment) were recorded throughout periodic weights at 1, 10, 22 and 42 days, in order to calculate the average daily gain (ADG) for each of the feeding periods and diets (starter, grower and finisher).

***Health status***

At of the end of the trial, broilers (n=5/group) were randomly selected for haematologic and metabolic sampling and determinations. Blood samples were collected from the bird's brachial vein, on EDTA and heparin tubes. Haematologic profile was performed by using an automatic analyser (Abacus Junior Vet 5, Diatron, Austria) and included: white blood cell (WBC), red blood cell (RBC), haemoglobin concentration (HGB), haematocrit (HCT), mean corpuscular volume

(MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC).

The blood samples collected on gel and clot activator tubes were centrifugated (3000 rpm, 15 min, SIGMA), and serum was collected in 1.5 ml Eppendorf tubes and stored at -20°C, for 7 days. Metabolic blood serum profile: glucose (Glu), total cholesterol (Cho), triglycerides (Tg), total protein (Tpro), albumin (Alb), total bilirubin (Tbil), creatinine (Cre), urea, uric acid (UA), alanine-aminotransferase (TGP), aspartate- aminotransferase (TGO), and gamma glutamil-transferase (GGT) were investigated using a semi-automatized analyser (StarDustMC15, DiaSys, Spain).

***Statistical analyses***

Data were analysed employing a mixed-effects model, using the GLM and ANOVA procedures with SPSS software (version 20 Inc. Chicago, IL, USA). Levels of SBY, Rh and their interactions were included in the statistical model. For growth performance (BWG and ADG), each pen was considered as the experimental unit. When comparing treatments means, Post hoc Tukey's multiple range test was carried out to assess any significant differences for the measured parameters. Differences were considered significant at a level of $P \leq 0.05$, while the tendency was set at $P \leq 0.10$.

## RESULTS AND DISCUSSIONS

***Nutrient composition of brewers spent yeast***

As expected, the SBY dietary supplement was found to have high levels of crude protein (39.6 ± 3.5%) and amino acids (Tables 3 and 4). Although, the amount of limitative amino acids such as methionine (1.18 ± 0.02 g/100 g of protein) and lysine (5.22 ± 0.03 g/100 g of protein) found in SBY, were lower than in the soybean meal (SBM), where lysine represents 6.38 g/100 g of protein and methionine accounts 1.26 g/100 g of protein, as previously reported by Saleh (2020).

The reported ether extract (EE) in SBY was lower than the basal energetic ingredient represented by corn, showing not more than 3.6% (Mateos et al., 2019). Furthermore, due to the low EE content, SBY might positively influence the visceral adiposity (Beisek et al., 2020), leading to a better health status and lowering the incidence of heart related disorders in broilers (Wang et al., 2021). However, considering the chemical composition of SBY, it is well known that the utilization in monogastric species, including humans, should be limited, due to the high levels of the nucleic acid ratio, that could cause a rise of the serum blood uric acid levels, damaging the tissues (Farcas et al., 2017).

Table 3. Nutritional basal composition of the dietary SBY supplement

| *SBY* | ME* (MJ/kg) | DM | EE | NDF | ADF | CP | Ash |
|---|---|---|---|---|---|---|---|
| | 2030±7.5 | 95.8±6.1 | 1.9±0.7 | 6.1±0.8 | 1.8±0.9 | 39.6±3.5 | 6.7±1.3 |

Data expressed as mean (n=3) ± standard deviation; *Metabolizable Energy value was calculated based on regression equations (NRC, 1994). DM – dry matter; EE – ether extract; NDF – non detergent fiber; ADF – acid detergent fiber ; CP – crude protein

Table 4. SBY amino acid profile

| Amino acid* (g/100 g protein) | | | |
|---|---|---|---|
| Arginine | 2.13±0.01 | Lysine | 5.22±0.03 |
| Cysteine | 0.49±0.02 | Methionine | 1.18±0.02 |
| Histidine | 0.93±0.1 | Phenylalanine | 1.64±0.01 |
| Izoleucine | 1.9±0.02 | Threonine | 1.88±0.01 |
| Leucine | 2.73±0.02 | Valine | 2.25±0.04 |

Data expressed as mean (n=3) ± standard deviation.

***Growth performance***

As reported in Table 5, there was no significant effect ($P < 0.05$) of the interaction between the main factors (SBY x Rh) on the growth performance of birds in the overall trial. Live body weight and average daily gain of broiler chick supplemented with 0.6 g/kg feed (groups 2 and 6) recorded similar values of live body weight and average daily gain as groups 1 and 5. Increased SBY levels (≥1 g/kg feed) in the broiler diet showed a linear (P<0.05) decrease at all ages. The highest concentration of SBY (groups 4 and 8) supplemented resulted in negative growth performances values, compared with all experimental groups. The Rh addition had no effects on broiler growth performance. Although, the dietary *Rhodotorula* spp. was proposed with concern to the desired appearance of the final product (meat colour) (Mata-Gomez et al., 2014; Barreiro et al., 2018; Duffose L., 2018) also with economic implications by using a natural low-cost alternative to the conventional synthetic colorant resource.

Similar to our findings, low levels of inactive yeast supplementation had no effects on broilers' growth performance (Wang et al., 2021). Current results are somewhat contrasting with reports from the literature, Ahiwe et al. (2020) and Sampath et al. (2021) studies showed that the increased levels of inactive yeast and yeast cell wall in the diets improved the broilers growth performances.

Other authors found that administrating live yeasts as probiotics, might enhance the live body weight in broiler chicks (Macelline et al., 2017) and quails (Sharif et al., 2018).

The growth-promoting effects of adequate levels of yeast supplementation on broiler chicks' diet could trigger a complex synergic system in boosting the immune and anti-inflammatory responses via vitaminic and amino acid abundance (Alagawany et al., 2021) along with balancing the nutrient availability and feed efficiency (Macelline et al., 2017).

Table 5. Effects of dietary SBY level and Rh addition on growth performance of broiler chickens[1]

| | | | BW(g) | | | | ADG (g) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Group** | **SBY[2]** | **Rh[3]** | **1st day** | **10th day** | **22nd day** | **42nd day** | **Starter** | **Grower** | **Finisher** | **Overall** |
| 1 | 0 | No | 43.42 | 268.88 | 953.44 | 2769.32 | 22.55 | 57.04 | 90.79 | 64.90 |
| 2 | 0.6 | No | 43.03 | 266.48 | 922.40 | 2688.00 | 22.34 | 54.66 | 88.28 | 62.98 |
| 3 | 1 | No | 42.88 | 259.80 | 892.44 | 2637.52 | 21.69 | 52.72 | 87.25 | 61.78 |
| 4 | 1.3 | No | 43.84 | 238.36 | 865.16 | 2501.68 | 19.45 | 52.23 | 81.83 | 58.82 |
| 5 | 0 | Yes | 43.13 | 270.92 | 952.84 | 2739.64 | 22.78 | 56.83 | 89.34 | 64.20 |
| 6 | 0.6 | Yes | 42.43 | 266.16 | 924.68 | 2672.68 | 22.37 | 54.88 | 87.40 | 62.63 |
| 7 | 1 | Yes | 43.46 | 254.80 | 865.24 | 2589.68 | 21.13 | 50.87 | 86.22 | 60.62 |
| 8 | 1.3 | Yes | 42.77 | 241.44 | 845.40 | 2467.88 | 19.87 | 50.33 | 81.12 | 57.74 |
| **SEM** | | | 0.366 | 2.672 | 9.235 | 33.687 | 0.270 | 0.786 | 1.808 | 0.803 |
| **Main effects** | | | | | | | | | | |
| **SBY level** | | | | | | | | | | |
| 0 | | | 43.28 | 269.90$^{a}$ | 953.14$^{a}$ | 2754.00$^{a}$ | 22.66$^{a}$ | 56.94$^{a}$ | 90.07$^{a}$ | 64.55$^{a}$ |
| 0.6 | | | 42.73 | 266.32$^{a}$ | 923.54$^{b}$ | 2680.34$^{ab}$ | 22.36$^{a}$ | 54.77$^{b}$ | 87.84$^{a}$ | 62.80$^{ab}$ |
| 1 | | | 43.17 | 257.30$^{b}$ | 855.28$^{c}$ | 2613.60$^{b}$ | 21.42$^{b}$ | 51.80$^{c}$ | 87.25$^{a}$ | 61.20$^{b}$ |
| 1.3 | | | 43.30 | 239.90$^{c}$ | 878.84$^{c}$ | 2484.78$^{c}$ | 19.66$^{c}$ | 51.28$^{c}$ | 81.48$^{b}$ | 58.13$^{c}$ |
| **Rh addition** | | | | | | | | | | |
| Yes | | | 42.95 | 258.33 | 897.04 | 2617.47 | 21.54 | 53.23 | 86.02 | 61.30 |
| No | | | 43.29 | 258.38 | 908.36 | 2649.13 | 21.51 | 54.17 | 87.03 | 62.04 |
| **p-value** | | | | | | | | | | |
| SBY effect | | | 0.366 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Rh effect | | | 0.180 | 0.979 | 0.085 | 0.185 | 0.876 | 0.093 | 0.427 | 0.191 |
| SBYxRh | | | 0.971 | 0.304 | 0.440 | 0.146 | 0.303 | 0.406 | 0.997 | 0.969 |

Different superscript within the same column is different (P<0.05). [1]Data are means of 40 broilers for each treatment. [2]SBY dietary supplementation g/kg diet. [3]*Rhodotorula spp.* lyophilised biomass 0.3kg/T feed. SEM - standard error of the mean. Starter period – from day 1 to 10th day of experimental trial. Grower period – between the 11th day to 22nd day. Finisher period, between the 23rd day to 42nd day of the trial. Overall period, the 42 days of experimental trial.

### *Haematologic profile*

Blood hematologic parameters are often employed to evaluate the clinical status of birds. Table 6 shows the broiler hematologic profile that was not affected by the dietary SBY or Rh supplementation (p>0.05).

The levels of blood constitutive elements were similar between all the experimental groups and fell within the physiologic range for broiler chicks at 42 d of age (Al-Nedawi, 2018). With the lack of statistical differences, we could highlight that tested inactive yeasts products did not affect the broiler's hematologic constituents. However, live yeast administration might have an effect on broiler immune and hematologic profiles via the synergic relation between the host and dietary live foreigner, enhancing blood white cells (lymphocytes and monocytes) and immunoglobulin G (Ahiwe et al., 2020).

Our results share similarities with Rafique et al. (2020), and Osita et al. (2020) studies that used *Saccharomyces cerevisiae* as a dietary supplement and observed no effect on the broilers hematologic profile.
In contrast to our findings, the inclusion of live yeast in broilers diets significantly improved the levels of hematologic components (Maoba et al., 2021; Mousa et al., 2018).

Feeding supplements such as SBY and Rh are generally considered safe (Farkas et al., 2020) and widely used in laying hens' diets (Sun et al., 2020; Thanapal et al., 2021) and other poultry species (Barreiro & Barredo, 2018).
Although, no studies are reporting the effects of active or inactive Rh biomass dietary supplementation on broiler blood profiles.

Table 6. Effects of dietary SBY levels and Rh addition on haematologic profile of broiler chickens[1]

| Group | SBY[2] | Rh[3] | WBC | RBC | HGB | HCT | MCV | MCH | MCHC |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | No | 24.63 | 2.35 | 6.94 | 33.48 | 114.36 | 46.56 | 26.04 |
| 2 | 0.6 | No | 25.33 | 2.39 | 7.05 | 34.22 | 113.98 | 47.76 | 25.84 |
| 3 | 1 | No | 25.08 | 2.36 | 6.83 | 33.76 | 113.32 | 43.98 | 25.38 |
| 4 | 1.3 | No | 24.96 | 2.45 | 6.95 | 34.92 | 112.62 | 46.02 | 26.15 |
| 5 | 0 | Yes | 25.19 | 2.29 | 6.90 | 34.31 | 112.88 | 43.68 | 25.20 |
| 6 | 0.6 | Yes | 25.39 | 2.39 | 6.92 | 34.96 | 113.60 | 45.55 | 25.74 |
| 7 | 1 | Yes | 25.24 | 2.42 | 7.07 | 34.05 | 113.76 | 46.38 | 24.97 |
| 8 | 1.3 | Yes | 25.37 | 2.37 | 6.94 | 34.83 | 113.28 | 45.64 | 25.95 |
| SEM | | | 0.401 | 0.064 | 0.309 | 1.283 | 1.336 | 1.137 | 0.832 |
| **Main effects** | | | | | | | | | |
| **SBY level** | | | | | | | | | |
| 0 | | | 24.91 | 2.32 | 6.92 | 33.90 | 112.95 | 45.12 | 25.17 |
| 0.6 | | | 25.16 | 2.39 | 6.95 | 33.91 | 113.54 | 45.18 | 25.62 |
| 1 | | | 25.16 | 2.39 | 6.95 | 34.59 | 113.62 | 45.83 | 25.79 |
| 1.3 | | | 25.36 | 2.41 | 6.98 | 34.87 | 113.79 | 46.66 | 26.05 |
| **Rh addition** | | | | | | | | | |
| Yes | | | 25.30 | 2.37 | 6.96 | 34.54 | 113.38 | 45.31 | 25.46 |
| No | | | 25.00 | 2.39 | 6.94 | 34.10 | 113.57 | 46.08 | 25.85 |
| **p-value** | | | | | | | | | |
| SBY effect | | | 0.760 | 0.542 | 0.998 | 0.829 | 0.929 | 0.507 | 0.760 |
| Rh effect | | | 0.303 | 0.636 | 0.631 | 0.937 | 0.347 | 0.842 | 0.514 |
| SBY effect x Rh effect | | | 0.401 | 0.064 | 1.283 | 0.309 | 1.137 | 1.336 | 0.832 |

[1]Data are means of 5 broilers for each treatment. [2] SBY supplementation in g/kg diet. [3] *Rhodotorula* spp. lyophilised biomass 0.3 kg/T feed.

***Serum biochemical parameters***

The interaction between the main factors (SBYxRh) did not affect the broiler blood serum profile (P>0.05) (Table 7). Serum biochemical constituents had similar values (P>0.05), except for the energetic profile.
A significant difference between the experimental SBY treated groups was recorded (P<0.05) for the serum glucose parameter. The linear decrease was displayed, having the lowest serum glucose values on groups 4 and 8, compared with all experimental groups (P<0.05). As a supposition, managing the broilers health by decreasing serum glucose it might increase insulin production (Chougule et al., 2020), that may enhance glycogenesis and improve glycolysis pathways (Givisiez et al., 2020). Previous studies (Rafique et al., 2020; He et al., 2021; Wang et al., 2021; Liu et al., 2021) had suggested that dietary yeasts supplementation decrease the broilers serum glucose, triglycerides and low density -lipids and enhances the high-density lipids values, while improving the lipid metabolism function.

Table 7. Effects of dietary SBY levels and Rh addition on blood biochemical profile of broiler chickens[1]

| Group | SBY[2] | Rh[3] | Glu | Cho | Tg | T-pro | Alb | T-bill | Cre | UA | Urea | TGP | TGO | GGT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | No | 278.67 | 124.67 | 59.33 | 2.53 | 1.07 | 0.20 | 0.70 | 5.70 | 2.00 | 11.33 | 267.33 | 50.33 |
| 2 | 0.6 | No | 266.33 | 127.33 | 57.00 | 2.60 | 1.00 | 0.20 | 0.63 | 4.90 | 2.00 | 9.33 | 205.00 | 48.33 |
| 3 | 1 | No | 247.33 | 149.00 | 55.00 | 2.97 | 1.07 | 0.20 | 0.57 | 5.17 | 2.00 | 9.33 | 229.33 | 52.67 |
| 4 | 1.3 | No | 235.00 | 123.47 | 54.33 | 2.67 | 0.97 | 0.27 | 0.57 | 5.40 | 2.00 | 11.33 | 265.00 | 57.67 |
| 5 | 0 | Yes | 301.00 | 136.33 | 48.67 | 2.70 | 1.03 | 0.23 | 0.63 | 6.00 | 2.33 | 10.00 | 200.00 | 48.00 |
| 6 | 0.6 | Yes | 252.67 | 123.67 | 54.67 | 2.57 | 1.03 | 0.20 | 0.63 | 5.67 | 2.00 | 11.00 | 311.67 | 43.00 |
| 7 | 1 | Yes | 244.33 | 127.67 | 56.00 | 2.60 | 1.07 | 0.20 | 0.60 | 5.23 | 2.00 | 9.00 | 239.00 | 51.67 |
| 8 | 1.3 | Yes | 260.67 | 134.00 | 58.67 | 2.77 | 1.03 | 0.20 | 0.53 | 5.43 | 2.00 | 11.00 | 263.33 | 51.67 |
| **SEM** | | | 6.064 | 2.734 | 1.199 | 0.059 | 0.030 | 0.011 | 0.018 | 0.180 | 0.042 | 0.364 | 11.33 | 1.832 |
| | | | | | | | **Main effects SBY level** | | | | | | | |
| 0 | | | 289.84[a] | 130.50 | 54.00 | 2.62 | 1.05 | 0.22 | 0.667 | 5.85 | 2.17 | 10.67 | 233.67 | 49.17 |
| 0.6 | | | 259.50[ab] | 125.50 | 55.83 | 2.58 | 1.02 | 0.20 | 0.583 | 5.28 | 2.00 | 10.17 | 258.33 | 45.67 |
| 1 | | | 245.83[b] | 138.33 | 55.50 | 2.78 | 1.07 | 0.20 | 0.583 | 5.2 | 2.00 | 9.17 | 234.17 | 52.17 |
| 1.3 | | | 247.84[ab] | 128.73 | 56.50 | 2.72 | 1.00 | 0.23 | 0.550 | 5.42 | 2.00 | 11.17 | 264.17 | 54.67 |
| | | | | | | | **Rh addition** | | | | | | | |
| Yes | | | 256.83 | 131.12 | 56.42 | 2.69 | 1.03 | 0.217 | 0.617 | 5.29 | 2.00 | 10.33 | 241.67 | 52.25 |
| No | | | 264.67 | 130.12 | 54.5 | 2.66 | 1.04 | 0.208 | 0.600 | 5.58 | 2.08 | 10.25 | 253.50 | 48.58 |
| | | | | | | | **p-value** | | | | | | | |
| SBY effect | | | 0.023 | 0.365 | 0.902 | 0.665 | 0.901 | 0.715 | 0.134 | 0.673 | 0.418 | 0.300 | 0.638 | 0.426 |
| Rh effect | | | 0.472 | 0.893 | 0.441 | 0.794 | 0.814 | 0.728 | 0.644 | 0.477 | 0.332 | 0.912 | 0.581 | 0.363 |
| SBY effect x Rh effect | | | 0.828 | 0.120 | 0.195 | 0.466 | 0.962 | 0.516 | 0.775 | 0.909 | 0.418 | 0.554 | 0.066[T] | 0.963 |

[a,b] Means within a column with no common superscript differ significantly ($P<0.05$). [T] - tendency, P<0.1.

[1]Data are means of 5 broilers for each treatment. [2]SBY dietary supplementation in g/kg diet. [3]*Rhodotorula* spp. lyophilised biomass 0.3 kg/T feed. Glu - glucose. Cho - total cholesterol. Tg - triglycerides. Tpro -total protein. Alb - albumin. Tbil - total bilirubin. Cre - creatinine. UA - uric acid. TGP - alanin-aminotransferase. TGO - aspartate-aminotransferase. GGT - gamma glutamiltransferase.

Opposite to these findings, Nelson et al. (2020) reported that the dietary inclusion of live yeast in the stressed broiler diets had no significant effect on serum protein profile.

There were no effects of SBY dietary supplementation, with or without Rh, on broilers protein, mineral or enzymatic serum profile. Although, a tendency (P = 0.066) was observed, due to the main factor's interaction as dietary supplements in broilers might increase the serum GOT enzyme.

Further studies are necessary to investigate the mechanism through which the SBY dietary supplement lowers serum glucose in broilers.

## CONCLUSIONS

The SBY dietary supplementation at levels of 0.6 g/kg feed, resulted in similar growth performance during the overall trial, compared with chicks reared on corn-soybean diet. The SBY in broiler chicks' diets decreased the serum glucose, which could increase the broilers energetic metabolism. Higher levels of SBY supplementation had negative effects on growth performance and tended to affect the enzymatic serum profile, as a result these interactions could cause hepatic disturbance. *Rhodotorula* spp. supplement had no favourable effect on BW, ADG, haematologic and serum biochemical profiles of the broilers.

Further studies are required to evaluate the impacts of dietary SBY supplementation with or without Rh on broiler meat quality and sensorial attributes of carcasses.

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# SIMULATION OF PIG PRODUCTIVITY UNDER FEED CONSUMPTION

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***Abstract***

*The aim of the research is to develop a computer model that makes it possible to predict the productivity of pigs based on the data on their consumption of the exchange energy of feed and the amount of feed loss during storage and operation of technological equipment. Based on the known results, regression relationships have been established between the value of the exchange energy of the given feed and the average daily gain in the live weight of animals, as well as the cost of feed per kilogram of gain. Due to the loss of feed nutrients and the feed itself during the storage of feed in warehouses, during preparation and delivery to animals, as well as throwing away part of the feed when it is eaten by animals, the amount of nutrients consumed by the animal and the accounting of feed consumption do not match.*

***Key words:*** *animal weight, feed effect, metabolic energy, pig weight gain model.*

## INTRODUCTION

Pig breeding is one of the oldest branches of agriculture, when animals were kept on pasture in the process of growing. To reduce the cost of meat production, this method of keeping pigs is sometimes used now, using the walking method of production (García-Gudino et al., 2021). However, obtaining a large volume of meat products requires the use of modern equipment and technologies. Timely and accurate assessment of the body weight of an animal when raising a pig affects the assessment of profit in its production, allowing decisions to be made on the timing of fattening animals to reduce labor and feed costs (He et al., 2021). Improving the technology of pork production is aimed at increasing the absorption of nutrients and reducing feed consumption (Pomar et al., 2021). Feeding is an important operation in the life of an animal, ensuring the efficiency of animal husbandry. The level of provision of an animal with nutrients significantly affects its behavior and the rate of weight growth (Jia et al., 2021; Misiura et al., 2021). Modern research in pig breeding has several directions.

In particular, the influence of feeding conditions on the state of health of the animal is revealed, and through this, on the indicators of its productivity (Misiura et al., 2021). Another focus is on the impact of different diet formulation methods in providing pigs with the required amount of nutrients while minimizing nutrient excretion and greenhouse gas emissions. In particular, it was found that the reduction of crude protein improves the absorption of available energy due to a decrease in energy losses for protein deamination. Due to growing concern for the environment, selection in pig breeding for nitrogen excretion is being studied. A number of researchers (Monteiro et al., 2021) consider the assessment of the life cycle of an animal to be a more promising indicator. Relationships between production traits and the impact of life cycle assessment of individual reared pigs on finishing (feed consumption per kilogram of body weight gain) were studied (using a modeling approach). Double and precision feedings were compared. This indicator appeared to be the best indicator of the impact on the life cycle assessment with a very high and positive correlation (r> +0.99) for both feeding programs. Some work is focused on studying the effect of supplements or changing the norm of individual nutrients. The aim of the study (Ewaoluwagbemiga et al., 2021) was to evaluate the behavior of animals during feeding with dietary protein restriction. A significant correlation coefficient was found between the predicted and observed values of protein (0.50), metabolic energy (0.70) and lipid (0.90).

The results of the researches show the relationship between the dynamics of feeding and the behavior and body weight of the animal. A model was used in which several breeds of animals, their age, feeding (behavior and feed intake) were used as factors. The results (Kavlak et al., 2021) show that the social interactions of animals in a group have a significant indirect genetic effect on the feeding, behavior and feed conversion rate of the pig, but not on the average daily weight gain and fat thickness. In an automatic feeding station for pigs, the features of animal behavior and interaction with other individuals were studied (Angarita et al., 2021) by recording the indicators of individual feeding of an animal. The results showed that both direct and social effects influenced the duration of eating at a single-site feeder. Animals that spent more time at the feeder per day

Investigated the effect of feeding frequency (one /M1/, three /M3/ and five /M5/ times a day) with the same daily feeding rations. The results of these studies show that the number of feed deliveries per day affects the digestion and absorption of proteins and fats in pigs, and does not affect the weight gain of animals. Final body weight, average daily weight gain in the M3 and M5 groups were significantly higher than in the M1 group, but the specific feed consumption was significantly lower than in the M1 group.

The researches consider the problems of animal productivity modeling. The limitations of the possibility of linear prediction and double exponential smoothing are noted. Often, uncertainty and correlation in the estimated features are not sufficiently taken into account. Alternative approaches to predicting individual growth or consumption response based on non-linear models (allometric, monomolecular, rational) and Bayesian methodology were developed and evaluated to fit the model to the original data and the ability to generate probabilistic forecasts. It was found that a good fit does not guarantee an accurate forecast, which has a quantitative value in the medium and long term. Forecasts from non-linear models gave more accurate results compared to the reference linear models.

An analysis of the methods used to model the studied livestock processes showed that pig breeding does not use methods typical of technical sciences (Bormotov et al., 2020; Bormotov et al., 2022), but mainly regression modeling and correlation or dispersion analysis are used. An exception is the work (Plaksin et al., 2021), where the simulation was performed on the basis of a theoretical analysis. This mathematical model takes into account the change in the consumption of the prepared feed mixture depending on the age of the suckling pigs during the operation of the technological equipment. Models that make it possible to comprehensively predict the productivity of pigs have not been identified.

The aim of the research is to develop a computer model that makes it possible to predict the productivity of pigs based on the data on their consumption of the exchange energy of feed and the amount of feed loss during storage and operation of technological equipment.

## MATERIALS AND METHODS

The research methodology included a regression analysis of the known production data for growing pigs for fattening to obtain adequate dependencies. The correlation coefficient of the initial and calculated values for the models was R=0.9321-0.9843. Based on the established functional dependencies, the indicators were modeled in the mathematical package MathCAD to determine the forecast of their values, taking into account the magnitude of feed losses during the operation of technological equipment.

## RESULTS AND DISCUSSIONS

An analysis of the results of the economic activity of pig-breeding enterprises (Tronchuk et al., 1990) made it possible to establish in tabular form the relationship between the amount of metabolic energy during fattening of pigs and the value of animal weight gain, feed costs per kilogram of live weight gain and the duration of pig rearing. The specified information allows us to evaluate the results of the economic activity of the enterprise and identify existing trends, however, they are insufficient for numerical modeling of the performance indicators for raising pigs. At a minimum, it is required to establish regression expressions that numerically show the relationship of these indicators. The established regression models

(Figure 1) were obtained on the basis of the data of the industrial rearing of pigs.

The applied expressions make it possible to use the well-known energy model of a living organism (Kill et al., 2013; Noblet et al., 2004), when part of the energy entering it (for example, Figure 1 a) is spent on maintaining a viable state (the minimum required amount, i.e. at Ec≤20 MJ), and the excess of this energy begins to be converted into additional products (deposited in the body as a reserve of nutrients and an increase in live weight with the growth of the animal, i.e. at 18 MJ≤Ec≤26 MJ).

If at the beginning of the increase in excess energy the animal receives a comfortable existence, and the amount of increase gradually increases, then after reaching a certain value, an intensive increase in animal weight gain is observed (26 MJ≤Ec≤31 MJ). After the realization of the body's ability to transfer food into the reserves of a living organism, there is a decrease in the efficiency of feed use (Ec≥31 MJ). That is, the proportion of food in transit through the animal increases. These losses worsen not only the efficiency of the feed, but also the ecology of production (De et al., 2018).

The results of the influence of the amount of expended metabolic energy on the specific feed consumption (Figure 1 b) and the duration of rearing (Figure 1 c) are interesting. In both regression models for Ec≤26 MJ indicators have the highest values.

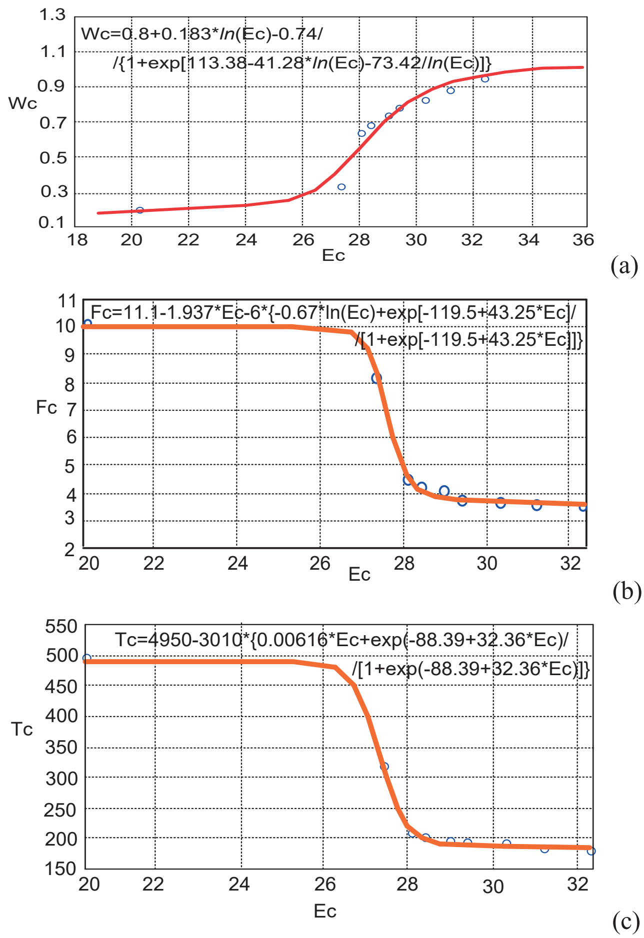


Figure 1. Influence of the average daily consumption of the exchange energy of feed (Ec, MJ) on:
a. - on the average daily gain in live weight (Wc, kg); b. - feed costs per kilogram of live weight gain (Fc, kg);
c. - the duration of rearing the fattening animal (Tc, days)

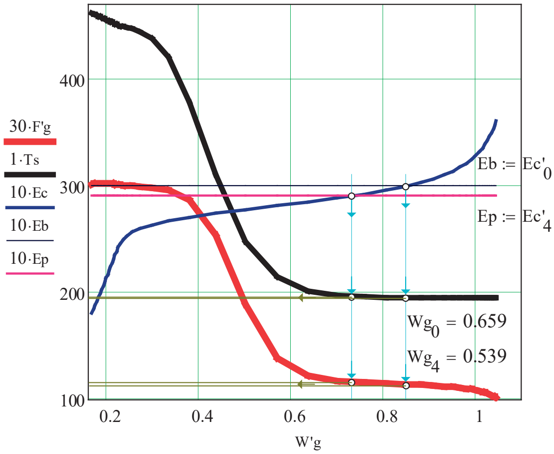


Figure 2. The relationship between the average daily gain in live weight (Wg, kg) and indicators: feed costs per kilogram of weight gain (Fg, kg); the duration of rearing the fattened animal (Ts, days); average daily consumption of the exchange energy of feed (Ec, MJ)

For the interval (26 MJ≤Ec≤31 MJ) is characterized by an intensive improvement in performance. For values of Ec≥At 31 MJ, stabilization of the indicators under consideration is again observed. As a result, a sharp reduction in the growing period stops, and the specific feed consumption decreases slightly.

Interesting information is obtained by changing the abscissa index (Figure 2). With a weight gain of more than 0.6 kg, both the growing period and the specific feed consumption do not improve significantly. At the same time, in the area Wg=0.3-0.9 kg, a linear increase in the consumption of the exchange energy of the feed is observed. This allows us to consider the further use of scaling by the proportionality factor Kw justified. With a further increase in the weight gain of animals, the costs of metabolic energy increase sharply. That is, the limited feeding of animals contributes to the efficient use of the exchange energy of the feed. Feeding ad libitum reduces the energy efficiency of feed. Thus, feeding animals with their consumption of metabolic energy corresponding to this transitional regime (according to Figure 2 - Wg = 0.9-1.0 kg), allows you to reduce the period of growing animals and effectively use the feed.

It should be noted the production conditions for obtaining the initial data of regression models. For the result, the important point is precisely the consumption of the right amount of feed by the animal, and not its issuance to the feeder or accounting for consumption. Since some of the energy can simply be lost. The amount of feed energy stored and taken into account in accounting does not always correspond to the actual energy sent to the livestock department. There is the effect of shrinkage, rotting, natural decline in feed quality, eating by rodents and theft by people. Feed mixtures are prepared from feed components in the workshops. In this case, part of the material is inevitably lost, and during heat treatment, the amount of metabolic energy available to animals increases. When eating food, animals instinctively throw out part of the feed from the feeder,

Losses in specialized feeders that follow the contours of the animal's head can be reduced to 0.3%, and in production - up to 2% (Baxter, 1989). Changes in the dimensions of pigs during the selection process also affect the rational parameters of the feeders (Condotta et al., 2018; Shneider et al., 2021; Neilson et al., 1996). A review (Konovalov et al., 2005) of the technical condition of technological equipment showed that: the loss of feed from dusting when unloading dispensers is about 0.5%, from scattering when falling into the feeder or on the floor - up to 5.2%, when eating - up to 10.3 %; daily fluctuations in the dose of feed on dry matter can be 20-25%, which additionally leads to a decrease in weight gain by 2-4%.

Three areas of the occurrence of losses are considered: 1 - loss of feed (and, accordingly, the exchange energy in it) in the process of eating the given portion by the animal; 2 - loss of exchange energy and feed in the shops of the livestock enterprise in the process of preparing the feed mixture, delivering and distributing it to animals (taking into account the change in exchange energy, the percentage of losses can be negative); 3 - loss of exchange energy and feed in the process of storage of raw feed components. Losses are measured as shares of the amount of feed and its energy according to the company's accounting records.

It should be borne in mind that different breeds of animals are used in different livestock enterprises, with different directions of cultivation and genetic inclinations. In this case, different types of feeding and different balances of nutrients are used (Panin et al., 2017) even if the specific values of exchange energy are equal. Therefore, it is required to create a model capable of predicting the considered indicators of the efficiency of pig production. Based on the fact that the general principal model of the influence of the amount of exchange energy on the efficiency indicators of pig breeding is preserved, we will model the weight gain of animals, taking into account the correction for the results of updated studies (Figure 3). Knowing the weight gain of fattening animals and the amount of metabolic energy consumed by them, as well as the percentage of feed losses, the value of the correction factor Kw is determined (Figure 3) recalculation of the weight gain chart (Figures 4, 5).

At the same time, losses are important. In the above model, the percentage of losses is indicated as notional values to show the logic of the process and the modeling technique. When conducting an updated experiment, the amount of feed given out and the value of weight gain are known (W=0.86 kg, Figure 3). The percentage of losses is conditionally taken as 2% (Flb1=0.02). Taking into account these losses, the amount of feed consumed by the animal was recalculated with the calculated exchange energy (taken as Ec=30 MJ). If these are the results of production work, then its specific percentages of losses are indicated. If these are the results of trial fattening of a group of animals, then the percentages of losses are indicated (for example, Flb2=0.03 and Flb3=0.05) corresponding to the loss conditions of the intended production (Flp2=0.03; Flp3=0.05; Flp1=0.03). When implementing the model, the calculated values are determined.

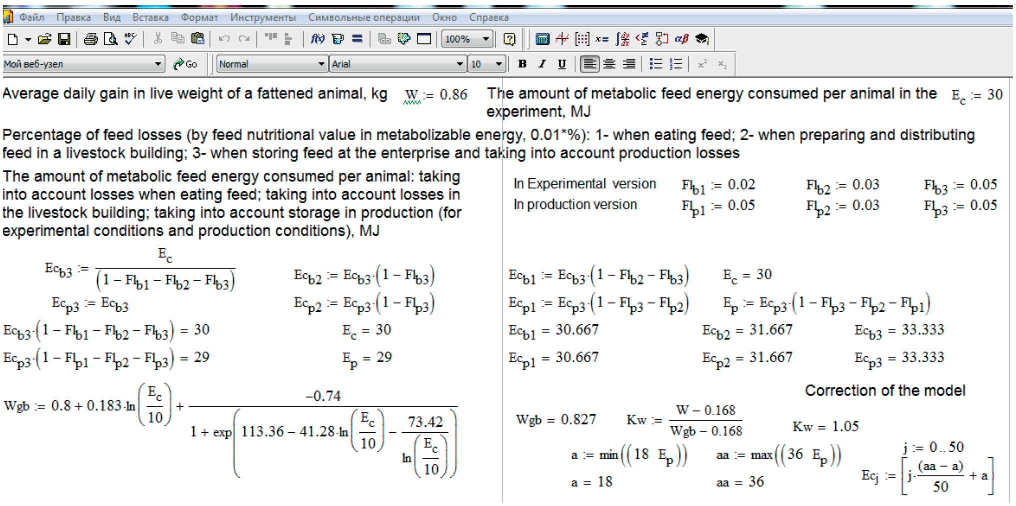


Figure 3. Process modeling (beginning - input of initial data and determination of the correction factor Kw)

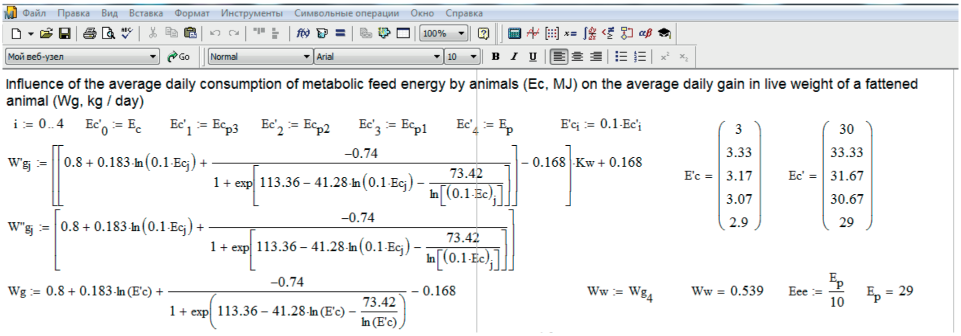


Figure 4. Modeling the process (continuation - calculation of the model to determine the weight gain of animals)

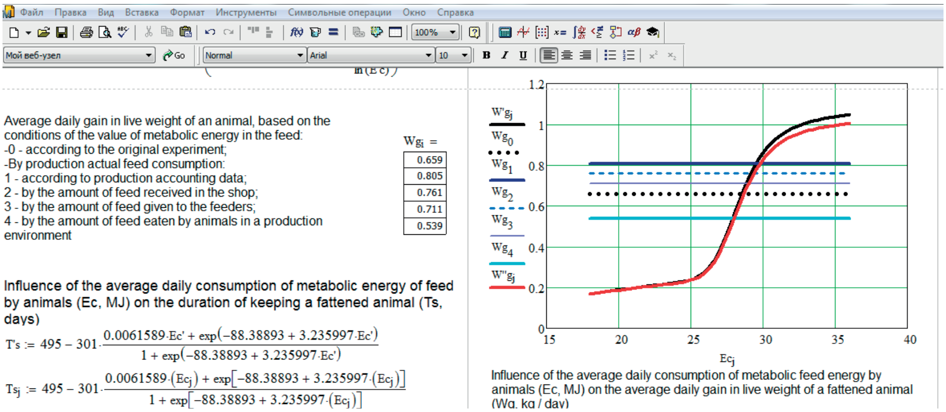


Figure 5. Modeling the process (continued - the graph of animal weight gain and the calculation of the model to determine the duration of rearing)

In Figure 5, the red curve shows the original weight gain model as an analogue of the graph in Figure 1a, and the black curve corresponds to the adjusted calculation model for the updated experiment data. Due to the value of Kw=1.05 (Figure 3), the graph of the calculation model has shifted upwards (Figure 5). Taking into account the percentage of losses, the values of the exchange energy Ec are determined (Figures 4 and 5). Metabolic energy in Figure 4 is given numerically (0 - for the animal in the updated experiment; 1 - according to production records; 2 - according to shop records; 3 - feed given out; 4 - feed consumed), and in the graph of Figure 5, the values are presented in in the form of horizontal lines crossing the weight gain curve of the animal. Due to feed losses, the value of available metabolizable energy decreases, and accordingly the point of intersection with the curve shows a decrease in the expected weight gain. A change in the initial value of the exchange energy of feed based on the results of updated experiments with the same loss values would show a non-linear change in weight gain. The main reference point is the weight gain in terms of the exchange energy of the consumed feed. At the initial value of the exchange energy, the calculated weight gain is Wg = 0.659, and according to the calculation model - 0.539 kg. If we reduce feed losses under production conditions, then we can increase weight gain at the same production costs of feed energy (additional values - 0.805; 0.761; 0.711). In Figure 6, models are implemented for the duration of rearing and specific feed consumption. Based on their values, adjusted graphs were constructed depending on the weight gain of animals, shown in Figure 2. Vertical blue projections on the abscissa (Wg0=0.659 end Wg4=0. 539) the intersection of the thin black horizontal 10·Eb (and the red horizontal 10·Ep) with the blue curve 10·Ec gives indicative values at the points of intersection with the red and black curves (marked with dots in Figure 2) of the specific feed consumption per kilogram of weight gain and duration of cultivation according to the initial and projected options. The interval of the difference between the values of indicators by options (vertically) will show the expected effect of the changes.

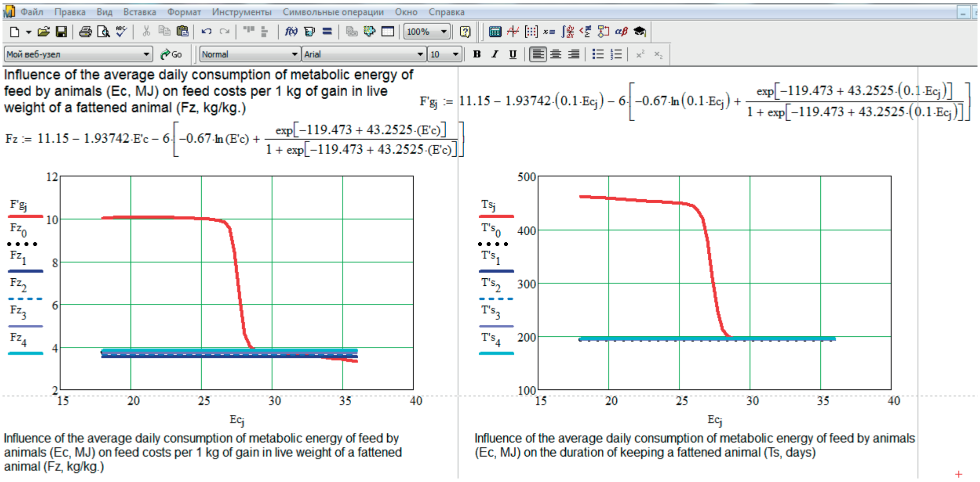


Figure 6. Simulation of the process (end - calculation of the model to determine the specific feed consumption, graphs of the duration of cultivation and specific feed consumption)

## CONCLUSIONS

Regression analysis of the known production data on the relationship between the amount of metabolic energy of fattening pigs and the weight gain of pigs, the cost of feed per kilogram of weight gain and the duration of rearing made it possible to obtain adequate functional dependencies of these indicators.

Modeling in the mathematical package MathCAD made it possible to predict the indicated production indicators of the efficiency of raising pigs, taking into account feed losses in the sections of the feed transportation technological chain.

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# RESEARCH ON SOME COMPOUND FEED RAW MATERIALS HAZARDS IN RELATION WITH FOOD SAFETY

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***Abstract***

*Food safety hazards associated with compound feed can be physical, chemical and biological. The paper aims to conduct a study during 2019 and 2020, on the production of compound feed in relation on food safety, by physical analysis of samples of raw materials (corn grains, wheat grains) used in the production of compound feed from two feed mills from Romania, called in the paper "unit A" respectively "unit B". Regarding the hazards identified in unit A, it was found that in 2019, 20 lots of corn grains (68.9%) and 9 lots of wheat grains (31%) were refused, the most common hazard identified (78.5%) being represented by their high humidity. In 2020, 17 batches of raw materials were rejected, represented by corn grains (52.9%) and wheat grains (47%); humidity was also the predominant potential hazard identified (70.5%). In the case of unit B, based on physical parameters, in 2019, 22 batches of corn grains (91.6%) and two batches of wheat grains (8.3%) were refused; the most frequently identified hazard (58.3%) was the presence of sprouted and moldy grains. In 2020, 53 batches of raw materials were rejected, represented by corn grains (79.2%) and wheat grains (20.7%); the most frequently identified hazard was beetle infestation (50.9%). It can be concluded that in the production process of compound feed it is mandatory the physical analysis of raw materials to determine potential hazards; this goal is achieved in the units studied, the results highlighting the effectiveness of specific food safety control processes.*

***Key words****: compound feed, food safety, hazards, raw materials.*

## INTRODUCTION

Food safety hazards associated with compound feed can be physical, chemical and biological. Each hazard is associated with certain sources and routes of contamination, and risk management must be based on a thorough understanding of these characteristics. Hazards may be introduced into the production process upon receipt of the raw materials or by contamination of the products during production, storage and transport. The presence of a hazard may also result from accidental or deliberate human intervention (fraud or bioterrorism) (FAO and IFIF, 2010).

The Codex Alimentarius Commission adopted the Code of Good Practice on Animal Nutrition (CAC / RCP 54-2004) which became a support for national authorities and the private sector (FAO / WHO, 2004a; FAO / WHO, 2004b).

In order to facilitate the application of the above-mentioned code, the Codex Commission has developed a document setting out guidelines for the application of feed risk assessment (CAC/GL 80-2013). In that document, risk assessment is one of the three components of the risk analysis framework, together with risk management and risk communication.

Physical hazards are auxiliary materials or foreign objects that are not normally found in food or feed, and which could cause injury or disease. Depending on their nature, physical hazards can be classified into three groups: minerals (earth, stones, dust, metals, glass, paint flakes, etc.); plants (weeds, leaves, stems); animals (mites, insects, rodents, birds). Contaminants from the three groups listed above may occur during the harvesting of raw materials, during their storage, as well as during the processing of the finished product.

Sources of contamination with physical hazards can be divided into: raw materials, water, floors, construction and building materials, personnel (Aladjadjiyan, 2006).

Physical hazards such as glass, metal, sharp objects, paper and plastics can be introduced into feedstocks through raw materials, in the manufacturing process, and can be controlled or removed by sieving and other means, such as magnets for detecting metals.

The materials used for packaging are of particular importance, especially those derived from waste and/or by-products; they may come from the packaging of ingredients used as raw materials and may be inadvertently introduced into the feed manufacturing process (FAO/WHO, 2015).

Purchased raw materials in bulk are the most common source of physical contamination. Proper pre-discharge inspection is the first step in minimizing the risk of contamination. Bulk raw materials can be transported several times, in different means of transport, before reaching feed mill and being transformed into finished products. Thus, the degree of contamination of cereal seeds with foreign seeds or foreign bodies will be identified. As the composition of the batches of cereals is seldom homogeneous and certain contaminants are unevenly distributed, a sufficient number of incremental samples must be taken and homogenized to form a aggregate sample from which the laboratory sample will be obtained later.

The National Consumer Seed Grading Commission has developed the “Consumer Seed Grading Manual”, approved by Order of the Minister of Agriculture and Rural Development no. 228/2017, which has the role of working tool for graders. Grading (classification) of consumer seeds is the operation of identifying and separating batches of cereals, legumes and oilseeds according to their appearance and physical condition. The structure of the grading manual for consumer seeds includes grading techniques and plans for wheat, durum wheat, rye, barley, oats, corn, flax seeds, beans, peas, sunflower, soybeans, rapeseed, saffron, seeds of mustard and rice.

The paper aims to conduct a study during the years 2019 and 2020, on the production of compound feed in relation with food safety, by physical analysis of raw materials (corn grains, wheat grains) used in the production of compound feed.

## MATERIALS AND METHODS

Methodologically, the results of the analyzes performed during the reception stage of raw materials from two feed mills representative for Romania in terms of production capacity, called in the paper "unit A" and "unit B" were processed, synthesized and interpreted; the research took place during 2019 and 2020.

The raw materials analyzed to determine the physical hazards were corn grains and wheat grains.

For physical analysis, was used as a standard "Grading Manual for Consumer Seeds" approved by Order of the Minister of Agriculture and Rural Development no. 228/2017; the analysis of the hazards of the raw materials under study was performed according to the following standards specified in the grading manual: SR EN ISO 24333:2010 - Cereals and cereal products. Sampling.; SR ISO 6639-1:1996 - Cereals and pulses. Determination of hidden insect infestation. Part 1: General principles; SR 5447:2013 Corn (*Zea mays*, L.). Specifications.; SR 13548 Common wheat (*Triticum aestivum* L.). Specifications.; STAS 6253-80 Seeds for consumption. Determination of organoleptic characteristics; SR EN 15587:2008 - Cereals and cereal products. Determination of impurities in wheat (*Triticum aestivum* L.), durum wheat (*Triticum durum* Desf.), Rye (*Secale cereale* L.) and fodder barley (*Hordeum vulgare* L.); SR fprEN 16378: 2013 - Cereals. Determination of impurities in maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L.).

The results obtained were compared with the values regulated by national legislation. The interpretation of the results led to the formulation of conclusions concerning the production of compound feeds in relation on food safety.

## RESULTS AND DISCUSSIONS

The results of the physical analyzes carried out in 2019 in unit A for the raw material samples (grain maize and wheat grain) were ordered according to the month in which they were identified (Table 1). Potentially unsafe products identified are those which did not meet the limits set by the standards in the Consumer Seed Grading Manual, or which did not comply with the quality requirements of the contracts concluded with suppliers, and which may have an negative effect on the safety of compound feed.

Table 1. Hazards identified at reception of raw materials from Unit A in 2019

| No. | Date | Product | Description of hazard | Correction |
|---|---|---|---|---|
| 1 | 02.2019 | Corn grain | Moldy grains | Refused product |
| 2 | 02.2019 | Corn grain | Moldy grains | Refused product |
| 3 | 04.2019 | Wheat grain | M = 15.1% | Refused product |
| 4 | 04.2019 | Corn grain | M = 16.3% | Refused product |
| 5 | 04.2019 | Corn grain | M = 15.3% | Refused product |
| 6 | 04.2019 | Corn grain | Moldy grains | Refused product |
| 7 | 05.2019 | Corn grain | Moldy grains | Refused product |
| 8 | 05.2019 | Corn grain | M = 15.0% | Refused product |
| 9 | 05.2019 | Corn grain | M = 15.3% | Refused product |
| 10 | 05.2019 | Corn grain | M = 15.4% | Refused product |
| 11 | 05.2019 | Wheat grain | M = 14.3% | Refused product |
| 12 | 05.2019 | Wheat grain | Impurities = 4.2% | Refused product |
| 13 | 05.2019 | Wheat grain | M = 15.2% | Refused product |
| 14 | 05.2019 | Wheat grain | M = 15.0% | Refused product |
| 15 | 06.2019 | Wheat grain | M = 15.0% | Refused product |
| 16 | 06.2019 | Corn grain | M = 14.7%; moldy grains | Refused product |
| 17 | 06.2019 | Corn grain | M = 14.7%; moldy grains | Refused product |
| 18 | 06.2019 | Corn grain | M = 15.8%; moldy grains | Refused product |
| 19 | 07.2019 | Wheat grain | M = 16.7% | Refused product |
| 20 | 07.2019 | Wheat grain | M = 15.0% | Refused product |
| 21 | 07.2019 | Corn grain | M = 14.8% | Refused product |
| 22 | 09.2019 | Wheat grain | M = 16.7 %; mold smells | Refused product |
| 23 | 09.2019 | Corn grain | M = 18.2 % | Refused product |
| 24 | 10.2019 | Corn grain | M = 18.4 % | Refused product |
| 25 | 10.2019 | Corn grain | M = 18.3 % | Refused product |
| 26 | 10.2019 | Corn grain | M = 21.2 % | Refused product |
| 27 | 11.2019 | Corn grain | Hot and burnt grains | Refused product |
| 28 | 11.2019 | Corn grain | Hot and burnt grains | Refused product |
| 29 | 12.2019 | Corn grain | Infestation with insects | Refused product |

M = moisture

The table above shows that in 2019, based on physical parameters, in the stage of receiving raw materials from unit A, 20 lots of corn grains (68.9%) and 9 lots of wheat were refused (31%). In the case of maize and wheat grains, the identified hazards were the presence of moldy, hot and burnt grains, infestation, impurities above the limit mentioned in the Grading Manual and humidity above the contractual limits.

The most commonly identified hazard (78.5%) was increased grain moisture. According to the contractual conditions established with the suppliers of raw materials, unit A imposed maximum limits on the moisture content for cereal seeds as follows: 14.5% for maize and 14% for wheat; batches of raw materials that have recorded a higher moisture content than provided for in contract, were refused. In May most batches of raw materials (n=8) were rejected during 2019. The results of the physical hazard analyzes performed in 2020 in unit A for raw material samples were presented in Table 2. In 2020, 17 batches of raw materials were rejected, represented by corn grains (52.9%) and wheat grains (47%). The identified physical hazards were represented by the presence of foreign bodies and moldy grains, grains attacked by insects, broken grains above the limit provided in the Grading Manual and moisture above the limit established in the contract with suppliers; moisture was the predominant hazard for both corn and wheat grains (70.5%).

Table 2. Hazards identified at reception of raw materials from Unit A in 2020

| No. | Date | Product | Description of hazard | Correction |
|---|---|---|---|---|
| 1 | 01.2020 | Corn grain | Foreign objects | Refused product |
| 2 | 06.2020 | Corn grain | M = 14.9%; moldy grains; infestation with insects | Refused product |
| 3 | 07.2020 | Wheat grain | M = 16.8% | Refused product |
| 4 | 07.2020 | Wheat grain | M = 15.3% | Refused product |
| 5 | 07.2020 | Wheat grain | Foreign objects | Refused product |
| 6 | 07.2020 | Wheat grain | M = 16.9% | Refused product |
| 7 | 07.2020 | Wheat grain | Foreign objects | Refused product |
| 8 | 07.2020 | Wheat grain | Foreign objects | Refused product |
| 9 | 07.2020 | Wheat grain | M = 16.2% | Refused product |
| 10 | 07.2020 | Wheat grain | M = 15.8% | Refused product |
| 11 | 08.2020 | Corn grain | Moldy grains; denaturated grains by insects | Refused product |
| 12 | 08.2020 | Corn grain | M = 17.7% | Refused product |
| 13 | 08.2020 | Corn grain | M = 15.2% | Refused product |
| 14 | 10.2020 | Corn grain | M = 21.4%; broken grains = 5.87% | Refused product |
| 15 | 11.2020 | Corn grain | M = 15.5% | Refused product |
| 16 | 12.2020 | Corn grain | M = 15.3%; broken grains = 7.22% | Refused product |
| 17 | 12.2020 | Corn grain | M = 15.3%; broken grains = 7.22% | Refused product |

M=moisture

The potentially unsafe products identified in Unit B in 2019 and 2020 are shown in Table 3 and Table 4.

In 2019, based on the physical parameters, in the stage of receiving raw materials from unit B, 22 batches of corn grains (91.6%) and two batches of wheat grains (8.3%) were refused. In the case of corn and wheat grains, the identified hazards were the presence of sprouted, moldy and spoiled grains, infestation, and the presence of foreign bodies above the limit set out in the Grading Manual.

The most frequently identified hazard (58.3%) was the presence of sprouted and moldy grains. In January most batches of raw materials (n=12) during 2019 were refused.

Table 3. Hazards identified at reception of raw materials from Unit B in 2019

| No. | Date | Product | n | Description of hazard | Correction |
|---|---|---|---|---|---|
| 1 | 01.2019 | Corn grain | 8 | Sprouted and moldy grains | Refused product |
| 2 | 01.2019 | Corn grain | 1 | Moldy grains = 0.91 % | Refused product |
| 3 | 01.2019 | Corn grain | 3 | Moldy, spoiled and sprouted grains | Refused product |
| 4 | 02.2019 | Corn grain | 1 | Spoiled grains = 0.68 % | Refused product |
| 5 | 02.2019 | Corn grain | 3 | Weevil infestation | Refused product |
| 6 | 05.2019 | Corn grain | 1 | Foreign objects above the allowed limit = 10.69 % | Refused product |
| 7 | 05.2019 | Corn grain | 1 | Foreign objects above the allowed limit = 6.07 % | Refused product |
| 8 | 05.2019 | Corn grain | 2 | Mold smells | Refused product |
| 9 | 06.2019 | Corn grain | 1 | Moldy grains | Refused product |
| 10 | 07.2019 | Corn grain | 1 | Mold smells | Refused product |
| 11 | 10.2019 | Wheat grain | 2 | Flour beetle infestation (*Tribolium confusum*) | Refused product |

n=number of batches refused

Table 4. Hazards identified at reception of raw materials from Unit B in 2020

| No. | Date | Product | n | Description of hazard | Correction |
|---|---|---|---|---|---|
| 1 | 01.2010 | Corn grain | 12 | Weevil infestation | Refused product |
| 2 | 02.2020 | Corn grain | 1 | Sprouted and moldy grains | Refused product |
| 3 | 02.2020 | Corn grain | 8 | Bulbs with moldy and sprouted grain | Refused product |
| 4 | 02.2020 | Corn grain | 1 | Altered grains | Refused product |
| 5 | 02.2020 | Corn grain | 2 | Weevil infestation | Refused product |
| 6 | 03.2020 | Wheat grain | 4 | Weevil infestation | Refused product |
| 7 | 04.2020 | Corn grain | 5 | Flour beetle infestation | Refused product |
| 8 | 04.2020 | Corn grain | 1 | Sprouted and moldy grains | Refused product |
| 9 | 06.2020 | Wheat grain | 6 | Weevil infestation | Refused product |
| 10 | 08.2020 | Corn grain | 2 | Sprouted and moldy grains | Refused product |
| 11 | 09.2020 | Corn grain | 1 | Sprouted and moldy grains | Refused product |
| 12 | 10.2020 | Corn grain | 1 | Sprouted and moldy grains | Refused product |
| 13 | 11.2020 | Corn grain | 1 | Sprouted and moldy grains | Refused product |
| 14 | 11.2020 | Corn grain | 1 | Burnt grains | Refused product |
| 15 | 11.2020 | Corn grain | 1 | Sprouted grains | Refused product |
| 16 | 11.2020 | Corn grain | 1 | Weevil infestation | Refused product |
| 17 | 11.2020 | Wheat grain | 1 | Weevil infestation | Refused product |
| 18 | 12.2020 | Corn grain | 1 | Weevil infestation | Refused product |
| 19 | 12.2020 | Corn grain | 2 | Mold smells | Refused product |
| 20 | 12.2020 | Corn grain | 1 | M =15.9 % | Refused product |

n = number of batches refused
M = moisture

In 2020, 53 batches of raw materials were refused, represented by corn grains (79.2%) and wheat grains (20.7%); the hazards identified were infestation with various insects, the presence of moldy, burnt and spoiled grains, and moisture. The most frequently identified hazard, for both maize and wheat, was the infestation with weevils (50.9%).

It was reported that based on physical parameters in 2020 were 54.7% more refused products of raw materials represented by corn and wheat than in 2019.

## CONCLUSIONS

In order to identify the hazards associated with the production of compound feed, the raw materials (maize grain and wheat grain) from two feed mills studied were analyzed and controlled during 2019 and 2020.

Regarding the hazards identified in unit A, in 2019 at the stage of receipt of raw materials, 20 batches of maize grains (68.9%) and 9 batches of wheat grains (31%) were refused, the most frequently identified hazard (78.5%) being represented by their high moisture. In 2020, 17 batches of raw materials were refused, represented by corn grains (52.9%) and wheat grains (47%); moisture was also the predominant potential hazard identified (70.5%). In comparison, in 2019 there were more refusals of raw materials (n=29) based on physical parameters, compared to 2020 (n=17).

In the case of unit B, based on the physical parameters identified in the stage of receipt of raw materials in 2019, 22 batches of maize grain (91.6%) and two batches of wheat grain (8.3%) were refused; the most frequently identified hazard (58.3%) was the presence of sprouted and moldy grains. In 2020, 53 batches of raw materials were refused, represented by corn grains (79.2%) and wheat grains (20.7%); the most frequently identified hazard was weevil infestation (50.9%). In comparison, in 2020 were 54.7% more non-compliant products refused than in 2019.

It can be concluded that in the production process of compound feed it is mandatory the physical analysis of the raw materials to determine the potential hazards; this goal is achieved in the units studied, the results highlighting the effectiveness of specific food safety control processes.

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# PREFERENCE TEST OF LOW CHOLESTEROL FUNCTIONAL CHICKEN MEAT

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***Abstract***

*This study was conducted with the aim of measuring the hedonic quality of low cholesterol functional chicken meat through a preference test. The study was conducted on 240day-old broilers with an average body weight of Lohman strain of 44.16 ± 3.72 grams using a 3x2 factorial completely randomized design with 4 replications. As factor A is a source of oil, namely A1 fish canning waste oil (FO), A2 coconut oil (CO), and A3 pure lauric acid (LA). Factor B is the level of oil on ration, namely B1 5%, and B2 8%. 6 treatment combinations apply. The feed was given until day 35. There was no interaction between the source of oil and the levels on all variable of hedonic test of chicken meat. The organoleptic test is an assessment measure using the sensory senses and meat quality parameters consisting of colour, aroma, taste, texture tests that are tested subjectively by the panelists. Panelists like the nature and quality of a material with organoleptic testing so that someone can give an assessment. Differences in oil sources and levels do not change consumer preferences for low-cholesterol functional chicken.*

***Key words****: hedonic quality of chicken meat, level of oil, oil sources.*

## INTRODUCTION

Along with increasing public awareness of healthy living, consumer demands for food are also increasingly shifting. Consumers began to pay attention to the food they consume to avoid the risk of disease. The main desire of consumers is to consume food that is safe and healthy, free from substances that can harm their health. This phenomenon gave birth to the concept of functional food. Designer food, functional food and fortified food are synonyms, referring to food that is fortified or enriched with nutrients already contained in the food or complementary nutrients.

Meat that has been damaged by bacteria is characterized by smell deviations and the appearance of mucus. Functional meat is meat which, apart from having high nutritional value, also contains compounds that can maintain the health of humans who consume it. Fast growth of broiler tends to result in fatty or fat accumulation in broilers due to high appetite (Jahanpour et al., 2015).

Functional food is defined as a compound that contains physiologically active compounds (bioactive compounds) and is used for the prevention or cure of a disease or to achieve optimal body health. The increase in the economic status of the community causes the consumption of animal products to increase as well. The essential components in the community's diet are meat and processed meat products. According to WHO in 2010, 25% of deaths in developing countries were caused by cardiovascular disease. Cardiovascular disease and type 2 diabetes are thought to be the result of increased consumption of animal products. Many studies have been conducted to refute this assumption. The results showed that total fat, saturated fatty acids and monounsaturated fatty acids were not associated with death from coronary heart disease, and consumption of saturated fatty acids was not associated with the risk of coronary heart disease, stroke and cardiovascular disease. Currently functional food is not only concerned with the high content of omega-3 fatty acids, but has shifted to the ideal ratio between omega-3 and omega-6. According to Simopoulus (2002), the ratio of omega-3 and omega-6 of 1:4 can reduce deaths from cardiovascular disease by 70%, while the ratio of 1:3 is very good for asthmatics. The imbalance of these fatty acids has a negative effect, including causing cardiovascular disease. Strengthening the supply of food of animal origin is in a dilemma because on the one hand the consumption of meat per capita is still low but on the other hand there is a tendency for certain consumers to limit the consumption of livestock meat because of the negative effect of food on health (Chen, 2009). This issue is certainly a challenge for animal husbandry experts on how to develop a business that can produce livestock commodities with carcasses that have a high edible meat portion as a source of safe and healthy food for consumers. Livestock commodities that are considered strategic for meeting animal protein needs are chicken meat.

Fish oil, like other oils, is composed mainly of triglycerides. Fish oil contains polyunsaturated fatty acids (PUFA) mainly consisting of DHA and EPA (Larsen, 2000). Fish oil showed as anti-aggregation and the addition of fish oil was effective in reducing plasma triglycerides and decreasing serum triglycerides caused by the oxidation of DHA and EPA. Dietary supplementation with fish oil can lower LDL. The beneficial effect of omega-3 essential fatty acids can help overcome coronary heart disease, hypertension, cancer, and a significant reduction of total cholesterol (Supadmo, 1997). Choct et al. (2000) explained that dietary supplementation with fish oil can lower LDL. In addition, the beneficial effect of omega-3 essential fatty acids is that it can help overcome coronary heart disease, hypertension, cancer and a significant reduction of total cholesterol. According to Miles & Jacob (1998), DHA and EPA are important for the health and normal growth of animals, where DHA is essential in brain membranes, spermatozoa, heart muscle, and cells in the retina of the eye. It is said further that fish oil is high in DHA, which is why it is called a good brain food. While EPA is a precursor of prostaglandin B and tromboxan A3 and leukotriene 5, substances that are very effective for anti-platelet aggregation (Brodeur, 2000). Schwartz & Weiss (1990) suggested that theoretically omega-3 PUFAs such as DHA and EPA are one of the main preventers of coronary artery disease through their effects on plasma lipids and platelet function. In addition, it is important in the prevention of coronary artery atherosclerosis and peripheral vascular disease through the same mechanism, namely cholesterol concentration, blood viscosity and platelet aggregation. It is also said that omega-3 PUFAs also have a role in the treatment of special diseases, namely hypertension and immune problems. Research using lemuru fish oil with a level of 3% in laying hens rations showed an increase in HDL-cholesterol in chicken eggs from 0.76 to 1.22 mg/100 ml and a decrease in LDL-cholesterol from 0.58 to 0.38 mg/100ml and a decrease in total cholesterol from 1.42 to 1.35 mg/100 ml (Londok, 2003). Rapid weight gain in broiler chickens is always followed by a large amount of fat and cholesterol deposits in broiler chicken meat (Supadmo, 1997). Cholesterol has various uses in embryonic development, including its role as a structural component of cell membranes and as a precursor to adrenal hormones, vitamin D, and bile acids (Leeson & Gonzales, 2000). According to Wiryanti (1990), fish has 5 to 20 percent fatty acids and almost all types contain unsaturated fatty acids that have double bonds, including omega-3.

Coconut oil is the processed product of coconuts. 92% of the fat in coconut oil is saturated fat. The medium chain fatty acids (ALRM) in coconut oil contain 47.7% lauric acid. The advantage of ALRM over long chain fatty acids (ALRP) is in its metabolic processes in the body. Crude fiber is one of the important food substances in poultry rations, because it functions to stimulate the peristaltic motion of the digestive tract so that the digestive process of food substances goes well. Poultry has limitations in digesting crude fiber because the fermenter organ is located at the end of the absorption organ. High crude fiber causes poultry to feel full, so it can reduce consumption because crude fiber is voluminous. The energy level in the feed will determine the amount of feed consumed. Broilers tend to increase their consumption when the metabolic energy content in the feed is low. Feed formulation by optimizing the use of fiber-rich feed ingredients can reduce the fat content of meat so that it becomes a safe/healthy meat product for consumers.

The resulting functional meat needs to be tested for its hedonic quality. The functional chicken meat as a result of this study is widely preferred because it is more chew and muscular, has less fat like broiler chickens. In Indonesia, many consume free-range chicken as a processed menu because the meat is not easily destroyed. Meat from chicken contains amino acids (21.88% non-essential and 19.96% essential). Carcass is the main product of slaughtering livestock which has high economic value (Soeparno, 1992). The most expensive component of carcass is meat and the largest part of the meat is found in the breast, so that the size of the breast is used as a measure to compare the quality of meat in broilers (Muchtadi et al., 2010). Body parts affect the amount of fat, breast meat is relatively lower than thigh meat (Pane 2006). This study was conducted with the aim of measuring hedonic quality of functional chicken meat contain low meat.

## MATERIALS AND METHODS

The study was conducted on 240 day-old broilers with an average body weight of Lohman strain of 44.16 ± 3.72 grams using a 3x2 factorial completely randomized design with 4 replications. As factor A is a source of oil, namely A1 fish canning waste oil (FO), A2 coconut oil (CO), and A3 pure lauric acid (LA). Factor B is the level of oil on ration, namely B1 5%, and B2 8%. 6 treatment combinations apply. Experimental broiler chickens were fed during 35 days of age by *ad libitum* system with feed mixture. Nutritional value of feed mixtures on this experiment was showed in Table 1. At the end of the study the chickens were fasted for 8 hours (all night) and the next morning the chicken was slaughtered for variable measurement. Slaughter was carried out on 1 broiler for each experimental unit. Furthermore, for the hedonic test using chicken breast meat. $35^{th}$ half-trained panelist tested the hedonic quality of chicken meat. The organoleptic difference test for colour, smell, and texture of chicken meat was based on a comparison scale transformed into a numerical scale (Table 2). Organoleptic testing on panelists was carried out by presenting six samples of chicken meat, each with labelled cut of meat. Data analysis used Minitab version 16. If there are significant differences between treatment combinations and their interactions or at least one treatment combination and their interactions are significantly different, it is followed by an honest real difference test (HSD).

Table 1. Composition of experimental diet (*as fed*)

| Ingredient | FO (%) | | CO (%) | | LA (%) | |
|---|---|---|---|---|---|---|
| | 5% | 8% | 5% | 8% | 5% | 8% |
| Yellow corn | 48 | 38 | 58 | 46 | 55 | 50 |
| Rice bran | 10 | 17 | 3 | 14 | 4 | 6 |
| Soybean meal | 18 | 18 | 18 | 22 | 18 | 18 |
| MBM | 17 | 17 | 14 | 8 | 16 | 16 |
| CaCO3 | 1 | | 1 | | 1 | |
| NaCl | 0.35 | | 0.35 | | 0.35 | |
| Premix | 0.6 | | 0.6 | | 0.6 | |
| DL-Methionine | 0.05 | | 0.05 | | 0.05 | |
| Fish canning oil | 5 | 8 | 0 | 0 | 0 | 0 |
| Coconut oil | 0 | 0 | 5 | 8 | 0 | 0 |
| Lauric acid | 0 | 0 | 0 | 0 | 5 | 8 |
| **Calculated Nutrient content** | | | | | | |
| ME (Kcal/kg) | 3124 | 3268 | 3052 | 3052 | 2997 | 3077 |
| Protein | 21.47 | 21.75 | 19.70 | 19.29 | 20.64 | 20.58 |

Table 2. Comparative scale and numerical scale of colour, juiciness, tenderness, flavour, and texture of breast meat of broiler

| Comparative scale | Numeric scale |
|---|---|
| really, really like | 5 |
| really like | 4 |
| like | 3 |
| do not like | 2 |
| very dislike | 1 |

Source: Soekarto (1985)

## RESULTS AND DISCUSSIONS

Result from hedonic test of breast meat of broiler after consume ration contain different source and level of oil were shown in Table 3. There was no interaction between the source of oil and the levels on all variable of hedonic test of chicken meat. The organoleptic test is an assessment measure using the sensory senses and meat quality parameters consisting of color, aroma, taste, texture tests that are tested subjectively by the panelists. Panelists like the nature and quality of a material with organoleptic testing so that someone can give an assessment. The organoleptic test assessment involved 35 semi-trained panelists to assess the broiler meat served. The average organoleptic test scores obtained can be seen in Table 3.

Colour organoleptic test is one of the factors that affect food when viewed visually, it affects consumer tastes. Bintoro (2008) states that the colour of processed meat can be obtained by processing methods and ingredients added. The colour of the meat shown in Table 3 based on the results of the hedonic test on the colour of the meat shows that there is no difference between the treatment with different sources of oil.

Table 3. Average of hedonic quality of chicken meat breast[1]

| Variable | Source of oil | Level of oil | |
|---|---|---|---|
| | | 5% | 8% |
| Colour | FO | 4.37±0.54 | 4.44±0.63 |
| | CO | 4.27±0.53 | 4.56±0.55 |
| | LA | 4.29±0.72 | 4.39±0.79 |
| Juiciness | FO | 3.17±1.32 | 2.63±1.32 |
| | CO | 2.71±1.05 | 2.98±1.27 |
| | LA | 2.93±1.31 | 3.07±1.29 |
| Tenderness | FO | 3.44±1.18 | 3.59±0.81 |
| | CO | 3.22±0,99 | 3.63±0.92 |
| | LA | 3.44±1.07 | 3.71±0.84 |
| Flavour | FO | 3.54±0.84 | 3.59±0.89 |
| | CO | 3.41±0.95 | 3.80±0.87 |
| | LA | 3.41±0.85 | 3.73±0.87 |
| Texture | FO | 3.22±1.11 | 3.34±0,73 |
| | CO | 3.22±1.08 | 3.59±1.00 |
| | LA | 3.34±0.96 | 3.63±0.83 |

[1]values are the means of 3 replications, values are expressed as mean±Stdev, $^{A-B}$different superscripts within row shows highly significantly different (p<0.01). $^{A-B}$different superscripts within column shows highly significantly different p<0.01). $^{a-b}$ different superscripts within column shows significantly different (p<0.05), $^{A-B}$different superscripts within row and column shows highly significantly different (p<0.01).

The assessment has been carried out by 35 (thirty-five) panelists giving a score that is not different from colour, namely an average score of 4.27-4.56. The highest average score for the panelists assessment was found in the CO treatment combination at the 8% level, namely 4.56±0.55, which was then followed by the FO treatment combination at 8%, LA treatment combination at 8% level, FO treatment combination at 5% level, and the FO treatment combination at the 5% level. LA treatment was at 5% level, and the lowest was CO treatment combination at 5% level with values respectively 4.44±0.63, 4.39±0.79, 4.37±0.54, 4.29±0.72 and 4.27±0.53 which ranged from white to yellowish white. This is because the addition of oil sources with different levels does not affect haemoglobin (Chartrin *et al*., 2006). The colour of the meat is determined by the processing method so that it produces colour from a non-enzymatic process (Permadi *et al*., 2012). Chicken meat is generally white in colour (Jaelani *et al*., 2014). Oil contains β-carotene which functions to produce bright colours in chicken meat, but differences in oil sources do not cause differences in chicken meat colour. Lawrie (2003) states that the pigment oxymyoglobin is an important pigment in fresh meat, this pigment is only found on the surface and describes the colour of meat that consumers want. Forrest *et al*. (1975) stated that normal chicken meat is grayish white to pale red or purple. In other words, the use of various sources of oil in the ration still gives normal colour to chicken meat. Good meat colour is thought to be supported by good handling so that chickens do not experience stress during slaughter. This is as stated by Soeparno (1994), that the factors that affect meat colour are feed, species, nation, age, sex, stress, (activity level and muscle type), pH and oxygen. All of these factors are the main determinants of the concentration of the meat myoglobin pigment (Soeparno, 1994). The provision of different sources and levels of oil in broiler rations did not have a negative effect on the colour of broiler meat.

Juiciness of meat. Juiciness is a sensory property related to the level of wetness of the meat. The results showed that the juiciness score of broiler meat fed with different sources and levels gave a non-significantly different effect. The juiciness score of broiler chicken that was fed with the addition of oil from different sources and levels ranged from a score of 2.63 to 3.17 which ranged from slightly juice to juice. The increasing level of added oil in the feed, the lower the fat content of broiler meat (Oktaviana, 2009) did not affect the sensory panelists on the juiceness of the meat, even though the presence of fat played a role in the juiciness characteristics of the meat (Williams and Damron, 1998). This is presumably because the effect of the liquid released during mastication (Soeparno, 2005) on broiler chicken meat with the addition of different oil sources in the feed is relatively the same so it does not affect the juiciness of the meat. Good quality meat contains relatively more juice than low quality meat (Soeparno, 2005).

Tenderness is the main parameter in determining the quality of meat that is tested sensory. The results showed that the addition of different sources and levels of oil in the feed gave a score of tenderness of broiler chicken meat that was not significantly different. The tenderness score of broiler chicken that was fed with the addition of oil sources and different levels ranged from a score of 3.22 to 3.71, which was tender. This can be influenced by the fact that less connective tissue is softer than muscle which contains a larger amount of connective tissue (Soeparno,

1991) and the higher the fat, the more marbling the meat will be (Dilaga and Soeparno, 2007). In addition, three main components of meat that contribute to tenderness or toughness, namely connective tissue, muscle fibers, and adipose tissue (Soeparno, 1991). Sindu (2006) revealed that when the meat is pressed with a finger, healthy meat will have a chewy to dense consistency. Tender meat is the most sought after by consumers (Komariah *et al*., 2004).

The evaluation of flavor is very dependent on the tastes of the panelists, because of the diversity between individuals in responding to the intensity and quality of a stimulus, causing the assessment of the smell and taste given to differ between the panelists because there is disagreement about the detailed aspects of taste and aroma. The results of the analysis of variance in the provision of alternative feeds had an effect on the organoleptic test of the taste of chicken meat fed with different sources and levels of oil, which gave no significant effect on the taste of chicken so that they had the same level of taste. From the results of the score assessment given by the panelists, it can be seen that the average value ranges from 3.41-3.80. The highest value was given to the treatment combination of 8% CO with a value of 3.80, which was then followed by the treatment of 8% LA, 8% FO, 5% FO, 5% LA and 5% CO, which ranged from good to very good. This result is evidenced by the results of Prayitno *et al*. (2010) showed that the taste score of broiler chicken fed with the addition of virgin coconut oil ranged from slightly savory to savory. The delicious and savory taste of broiler chicken meat is caused by the addition of different sources and levels of oil in the feed that does not affect the volatile substances contained in the meat as small molecules released by food (during heating, chewing, etc.) receptors in the mouth or nasal cavity (Soeparno, 2005; Prayitno *et al*., 2010).

Texture. Texture is a sensory property of meat related to the level of smoothness of the meat. The average value of cooked free-range chicken meat texture that was fed commercial feed with coconut pulp flour substitution was 2.33, meaning that the panelists preference for cooked free-range chicken meat was still in the like area. The increase in substitution of oil levels in commercial feed did not affect the panelists texture. This is influenced by the tenderness of the meat which is still preferred by the panelists and is supported by the young age of the chickens. Soeparno (2005) added that the level of texture roughness increases with age, muscles with small muscle fibers do not show a significant increase in texture hardness with increasing age (Warris, 2000). Three main factors are known to influence the texture of meat, including the length of the sarcomere, the amount of connective tissue and cross-linking and the degree of proteolytic changes that occur during withering. Muscle texture can be divided into two categories, coarse texture with large fiber bundles and fine texture with small fiber bundles (Soeparno, 2005).

## CONCLUSIONS

Low-cholesterol functional chicken meat resulting from the provision of different sources and levels of fat in the ration provides the same level of preference by consumers.


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# NUTRIENTS AND PHYTOCHEMICALS OF WELSH ONION (*ALLIUM FISTULOSUM* L.) AND THEIR IMPORTANCE IN NUTRITION OF POULTRY IN THE FUTURE – A REVIEW

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***Abstract***

*Allium fistulosum L., commonly called spring onion, welsh onion, or Japanese bunch onion, is a clumping, slowly spreading, evergreen perennial type that is mainly grown as a vegetable because of its onion-scented leaves. This species is very similar in taste and smell to the related common onion, Allium cepa, but does not develop bulbs. Welsh onion were detected to contain volatile compounds containing sulphur and polyphenolic compounds which exhibit various biological activities, and which have anti-fungal, anti-oxidative, anti-hypertensive, anti-platelet, regulation of immune function and anti-obesity effects. This plant is much less known in the world than the traditional onion, A. cepa. This review intends to describe and summarize recent advances the nutrients and phytochemicals of welsh onions for using as a poultry alternative feed additive, their beneficial effects, and the mechanisms underlying their involvement for future in poultry nutrition.*

***Key words**: nutrients, phytochemicals, poultry, welsh onion.*

## INTRODUCTION

Allium is a genus of the Liliaceae family that is easily found in Asia, Europe and America (Gitin et al., 2012). This genus has more than 700 species, including: *A. cepa* L., *A. sativum* L., *A. fistulosum* L., *A. schoenoprasum* L., *A ursinum* L., *A. flavum* L., *A. scorodoprasum* L., *A. vineale* L., *A. atroviolaceum* Boiss., *A. psekemense* B. Fedtsch., *A. kurtzianum*, *A. chinense* and *A. rubellum* and many other species from other countries (Verma et al., 2015; Verma et al., 2008). According to Rose et al. (2005), Allium species are well-known as foodstuffs that are widely used worldwide. Approximately 800 species, including welsh onion (*A. fistulosum*), garlic (*A. sativum*), and onion (*A. cepa*), are known.

*Allium fistulosum* L., known as Japanese bunching onions, welsh onions or spring onions is one of Allium species known for originating from Romania which has similarities to scallions and smells and tastes similar to *A. cepa* L. Unlike other species, this plant does not form bulbs and has hollow leaves (Immaculate et al., 2020). In Japan and China, this plant is used as a vegetable or as a traditional medicine to improve the function of internal organs and metabolism and treat several diseases such as headaches, diarrhea, stomach pains and colds (Bede and Zaixiang, 2020; Hirayama et al., 2019; Newenhouse, 2011). Welsh onion (*Allium fistulosum* L.) is a major vegetable product widely cultivated from Siberia to tropical Asia as well as in Japan, Korea and China (Hirayama et al., 2019).

According to Waghulde et al. (2021) welsh onion is a perennial species originated from Eastern Asia. Its leaves have nutritional value, and they can be fresh consumed all over the year, still green over the winter. According to Wang et al. (2005) leek is popular in Western Europe and Japanese bunching onion (synonim: Welsh onion) is common in China, Japan, Korea and Taiwan. However, this plant has much less known in the world than the traditional onion, *A. cepa* (Tendaj, 2003). Welsh onion is a clumping, slowly spreading, evergreen perennial type that is mainly grown as a vegetable because of its onion-scented leaves. This species is very similar in taste and smell to the related common onion, *Allium cepa*, but does not develop bulbs. It somewhat has hollow leaves (fistulosum meaning "hollow") and scapes. Welsh onion can reproduce by forming an evergreen clump (Thompson, 1995).

Phytochemical studies reported that Welsh onions contain organosulfur compounds (Zhou et al., 2011) and polyphenolic compounds (Vlase et al., 2013). Welsh onions (*Allium fistulosum* var. maichuon) and shallots (*Allium fistulosum* var. caespitosum) were detected to contain volatile compounds containing sulfur (Kuo et al., 1990). Sulfur-containing compounds from the Allium plant exhibit various biological activities. Several studies have shown Welsh onions have anti-fungal, anti-oxidative, anti-hypertensive, anti-platelet, and anti-obesity effects (Sung et al., 2011; Yamamoto et al., 2005; Sang et al., 2002; Chen et al., 2000).

Welsh onions are mainly used in traditional Chinese medicine because of its natural chemical compounds which are beneficial for human health. Bulbs, pseudo stem juice, leaves, flowers, seeds and roots have medicinal values, as antibacterial, antitumor, antioxidant, antihypertensive, antiobesity, antiplatelet aggregation, and regulation of immune function (Liang et al., 2021; Nohara et al., 2021; Tigu et al., 2021; Hirayama et al., 2019; Sung et al., 2018). The main active compounds include essential oils which mostly contain sulfides, oleic acid, linoleic acid, pectin, allicin, and vitamin C (Nohara et al., 2021; Tigu et al., 2021; Sung et al., 2018; Zhang et al., 2017; Tian et al., 2016). It was reported that the stem, leaf and root extracts of Welsh onions all had an antioxidant effect, and the stem extract showed the strongest antioxidant effect (Wang, 2017). This article aims to review the nutrients and phytochemicals of welsh onion and to determine the possible use as alternative poultry feed additive and feed ingredients, their beneficial effects, and the mechanisms underlying their involvement for future in poultry nutrition.

## MATERIALS AND METHODS

A literature review which search from the Google of the potential of Welsh onions as alternative feed additives and feed ingredients for broiler chickens is presented in this paper. This review summarized the existing literature and gathers information to present the current relevance of welsh onions as an effective poultry feed additive, discussing the various compounds present in welsh onions in terms of their bio-functionality. Emphasis is placed on the effects of the Welsh onion diet, growth performance, immunomodulatory properties, gut microbiota and gut morphology, and quality product in poultry. Although the selection of publications to improve upon for optimal application of welsh onions to poultry is still very limited, the review remains based on scientific results.

## RESULTS AND DISCUSSIONS

### Overview of Bioactive Compounds in Welsh Onion

Welsh onion, synonim green onion (*Allium fistulosum* L.), is an important spice in the world (Wang et al. 2020) with high nutritional value (Gao et al., 2021). Rouphael et al. (2012) reported that spice plants as green onions contain high concentrations of minerals and bioactive ingredients. Welsh onion are indicated to have antioxidant, antifungal, and antihypertensive activity (Singh et al., 2020).

Phytochemical analysis in both *Allium fistulosum* and *Allium ursinum* species indicated the presence of p-coumaric and ferulic acid in phenol carboxylic acid pattern. Isoquercitrin and quercitrin were found only in *A. fistulosum*. Quercetol and kaempferol were identified before and after hydrolysis in *A. fistulosum*, whereas kaempferol was only after hydrolysis in *A. ursinum*. Allicin was identified in all extracts, higher amounts were found in *A. ursinum*. Sitosterol and campesterol were identified in both species, and stigmasterol only in *A. fistulosum* (Vlase et al., 2013).

Udjaili et al. (2015) reported that the phenolic extract of welsh onion root contained total phenolic, flavonoid and tannin as well as the best free radical scavenging activity to ward off DPPH free radicals. The activity test using the

DPPH test showed that the percentage value of the phenolic extract of dry welsh onion was higher than that of the phenolic extract of fresh welsh onion. GC-MS analysis of the ethanol extract welsh onion revealed the presence of 31 compounds, including flavonoids, terpenoids, fatty acids, fatty alcohols and compounds containing sulfur. Therefore, welsh onion may have chemopreventive, anticancer, anti-microbial activity, antioxidant and antidiabetic activity, anti-inflammatory, antibacterial, antifungal, skin conditioning properties due to the presence of secondary metabolites in the ethanolic extract (Monika & Sakthi, 2018).

Tabassum et al. (2016) stated that green onion (syn. scallion and welsh onion) (*Allium fistulosum*) is used as an important spice worldwide. It have high concentrations of allicin, flavonoids, vitamins and other organosulfur compounds (Yin et al., 2003). Phytochemical studies reported that welsh onion contain organosulfur compounds (Zhou et al., 2011) and polyphenolic compounds (Vlase et al., 2013). Allicin, one of the most characteristic organosulfur compounds, is responsible for the pungency and flavor in the monocotyledonous flowering plant Allium (Block et al., 1992). Welsh onion has antioxidant and antibacterial properties and has the potential to benefit the cardiovascular system (Chan et al., 2013), also have antifungal, antihypertensive, antiplatelet, and antiobesity effects (Sung et al., 2011; Yamamoto et al., 2005; Sang et al., 2002; Chen et al. al., 2000).

Welsh onion is a perennial herb widely cultivated throughout the world, especially in China, Japan and Korea. Strong antimicrobial agents such as fistuloside A, B, and C have been isolated from the edible parts of welsh onion. Their antimicrobial activity was evaluated by pathogenic microorganisms or food spoilage based on disc diffusion assays, minimal inhibition concentration (MIC) and determination of minimal fungicide concentration (MFC). Fistuloside A and fistuloside C showed strong antifungal and anti-proteus activity, whereas fistuloside B was only effective against fungi. Fistuloside C showed prominent antifungal activity with 3.1-6.2 ttg/ml MIC and MFC. That fistuloside C has prominent antifungal activity and supports the use of welsh onion to treat microbial infections (Sohn et al., 2006).

Welsh green onions are a powerful antioxidant food comparable to yellow onions, and are a good source of kaempferol. The increase in antioxidant activity and the decrease in flavonoid content (especially kaempferol) during boiling may have some relationship. Consequent studies should be carried out to clarify the reasons for the thermal instability of flavonoids during heating, and the mechanism of thermal enhancement of antioxidant activity in welsh green onions (Aoyama & Yamamoto, 2007).

Welsh onion is also a common Allium plant in Eastern Europe, with antifungal and antimicrobial properties, due to its high concentration of sterols and sulfate compounds (Vlase et al., 2013). Ethanolic extracts 30% of *Allium fistulosum* L. and *Allium sativum* L. were investigated to evaluate their antioxidant and antimicrobial capacity. The highest DPPH scavenging activity in *A. fistulosum* L. leaves was IC50 of 14.61 $g.mL^{-1}$. The highest antioxidant activity using the TEAC test and total phenolic content were observed in stems of *A. fistulosum* L. That the DPPH IC50 value was significantly correlated with the total phenolic content and antioxidant activity using the TEAC assay. The stem extract of *A. fistulosum* L. was more active against *Bacillus subtilis*, with MIC and MBC. That extract Allium spp. can be used as a potential source of natural antioxidants and antimicrobial agents (Chang et al., 2013). Stajner et al. (2002; 1999; 1998) concerning the antioxidant abilities of different Allium species showed that they possess antioxidant abilities in all organs, but especially in the leaves.

*Allium fistulosum* L. contained GSH (reduced glutathione) 0.497 nmol/mg protein, flavonoids 465.87 mg/g, vitamin C 0.161 mg/g, soluble proteins 7.30 mg/g, carotenoids 2.87 mg/g, and scavenging activity 82.15%. According to the data that the scavenger activity of *Allium fistulosum* L. was high, the generation of OH radicals (the most toxic oxygen species) was reduced by 87.09%. Other results regarding *Allium fistulosum* L. support this assessment due to the high activity of all antioxidant enzymes SOD, CAT, GPX and GP, low concentrations of O2, OH and MDA, and high amounts of GSH, flavonoids, vitamin C and soluble proteins, such as carotenoid content. That *Allium fistulosum* L. was the most promising source of natural, non-toxic antioxidants that can be used in the food, pharmaceutical and cosmetic industries (Stajner et al., 2006). The physicochemical properties of the welsh onion reported by several references showed in Table 1 and phytochemical of welsh onion reported by several authors showed in Table 2.

Table 1. The physicochemical properties of the Welsh Onion by Several References

| Proximate Composition (%) | Reference | |
|---|---|---|
| | Adeyeye (2020) | https://rxharun.com/allium-fistulosum-nutritional-value-health-benefits-recipes/ |
| Moisture | 89.55 | NR |
| Ash | 0.82 | NR |
| Crude Oil | 0.64 | NR |
| Crude Protein | 1.82 | NR |
| Crude Fiber | 1.65 | NR |
| Carbohydrate | 5.54 | |
| Vitamin K (%) | NR | 161.17 |
| Vitamin C, (%) | NR | 30.00 |
| Vitamin B2, (%) | NR | 6.92 |
| Vitamin B6, (%) | NR | 5.54 |
| Fe, (%) | NR | 15.25 |
| Cu, (%) | NR | 7.78 |
| P, (%) | NR | 7.00 |
| Mg, (%) | NR | 5.48 |
| Mn, (%) | NR | 5.96 |
| Total Dietary Fiber, (%) | NR | 6.32 |

*NR = not reported*

Chemical composition of welsh onion seeds according to Golubkina et al. (2015): oil content 7.1%, protein 23.8%, total phenolic 3.8 mg $g^{-1}$ f.w, Selenium 476 µg $kg^{-1}$ f.w. The advantages of Japanese bunching onion are high nutritive value and unique flavour (Tendaj & Mysiak, 2007). Higashio et al. (2007) reported that Japanese bunching onion is abundant in vitamin C, and contains other valuable compounds such as carotenoids, macronutrients and micronutrients, especially Ca and K, as well as flavonoids, which are potent antioxidants (Aoyama & Yamamoto, 2007; Mysiak & Tendaj, 2006, 2008). The leaf blades contain more vitamin C, carotenoids, vitamins B1, B2, niacin and minerals than the pseudostem. The specific odour of the crop is attributed to volatile allyl sulphides (Warade and Shinde 1998).

Several studies reported that *A. fistulosum* leaf extract contains flavonoids (myricetin, quercetin, rutin, kaempferol, naringenin and hesperetin), polyphenols (benzoic acid, salicylic acid, ferulic acid, caffeine, p-coumaric acid, coumarin, vanillic acid, gallic acid and cinnamic acid) (El-Hadidy et al., 2014). Also contains apigenin (41,5,7-trihydroxy-flavone) (Immaculate et al., 2020), and dichloroacetic acid, 1-buten-3-yne, 1-chloro-, (Z)-,-pinene, D-limonene, thymol (Ajayi et al., 2019). Besides, kujounin A3, kujounin B1, kujounin B2, kujounin B3, allium sulfoxide A2, allium sulfoxide A3, kujounin A1 (Fukaya et al., 2019), onionin A1, and onionin A2 and onionin A3 (Nohara et al., 2014).

Table 2. Phytochemical of Welsh Onion Reported by Several Authors

| Phytochemical | Authors | | |
|---|---|---|---|
| | Waghulde et al. (2021) | Siregar et al., 2015* | Tigu et al (2021) |
| Saponin, g/100 g | 0.26 | NR | NR |
| Tannin, g/100 g | 2.55 | √ | NR |
| Cardiac glycosides, g/100 g | 1.85 | NR | NR |
| Flavonoids, g/100 g | 0.08 | √ | NR |
| Alkaloids, g/100 g | 0.18 | √ | NR |
| Phenolics, g/100 g | NR | √ | NR |
| Steroids, g/100 g | NR | √ | NR |
| Toxicity test, ppm | NR | 603.66 (mild) | NR |
| Quercetin, µg/mL | NR | NR | 26 |
| Quercitrin, µg/mL | NR | NR | 95 |
| Isoquercitrin, µg/mL | NR | NR | 280 |
| Kaempferol, µg/mL | NR | NR | 30 |
| Rutin, µg/mL | NR | NR | 215 |
| Ferulic ac., µg/mL | NR | NR | 230 |
| Alliin, µg/mL | NR | NR | 145 |
| Allicin, µg/mL | NR | NR | 20 |

**Phytochemical screening; NR = not reported*

El Hadidy et al. (2014) reported that there were three main compounds isolated from the leaf extract of *A. fistulosum*, namely myricetin, quercetin and rutin. Myricetin was the most abundant compound in Giza 6 and photon varieties, among the three compounds, which was 38.75%. Antioxidant activity test using the DPPH method showed a decreasing in activity after three months of storage based on the percentage of antioxidants. Myricetin was also isolated from *A. fistulosum*. This compound is classified into the flavonoid group, which has six hydroxyl groups at positions 3, 5, 7, 31, 41 and 51 (Yao et al., 2014). The presence of a hydroxyl group at position 51 in ring B greatly affects its antioxidant activity so that it becomes stronger with the IC50 (4 μM) and 463.40 μM in testing using DPPH radical scavenging activity (Ahmadi et al., 2020; Sim et al., 2007; Seyoum et al., 2006).

Kaempferol can be found in fruits and vegetables (Singh & Kumar, 2017; Kruzlicova et al., 2012). This compound is also easily found in some Allium species (Bilyk et al., 1984). Several studies had reported the presence of kaempferol in *A. fistulosum*, *A. ursinum*, *A. schoenoprasum*, *A. sativum* and other species (Amabye, 2015; Shakurfow et al., 2015). Farkas et al. (2004) reported that kaempferol has antioxidant activity in inhibiting heat-induced oxidation in a β-carotene-linoleic acid-model-system (65.3%).

Okungbowa et al. (2017) reported that the leaf extracts of *A. fistulosum*, *B. pinnatum*, *C. citratus* and *H. crinita* possess potent antioxidant activity, phytochemical and nutritional benefits, with *B. pinnatum* having the highest phytochemical and nutritional content. Xu et al. (2005) reported that Welsh onion had 23.6 mg of quercetin per kg of fresh weight (FW), with no other flavonols detected. Miean & Mohamed (2001) reported that Welsh onion leaves had a total flavonol (TF) content of 2720.5 mg/kg of dry weight (DW), with 1497.5 mg/kg of quercetin, 391 mg/kg of luteolin, and 832 mg/kg of kaempferol.

Welsh onion leaves contain high levels of quercetin, a flavonol compound with potential benefit to human health. Quercetin was reported to have protective effects in reducing the risk of cardiovascular disease and act as anti-cancer and antioxidant agents due to its antiprostanoid and anti-inflammatory responses and decreased rate of DNA degradation (Crystal et al., 2003). Chinese scallions (*Allium fistulosum*) were characterized by their high content of the antioxidant allicin (Wang et al., 2020).

Analysis of the aqueous extract *A. fistulosum* with GC-MS identified D-Limonene, a cyclic monoterpene, as the most abundant bioactive compound in *A. fistulosum* with approximately 99% of the total yield. Also minor bioactive constituents present in the plant include dichloroacetic acid (0.48%), α-pinene (0.36%), 1-Buten-3-yne, 1- chloro-, (Z)- (0.14%) and thymol, TMS derivative (0.07%). D-Limonene has been known to be commonly present in citrus peels, however, it is the first time this compound will be identified by GC-MS analysis as the major bioactive compound in *A. fistulosum* (Ajayi et al., 2019). D-Limonne has been reported to possess anti-oxidant (Yu et al., 2017), anti-inflammatory (Yilmaz & Özbek, 2018; Souza et al., 2003) and anti-carcinogenic (Crowell and Gould,1994) properties.

## Overview of Fatty Acids Compounds in Welsh Onion

Linoleic and oleic acids were the most abundant of the total fatty acids in welsh onion and also of the total unsaturated fatty acids with the two totalling 70.44% of all fatty acids. Palmitic and stearic acids were the two most abundant saturated fatty acids, totalling 18.61% of all fatty acids. The total unsaturated fatty acids (77.35%) predominated the total saturated (22.63%), while the percentage poly-unsaturated (56.34%) was far greater than mono-unsaturated (21.04%) (Table 3).

Table 3. The Fatty Acid Profile of the Welsh Onion Reported by Several Authors

| Fatty Acid (%) | Authors | |
|---|---|---|
| | Adeyeye (2020) | Golubkina et al., 2015 |
| Linoleic, C18:2 | 52.87 | 72.34 |
| Oleic, C18:1 | 17.57 | 18.30 |
| Palmitic, C16:0 | 9.80 | 5.25 |
| Stearic, C18:0 | 8.81 | 1.47 |
| Linolenic, C18:3 | 2.88 | 0.22 |
| Palmitoleic, C16:1 | 2.84 | 0.07 |
| Myristic, C14:0 | 1.28 | 0.18 |
| Behenic, C22:0 | 1.23 | 0.33 |
| Lauric, C12:0 | <1.00 | NR |
| Arachidonic, C20:4 | <1.00 | NR |
| Lignoceric, C24:0 | <1.00 | 0.08 |
| Total saturated (SFA) | 22.63 | 7.93 |
| Total unsaturated | 77.38 | NR |
| Total mono-unsaturated (MUFA) | 21.04 | 19.01 |
| Total poly-unsaturated (PUFA) | 56.34 | 73.05 |
| Essential fatty acids (EFA) | 55.75 | NR |

NR = not reported

The high level of essential fatty acids in the plant oil is an advantage in food consumption and the good total unsaturated/saturated (P/S) ratio makes the fruit oil nutritionally very useful to be adopted for domestic purposes (Adeyeye, 2020). *Allium fistulosum* plant oil is characterized by a reasonable polyunsaturated/ saturated (P/S) ratio of 3.42. This ratio determines the detrimental effect of dietary fat. A high P/S ratio supports the reduction of serum cholesterol and atherosclerosis and prevention of heart disease (Oomah et al., 2000). The many saturated fatty acids present in plant oils have several important uses. Lauric acid is believed to have antimicrobial properties (Muhammad and Ajiboye, 2010). Palmitic acid is the first fatty acid produced during fatty acid synthesis and from which longer chain fatty acids can be synthesized (Muhammad & Ajiboye, 2010).

**Welsh Onion as Poultry Feed Additives**

Minh et al. (2017) reported that the growth rate and feed conversion of local chickens increased linearly when fed diet supplemented with chives (= welsh onion) 0 to 2% (DM basis). There was no effect of chive supplementation on hematological and biochemical indices in blood. *E. coli* was shown to be susceptible to chive extract in MIC test. However, supplementation with chives up to 20 g/kg DM diet had no significant effect on fecal *E. coli* counts. That chives can be considered as a prebiotic for natural growth promoter in chickens. Welsh onions is less studied than garlic. Welsh onion have been well known for their medicinal properties, however, there is very little information emphasizing their effect on growth performance of chickens.

Sung et al. (2018) reported the utilization of welsh onion in mice diet that HPLC showed both ethanolic extracts and aqueous extracts of welsh onion contain ferulic acid and quercetin. Oral administration of aqueous extracts and ethanolic extracts of welsh onion to HFD-fed mice decreased body weight, liver, adipose tissue weight and adipocyte size. Serum lipid profiles and adiponectin levels were improved in HFD-fed mice treated with ethanolic extracts of welsh onion but not aqueous extracts of welsh onion. However, both aqueous extracts of welsh onion and ethanolic extracts of welsh onion significantly attenuated HFD-induced changes in serum leptin and insulin-like growth factor 1 levels, liver expression of AMPK, and adipose tissue expression of UCP2. The findings suggested that welsh onion extracts have potential as functional food materials for weight control in obesity.

A study was conducted to examine the effects of Chongbaek (= welsh onion) aquaeos extract (*Allium fistulosum*) and Chongbaek 30% ethanol extract on bone growth using an animal model (rat) lacking calcium and vitamin D. Serum analysis showed that Chongbaek extract promoted bone growth based on the osteogenic markers ALP, calcium, osteocalcin, and type 1 collagen and increase bone mineral content, bone mineral density, and growth plate length. Overall, the results suggest that Chongbaek aquaeos extract and Chongbaek ethanol extract can be used to facilitate bone growth and increase bone mineral density in children and adolescents by lengthening the growth plate without any adverse side effects, such as metabolic disturbances or release of trigger hormones obesity (Ryuk et al., 2021).

Welsh onion is an important ingredient of cuisine. It has nutrients such as carbohydrates, proteins, lipids, minerals (magnesium, calcium, potassium and iron), vitamins (A, C, E, K) and a lot of fiber that facilitate digestion and avoid different problems like colon diseases and constipation (Sakakibara, et al., 2003).

Recently, plant-derived feed additives have gained considerable interest as sustainable substitutes in poultry diets (Habibi and Ghahtan, 2019; Sugiharto, 2016). Plant-derived additives that are effective in poultry are expected to stimulate feed consumption, increase secretion of digestive enzymes, activate the immune system, modulate gut microbiota, and have antibacterial, coccidiostatic, antiviral, antioxidant and/or anti-inflammatory activities (Habibi & Ghahtan, 2019; Sugiharto, 2016; Toghyani et al., 2011).

Research on the use of welsh onion in diet of animal model that have a significant effect on growth performance and health is expected if applied to poultry will have a significant effect on performance and health of poultry.

## CONCLUSIONS

From the review, linoleic and oleic acid were the most abundant of the total fatty acids in welsh onion (*A. fistulosum* L.), palmitic and stearic acids were the two most abundant saturated fatty acids. Welsh onion also rich in nutrients and secondary metabolites. This plant showed powerful of bioactivities such as antioxidant, antibacterial, antifungal, anti-inflammatory and others, and have a great role in the health field and the growth performance. So, it is possible use as alternative poultry feed additive and feed ingredients for future in poultry nutrition.

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# CARCASS AND CUTTING YIELDS, MEAT QUALITATIVE TRAITS AND SENSORY EVALUATION OF BROILER CHICKENS FED DIET CONTAIN CLOVE AND TREATED OF CARROT IN DRINKING WATER

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***Abstract***

*This study was conducted to investigate the carcass and cutting yields, meat qualitative traits and sensory evaluation of broiler chickens fed diet contain clove and treated of carrot in drinking water. A total of 200 D.O.C of broilers were used. The experiment utilized a completely randomized design with 4 treatments and 5 replications. Treatments were carrot juice consist of 0, 10, 20, 30 ml/liter water, respectively. Based diet consist of commercial diet 73%, corn 23%, clove meal 1%, and palm oil 3%. Results showed that water intake was significantly decreased, carcass weight, slaughter weight cutting yield, and giblet were non significantly difference but gizzard was significantly increased. Blood triglyceride was non significantly difference, HDL-cholesterol was significant increased, LDL-cholesterol, blood glucose and SGOT were significantly decreased. Cooking loss, WHC and water content of meat were non significantly difference but tenderness was significantly decreased. The color, aroma, texture and taste of meat were non significantly difference. It can be concluded that carrot juice in drinking water could be acceptable up to 30 ml per liter water when given to broiler chickens fed diet contain clove meal.*

***Key words:*** *broiler chickens, carrot, clove, drinking water, meat.*

## INTRODUCTION

In recent years, poultry welfare and food safety have become a priority for poultry producers, because more and more consumers prefer products from animals raised in free-range or organic farming systems, which they believe provide more convenience and guarantee safe and healthy food products. In addition to prebiotics, probiotics and organic acids, there is increasing interest in preparations of plant origin (phytobiotics), which are considered natural and safe additives characterized by their positive effects on animals, such as antioxidants, antibacterial, antiviral, antifungal, immunostimulants, and stimulating the secretion of digestive enzymes. In view the above, it is necessary to conduct research to test the hypothesis whether herbal diet or external access has a positive effect on the quality of broiler meat.

Herbs are also natural immunostimulants (Nasir & Grashorn, 2010). The positive effect on the health status of poultry is also produced by hypocholesterolemic herbs, as in essential oils in plants were found to inhibit the activity of a liver enzyme (HMG-CoA reductase) which regulates the amount of cholesterol synthesized and thereby reduces its level in the blood (Bölükbaşı et al., 2008). Stimulation of the immune system and antioxidant defense in poultry has become a topical issue since the introduction of the prohibition on the use of antibiotics as growth promoters in animal feed. The use of alternative feed additives that promote animal growth without negative side effects such as antibiotic resistance from pathogenic strains is being taken into account. Herbs and spices contain many active ingredients that can exert various bactericidal, immunomodulatory and antioxidant effects on animals, and ultimately can affect the health status and productivity of animals as well as the quality of animal products (Madhupriya et al., 2018).

Carrot (*Daucus carota*), from the Apiacea family, is a commonly consumed vegetable that grows in temperate climates in Europe, Asia and Africa (Hammam, 2014). Carrot meal has been tested for its potential as a food ingredient in the livestock industry (Steenfeldt et al., 2007). There were many contain active ingredients such as steroids, tannins, flavonoids, and carotenes (Vasudevan et al., 2006; Jasicka-Misiak et al., 2005). According to Febrina (2012) carrots have high content α- and β-carotene, both types of carotene are important in nutritional needs as provitamin A. In addition to the high content of provitamin A, carrots also contain vitamin C and vitamin B and contain minerals, especially calcium and phosphor. Vitamin A in carrots has functions for cell differentiation, digestion, immunity and increasing feed efficiency. Calcium plays a role in various stages of metabolism, especially as a cofactor in enzyme activity. The balance of calcium and other mineral ions in the body is necessary for the regulation of enzyme activity. Calcium as a catalyst for biological reactions such as absorption of B vitamins which are used for protein metabolism.

Al-Snafi (2017) stated in his reviewed article that nutritional analysis of carrot juice showed protein 1.067%, crude fat 0.367%, crude fibre 1.167%, carbohydrates 6.100 %, many vitamins and minerals, such as: ascorbic acid 16.667 mg/100 g, Ca++ 55.000 mg/100 g, Fe++ 1.667 mg/100 g, PO4 44.333 mg/100 g, thiamine 0.057 mg/100 g, niacin 0.300 mg/100 g, riboflavin 0.100 mg/100 g, β-carotene 2730, and vitamin A 2805. The pharmacological studies revealed that the plant possessed cytotoxic, antioxidant, antidiabetic, antimicrobial, smooth muscle relaxant, hypotensive effect and decrease intraocular pressure, gastro-protective, nephro-protective, hepato-protective, cardio-protective anti-depressant memory enhancement, anti-inflammatory, reproductive, wound healing and hear induction and many other effects. Sahin et al. (2009) stated that vitamin A plays a role in several body functions, including the differentiation of digestive epithelial cells and has an effect on the immune function of poultry, as well as being able to increase feed efficiency and increase body weight. The calcium content in carrots is quite high which functions as a coenzyme in protein metabolism.

Arkoub-Djermoune et al. (2020) reported about two varieties of carrot that determined before storage of the samples and after storage every three days: after 3, 6, 9, and 12 days, that the Touchon variety is richer in phenolics, flavonoids, and carotenoids and presents higher antioxidant activity in comparison with the Supermuscade variety. At the end of storage, the bioactive compound content and antiradical activity increased significantly. Also, an extremely significant correlation was observed between the antioxidant contents and the antioxidant capacities of aqueous carrot extracts. Suplementation dried carrot waste up to 5% in broiler diets enhanced the productive performance and economic efficiency (Hashem, 2012). Muzaki et al. (2017) stated the use of waste carrot meal more than 4% reduced body weight, but were not significantly different in feed consumption and feed conversion ratio. The use of carrot waste in broiler diets should not be more than 2%. A mixture of carrot and fruit waste juices can be used up to 20% in broiler diet replacing 40% of maize effectively. The high crude fiber content in mixed carrot and fruit waste juices limited their use in broiler feeds (Rizal et al., 2010).

The use of carrot waste meal up to 6% in the ration did not affect protein consumption and protein efficiency ratio, but it could increase calcium retention. Therefore, carrot waste meal can be used as feed for broiler chickens (Prasetyo et al., 2018). Addition of cold-press carrot seed oil to the basal diet increased weight gain, hot carcass weight and carcass yield, and had resulted in positive changes on lactic acid bacteria count and breast tissue shelf life. As a result, carrot seed oil can be added in the diet of broilers as a beneficial dietary source of natural antioxidant for broilers (Ürüşan et al., 2018). Dried carrot meal prepared from fresh carrots was found to be a good source of xanthophyll (54 g/kg), a moderate source of protein (188.3 g/kg) and energy (2510 kcal/kg) with a low fiber content (80 g/kg). The use of 8% dry carrot meal added in the based diet significantly improved egg yolk color, and 4% dried carrot meal increased egg yolk score, however, weight gain, egg production and feed conversion were not significantly affected by the addition of dried carrot meal in diet of laying hen (Sikder et al., 1998).

Supplementation carrot meal 20-100 g per kg dry matter of feed did not affect the growth rate, live weight and carcass characteristics of female broiler chickens aged 22-42 days, but increased feed consumption, feed conversion ratio, metabolic energy consumption and nitrogen retention (Ng'ambi et al., 2019). Carrots are one of the potential and multipurpose horticultural commodities in livestock as a source of feed. Supplementation of carrot tuber juice 52.5 ml/head/day increased the daily average body weight gain of broiler chickens (Paramita et al., 2019).

Clove (*Syzygium aromaticum*; synonym: *Eugenia cariophylata*), is a plant from the Myrtaceae family native to the Maluku islands in Eastern Indonesia (Kamatou et al., 2012). Cloves are aromatic flower buds from an evergreen tree (Mbaveng and Kuete, 2017), considered a spice, contain 10% essential oil which is mostly eugenol, a substance that has an anesthetic effect (Prashar et al., 2006), also contains vitamins B and C (Merrill and Perry, 2009). Vitamin C is involved in the synthesis of stress hormones so that it has a major role in lowering body temperature by increasing heat dissipation through the blood vessels that surround the body to maintain a relatively constant temperature (Cheng, 1990). Cloves contain phenolic compounds that are antibacterial (Dorman & Dean, 2000).

Consumer refusal to synthetic additives has increased in recent times (Kirkpinar et al., 2014). Therefore, supplementation of poultry diet with natural components to increase production has been widely applied in the world (Abou-Elkhair et al., 2020). Consequently, herbaceous spices and their bioactive constituents are becoming important in poultry production due to their valuable impact on growth, production, immune status and meat quality without leaving residues on the product or rearing environment (Hussein et al., 2020; Abd El-Hack et al., 2016).

Final body weight and weight gain of rats were not significantly different between treatments. Administration of conventional emulsion of clove essential oil, clove oil microemulsion, or eugenol microemulsion resulted in significant improvement in fatty liver inflammation (steatohepatitis) and dyslipidemia with consequent prevention of cardiovascular disease and other complications of steatohepatitis (Al-Okhbi., 2014). Clove, and its essential oil, is one of the plant extracts found useful in poultry to improve growth performance by enhancing the intestinal microbiota population (Mohammadi et al., 2014). Supplementation of 450 ppm clove essential oil in broiler ration significantly increased body weight gain and feed conversion ratio during the experimental period (0-42 days) and decreased total cholesterol composition at 21 days of age. Clove essential oil can be considered as a potential growth promoter for poultry (Mehr et al., 2014).

The most common product from a chicken slaughterhouse is a whole chicken. However, in recent years there has been a shift from sales of whole chickens to sales of cut chicken parts and convenience products as these products have a higher value (FAO, 2010). Breast meat makes up about 30% of the edible carcass meat and 50% of the edible protein (Summers et al., 1988). The criteria for breast meat quality include color, pH, water holding capacity, tenderness, and sensory acceptance (Barbut, 2009; Van Laack et al., 2000). The quality of broiler meat is a major issue for the poultry industry.

There is very little information available in the literature on the use of carrot juice in drinking water and the formulation of diets containing cloves, therefore, the aim of the present study was to determine the effect of water supplementation with carrot juice on carcass and cutting yields, meat qualitative traits and sensory evaluation of broiler chickens fed diet contain clove and for the reason of improving chicken welfare and food product safety.

## MATERIALS AND METHODS

The study was conducted using 200 of one-day-old broiler chickens. Treatments were carrot juice consist of 0, 10, 20, 30 ml/liter water, respectively, and the diets formula shown in Table 1. Feed and water were provided *ad libitum*. The preparation of carrot juice based on Alom (2013). Carrots were cleaned, cut into small pieces and added with water in a ratio of 1:10, and then was blended. Stored in the refrigerator at a temperature of 4°C to retain the active ingredients of the juice.

The variables measured were carcass and cutting yields, giblet, blood lipid profiles, physical traits and sensory evaluation of meat: cooking loss, water holding capacity, water content and tenderness of meat; colour, aroma, texture and taste of meat. At the end, d-35 of the experiment 5 birds per treatment were randomly selected and processed to determine processing yields. Birds were weighed, killed by cervical dislocation after 9 h of feed and water deprivation, bled, scalded, and defeathered. Data from carcass weight, cut parts (breast, wings, leg, thighs and drumsticks) and internal organ (liver, heart, gizzard, spleen, bile) were recorded.

The value of carcass percentage was obtained by comparing the weight of the carcass (g) with the slaughter weight (g) multiplied by 100%. Water holding capacity was determined by the method of Hamm, cooking loss, and tenderness of the meat with the shear press method, as a modified Warner-Bratzler method as reported by Soeparno (2011). Meat sensory tests were conducted with a sample of meat that had been cooked without salt or spices. Testing was undertaken using the meat sensory panelists, including as many as 35 individuals who are not trained with the scoring method. Each panelist evaluated the samples presented to them at random, and evaluated color, aroma, texture, and taste. All sensory attributes were scored on a scale ranging from 1 (least intense) to 7 (most intense).

Tabel 1. Composition and Nutrients Content of the Diets

| Feedstuff | 2-3 Weeks | 4-5 Weeks |
|---|---|---|
| Commercial Diet 1 (%) | 73 | 0 |
| Commercial Diet 2 (%) | 0 | 73 |
| Corn (%) | 23 | 23 |
| Clove Meal | 1 | 1 |
| Coconut Oil (%) | 3 | 3 |
| Total | 100 | 100 |
| Chemical Composition: | | |
| Crude Protein (%) | 19.82 | 17.09 |
| Crude Fiber (%) | 3.37 | 3,10 |
| Fat (%) | 3.01 | 2.83 |
| Ca (%) | 5.25 | 1.79 |
| P (%) | 0.57 | 0.25 |
| GE (Kcal/kg) | 4153.52 | 4054.05 |
| ME (Kcal/kg) | 3115.14 | 3040.54 |

This study used a completely randomized one-way design (CRD) (Steel and Torrie, 1982) consisting of 4 treatments and 5 replications. Data for all parameters were subjected to an analysis of variance. The treatments mean with significant differences at P<0.05 were compared using Duncan's Multiple Range Test. The data was then analyzed using IBM SPSS 24 software.

## RESULTS AND DISCUSSIONS

Results showed that water intake was significantly decreased, and carcass weight, slaughter cutting yield weight (Table 2), and internal organ were non significantly difference but gizzard (Table 3) was significantly increased. Blood triglyceride was non significantly difference, HDL-cholesterol was significantly increased, LDL-cholesterol, blood glucose and SGOT (Table 4) were significantly decreased. Cooking loss, WHC and water content of meat (Table 5) were non significantly difference but tenderness was significantly decreased. The color, aroma, texture and taste of meat (Table 5) were non significantly difference.

Water quality has the potential to affect digestion and absorption of nutrients, as well as flock health. Because of its hydrogen bonding capabilities, water is a universal solvent and, as a result, may contain numerous dissolved minerals and other compounds. Water intake in birds were roughly twice the weight of feed intake. However, during periods of extreme heat stress, water intake may triple or even quadruple. Pesti et al. (1985) reported that chickens will consume drinking water twice or more than feed intake. Although it varied, the proportion of water: feed = 2 : 1, especially in summer.

Water intake in this study (Table 2) was lower than the standard broiler drinking water intake (around 190 ml/head/day in the 3$^{rd}$ and 4$^{th}$ weeks) (Lesson and Summer, 2005) when treated by carrot juice 10-30 ml. The addition of 20% of carrot juice and more caused the decreasing of drinking water. This may be due to a slightly bitter taste in drinking water because of the tannin content so that chickens do not like it.

Serrano et al. (2013) observed an increased water intake with pelleted diets compared to mash diets, whereas water to feed ratio was not affected. Water intake is highly correlated with the quality and amount of feed consumed by chickens, and also with poultry age, body weight, environmental temperature, and others (May et al., 1997).

Table 2. Effect of Carrot Juice in Drinking Water on Carcass and Cutting Yields

| Variables | Treatments in Drinking Water | | | | SEM | p Value |
|---|---|---|---|---|---|---|
| | 0 ml CJ | 10 ml CJ | 20 ml CJ | 30 ml CJ | | |
| Water Intake, ml, head$^{-1}$ day$^{-1}$ | 195.14$^{b}$ | 186.54$^{b}$ | 177.23$^{ab}$ | 163.96$^{ab}$ | 4.2 | 0.03 |
| Total Water Intake, ml, head$^{-1}$ | 5463.76$^{b}$ | 5222.96$^{b}$ | 4962.36$^{ab}$ | 4602.08$^{a}$ | 115.2 | 0.04 |
| Feed Intake, g, head$^{-1}$ day$^{-1}$ | 79.75$^{ab}$ | 84.72$^{c}$ | 80.13$^{ab}$ | 78.37$^{a}$ | 1.1 | 0.11 |
| Slaughter Weight, g$^{ns}$ | 1512.8 | 1532.0 | 1451.40 | 1442.60 | 21.3 | 0.38 |
| Carcass Weight, g$^{ns}$ | 1063.40 | 1082.00 | 1047.80 | 1010.00 | 16.9 | 0.52 |
| Left Breast, % $^{ns}$ | 14.64 | 15.41 | 14.41 | 14.76 | 0.29 | 0.67 |
| Right Breast, % $^{ns}$ | 14.17 | 15.20 | 14.49 | 14.10 | 0.27 | 0.50 |
| Left Thigh + Drumstick, % $^{ns}$ | 16.12 | 16.15 | 15.93 | 16.33 | 0.17 | 0.88 |
| Right Thigh + Drumstick, % $^{ns}$ | 16.51 | 16.24 | 15.95 | 16.47 | 0.16 | 0.62 |
| Left Wing, % $^{ns}$ | 5.20 | 5.29 | 5.16 | 5.32 | 0.07 | 0.84 |
| Right Wing, % $^{ns}$ | 5.22 | 5.58 | 5.21 | 5.30 | 0.10 | 0.63 |
| Leg, % $^{ns}$ | 6.56 | 6.57 | 6.35 | 7.40 | 0.19 | 0.20 |

$^{CJ}$carrot juice; $^{ns}$non significant; $^{abc}$different superscript at the same raw indicated significantly different(P<0.05).

The carcass traits of broilers such as carcass yield and cutting yield were tested. Analysis of variance showed no significant differences in relative weight of breast, thigh + drumstick, wing, and leg among experimental groups. As illustrated in Table 2, no notable difference in carcass was revealed between groups. The present results of carcass were in agreement with those noticed by several researchers who noted that the addition of lime juice in drinking water at the level of 1% was able to reduce abdominal fat without affecting the appearance of the carcass (Rakhamansyah et al., 2019). The use of betel leaf extract up to a level of 2% in drinking water did not affect the final weight, carcass percentage and abdominal fat of broiler chickens slaughtered at the age of five weeks (Pahlepi et al., 2015). On the contrary with Haroen and Budiansyah (2018) stated that the use of fermented ginger (*Zingiber officinale*) extracts in the drinking water up to 8 ml increase the carcass quality dan feed intake but decreased cholesterol carcass dan abdominal fat of broiler chicks.

Carrot juice supplementation had no effect on carcass, breast, thigh+drumstick, wing, leg, liver, heart, spleen, but gizzard and bile weights of broiler chickens. It is interesting to notice that, gizzard weight (%) was significantly decreased when drink 10 ml carrot juice compared to control, however, significantly increased with increasing the level of carrot juice till 30 ml in broiler drinking water (Table 3). These results agreed with those of Abdel-Azeem & Hemid (2006) who found a gradual decrease in the abdominal fat, gizzard fat and total non-carcass fat, while the relative weight of gizzard was increased by increasing barley radicel levels in the broiler diets. Ürüşan et al. (2018) reported increase in hot carcass weight and carcass yield of broiler chickens feed carrot seed oil indicated that increase carcass weights occurred because of the appetizer properties of plant extracts by increasing the gastric digestion liquor.

Table 3. Effect of Carrot Juice in Drinking Water on Internal Organ

| Variables | Treatments in Drinking Water | | | | SEM | p Value |
|---|---|---|---|---|---|---|
| | 0 ml CJ | 10 ml CJ | 20 ml CJ | 30 ml CJ | | |
| Liver (%)$^{ns}$ | 1.75 | 1.51 | 1.59 | 1.56 | .06 | .52 |
| Gizzard (%) | 1.38$^{ab}$ | 1.30$^{a}$ | 1.34$^{ab}$ | 1.55$^{b}$ | .04 | .09 |
| Heart (%)$^{ns}$ | 0.46 | 0.43 | 0.48 | 0.53 | .02 | .19 |
| Spleen (%)$^{ns}$ | 0.09 | 0.09 | 0.09 | 0.10 | .01 | .90 |
| Bile | 0.16$^{b}$ | 0.13$^{ab}$ | 0.15$^{ab}$ | 0.10$^{a}$ | .01 | .08 |

$^{CJ}$carrot juice; $^{ns}$non significant; $^{abc}$different superscript at the same raw indicated significantly different(P<0.05).

In this study, blood triglyceride was non significantly difference, HDL-cholesterol was significantly increased, LDL-cholesterol, blood glucose and SGOT (Table 4) were significantly decreased. Sigolo et al. (2021) reported that dietary supplementation with thyme extract at 300 mg/L drinking water level improved broiler chicken carcass traits in terms of carcass and drumsticks yields, and blood serum parameters such as total protein, albumin, urea, total cholesterol and HDL.

Table 4. Effect of Carrot Juice in Drinking Water on Serum Biochemical

| Blood Lipid Profiles | Treatments in Drinking Water | | | | SEM | p Value |
|---|---|---|---|---|---|---|
| | 0 ml CJ | 10 ml CJ | 20 ml CJ | 30 ml CJ | | |
| Triglyceride, mg.dL$^{-1}$ ns | 62.5 | 41.8b | 47.2 | 45.5 | 3.6 | .19 |
| Total Cholesterol, mg.dL$^{-1}$ | 136.5$^{ab}$ | 158.0$^{c}$ | 131.5$^{a}$ | 150.0$^{bc}$ | 3.32 | .006 |
| HDL-Cholesterol, mg.dL$^{-1}$ | 96.0$^{a}$ | 114.0$^{b}$ | 107.5$^{b}$ | 108.5$^{b}$ | 1.78 | .000 |
| LDL-Cholesterol, mg.dL$^{-1}$ | 25.0$^{b}$ | 33.0$^{d}$ | 18.5$^{a}$ | 30.0$^{c}$ | 1.31 | .000 |
| SGOT, mg.dL$^{-1}$ | 206.0$^{bc}$ | 210.5$^{c}$ | 195.5$^{ab}$ | 185.5$^{a}$ | 3.09 | .008 |
| Blood Glucose, mg.dL$^{-1}$ | 219.0$^{b}$ | 223.0$^{b}$ | 210.0$^{a}$ | 207.0$^{a}$ | 1.69 | .000 |

$^{CJ}$carrot juice; $^{ns}$non significant; $^{abc}$different superscript at the same raw indicated significantly different (P<0.05).

The effect of the carrot juice in drinking water on sensory evaluation of breast meat of broiler chicken was shown in Table 5.

Table 5. Effect of Carrot Juice in Drinking Water on Physical and Sensory Evaluation of Meat

| Variables | Treatments in Drinking Water | | | | SEM | p Value |
|---|---|---|---|---|---|---|
| | 0 ml CJ | 10 ml CJ | 20 ml CJ | 30 ml CJ | | |
| Cooking Loss (%)$^{ns}$ | 22.3 | 21.7 | 23.5 | 23.6 | .48 | .25 |
| WHC (%)$^{ns}$ | 45.5 | 49.8 | 47.6 | 49.0 | .84 | .11 |
| Water Content (%)$^{ns}$ | 64.4 | 66.5 | 65.1 | 64.3 | .60 | .26 |
| Tenderness (g/cm2) | 195.0$^{c}$ | 185.0$^{a}$ | 195.5$^{d}$ | 191.0$^{b}$ | .97 | .000 |
| | | | | | | |
| Color | 4.67 | 4.77 | 4.53 | 4.53 | .059 | .44 |
| Aroma | 4.93 | 4.83 | 5.03 | 5.10 | .079 | .66 |
| Texture | 5.07 | 5.07 | 5.00 | 5.00 | .084 | .99 |
| Taste | 4.87 | 5.00 | 5.00 | 5.17 | .082 | .64 |

$^{CJ}$carrot juice; $^{ns}$non significant; $^{abc}$different superscript at the same raw indicated significantly different (P<0.05).

There was no significant difference in taste, aroma, texture, and color. Soeparno (2011) stated that an important sensory quality element for processed meat is the appearance of meat color. Even though the smell, taste and texture are attractive, if the color display is not attractive then the taste for the food will be reduced. The color of chicken meat is white, because the concentration of myoglobin in the muscles is about 0.025%. Chicken meat is very unique, because it has a striking color difference. The normal color of raw breast meat is pale pink, while the thighs and drumstick are dark red. Kristensen et al. (2002) found a relationship between diet and texture of meat. Also, Cisneros et al. (1996) and Smith et al. (2002) reported a relationship between feed and the colour of meat. The main differences in meat quality between free-range and conventionally-reared chickens are related to colour, flavour, and texture. However, breast meat yield was little affected by diet composition (Summers et al., 1988).

Carrot juice supplementation affected tenderness broiler chicken meat. The carrot juice in drinking water had significantly (P<0.01) effect the tenderness value when compared to control. Supplementation 20 ml of carrot juice improved meat tenderness. Meat physical attribute values of cooking loss, WHC, and water content on meat (Table 5) were not affected by the carrot juice in which no significant difference (P>0.05) was observed between the dietary groups when compared to control.

## CONCLUSIONS

Carrot juice supplementation did not have any effect on slaughter weight, carcass weight, carcass cutting, meat cooking loss, WHC, and water content but tenderness of broiler chickens. Carrot juice supplementation decreased water intake and feed intake of female broiler chickens. As a result, carrot juice can be added in the drinking water of broilers as a beneficial feedstuff supplement which contains natural antioxidants. Optimal improvements of feed intake, slaughter weight, carcass and tenderness of meat were achieved at different carrot juice supplementation levels. Thus, carrot juice levels for optimal productivity will depend on the parameter in question. This has a lot of implications in drinking water formulations where carrot juice is included.

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# PERFORMANCE AND EGG QUALITY OF LAYING HENS FED WITH DIETARY RAW MATERIALS RICH IN PUFA Ω:3

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***Abstract***

*The effect of dietary flaxseed meal, rapeseed meal and fodder peas on layers' performances and egg quality was investigated in a 6-wk feeding trial on 168 Tetra SL layers (65 weeks) assigned to 4 groups (C, E1, E2, E3). The commercial (C) diet had 2750 kcal ME and 16.4% CP. Compare to diet C, the experimental groups were fed with flaxseed meal (3%, E1), flaxseed meal-fodder peas (3%; 10%; E2), flaxseed meal-rapeseed meal (3%; 10%; E3) which increased the dietary level of the total polyunsaturated fatty acids PUFA n-3 (% of total fat) to 5.72(E1), 6.87 (E2) and 5.65 (E3) compared to group C (1.19), in diet. At the end of the trial, 18 eggs/group were collected to determine the eggs nutritional and quality parameters. The results showed that egg intensity was lower ($P \leq 0.05$) in all experimental groups. Egg weight (g) in E1 (65.39) was higher ($P \leq 0.05$) compared to all other groups. Also, yolk colour intensity increased significantly ($P \leq 0.05$) in all experimental groups compared to C group. Similarly, the results showed that PUFA n-3 acid content was higher in all experimental groups (3.14%; 3.38% and 3.53%) compared to C group (1.13%). In conclusion, using dietary raw materials rich in PUFA n-3 had a positive influence on laying hens' egg quality and improved the nutritional quality indices of the lipids in egg yolks.*

***Key words***: *eggs quality, flaxseed meal, fodder peas, rapeseed meal, layers' performance, PUFAn-3 acid, yolk indices.*

## INTRODUCTION

Eggs are consumed by millions of people around the world, being considered a complete food for the human diet due to the large amounts of essential nutrients they hold, such as protein, lipoproteins (ovalbumin, ovotransferine, HDL and LDL), a wide variety of minerals (potassium, phosphorus, calcium, iron, magnesium), vitamins (A, D, E, K, B6, B9, B12, riboflavin), lipids (MUFA, PUFA, carotenoids, choline and phospholipids) and other bioactive compounds. Genetic factors and diet can alter the chemical composition of chicken eggs(Layman & Rodriguez, 2009; Ruxton et al., 2010; Conrad et al., 2017; Franco et al., 2020). Omega-3 polyunsaturated fatty acidsenriched eggs provide the consumer with a value-added product that shows a clear and functional benefit for an increasingly health-conscious population. Humans require a ratio of omega-6 fatty acids: omega-3 of 4:1 (Sittiprapaporn, 2020). In the recent years people have become more conscious of the relation between food and their health and started to show more interest in the quality of eggs that they consume (Siro et al., 2008). The main concern of consumers is that the food they consume to be safe and healthy, to have reduced content of substances which can pose risk to human health and to have additional benefit by enriching with substances beneficial for their health (Carocho et al., 2014; Sireesha and Prasanna, 2019, Untea et al. 2021).

Functional foods are designed to encourage the consumer to change their diets, instead of taking pills or capsules that could harm the body (Karelakis et al., 2020). Poultry eggs have huge potential in this aspect (Fernandez & Lemos, 2019). The most common practices to obtain such food products is to include flaxseed, rapeseed, canola or their by-products into poultry feeding with the purpose to increase the concentration of polyunsaturated fatty acids (Gheorghe et al., 2019; Świątkiewicz et al., 2020). From the polyunsaturated fatty acids (PUFA) linoleic and α-linolenic acids are lipids with important physiological roles considered essential to adults. Clinical studies have shown that replacing saturated fatty acids by polyunsaturated fatty acids produces beneficial effects on the cardiovascular system (Turcu et al., 2019).

In this regard many attempts have been made to focus on different methods of improving the nutritional quality of poultry eggs by enhancing levels of omega-3 fatty acids content, to obtain functional eggs through poultry feeding manipulation (Tocher et al., 2019; Goldberg et al., 2013; Panaite et al. 2021). Moreover, it was reported that current Western diets are generally deficient in PUFA, especially omega-3 fatty acids. For this reason, consuming foods enriched in omega-3 fatty acids, such as eggs, it is essential for human health nutrient deficiency (Simopoulos, 2002; Panaite et al., 2019). Enrichment of eggs in omega-3 PUFA presents increased susceptibility of yolk lipid peroxidation, which could affect the quality of eggs and may have deleterious effects on humans (Alagawany et al., 2019; Panaite et al., 2021). The fatty acids composition of eggs is dependent on the fatty acid composition of the feed given to the hens which plays an important role on in the prevention and regulation of different disorders and can modulate lipid metabolism in a beneficial way (Vlaicu & Panaite, 2021).

The richest sources to obtain polyunsaturated fatty acids enriched eggs, are those with a high content of alpha linolenic acid as rapeseed (respectively rapeseed meal), soya (soyabean meal), walnuts and flax (Harris et al., 2009), or flaxseed meal (Khan et al., 2019). To obtain polyunsaturated fatty acids enriched eggs, these raw materials enriched in omega-3 polyunsaturated fatty acids must be added into animals' diet (Franczyk-Żarów et al., 2019).

The aim of this paper is to evaluate the performance and egg quality of laying hens fed with dietary raw materials rich in polyunsaturated fatty acids.

## MATERIALS AND METHODS

### *Birds, housing and experimental diets*

The efficiency diets' evaluation with vegetable raw materials rich in polyunsaturated fatty acids was carried out by an experimental study conducted during 6 weeks on 168 Tetra SL laying hens (65 weeks of age), individually weighed and randomized into 4 experimental groups (C, E1, E2 and E3).

The experiment was conducted in accordance with the Romanian legislation (Law 206/2004, Ordinance 28/31.08.2011, Law 43/11.04.2014, Directive 2010/63/EU according to an experimental protocol approved by the Ethics Committee of IBNA.

The hens were accomodated in cages (2 hens/cage; 21 cages/group) Big Dutchman two-sided with 3-tier cages (length x width x height; 50×50×40 cm), benefited from the same conditions of controlled microclimate (temperature: 23.08±0.98$^{0}$C; humidity 66.35±5.68%; ventilation: 1.70±0.14% and a lighting program of 16 h/24 h).

The laying hens had free access to feed and water. For dietary feed formulation, the following were considered: the experiment objective, species, hybrid, age and nutritional requirements of the hybrid Tetra SL (Tetra-SL commercial Layer Management Guide, 2017).

The diets basic structure (Table 1) was the same for the 4 experimental groups, characterized by: 2750 kcal/kg metabolizable energy; 17.5% crude protein; the difference between the control group (M) and the experimental groups was given by the flaxseed meal inclusion (3%) as a source of polyunsaturated fatty acids.

The E1 diet included only flaxseed meal (3%) while E2 and E3 groups included, in addition to flaxseed meal, 10% peas (E2) and 10% rapeseed meal (E3), respectively. A nutritional optimization programme was used for diets' formulation (Table 1) (HYBRIMIN® Futter 5) in agreement with the feeding requirements of laying hens as given by NRC (1994).

The diets (table 1) were isocaloric and isonitrogenous.

Throughout the experiment were monitored the daily feed intake (DFI; g/day/layer), the feed conversion ratio (FCR; g feed/g egg), the laying intensity rate (LIR; %) and egg weight (EW; g).

Table 1. The experimental diets'structure

| Specifications | C | E1 | E2 | E3 |
|---|---|---|---|---|
| *Flaxseed meal, %* | - | *3.00* | *3.00* | *3.00* |
| *Peas, %* | - | - | *10.00* | - |
| *Rapeseed meal, %* | - | - | - | *10.00* |
| Corn, % | 39.31 | 38.69 | 35.20 | 37.71 |
| Wheat, % | 20.00 | 20.00 | 20.00 | 20.00 |
| Soyabean meal, % | 16.26 | 16.36 | 17.39 | 14.28 |
| Sunflower meal, % | 10.00 | 7.00 | - | - |
| Vegetal oil, % | 2.48 | 2.86 | 2.32 | 3.17 |
| Lysine, % | 0.09 | 0.13 | 0.09 | 0.05 |
| Methionine, % | 0.14 | 0.18 | 0.16 | 0.12 |
| $CaCO_3$, % | 9.01 | 9.02 | 9.05 | 8.89 |
| Monocalcium phosphate., % | 1.29 | 1.33 | 1.36 | 1.34 |
| Salt, % | 0.38 | 0.38 | 0.39 | 0.39 |
| Choline, % | 0.05 | 0.05 | 0.05 | 0.05 |
| Premix A5, % | 1.00 | 1.00 | 1.00 | 1.00 |
| Total | 100 | 100 | 100 | 100 |
| *Calculated analysis,* | | | | |
| Metabolizable energy., kcal/kg | 2750 | 2750 | 2750 | 2750 |
| Lysine, % | 0.80 | 0.80 | 0.80 | 0.80 |
| Met.+cist, % | 0.71 | 0.71 | 0.71 | 0.71 |
| *Chemical analysis, %* | | | | |
| Dry matter | 90.79 | 90.88 | 90.33 | 90.87 |
| Crude protein | 17.25 | 17.27 | 17.37 | 17.21 |
| Ether extract | 4.01 | 4.60 | 4.05 | 5.02 |
| Crude fiber | 5.29 | 5.20 | 4.47 | 4.88 |
| Ash | 13.52 | 14.27 | 13.80 | 14.84 |
| Non-nitrogen extractive substances | 50.72 | 48.54 | 50.64 | 47.92 |
| • *Fatty acid (% of the sum fatty acids)* | | | | |
| Linoleic acid (C 18:2n6), | 51.44 | 48.96 | 47.50 | 48.03 |
| Linolenic α acid (C 18:3n3) | 0.96 | 5.55 | 6.73 | 5.58 |
| PUFA, of which: | 52.75 | 54.94 | 54.56 | 53.80 |
| Ω3 | 1.19 | 5.72 | 6.87 | 5.65 |
| Ω6 | 51.56 | 49.22 | 47.69 | 48.15 |
| Ω6/Ω3 | 45.29 | 8.63 | 6.99 | 8.52 |

where: C- conventional diet; E1- conventional diet + 3% flaxseed meal; E2 - conventional diet + 3% flaxseed meal + 10% peas; E3 - conventional diet + 3% flaxseed meal + 10% rapeseed meal;
*1kg 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.

***Sampling collection and measurements***

After experimental feed was manufactured, sample of 500 g were extracted from each batch for chemical analysis then the bags were packed, labelled and stored in optimal conditions for the conduct of the experiment. At the end of the experiment (week 6), 18 eggs/group were collected randomly from each group and used determine the internal and external quality parameters of the egg: the whole egg weight and its components: egg white, yolk, shell (Kern balance, accuracy 0,001); colour intensity expressed in value on a scale of 1 to 15 measured with an egg Analyser TM analyser and albumen pH and yolk measurements (using mobile pH-meter). After all internal and external quality measurements were determined 6 samples of yolk/group were formed (3 eggs/sample) dried for 48 h (ECOCELL oven), at $65^0$C, and chemical composition were assayed (using the methods from Regulation (CE) 152/2009), fatty acids (FA) concentration (determined by gas chromatography described by Panaite et al, 2016), and cholesterol concentration.

***Health lipid indices of yolk eggs***

The fatty acid profile of yolk fat is important for the nutritional quality of lipids in yolk eggs.Thehealth lipid indices such as UFA/SFA, PUFA/SFA, PUFA n-6/n-3, the hypocholesterolemic/hypercholesterolemic ratio (h/H), index of atherogenicity (IA), the index of thrombogenicity(IT), peroxidizability index (PI) ratios, hypocholesterolemic acids (DFA), hypercholesterolemic acids (OFA), and nutritive value (NVI) indices, are widely used to evaluate the nutritional value of yolk fat.

They were calculated according to the following equations:

1) IA = (C 12:0 + 4 x C 14:0 + C 16:0)/∑UFA (Ulbricht and Southgate, 1991; Senso et al., 2007).
2) IT = (C 14:0 + C16:0 + C18:0)/[(0.5 x MUFA) + (0.5 x ∑ n-6) + (3 x ∑ n-3) + (∑ n-3/∑n-6)] (Ulbricht and Southgate, 1991;Senso et al., 2007).
3) h/H = [(C 18:1 n-9 + C 18:1 n-7 + C 18:2 n-6 + C 18:3 n-6 + C 18:3 n-3 + C 20:3 n-6 + C 20:4n-6 + C 20:5 n-3 + C 22:4 n-6 + C 22:5 n-3 + C22:6 n-3)/(C 14:0 + C 16:0)] (Fernandes et al., 2014).
4) PI = (monoenoic acid x 0.025) + (dienoic acid x 1) + (trienoic acid x 2) + (tetraenoic acid x 4) + (pentaenoic acid x 6) + (hexaenoic acid x 8) (Erickson, 1992).
5) NVI = (C 18:0 + C18:1)/C 16:0 (Chen et al., 2016).
6) DFA = (UFA+C18:0) (Medeiros et al., 2014)
7) OFA = (SFA-C18:0) (Skiepko et al., 2016)

***Statistical analysis***:

The measurements of all groups wereanalysed by the one-way analysis of variance (ANOVA) procedure of the SPSS version 20 (Inc., Chicago IL, USA), according to the following linear model:

$$Yij = \mu + Aj + eij$$

Where: Yij = value of trait (the dependent variable); μ = overall mean; Aj = the treatment effect; and eij = random observation error.We using a Tukey test to compare differences among treatment means and probabilities lower than 0.05 were considered as statistically significant ($P < 0.05$).

## RESULTS AND DISCUSSIONS

The chemical composition presented in Table 1 shows that all diets were isoproteic and isoenergetic. The raw materials rich in PUFA-3 inclusion into the dietary feeding structure resulted in an increase in ALA content in all experimental groups compared to the control group, mainly in the E2 group which included flaxseed meal mixed with peas (Table 1). Of the experimental groups, the E2 diet (3% flaxseed meal+ 10% peas) administered to the laying hens had the highest PUFA-3 content, corroborated with a much-improved ratio of PUFA-6/3 fatty acids compared to C group.

Tabelul 2. Effect of dietary raw materials rich in PUFA ω:3 on laying hens performance and egg size classification (average values/group)

| Specification | Experimental groups | | | | SEM | P-value |
|---|---|---|---|---|---|---|
| | C | E1 | E2 | E3 | | |
| | n=42 | n=42 | n=42 | n=42 | | |
| Daily feed intake (g/day/layer) | 129.06$^{a}$ | 121.69$^{b}$ | 125.75$^{ab}$ | 124.45$^{ab}$ | 0.073 | 0.0505 |
| Feed conversion ratio (g feed/g egg) | 2.17 | 2.11 | 2.23 | 2.23 | 0.021 | 0.1521 |
| Egg weight (g). | 64.08$^{b}$ | 65.39$^{a}$ | 63.57$^{c}$ | 63.75$^{bc}$ | 0.102 | <.0001 |
| Laying intensity rate (%) | 93.67$^{a}$ | 89.56$^{b}$ | 90.67$^{b}$ | 90.03$^{b}$ | 0.419 | 0.0018 |
| *Eggs classification** | | | | | | |
| "XL" (>73 g), % | 3.11 | 7.20 | 4.73 | 1.35 | | |
| "L" (63 - 73 g), % | 53.03 | 59.61 | 48.08 | 56.32 | | |
| "M" (53 - 63 g), % | 43.27 | 33.06 | 44.84 | 42.07 | | |
| "S" (< 53 g), % | 1.18 | 0.38 | 2.36 | 0.97 | | |

where: C- conventional diet; E1- conventional diet + 3% flaxseed meal; E2 - conventional diet + 3% flaxseed meal + 10% peas; E3 - conventional diet + 3% flaxseed meal + 10% rapeseed meal; n=hens per group;

$^{a,b,c}$ Means within a row with different superscripts differ significantly, $P \leq 0.05$.

* C.E Regulation no. 852/2004 on the general rules of food hygiene, with subsequent amendments and completions and Directive 2000/13 / C.E.

Concerning productive performances (Table 2), E1 group supplemented by 3% flaxseed meal recorded a significantly lower feed consumption ($P<0.05$) compared to C group corroborated with a low specific consumption (kg NC/kg egg). Following egg weight (g/egg) registration, the entire E1 group recorded the highest value of the egg weight, the differences being significant (P<0.05) compared to both experimental groups and C group, but laying intensity percentage decreased by 4.38% compared to C. Significant differences (P <0.05) were also recorded between E1 compared to E2 and E3 groups. In literature, the results obtained for the productive parameters and the eggs chemical composition are extremely contradictory and varied following the use of flax as a source of polyunsaturated fatty acids in the laying hen's nutrition. For example, Hayat (2009) showed that the flaxseed utilization in laying hens reduced the feed ingesta, while Caston et al. (1994) noticed the opposite. With regard to egg weight some studies show a decrease of this parameter (Scheideler and Froning, 1996), while in other studies there are no changes in the same parameter (Aymond & Van Elswyk, 1995). Some researches has also shown an increase in egg weight (Rizzi & Simioli, 2009). With regard to the percentage of laying as in the case above, the results obtained are variable and contradictory. Thus, Aymond & Van Elswyk (1995) demonstrate the decrease of this parameter; Scheideler & Froning (1996) demonstrated egg laying percentage increasing while other researchers (Bean & Leeson, 2003) believed that flaxseed utilization laying hens diet does not alter the laying intensity. Regarding the dietary peas in laying hens diets, existing studies in the literature have shown that a large amount of peas inclusion into diets has a detrimental effect on their performance (Igbasan & Guenter, 1997; Świątkiewicz & Koreleski, 2006).

At the end of the experiment, eggs collected throughout the whole experimental period were classified in accordance with Regulation No. 852/2004 of the General Rules on Food Hygiene, as subsequently amended and supplemented and Directive 2000/13/EC (table 2). Thus, most of the eggs were of classes "L" and "M". The highest percentage of 'L' eggs was recorded on groups E1 and E3, while for 'M' eggs the highest percentage was recorded in lot E2 followed by C, E3 and E1 groups. With regard to eggs of category "S" the highest percentage was recorded in lot E2 and the lowest value recorded in lot E1 with a percentage of 0.38%. The highest value for the category "XL" was recorded in lot E1, and the lowest value was seen in lot E3. This eggs classification is extremely important for the farmer, as there is a great emphasis on the egg price, which varies depending on its size. Economically, the best-selling eggs are those in categories "L" and "M". The EU has also created marketing standards for eggs. They have been designed to ensure consistent high product quality, protect consumers and ensure consistency of standards in the EU market. The EU Regulations (EC No 589/2008 of 23/06/2008) stipulates detailed rules that eggs must comply with in order to be marketed. Printing of such standards shall also specify the quality classes, that is, eggs of category AA or very fresh, eggs of category A or fresh eggs, eggs of category B or eggs of second quality.

Table3. Effect of dietary raw materials rich in PUFA ω:3 on egg quality parameters

| Egg quality parameters | Experimental groups | | | | SEM | P-value |
|---|---|---|---|---|---|---|
| | C | E1 | E2 | E3 | | |
| | n=18 | n=18 | n=18 | n=18 | | |
| Egg weight, g | 65.03 | 64.87 | 65.07 | 64.88 | 0.079 | 0.7469 |
| Egg albumen weight, g | $37.42^{c}$ | $38.72^{b}$ | $39.51^{ab}$ | $37.89^{c}$ | 0.206 | 0.0010 |
| Egg yolk weight, g | $18.22^{a}$ | $17.01^{b}$ | $16.87^{b}$ | $17.62^{b}$ | 0.167 | 0.0140 |
| Eggshell weight, g | 9.29 | 9.24 | 8.86 | 9.21 | 0.099 | 0.4042 |
| pH albumen(value) | 9.13 | 9.08 | 9.06 | 9.05 | 1.125 | 0.4039 |
| pH yolk (value) | $6.27^{ab}$ | $6.25^{b}$ | $6.33^{a}$ | $6.23^{b}$ | 0.013 | 0.0377 |
| Yolk colour intensity | $3.94^{b}$ | $4.50^{a}$ | $4.44^{a}$ | $4.47^{a}$ | 0.075 | 0.0205 |

where: C- conventional diet; E1- conventional diet + 3% flaxseed meal; E2 - conventional diet + 3% flaxseed meal + 10% peas; E3 - conventional diet + 3% flaxseed meal + 10% rapeseed meal;
$^{a,b,c}$ Means within a row with different superscripts differ significantly, P ≤ 0.05.

The data presented within Table 3 showed that eggs collected at the end of the experiment and analysed for internal and external quality parameters did not show significant differences (P>0.05) of their average weight. However, in the experimental groupsthe albumen weight showed the highest values. For E1 and E2 groups, the differences were significant (P<0.05) compared to C group. Group E3 differed significantly (P<0.05) only from E2 group. For yolk weight values significant differences (P<0.05) were recorded only in the case of E1 and E2 groups compared to C group. The data obtained by us are inconsistent with those obtained by Cherian et al., (2016) when using camelina as a source of polyunsaturated fatty acids. The results of the study showed that the egg albumen size and mass were smaller when camelina was used, with no differences observed for the Haugh unit, the yolk: albumen ratio and the yolk weight (Cherian et al., 2016). For the shell weight no significant differences (P>0.05) were recorded in any of the groups. In case on E2 group pH yolk recorded significant values (p<0.05) compared to E1 and E3 groups. Yolk colour registered a significant increase (p<0.05) in all experimental groups of this compared with the values recorded for C group. Similar results were obtained by Świątkiewicz & Koreleski (2006) in an experiment on Lohman Brown laying hens, who received a peas by-product (5, 10, 15 and 20%) achieving an egg yolk increase intensity. Other studies (Roberson et al., 2005) reported improved yolk color intensity at a rate of 5% and 10% dietary inclusion of the peas by-product, while a dietary level inclusion of 15% had no influence on its color intensity.

Table 4. Fatty acid composition in total lipids of eggyolks (avarage values/group)

| Fatty acid (g FAME/100 g Total FAME) | Experimental groups | | | | SEM | P-value |
|---|---|---|---|---|---|---|
| | C | E1 | E2 | E3 | | |
| | n=6 | n=6 | n=6 | n=6 | | |
| C14:0 | 0.253 ab | 0.240 ab | 0.259 a | 0.222 b | 0.009 | 0.027 |
| C15:0 | 0.064 b | 0.056 c | 0.067 ab | 0.074 a | 0.002 | <0.0001 |
| C16:0 | 22.995 a | 22.700 a | 22.539 a | 21.578 b | 0.160 | <0.0001 |
| C17:0 | 0.153 | 0.149 | 0.169 | 0.175 | 0.0124 | 0.421 |
| C18:0 | 11.066 | 11.016 | 10.849 | 11.318 | 0.321 | 0.778 |
| ∑ SFA | 34.532 | 34.161 | 33.882 | 33.366 | 0.387 | 0.217 |
| C14:1 | 0.044 a | 0.043 a | 0.044 a | 0.023 b | 0.003 | <0.0001 |
| C15:1 | 0.154 a | 0.144 ab | 0.107 ab | 0.115 b | 0.011 | 0.024 |
| C16:1 | 2.665 ab | 2.655 ab | 2.891 a | 2.374 b | 0.086 | 0.004 |
| C17:1 | 0.068 | 0.081 | 0.068 | 0.098 | 0.012 | 0.297 |
| C18:1n9c | 35.885 | 35.665 | 36.038 | 35.054 | 0.431 | 0.411 |
| C22:1n9 | 0.087 | 0.068 | 0.084 | 0.071 | 0.009 | 0.377 |
| C24:1n9 | 0.338 a | 0.242 b | 0.255 b | 0.277 b | 0.011 | <0.0001 |
| ∑ MUFA | 39.242 | 38.898 | 39.487 | 38.013 | 0.500 | 0.206 |
| C18:2n6 | 18.686 b | 18.938 b | 18.044 c | 20.160 a | 0.118 | <0.0001 |
| C18:3n6 | 0.106 | 0.087 | 0.113 | 0.092 | 0.008 | 0.092 |
| C18:3n3 | 0.187 c | 0.690 b | 0.793a | 0.781ab | 0.025 | <0.0001 |
| C20:2n6 | 0.176 a | 0.141 b | 0.167 b | 0.177 b | 0.006 | 0.002 |
| C20:3n6 | 0.279 | 0.249 | 0.309 | 0.290 | 0.019 | 0.194 |
| C20:3n3 | 0.217 | 0.296 | 0.269 | 0.261 | 0.029 | 0.309 |
| C20:4n6 | 4.249 | 3.818 | 3.966 | 3.863 | 0.187 | 0.384 |
| C22:4n6 | 1.572 a | 0.549 b | 0.483 b | 0.483 b | 0.029 | <0.0001 |
| C22:5n3 | 0.065 b | 0.171 a | 0.135 a | 0.164 a | 0.009 | <0.0001 |
| C22:6n3 | 0.658 b | 1.986 a | 2.188 a | 2.322 a | 0.096 | <0.0001 |
| ∑ PUFA | 26.193 b | 26.925 b | 26.468 b | 28.593 a | 0.187 | <0.0001 |
| ∑ PUFA n-3 | 1.126 b | 3.143 a | 3.385 a | 3.528 a | 0.098 | <0.0001 |
| ∑ PUFA n-6 | 25.067 | 23.782 | 23.083 | 25.065 | 0.119 | <0.0001 |
| ∑ UFA | 65.435 a | 65.823 b | 65.955 c | 66.606 a | 0.387 | 0.224 |
| Other fatty acids | 0.030a | 0.029a | 0.160b | 0.030a | 0.016 | <0.0001 |

where: C- conventional diet; E1- conventional diet + 3% flaxseed meal; E2 - conventional diet + 3% flaxseed meal + 10% peas; E3 - conventional diet + 3% flaxseed meal + 10% rapeseed meal; n=egg yolk samples

a,b,c Means within a row with different superscripts differ significantly, P ≤ 0.05.

Abbreviations: SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; UFA, unsaturated fatty acid;

The egg yolk FA composition for the four experimental treatments is summarized in Table 4. The results showed a significant increase (P<0.05) in their content in all experimental groups compared to C group. Significant concentrations (P<0.0001) of α-linolenic acid (ALA) and docosahexaenoic acid (DHA) were recorded in E2 and E3 groups compared to C group. Long chain omega-3 polyunsaturated fatty acids (PUFA n-3), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are essential components for both human and animal nutrition, with well recognized beneficial effects for human health (Roy et al., 2020).

The fact that the new feed solutions tested within this study led to a total content PUFA n-3 enrichment, and also an ALA and DHA increasing concentrations in egg yolk, is of particular importance for improving the nutritional egg quality, and implicitly for human nutrition. For fatty acids according to the degree of non-saturation (Table 4), the concentration of PUFA n-3 increased significantly (P<0.0001) in all three experimental groups compared to C group, leading to a significant improvement (P<0.0001) in the ratio of PUFA n-6/n-3 fatty acids (Table 5).

Table 5. Nutritional quality indices of the lipids inegg yolks.

| Item | Experimental groups | | | | SEM | *P*-value |
|---|---|---|---|---|---|---|
| | C | E1 | E2 | E3 | | |
| | n=6 | n=6 | n=6 | n=6 | | |
| Total yolk fat, (%) | 26.05 | 26.05 | 26.76 | 26.35 | 0.163 | 0.378 |
| Cholesterol, (g/100g whole egg) | 0.234 | 0.199 | 0.214 | 0.229 | 0.014 | 0.300 |
| UFA/SFA | 1.896 | 1.930 | 1.947 | 1.999 | 0.034 | 0.214 |
| PUFA/SFA | 0.759$^{b}$ | 0.789$^{b}$ | 0.78$^{b}$ | 0.858$^{a}$ | 0.008 | <0.0001 |
| PUFA n-6/n-3 | 22.39$^{a}$ | 7.620$^{b}$ | 6.826$^{b}$ | 7.153$^{b}$ | 0.432 | <0.0001 |
| DFA | 76.500$^{b}$ | 76.839$^{b}$ | 76.803$^{b}$ | 77.924$^{a}$ | 0.159 | <0.0001 |
| OFA | 23.466$^{a}$ | 23.145$^{a}$ | 23.034$^{a}$ | 22.048$^{b}$ | 0.163 | <0.0001 |
| DFA/OFA | 3.268$^{a}$ | 3.321$^{a}$ | 3.335$^{a}$ | 3.536$^{b}$ | 0.031 | <0.0001 |
| NVI | 2.042$^{b}$ | 2.057$^{b}$ | 2.080$^{ab}$ | 2.150$^{a}$ | 0.020 | 0.005 |
| IA | 0.367$^{a}$ | 0.360$^{a}$ | 0.357$^{a}$ | 0.337$^{b}$ | 0.004 | <0.0001 |
| IT | 0.964$^{a}$ | 0.830$^{b}$ | 0.809$^{b}$ | 0.785$^{b}$ | 0.013 | <0.0001 |
| h | 61.624$^{b}$ | 62.20$^{ab}$ | 62.03$^{ab}$ | 63.180$^{a}$ | 0.336 | 0.025 |
| H | 23.249$^{a}$ | 22.940$^{a}$ | 22.798$^{a}$ | 21.800$^{b}$ | 0.162 | <0.0001 |
| h/H | 2.654$^{b}$ | 2.711$^{b}$ | 2.723$^{b}$ | 2.902$^{a}$ | 0.032 | <0.0001 |
| PI (%) | 50.355$^{b}$ | 57.078$^{a}$ | 58.281$^{a}$ | 61.076$^{a}$ | 1.320 | <0.0001 |

where: C- conventional diet; E1- conventional diet + 3% flaxseed meal; E2 - conventional diet + 3% flaxseed meal + 10% peas; E3 - conventional diet + 3% flaxseed meal + 10% rapeseed meal; n = egg yolk samples
a,b,c Means within a row with different superscripts differ significantly, P ≤ 0.05.
Abbreviations: NVI, nutritive value index; AI, atherogenic index; TI, thrombogenic index; h/H, hypocholesterolemic/hypercholesterolemic index; PI, peroxidizability index; DFA,hypocholesterolemic acids; OFA, hypercholesterolemic acids;

Similar results were obtained by Franco et al. (2020), who tested three diets: conventional feed (CF), corn/pea/triticale mixture (CPT) and corn/wheat mixture (CW) on two hybrids of laying hens: Mos (native breed) and ISA Brown (commercial hybrid). The fatty acid profile was influenced by the type of diet, mainly the content of oleic and linoleic acids. Aguillón-Páez et al., 2020 conducted a laying hen study to evaluate sunflower or flaxseed seeds effects on performance, egg quality and fatty acids profile in yolk. The dietary inclusion of flaxseed (13.5%) resulted in a significant increase (P<0.05) of PUFA n-3 content and reduced the n-6:n-3 ratio without affecting performance parameters.Another study evaluated (Moghadam et al., 2020), the effects of dietary flax supplementation and flax in addition with enzymes laying hens feed. The new feeding solutions tested included: 15 g raw whole flaxseed/100 g feed, or 15 g heated whole flaxseed/100 g feed; flaxseed in addition with 0,1% enzyme. No dietary effects (P>0.05) on the total fatty acid content, respectively palmitic, stearic, α-linolenic, eicosapentaenoic acid, docosahexaenoic or arachidonic acid was observed. However, oleic acid and total monounsaturated fatty acids concentrations (mg/egg) were higher (P< 0.05. Concerning the eggs coming from hens which received only dietary flaxseed, compared to other experimental groups, the researchers concluded that heating flaxseed before consumption increases egg production while reducing the content of oleic acid and linoleic acid in the egg, but had no effect on egg weight or the level of α-linolenic acid in the eggs.

The nutritional value of dietary fat of poultry eggs can be enhanced by nutritional manipulation, changing the fatty acids ratio, especially those of PUFA n-3 (Franczyk-Żarów et al., 2019).

From the results presented in Table 5, although there was a decrease in cholesterol concentration in the experimental groups compared to C group, this was not statistically assured (P = 0.300). The most effective feeding diet concerning lowering the cholesterol levels in relation to the whole egg was found to be in E1 group in which flaxseed meal was included, the obtained results being in agreement with Basmacıoğlu et al. (2003).

Of the experimental groups, but also compared to C group, E3 presented the best PUFA/SFA ratio (0.858; P<0.0001), the results being in agreement with those obtained by Tomaszewska et al., (2021). At the same time, it showed a significantly higher content (77.924; P<0.0001) of cholesterol-lowering FA (DFA) and the lowest (22.048; P<0.0001) amount of hypercholesterolemic FA (OFA), leading to a significant increase (3.536; P < 0.0001) of DFA/OFA ratio. Similar results were obtained by Skiko et al., (2016); Walczak et al., (2017).

Examining the values reported in Table 5, it can be shown that both atherogenic indices (AI), considered pro-atherogenic with a role in coronary heart disease, and thrombogenic indices (TI) ones considered pro-thrombogenic with a tendency to form clots in blood vessels, recorded decreases compared to C group registered values. However, a significant decrease was recorded only for E3 in AI case (0.337; P<0.0001), while for TI all 3 experimental groups differed (P< 0.0001) significantly from C group. Similar results were obtained by other researchers (Omri et al., 2019; Dedouusi et al., 2022).

With regard to the h/H ratio, the data presented in Table 5 shows an increasing trend of the recorded values for E1 and E2 groups, while for E3 group the differences are significant compared to C (2.902 vs. 2.654; P<0.0001). The results are consistent with those obtained by Dedouusi et al, (2022), but contradictory to those obtained by Omri et al., (2019), (Panaite et al., 2020)

The peroxidability indices (PI) was significantly (P<0.0001) higher in all experimental groups (E1, E2, E3) compare with C group.Our results were similar with those recorded by Vlaicu et al. (2021), but contrary with those registrated by Zita et al. (2022)

## CONCLUSIONS

In conclusion, the utilization of raw materials rich in polyunsaturated fatty acids into laying hens diet increased the polyunsaturated fatty acids concentration within the egg and improved the nutritional quality indices of the lipids in egg yolks by decreasing the content of undesirable hypercholesterolemic acids and increasing the proportion of essential cholesterol acids, as well as DFA/OFA and UFA/SFA ratios, increasing the peroxides index and decreasing the hypercholesterolemic index.

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# *IN VITRO* EVALUATION OF *ENTEROCOCCUS FAECIUM* AS PROBIOTIC POTENTIAL IN POULTRY PRODUCTION

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***Abstract***

*Probiotics are important bacteria species due to their benefits to animal health. This study aimed to evaluate some characteristics of Enterococcus faecium (NCIMB 10415) and evaluated its survivability and capacity as a probiotic product. Gram-positive, catalase, antibiotics and haemolysis tests were screened using selective media. The strain was phenotypically characterized and biochemical profile using API 20STREP and identification by apiwebTM (Biomerieux (France) software were done (99.2% very good identification). After 24 h of incubation at 37°C, in aerobic conditions, E. faecium exhibits 11.88 $Log_{10}$ with an optical density (OD 600 nm) yield reaching a maximum from 0.2 at the beginning of the exponential growth phase to 1.7 value. The safety of the strain was confirmed by non-haemolytic activity on TSA agar medium. The impact of 16 antibiotics on our strain ranged from intermediate (75%) to susceptible (12.5%), with resistance to colistin sulphate and erythromycin. Regarding the importance of antibiotics such as vancomycin, the analysis of the E. faecium profile revealed intermediate activity. These data suggested that these bacteria do not create a risk to animal health and may be considered a reliable candidate as probiotic source for application in poultry nutrition.*

***Key words***: *Enterococcus, poultry, probiotics.*

## INTRODUCTION

Enterococci represent commensal microorganisms from intestinal microflora of animals and humans (Holzapfel et al., 2018; Lee et al., 2019), but they can also survive in several ecosystems such as soil, vegetables, water surfaces, food and feed (Zommiti et al., 2018).
Belong to the lactic acid bacteria (LAB) group, Enterococci occur important place due to their involvement in feed spoilage and fermentation processes (Zhang et al., 2016), as well for their utilization as probiotics in animal nutrition and also with successful utilization in human health (Franz et al., 2011).
Enterococci probiotics consumption was considered advantageous due to their capacity to produce multiple beneficial metabolites that also contribute to the stability of microorganisms in the gastrointestinal tract (GIT), considering that they are natural gut commensals (Fugaban et al., 2021). Some species of *Enterococcus* were used as probiotics in many countries due to their high ability to produce bacteriocins (Franz et al., 2011).
The application of enterococci in animal nutrition is still under discussion. A proposed classification is necessary to be feasible to divide pathogenic strains from well-evaluated beneficial *Enterococcus* strains (Fugaband et al., 2021). A part from enterococcal strains is successfully used as probiotic to improve animal health; on the other hand, other enterococcal types are associated with nosocomial infections (Franz et al., 2011).
Further, *Enterococcus* species have the capacity to resist in extreme temperatures fluctuation (10-45°C), wide pH gradients (4.5-10.0), high NaCl levels (6.5%), survive heat exposure (up to 80°C, more than 33 min) and grow in the presence of bile salts (Wieland et al., 2017).
It is known that some species from the *Enterococcus* genus ensure a significant place as probiotic bacteria in the host with a strong contribution to protection against pathogens and infectious diseases (Revajová et al., 2022).

Generally, probiotics in animal feed improve performance and health. In addition, the administration of enterococci in animal feed can prevent diseases, inducing the gastrointestinal micro populations or stimulating the immune system (Franz et al., 2011). Further, some representative strains from the genus *Enterococcus* have been studied regarding their application as starter cultures for exploring and obtaining the status of probiotics (Holzapfel et al., 2018) with significant results in poultry digestive function (Zhang et al., 2021). Usually, *E. faecium, E. faecalis and E. durans* are species from the genus *Enterococcus* used as veterinary feed supplements (Foulquié Moreno et al., 2006).

*E. faecium* has many biological traits (Mao et al., 2020), such as the capacity to survive in body conditions (low pH, bile salts concentration, inhibition and control of the growth of pathogenic bacteria), which makes it to displaying a potential source in feed preservation and in the prevention or treatment of other diseases in hosts (Saelim et al., 2012; Mao et al., 2020).

Therefore, the aim of the present study was conducted to assess some properties of *E. faecium* NCIMB 10415 based on its phenotypical profile as a probiotic candidate culture in poultry nutrition.

## MATERIALS AND METHODS

*Materials, reagents and strain*

To conduct this study, the strain *Enterococcus faecium* NCIMB 10415 (cultivated from Cylactin, DSM, Heerlen, Netherlands) was subjected to several tests; culture media and antibiotics disks were provided from Oxoid Basingstoke (Hampshire, UK).

*Culture media, growth conditions and morphological traits*

*E. faecium* NCIMB 10415 was cultivated in Brain Heart Infusion (BHI) broth medium (g/L) containing: beef heart (5.0), calf brains (12.5), D (+) glucose (2.0), sodium chloride (5.0), disodium phosphate (2.5), and peptone (10.0) with a final pH 7.4 ± 0.2.

After 24 h of incubation at 37°C, in aerobic conditions, the colonies of culture strain (broth and agar media) were evaluated for physiological characteristics (colonies morphology, Gram staining, catalase test) according to Bergey's Manual of Systematic Bacteriology (Hammes and Hertel, 2009).

*Colony-forming unit (CFU) and optical density*

The growth rate of strain was evaluated after cultivation in BHI broth at 37°C, for 24 h, aerobically.

The optical density (**OD**) was measured at 600 nm to estimate the *E. faecium* cells concentration at 4, 8, 12 and 24 h.

*API 20 STREP- biochemical characterization*

Biochemical characterization was done by analysing carbohydrate fermentation profiles using API 20STREP tests, a bacterial identification system (Bio Merieux, S.A., France). According to manufacturer instructions, the interpretation was done at 4 and 24 h, at 37°C using online software API 20 V 5.1.

*Haemolytic observation*

Blood agar plates [Trypticase soy agar (TSA, Sanimed) containing 5% (w/v) sheep blood] were used to test haemolysis activity. The strain was performed for the presence of clear zones surrounding the colonies. The interpretation was noted after incubation at 37°C, for 24 h as follows: clear zones corresponding to β-haemolysis, greenish zones as α-haemolysis and the absence of zones indicating no haemolysis which is known as gamma haemolysis (Dumitru et al., 2018; Bazireh et al., 2020).

*Antibiotic test*

The antibiotic susceptibility of *E. faecium* NCIMB 10415 to different antibiotics including amoxicillin (AMX 25 μg), gentamicin (GN, 10 μg), kanamycin (K, 30 μg), lincomycin (MY, 10 μg), tetracycline (TE, 30 μg), penicillin (P, 10 μg), vancomycin (VA, 5 μg), colistin sulphate (CT, 10 μg), clindamycin (DA, 2 μg), erythromycin (E, 15 μg), amikacin (AK, 30 μg), chloramphenicol (C, 30 μg), oxytetracycline (OT, 30 μg), enrofloxacin (ENR, 5 μg), streptomycin (S, 10 μg), and tilmicosin (TIL, 15 μg) was determined by agar disc diffusion method on BHI plates BHI.

*Statistical analysis*

The results are presented as mean values of three determinations. The graphics for strain viability during 24 h of incubation was performed with SigmaPlot V.11 software (San Jose, CA, USA).

## RESULTS AND DISCUSSIONS

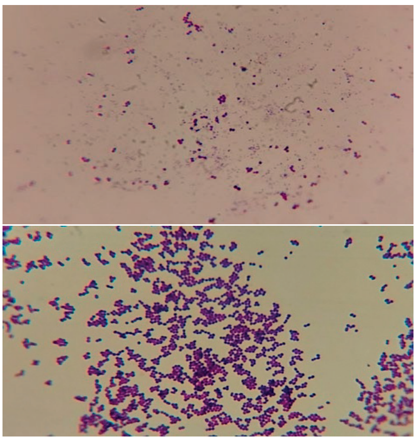
*Culture media, growth conditions and morphological traits*

The present strain has the origin from faeces of a healthy breast-fed newborn baby (Asplund, 1991).

The basic parameters examined in terms of effectiveness of probiotic candidates in chicken broiler are some phenotypical traits (micro- and macroscopically analysis, Gram staining, catalase).

It was observed that *E. faecium* NCIMB 10415 in broth medium, on agitation, involves homogeneous turbidity, with an abundant deposit, without surface formations. Regarding the agar BHI medium, the strain presented S-type colonies, whitish, with regular edges and a diameter of 1.5-2.0 mm.

Also, the colonies developed in broth and agar medium were verified by performing smears stained by Gram method, and the characteristic aspects (shape, type of Gram staining, grouping mode, ability of sporulation) allowed the differentiation of the genus *Enterococcus* from other bacterial species (Figure 1).


a

b

Figure 1. Cultural aspects of *E. faecium* in BHI medium (a: broth, b: agar) by Gram staining x 1000

Following the microscopical examination, the strain was observed as Gram-positive cocci which is a characteristic belonging to the family *Enterococcaceae*, diplo, in short chains and in small staph groups (on solid media).

Regarding the catalase test of *E. faecium* NCIMB 10415, the result was noted as negative, without effervescent at the addition of 3% $H_2O_2$. Over the last decades, probiotics occur a higher interest due to their multiple health benefits (Bazireh et al., 2020).

*E. faecium* is an important opportunistic pathogen easily transmitted between sick and healthy animals. It is known that the present analysed strain belongs to a pioneer type of bacteria, i.e. the first lactic acid bacteria transmitted from the mother's milk to the newborn, allowing the evolution of a healthy microbiome in the gastrointestinal tract (Wopereis et al., 2014). Further, Enterococci are among the most common human intestinal LAB, which harbours numerous useful biotechnological properties, such as the secretion of bacteriocins (enterocins) (Franz et al., 2011).

As a result, *E. faecium* is known to be a significant etiological agent of acute and chronic infections with a high degree of spread in hospital-acquired infections (Bazireh et al., 2020).

*Colony-forming unit (CFU) and optical density*

The growth of *E. faecium* NCIMB 10415 was drawn under the optimal conditions (pH 7.0, 37°C, 24 h, aerobic) and exhibited a good viability rate, around 11.88 $Log_{10}$ corresponding to a load of 7.6 x $10^{11}$ CFU/ml.

Regarding the optical density, after washing with SFS, the biomass of *E. faecium* NCIMB 10415 resulting from the fermentative medium was well homogenized with SFS, and the OD was read at a wavelength of 600 nm at 4, 8, 12 and 24 hours (Figure 2).

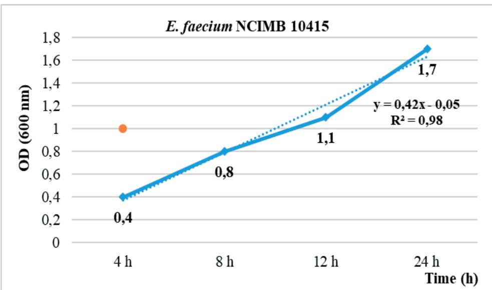



Figure 2. The optical density of *E. faecium* NCIMB during 24 h

*E. faecium* is one of the most important bacteria producing lactic acid belonging to the native microbiota of the human and animal gastrointestinal tract (Wu et al., 2019).

Exploring the growth characteristics and optimal growth conditions of probiotic strains is mandatory before they can be used in large-scale industrial production (Mao et al., 2020).

Comparatively to Mao et al. (2020) study, our strain involves a significant OD at 600 nm for 24 h, at 37°C, exhibiting a 1.7 value in the stationary phase (12-24 h). Further, the growth of *E. faecium* evaluated in the author's research did not exceed the 1.0 value of OD during the stationary or ageing phase (after 24 h) comparatively with *E. faecium* NCIMB 10415 strain which involves a best proliferation and a higher concentration in the condition of pH value (7.0) of the selective medium tested.

Generally, *E. faecium* has a considerable environment adaptability, with a strong proliferation and colonization of the animal GIT when it is added as probiotic. Moreover, feed supplementation with *E. faecium* has also been shown to offer a number of benefits, especially against the pathogen *E. coli* (Lodemann et al., 2017) and could provide an alternative source to enterococci for future probiotic development (Saelim et al., 2012).

*API 20 STREP- biochemical characterization*

The biochemical characteristics of *E. faecium* NCIMB 10415 is shown in Table 1. The capacity of fermentation was based on the strain's ability to ferment the substrates from API 20STREP (Figure 3). Sugar fermentation patterns confirmed that the presence of cocci belonged to *Enterococcus* genus.

*E. faecium* is the most common species of *Enterococcus* found in animal intestines and has similar properties and biochemical traits, with the capacity to use arabinose as carbon source (Mao et al., 2020). In addition, the strain was able to hydrolyse sodium pyruvate, bile esculin, pyrrolidinyl aryl amidase (at 24 h), α and β galactosidase, leucine aminopeptidase, L-arginine, D-ribose, D-arabinose, D-mannitol, D-lactose, D-trehalose, starch (at 24 h), but negative for hippuric acid, β-glucuronidase, D-sorbitol, inulin, raffinose, and glycogen.

Table 1. Assessment of biochemical characterization of *E. faecium* NCIMB 10415 by API 20STREP kit

| **Test** | **Fermentation Substrates** | **Strain 4 h** | **Strain 24 h** |
|---|---|---|---|
| VP | Sodium pyruvate | + | + |
| HIP | Hippuric acid | - | - |
| ESC | Esculin | + | + |
| PYRA | Pyrrolidinyl arylamidase | - | + |
| αGAL | α-galactosidase | + | + |
| βGUR | β-glucuronidase | - | - |
| βGAL | β-galactosidase | + | + |
| PAL | 2-naphthyl phosphate | - | - |
| LAP | Leucine aminopeptidase | - | + |
| ADH | L-arginine | + | + |
| RIB | D-ribose | + | + |
| ARA | D-arabinose | + | + |
| MAN | D-mannitol | + | + |
| SOR | D-sorbitol | - | - |
| LAC | D-lactose | + | + |
| TRE | D-trehalose | + | + |
| INU | Inulin | - | - |
| RAF | Raffinose | - | - |
| AMD | Starch | - | + |
| GLYG | Glycogen | - | - |

- = negative; += positive; ?= doubtful, weakly positive;

Figure 3. API 20 STREP inoculated with *E. faecium* NCIMB 10415

The percentage of identification (ID) was obtained according to the manufacture protocol API Biomerieux (France) and was registered as 99.2% that corresponding to a very good identification (Figure 4).

According to the manufacturer's instructions, the identification of *Enterococcus* strains at the species level was divided into four subgroups: (a) excellent species identification, ID ≥ 99.9%; (b) very good species identification, ID ≥ 99.0%; (c) good species identification, ID ≥ 90.0% and (d) acceptable species identification, ID ≥80.0%. In the present case, the enterococcal strain showed an ID ≥ 99.2%.

Based on the literature data, the present strain is in accordance with the results obtained by (Sanlibaba et al., 2018).

| VERY GOOD IDENTIFICATION | |
|---|---|
| Strip | API 20 STREP V7.0 |
| Profile | 5 3 5 7 5 1 1 |
| Note | POSSIBILITY OF Ent.gallinarum OR Ent.casseliflavus IF VancoR |

| Significant taxa | % ID | T | Tests against | | | |
|---|---|---|---|---|---|---|
| Enterococcus faecium | 99.2 | 0.98 | | | | |

| Next taxon | % ID | T | Tests against | | | |
|---|---|---|---|---|---|---|
| Enterococcus durans | 0.4 | 0.54 | ARA 15% | MAN 2% | | |

| Complementary test(s) | YELLOW | IRHAMNOSE | GLYCEROL | |
|---|---|---|---|---|
| Enterococcus casseliflavus | + | + | + | |
| Enterococcus faecium | - | v | - | |
| Enterococcus gallinarum | - | - | - | |

Figure 4. Percentage of *Enterococcus* identification by software Biomerieux using version API 20 V 5.1 (http://apiweb.mediclim.ro/)

The identification percentage generated by the Biomerieux online program for the analysed strain is similar to the ID of the *E. faecium* strain of IBNA 10 which was isolated according to the data presented by Sorescu et al. (2019) of the ileal contents of a turkey (age 73 days).

*Hemolytic activity*

Regarding the strain *E. faecium* NCIMB 10415, in 2011, the European Union (EU) approved their utilization as feed additive for different animals. An essential property until to use a strain as probiotic product is based on hemolytic activity. Our strain was found to be α-hemolytic without any clear area around colonies on TSA agar plates (Figure 5).

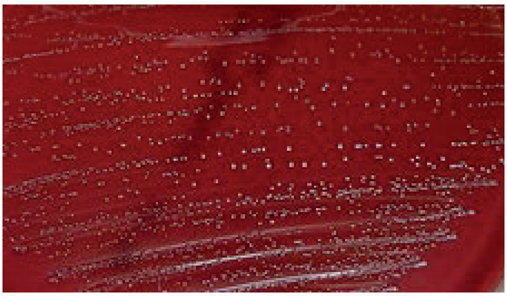


Figure 5. Hemolysis test of *E. faecium* NCIMB 10415

The assay is based on the ability of strain to cleave blood cells from the composition of culture medium, due to the presence of sheep blood (Dumitru et al., 2019). More, if the strain exhibited a transparent zone around the developed colonies which correspond to a hemolytic type (β with a clear hemolysis or γ with colonies that involve a green halo), the respective strain must be eliminated. Only strain which showed non-hemolytic activity were used for further experiments (Bazireh et al., 2020).

The main concern for *Enterococcus* spp. as a probiotic source is their pathogenicity based on the horizontal transfer of virulence factors, which is why hemolytic evaluation should not be neglected (Ben Braïek & Smaoui, 2019). Hemolytic activities α and β are considered a disadvantage for the probiotic potential (Halder et al., 2017). Thus, the hemolysis test is an extremely important safety parameter to develop new probiotic strains for use as supplements in animal feed.

In general, the absence of hemolytic activity is an advantage, which is why the present strain of *Enterococcus* proved to be non-hemolytic and thus can be used safely as a source of probiotic product in poultry nutrition.

It is known that many studies present a positive influence of enterococcal probiotics in poultry diets. For example, Vahjen et al. (2002) showed that a probiotic based on *E. faecium* SF68 in turkey feed led to increase the level of lactic acid bacteria from intestinal tract, respectively from ileum area. Later, in 2010, Samli et al. investigated the effect of the current strain, *E. faecium* NCIMB 10415 on broiler chickens' performance, where were observed significant results (weight gain and feed conversion ratio).

*Antibiotic test*

The results of the antibiotics resistance are reported in Table 2. The strain exhibited resistance to colistin sulphate and erythromycin, sensitivity for chloramphenicol and enrofloxacin, and intermediate resistance for the rest of antibiotics analysed.

Table 2. Antibiotics resistance of *E. faecium* NCIMB 10415

| Strain | Antibiotics discs | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **AMX** | **GN** | **K** | **MY** | **TE** | **P** | **VA** | **CT** | **DA** | **E** | **AK** | **C** | **OT** | **ENR** | **S** | **TIL** |
| | I | I | I | I | I | I | I | R | I | R | I | S | I | S | I | I |

amoxicillin (AMX) 25 µg; gentamicin (GN) 10 µg; kanamycin (K) 30 µg; lincomycin (MY) 10 µg; tetracycline (TE) 30 µg; penicillin (P) 10 µg; vancomycin (VA) 5 µg; colistin sulphate (CT) 10 µg; clindamycin (DA) 2 µg; erythromycin (E) 15 µg; amikacin (AK) 30 µg; chloramphenicol (C) 30 µg; oxytetracycline (OT) 30 µg; enrofloxacin (ENR) 5 µg; streptomycin (S) 10 µg; tilmicostin (TIL) 15 µg. Resistant (R): 0–5 mm; Intermediate (I): 6-25 mm; Sensitive (S): 26–35 mm; *E. faecium* NCIMB 10415.

Regarding the vancomycin resistance, the strain showed an inhibition spectrum below 1 cm, thus being characterized by a variation from resistant to intermediate. However, enterococcal resistance to vancomycin has been reported in several studies (Kolář et al., 2002; Vignaroli et al., 2011). For safety reasons, the sensitivity of commercially exploitable strains to commonly used antibiotics is desirable for use as coculture or early crops (Vignaroli et al., 2011). Also, in many cases, antibiotic treatment is indispensable, having a devastating effect on the balance of the intestinal flora (Stoica & Stoica, 2001). Instead, antibiotics, such as tetracycline, are responsible for gastrointestinal imbalances.

The trait of antibiotic resistance is an important property associated with probiotics because it helps selected microorganisms for probiotic purposes to survive in the gastrointestinal tract of the host organism during antibiotic treatment (Mishra and Ghosh, 2018; Choeisoongnern et al., 2021).

These findings were similar to previous studies in which bacterial isolates demonstrated a typical pattern of antibiotic susceptibility to *Enterococcus*, especially colistin (Mishra and Ghosh, 2018). In addition, enterococcal bacteria are part of the natural microflora of humans and animals (Golob et al., 2019), being considered natural commensal microorganisms that contribute to the stability of the host bacterial population in GIT (Fugaban et al., 2021).

## CONCLUSIONS

The results obtained in present study showed that. *E. faecium* NCIMB 10415 present relative probiotic properties to be used as an effective probiotic. In addition, the safety characteristics, high survivability, capacity to ferment sugars, absence on hemolysis activity, and antibiotic resistance suggested that, *E. faecium* NCIMB 10415 is a potent probiotic candidate with the ability to proliferate, survive and colonize in the host GIT. Furthermore, as suggested in the EFSA guidelines on the use of *Enterococcus* spp. as feed additives, the present strain may serve as good supplement, starter fermentation culture or probiotic product in poultry feed.

Further investigation is necessary to test and validate the impact of a probiotic based *on E. faecium* NCIMB 10415 on poultry health and growth in farm settings, as well as to find out the optimum level of inclusion.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# A COMPREHENSIVE REVIEW ON ALGAE AND PROPOLIS-CHARACTERISATION AND THE IMPLICATIONS OF THEIR USE IN THE LAYING HEN DIET

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***Abstract***

*Microalgae represent a new field of interest for laying hens' nutrition as they constitute a novel and valuable nutrient source, due to their nutritional composition and richness in polyphenols, polysaccharides and fatty and amino acids. Many studies have studied the effect of using microalgae in laying hen nutrition and their ability to improve health, production and egg quality. Propolis, like microalgae, is a natural source of nutrients with a long tradition in natural medicine. The literature has shown many benefits of using propolis in the diets of laying hens, such as improved productive performance and egg production, health, egg quality. This review makes it clear that including microalgae and propolis in laying hen diet can be an undeniable future nutritional strategy, enhancing standard feed formulations to the benefit of health and egg quality.*

***Key words****: diet, egg quality, laying hen, microalgae, nutrients, propolis.*

## INTRODUCTION

Nowadays there is an increasing demand for functional foods for human consumption that provide various benefits in addition to the nutrients. The farming industry has thus become interested in using natural forms of vitamins and minerals instead of synthetically produced ones. Considering these dynamics, the possibility of using microalgae as a new source of nutrients and health additives in animal feed formulations has been evaluated. Eggs can be enriched with certain nutrients through dietary manipulation to create products that could possibly provide health benefits for humans (Saracila et al., 2017; Panaite et al., 2021).

'Algae' is a generic term that groups brown, green, and red types of both macro- and microalgae (Coudert et al., 2020). Global demand for macroalgae and microalgae is growing. There is substantial evidence for health benefits, but it is challenging to study the effects of including them in poultry feed as natural sources of vitamins and minerals. In general, the growing trend in global nutritional demand for algae comes from a greater focus on health. In addition to their nutritional value, algae are increasingly being marketed as functional foods or nutraceuticals. In recent years, much interest has been focused on the potential of microalgae biotechnology, mainly due to the identification of several substances synthesized by these microorganisms (Andrade et al., 2018). Chlorella and Spirulina are two of the best-known genera of microalgae (Andrade et al., 2018). These marine plants may play a key role in the future for poultry production, as they constitute a new and valuable nutrient source, thanks to their nutritional composition and richness in as polyphenols, polysaccharides and fatty and amino acids. Algae are a valuable source of B vitamins (especially B1, B12), as well as vitamin A (derived from the β-carotene carotenoid) and vitamin E. Algae provide one of the few plant alternatives to cobalamin (vitamin B12) in the diet (Andrade et al., 2018). Microalgae produced through fermentation contains high levels of DHA (Zeller et al., 2001) and could be potentially used in animal diets. Ao et al. (2015) showed that supplements of 2 or 3% microalgae significantly improved the color and nutritional quality of egg yolk.

The global interest and the growing awareness of consumers, especially in terms of the nutritional and medicinal value of what they eat or drink, arouse the concept of a return to natural products, especially bee products. Propolis has attracted a lot of attention from food supplements and food processing industries due to its high value for health.

Propolis (bee glue) is a natural product collected by bees from various plants, particularly from flowers and leaf buds (Abdel-Kareem & El-Sheikh, 2015). Propolis contains resin and vegetable balsam (50%), wax (30%), essential and aromatic oils (10%) as well as both pollen and other substances (5%) as organic debris (Burdock, 1998). Literature showed many benefits of using propolis in laying hen diets such as improved productive performance and egg production (Abdel-Kareem & El-Sheikh, 2015), health promoting effect (El-Neney et al., 2014), egg quality (Casagrande et al., 2021).

This review describes the main nutritional characteristics of microalgae and propolis and the current knowledge on their effects in laying hen production, impacts on health, performance and egg quality.

## MATERIALS AND METHODS

In this review, we used 45 specialized articles, using databases such as Google academic, Science direct, etc. Recent research papers (last 5-10 years), well-designed experimental design, large number of animals taken in the experiment were the search criteria.

## RESULTS AND DISCUSSIONS

### *1. Microalgae - chemical characterisation and implication in laying hen diet*

Microalgae represent a new field of interest for animal nutrition and health, both biologically and economically. The economic importance is related to the wide range of applicability of microalgae worldwide (Andrade et al., 2018) and the number of publications devoted to or related to this subject in recent years has increased regularly (Coudert et al., 2020). Microalgae, microscopic single-celled organisms, can be used to produce a wide range of metabolites, such as proteins, fats, carbohydrates, vitamins, and organic minerals. The cultivation of microalgae was carried out with the aim of producing biomass both for food and also for obtaining value-added natural compounds. These natural compounds include polyunsaturated fatty acids, carotenoids, polysaccharides, vitamins, sterols, and many natural bioactive compounds, such as antioxidants, that can be used primarily for functional food production. Chlorella and Spirulina are two of the best-known genera of microalgae (Andrade et al., 2018).

### *1.1. Microalgae - chemical characterisation*

Chlorella and Spirulina microalgae live in freshwater and are rich in bioactive compounds such as proteins, vitamins, pigments, long chain polyunsaturated fatty acids, sterols and other compounds that make these microalgae very interesting in terms of health benefits (Andrade et al., 2018).

Vitamins are essential organic micronutrients that an organism cannot synthesize directly in sufficient quantities and therefore must be obtained from the diet. Algae are a source of B vitamins (especially B1, B12), as well as vitamin A (derived from the β-carotene carotenoid) and vitamin E (tocopherol) (Andrade et al., 2018). Algae provide one of the few plant alternatives to cobalamin (vitamin B12) in the diet. Chlorella and Spirulina microalgae produce vitamin A (beta-carotene), vitamin C, vitamin E, thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6), folic acid (B9) and cobalamin (B12). These vitamins are used to nourish the body, detoxify and normalize bowel function, as well as stimulate the immune system and regenerate cells (Andrade et al., 2018). Chlorella biosynthesizes vitamin A with a concentration of 30.77 mg/100 grams dry mass, while spirulina contains 0.34 mg/100 g dry mass. In fact, vitamin A is the most abundant vitamin produced by microalgae. Niacin (vitamin B3) is also abundantly biosynthesized by microalgae. Chlorella contains 23.8 mg Vitamin B3/100 g dry mass, and spirulina contains 12.08 mg vitamin B3/100 g dry mass (Andrade et al., 2018). Vitamin B12 (cyanocobalamin) is present in microalgae at a low level (0.1 μg) Vitamin B12 in microalgae from Chlorella sp. has better bioavailability than Spirulina microalgae (Solomons, 2012). Chlorella and Spirulina microalgae have a high concentration of folic acid (94 μg/100 g dry mass), which is necessary for cell formation and maintaining metabolism, preserving the skin and mucous membranes and for the normal development of bones and teeth (Andrade et al., 2018). Compared to Chlorella, Spirulina are richer sources of vitamin E, vitamin B1 and vitamin B7. Chlorella contains substantial amounts of vitamins D2 and B12, both of which are well known to be absent in plants. Commercially available Chlorella (*C. vulgaris*) products contain higher amounts of folate (approximately 2.5 mg/100 g dry weight) than spinach (Woortman et al., 2020). Vitamin D, a major regulator of calcium absorption, reduces the risk of osteomalacia in adults and rickets in children (Taofiq et al., 2017). The two main food forms of vitamin D are vitamin D2 and D3, which are found in mushrooms and foods of animal origin, such as fish and fish products, respectively. Ingestion of small amounts of microalgae (biomass) can help meet all vitamin requirements in both animal feed and human food. Vitamins from microalgae can increase the nutritional value of algae - applied as a supplement.

Algae contain significant amounts of iodine and iron (Wells et al., 2017). In particular, Chlorella contains substantial amounts of iron (104 mg/100 g dry matter) and potassium (986 mg/100 g dry matter), of which adequate intake prevents anaemia and hypertension, respecttively. Spirulina contains minerals such as iron, magnesium, calcium and phosphorus (Soni et al., 2017). The iron, calcium and phosphorus content of Spirulina are 1.7, 15 and 10 mg/ sample, respectively (Deasy Liestianty et al., 2019). Spirulina is a splendid source of iron that contains 20 times more iron than 1 gram of wheat (Soni et al., 2017). Microalgae can accumulate Se in high concentrations (100 μg Se/g dry weight) (Doucha et al., 2009). It is essential for many algae and works to protect them from oxidative damage. Selenium is an essential oligomineral that serves as a fundamental nutrient for human health. It is a component of selenoproteins such as thioredoxin reductase and glutathione peroxidases and protects against intercellular oxidative damage.

### *1.2. Microalgae-implication in laying hen diet*

Microalgae have been recognized as natural, sustainable and economically feasible resources of unconventional ingredients capable of promoting the benefits of animal husbandry and improving meat quality (de Medeiros et al., 2021). About 30% of the world's algae are currently used in animal feed (Li et al., 2002).

Once microalgae biomass is included in animal feed, these compounds meet the energy-protein needs for breeding animals with satisfactory performance. In parallel with the positive impact on the nutritional, technological and sensory quality of meat, microalgae biomass improves feed digestibility and improves the animal's immune response (Kibria and Kim, 2019). Microalgae have been introduced into animal feed mainly by incorporating the entire biomass of microalgae in the form of dry powder, high moisture extruded biomass or microalgae extracts (Van Vo et al., 2020).

Table 1 presents a summary of microalgae applications in hen diet. Literature research revealed that microalgae supplementation in laying hen diet improve productive parameters. Zheng et al. (2011) showed that dietary supplementation in 80-week-old Hy-Line Brown layers with 2% of *Chlorella vulgaris* increased egg productivity from 55.4% in the control to 59% in the supplemented group. An et al. (2014) revealed that 1% conventional or lutein-fortified Chlorella improved egg production.

Some microalgae are able to positively influence egg physical and nutritional quality (Table 1). Providing dietary 1.25% *C. vulgaris*, Englmaierová et al. (2013) showed an increase in egg weight (62.3 g vs. 61.1 g for control hens), shell weight (6.1 g vs. 5.9 g for control hens), and yolk colour, which was more intense (increased redness and yellowness) for the supplemented group.

Ao et al. (2015) showed that supplements of 2 or 3% All-G-RichTM significantly increased redness (a*) and decreased lightness (L) of egg yolk. Dietary 1% or 1.25% of *Chlorella vulgaris* in 56- to 63-week-old and 25- to 39-week-old ISA brown laying hens, significantly increased lutein, zeaxanthin and beta-carotene in egg yolk (Kotrbáček et al., 2015). Several studies have reported that microalgae supplementation significantly change the fatty acid composition of eggs. Adding All-G-RichTM in layer diets can produce DHA-enriched eggs with no negative impact on egg quality (Ao et al., 2015). Similar results were found by using Spirulina microalgae instead of Chlorella (Luo et al., 2015). Fraeye et al. (2012) showed that hens fed microalgae had a n-3 LC FA content 3.8- to 7.0-fold higher in eggs. Although enrichment in LC-PUFA accelerates the oxidative processes of lipids, some authors have shown that Yolk fatty-acid oxidation, as measured by thiobarbituric acid reactive substances (TBARS), was not affected by All-G-RichTM in eggs stored up to 30 days at 4°C (Ao et al., 2015).

Table 1. Summary of microalgae applications in hen nutrition

| Type of microalgae | Animals | Dose of inclusion | Effect | Reference |
|---|---|---|---|---|
| All-G-RichTM | | 2 or 3% | - increased redness (a*) and decreased lightness (L) of egg yolk<br>- enriched eggs yolk in DHA | Ao et al. (2015) |
| *Spirulina platensis* | Hy-line W36 hens, 63-67 wk | 1.5, 2.0, 2.5% | Increased yolk colour | Zahroojian and Morajev (2013) |
| *Chlorella vulgaris* | ISA brown hens, 25-39 wks | 1.25% | Increased egg weight, shell quality, yolk colour, lutein and zeaxanthin concentrations | Englmaierová et al. (2013) |
| *Chlorella vulgaris* | Laying hens, 56-63 wks | 1.0% | Increased yolk carotenoids (lutein, beta-carotene and zeaxanthin) | Kotrbáček et al. (2015) |
| *Spirulina platensis* - Supercritical Extract | ISA Brown, 36 weeks of age | 0.2% | increase the concentration of DPA, EDA reduce the content of saturated fatty acids such as pentadecanoic acid | Michalak et al., (2020) |
| Lutein-fortified Chlorella | Laying hens, 70-72 wk (Exp. 1), 60-62 wk (Exp. 2) | 0.1 or 0.2% | 1% conventional or lutein-fortified Chlorella improved egg production, yolk colour and lutein content in the serum, liver and growing oocytes. 0.2% lutein-fortified quality, lutein of lutein- fortified Chlorella increased egg weight, yolk colour and lutein content in egg | An et al. (2014) |
| Fermented Chlorella biomass | Laying hens, 80-86 wk | 0.1 or 0.2% | -improved egg production, yolk colour, Haugh units and lactic intestinal acid bacteria cecal population | Zheng et al. (2012) |

Some microalgae may influence egg lipid composition. Several studies have reported that microalgae supplementation can significantly reduce egg cholesterol (Table 1). This was the case for supplementation with *Spirulina platensis* (1.5, 2 and 3% in feed) in 63- to 67-week-old Hy-line W36 hens (Zahroojian & Morajev, 2013), with *Chlorella vulgaris* in 80-week-old Hy-line Brown layers (Zheng et al., 2011). Few studies revealed that microalgae supplementation in laying hen diet could improve gastrointestinal health (Table 1). Zheng et al. (2011) showed that 0.1 or 0.2% of fermented Chlorella biomass supplementation in laying hen diet increased the number of lactic acid bacteria in the cecum.

### *2. Propolis - chemical characterisation and implication in laying hen diet*

The application of propolis in the formulation of feed is ongoing (Kostić et al., 2020). In recent decades, the use of natural products has been promoted to improve the performance and meat quality (Saracila et al., 2021a; 2021b; Untea et al., 2021). Therefore, propolis is one of the natural candidates for this purpose.

Propolis is considered a valuable ingredient for animal nutrition due to its active components that have significant health properties (Abdel-Kareem & El-Sheikh, 2017). It is generally marketed as a functional and affordable food with promising future industrial potential.

### *2.1. Propolis - chemical characterisation*

Propolis is collected from buds, leaves and similar parts of trees and plants like pine, oak, eucalyptus, poplar, chestnut, etc. by bees and mixed with wax (Valle, 2000). Chemically propolis is composed of more than 180 different types of chemicals (Kuropatnicki et al., 2013). Propolis contains resin and vegetable balsam (50%), wax (30%), essential and aromatic oils (10%) as well as both pollen and other substances (5%) as organic debris (Burdock, 1998). Generally, the known major components of propolis are aromatic acids, flavonoids, diterpenoid acids, phenolic compounds and triterpenoids (Elnakady et al., 2017). Cecere et al. (2021) showed a content in total phenolic compounds of 916.28±23.22 mg equivalent in gallic acid/mL; 158.15±4.47 µg/mL antioxidant activity (IC50); and concentrations of gallic acid of 0.57 mg/mL, p-coumaric acid of 3.10 mg/mL, and chlorogenic acid of 1.41 mg/mL.

Devequi-Nunes et al. (2018) analysed three varieties of propolis (red, green and brown) and showed a concentration of protein that ranges from 2.12-2.49%, of lipids 8.19-15.61% and fiber 68.72-70.82%. The same authors analysed the main chemical classes present in propolis. They are flavonoids, phenolics, and aromatic compounds. The content of phenolic compounds varied from 113.41±0.01 (Brown SCO2) to 481.59±0.02 mg EAG/g (Red EtOH), whereas the content of flavonoids varied from 29.67±0.01 (Brown EtOH) to 186.96±0.01 mg EQ/g (Red EtOH) among other samples, and the antioxidant capacity varied from 371.12±0.01 (Brown SCO2) to 89.90±0.02 (Red EtOH) (IC50).

### *2.2. Propolis - implication in laying hen diet*

In general, the use of propolis is pronouncedly increasing in medical science, but very limited data is available regarding its use in the field of poultry production. This subsection aimed to present the effect of supplementing the diets of laying hens.

Table 2 presents a summary of propolis applications in hen diet. Some researchers showed a beneficial influence on daily gain, feed intake and conversion in different animal species, including poultry (Guclu-Kocaoglu, 2010; Mathivanan et al. 2013). Galal et al. (2008) found that the feed consumption for laying hens fed diets that contained 100 and 150 mg propolis/kg diet increased significantly compared with control group.

Table 2. Summary of propolis applications in hen nutrition

| Type | Animals | Dose of inclusion | Effect | Reference |
|---|---|---|---|---|
| Propolis | 28-weeks-old Lohmann LSL hybrid layers | 250, 500 and 1000 mg/kg diet | - improved egg production, blood constituent and haematological parameters of the commercial laying hens. | Abdel-Kareem and El-Sheikh (2017) |
| Green propolis | brown Hy-line laying hens | 10 20, 30 g/kg of feed | - reduced bacterial contamination in the eggshells reduced the lipid peroxidation of fresh and stored eggs | Casagrande et al. (2021) |
| Propolis | 55-wk-old Isa Brown® layers | 0, 1, 2 and 3% | - did not improve performance and worsened the eggs' quality improves the integrity of the gastric tract | Belloni et al. (2015) |
| Propolis | Hy-Line White strain 46-54wk | 50, 100 and 150 mg | - 100 or 150 mg is beneficial for improving the performance and immunity and for exploiting the full genetic potential of the commercial laying hens. | Galal, et al. (2008) |
| Propolis | Hyline White Leghorn, 42-week-old (chronic heat stress) | 3 g/kg | - exhibited the same efficiency than antibiotic for restoring performances, nutrient digestibility and egg qualities in laying hens chronically exposed to heat stress. | Seven et al. (2011) |
| Propolis | White Leghorn layer hens | 0.5, 1, 3, and 6 g/kg of diet | - 3 g/kg of diet may have a positive effect on humoral immunity of laying hens. | Çetin et al. (2010) |

Contradictory results were observed by Abdel-Kareem and El-Sheikh (2017), respectively the final body weight and egg weight of hens that received different levels of propolis were not significantly different in comparison with control group. Belloni et al. (2015) also showed that the propolis supplementation did not improve performance and worsened the eggs' quality.

Regarding the egg production, the result showed that dietary propolis supplementation improved egg numbers and egg production rate for hens treated with propolis at 100 and 150 mg/kg diet significantly than those of the control group (Galal et al., 2008). The same authors showed that the averages of increased while the eggshell thickness for eggs produced from treated laying hens was significantly higher as compared to the control group. Dietary supplementations of laying hens with flavomycin or propolis have significantly reduced the negative effects of heat stress on performances, nutrient digestibility and eggshell characteristics (Seven et al., 2011).

Dietary propolis supplementation has an effective role in improve laying hen's health. In this line, El-Neney et al. (2014) showed that plasma cholesterol was significantly reduced in Dokki 4 laying hens fed propolis compared to control. Plasma total protein, albumin and globulin were significantly lower for control than those fed propolis. Using different dietary propolis levels of treated groups led to a significant increase in RBC and WBC, Hb, lymphocytes, eosinophils and monocytes percentages, while the basophils percentage was insignificantly affected. Due to the antibacterial and antioxidant activity, some studies showed that propolis can reduce the bacterial contamination and delay the lipid oxidation process in eggs (Casagrande et al., 2021).

## CONCLUSIONS

It is undeniable that there is an increasing demand for functional foods for human consumption that offer various benefits in addition to nutrients. Dietary microalgae and propolis could be effective in improve the nutritional quality of eggs without affecting laying hen's performance. This observation result from the valuable chemical composition: microalgae contain important percent of vitamins (especially B1, B12, A), carotenoids, minerals (Fe, K, Ca, Se) and propolis is characterised by aromatic acids, flavonoids, diterpenoid acids, phenolic compounds and triterpenoids with high antioxidant capacity. Many studies confirmed the advantages of using microalgae and propolis in laying hen nutrition and their ability to improve health, productive parameters, egg nutritional quality (fatty acids, carotenoids, reduced cholesterol) and to inhibit lipid oxidative processes in eggs.


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# DIETARY INCLUSION OF *SACCHAROMYCES CEREVISIAE* FERMENTED RAPESEED MEAL MODULATED IMMUNE, OXIDANT AND ANTIOXIDANT INDICES IN PIGLETS AFTER WEANING

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***Abstract***

*Fermented rapeseed meal could be an attractive feed source for piglets after weaning due to its high level of protein with special amino acids and bioactive compounds (polyphenols, PUFA, vitamins B, minerals, fiber) known for their antimicrobial, antioxidative and immunostimulatory effect and lower antinutrients. Lactic acid bacteria were mainly used for rapeseed meal fermentation while yeasts have been less used. The present study evaluated the effect of a diet including 10% rapeseed meal fermented with Saccharomyces cerevisiae on inflammation, oxidative and antioxidative response in spleen of pigs after weaning. The fermentation reduced efficiently glucosinolates level by half in fermented compared to unfermented rapeseed meal and enriched the rapeseed meal in several bioactive compounds such iron, zinc, manganese, n-6 and n-3 unsaturated fatty acids and fiber. Fermented diet reduced the concentration of two important mediators of inflammation, interleukine-1β (-10.28%) and interleukine-6 (-10.92%) and increased the concentration of IFN-γ (+15.36%), a cytokine involved in the antiviral and antibacterial response. An increase in antioxidant enzymes activity and a reduction in lipid peroxidation was also found in spleen of piglets fed fermented diet.*

***Key words**: antioxidant status, inflammation, fermentation, pig, rapeseed meal.*

## INTRODUCTION

During weaning period, piglets have to defy many challenges such as the replacement of breast milk with solid feed, adaptation to another environment, insufficient development of the immune and enzymatic system etc. In general, these events have been overcome by the use of antibiotics at sub therapeutic level (Magnoli et al., 2022). Their interdiction (2006) has led to the necessity to find new alternatives of antimicrobial compounds and this has opened up many opportunities for animal nutrition research. Rapeseed meal could be such an alternative source rich in protein with important particularities due to its content in amino acids like arginine, cysteine and methionine as well as bioactive compounds such as polyphenols, unsaturated fatty acids, minerals, vitamins etc. (Chen et al., 2019) (Wickramasuriya et al., 2015). In animal nutrition rape seed meal was manly an important source of protein. In young pigs, it has been used sparingly due to anti-nutrients that it contains (e. g. glucosinolates) which could affect piglets' performance, nutrient digestibility (Chen et al., 2019) especially during the sensitive period of weaning. (Pérez de Nanclares et al., 2019) showed that the replacement of soybean meal by expeller rapeseed meal with up to 30% (10%, 20%, 30%) had no effect on growth performance and nitrogen metabolism but affect the apparent total tract digestibility.

The research from recent years has focused on the reduction of anti-nutrients from rapeseed meal by different procedures. Fermentation is the most widely used biotechnological process for this purpose resulting in an enrich in protein levels and a decrease in the concentration of glucosinolates, phytic acid, NDF, etc. For all these reasons fermented rapeseed meal can be an attractive source of feed for piglets. Until now the fermentation has been done manly with lactic acid bacteria (different species of *Lactobaccilus* sp., *Aspergilus* sp.) or a mix with yeasts (Shi et al., 2016) (Plaipetch & Yakupitiyage, 2011; 2013). The fermentation enhanced also rapeseed meal in bioactive nutrients (peptides, polyphenols, PUFA, vitamins B, minerals, fiber etc) known for their antimicrobial, antioxidative and immunostimulatory effect. For example, fermentation of rapeseed meal with *Bacillus subtilis* resulted in an increase in peptides with antioxidant activity which demonstrated *in vitro* inhibitory activity on lipid peroxidation and iron ion chelator (Wang et al., 2003). This fermentation product has a high nutritional value, having a high content of essential amino acids: Histidine, Tyrosine, Methionine, and Cysteine (11.59% of the total amino acids). Studies with fermented rapeseed meal in different farm animal species (chicken, turkey, pig) have shown an improve in growth performance, nutrients digestibility, gut morphology and microbiota as well as in antioxidant and health indices (Chen et al., 2019; Drazbo et al., 2018; Hu et al., 2016; Satessa et al., 2020). Fermentation with *Saccharomyces* sp. has been less used. Utilization of yeasts for the fermentation of by-products could be of interest as the cell wall of yeasts contain polysaccharides manly ß-glucan and mannan with antioxidant properties due to their polymeric structure which can trap free radicals through their capacity to encapsulate toxins like lipopolysaccharide endotoxin (LPS) reducing then its negative effect into the cells (Chuang et al., 2021). The use of rapeseed meal fermented with yeasts as a source of biologically active molecules which could support the transitional post-weaning period has been scarcely investigated in pig. That is why the present study evaluated the effect of a diet including 10% rapeseed meal fermented with *Saccharomyces cerevisiae* on several indices of immune, oxidant and antioxidant response in spleen of pig after weaning. The analysis at the spleen level is relevant as it is one of the most secondary important immune organs where the immune response is built.

## MATERIALS AND METHODS

### *Ethical statement*

The experimental protocol was approved by the Ethical Committee (no. 118/2019) of the National Institute of Research and Development for Biology and Animal Nutrition, Balotesti, Romania. Animals were carried on the basis of European and Romanian legislation (The EU Council Directive EC/63/2010 and the Romanian Law 43/2014) for handling and protection of animals used for experimental purposes.

### *Diets*

The three groups of piglets were assigned to three experimental diets as followed: 1) control diet: a starter diet based on corn-soybean meal; 2) experimental diet 1: control diet with 10% unfermented rapeseed meal (RSM diet); 3) experimental diet 2: control diet with 10% fermented rapeseed meal (FRSM diet). Rapeseed meal replace 10% soybean meal (Tables 1 and 2). Diets were formulated to meet the NRC (2012) requirements for pigs after weaning.

Table 1. Ingredients and calculated nutrient content of experimental diets

| Ingredients (%) | Weaned phase[1] | | |
|---|---|---|---|
| | Control diet | RSM diet | FRSM Diet |
| Corn | 65.76 | 64.96 | 64.96 |
| Soybean meal | 24.00 | 15.00 | 15.00 |
| Rapeseed meal | - | 10.00 | - |
| Fermented rapeseed meal | - | - | 10.00 |
| Corn gluten | 1.5 | 1.5 | 1.5 |
| Monocalcium phosphate | 0.83 | 0.48 | 0.48 |
| Limestone | 1.40 | 1.45 | 1.45 |
| NaCl | 0.10 | 0.10 | 0.10 |
| DL-Metionină | 0.07 | 0.06 | 0.06 |
| L-Lisine | 0.23 | 0.34 | 0.34 |
| Choline premix | 0.10 | 0.10 | 0.10 |
| Mineral vitamin-premix[2] | 1.00 | 1.00 | 1.00 |
| **TOTAL** | **100.00** | **100.00** | **100.00** |

Table 2. Calculated nutrients content of experimental diets

| Calculated Nutrient content | Weaned phase | | |
|---|---|---|---|
| | Control diet | RSM diet | FRSM diet |
| Crude Protein (%) | 19.00 | 18.95 | 18.95 |
| Digestible protein (%) | 12.07 | 11.94 | 11.94 |
| Crude Fat (%) | 2.97 | 2.95 | 2.95 |
| Crude fiber (%) | 3.33 | 3.70 | 3.70 |
| ME (Kcal/kg) | 3294 | 3228 | 3228 |
| Lysine (%) | 1.20 | 1.20 | 1.20 |
| Digestible Lysine (%) | 1.01 | 1.01 | 1.01 |
| Met + Cys (%) | 0.72 | 0.72 | 0.72 |
| Calcium (%) | 0.90 | 0.90 | 0.90 |
| Phosphorus (%) | 0.69 | 0.69 | 0.69 |

Rapeseed meal was fermented with commercial dry yeast *Saccharomyces cerevisiae* using the protocol described by (Plaipetch & Yakupitiyage, 2011; 2013) for 24 hours. The fermentation time (24 h) of rapeseed was chosen after performing two-time tests (24 and 72 hours). No changes in chemical composition were observed.

***Animals and samples collection***

The nutritional trial was performed on the IBNA experimental farm on 24 weaned TOPIG hybrid [(Landrace × Large White) × (Duroc × Pietrain) NORVEGIAN] piglets (8 piglets/group/two replicates per group and 4 pigs per replicate) with an average initial weight of 9.04 ± 0.19 kg for 21 days. After weaning (at 35 days) piglets housed in pens were acclimatized for one week before being used in the experimental protocol. They were individually eartaged and divided in three groups according with their body weight. During the experimental period feed and water was given *ad libitum* to the animals. At the end of experimentally 21 days pigs were euthanized and samples of organs were collected. Spleen samples were perfused with ice-cold physiological serum to remove blood and stored at -80$^0$C until analysed.

***Feed chemical analysis***

Chemical composition consisting in: dry matter, crude protein, crude fat, crude cellulose and ash, trace elements (calcium, sodium, potassium, magnesium, iron, zinc manganese, copper) of unfermented and fermented rapeseed meal as well as of the complete feed used in the experiment (control, RSM and FRSM) was analysed according to the International Standard Organization methods (SR ISO 6496/2001, Standardized Bulletin 2010, www.asro.ro), flame atomic absorption spectrophotometry with Zeeman background correction and graphite furnace (Pye Unicam, Thermo Electron, Solaar M6, Cambridge, UK). Fatty acids (SFA-PUFA) was determined by gas chromatography (Perkin Elmer, Clarus 500 USA) as described by (Taranu et al., 2018) and (Untea et al., 2012). Polyphenols were extracted in acetone 80% and methanol. Total polyphenols concentration was detected by using Folin-Ciocalteu method (Taranu et al., 2018).

Anti-nutrients such as intact glucosinolates and 3-butenyl isothiocyanate were extracted with water. After ultrasonication and centrifugation sample supernatants were diluted with water and injected into HPLC (Agilent Technologies 1200 Series, Morge, Switzerland) with G1315D DAD detector and G1316B TCC SL column thermostat. The chromatographic data were collected and processed using ChemStation software (version B.04.01, Waldbronn, Germany). The results were expressed as mg sinigrin/g dry sample. Sinigrin is a glucosinolate which was used as external standard.

***Measurement of molecular mediators of immune response***

Several important molecular mediators of immune response such as interleukine-1 beta (IL-1ß), interferon gamma (IFN-γ), interleukine-6 (IL-6), interleukine-8 (IL-8), tumour necrosis factor alpha (TNF-α) was measured in splenic lysates by ELISA using commercially kits (R & D Systems, Minneapolis, MN 55413, USA), according to the manufacturer's instructions. The lysates were prepared by homogenizing frozen spleen samples in buffer phosphate containing 1% IGEPAL, 0·5% sodium deoxycholate, 0.1% SDS and complete protease inhibitor cocktail tablets (EDTA-free). The supernatant obtained by centrifuging the homogenates was used to measure the total protein concentration with Pierce BCA Protein Assay Kit (Thermo Fisher Scientific). For the detection of interleukines in undiluted lysate supernatant, primary capture antibodies (anti-swine cytokines IL-1ß, IFN-γ, IL-6, IL-8, TNF-α) in conjunction with anti-swine biotinylated secondary antibodies, streptavidin-HRP (Biosource, Camarillo, USA) and TMB (tetramethylbenzidine, Sigma-Aldrich, S Louis, MO, USA) were used. Recombinant swine standard protein for IL-1ß, IFN-γ, IL-6, IL-8, TNF-α) diluted according to the manufacturer's instructions was used for standard curve generation. Results were expressed as picograms of cytokine/mL. The absorbance was measured using a Tecan microplate reader (Tecan, SunRise, Austria).

***Measurement of oxidative and antioxidative response***

Activity of antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GpX) was measured by using Cayman kits (Michigan, USA) as described by Taranu et al., (2022). Spleen samples (0.2g) were homogenized in specific chilled phosphate buffer indicated by the instruction of each Cayman kit, centrifuged and resulted supernatants were used for enzyme activity measurement. The absorbance was read at 540 nm (CAT), 440-460 nm (SOD) and 340 nm (GPx) using a microplate reader (Tecan Infinite M200, Salzburg, Austria). Lipid peroxidation was assessed by the determination of TBARS-MDA (thiobarbituric acid-reactive substances-malondialdehide). Briefly, spleen sample (0.2 g) homogenized with phosphate buffer was incubated at 95°C for 15 min to form TBARS adducts whose fluorescence was measured with a Tecan Sunrise, Austria and expressed as nmol/g spleen.

***Statistical analyses***

The results are presented as mean ± standard error of the mean (SEM). One-way ANOVA and *t-test* analysis (SAS Analytics, USA) followed by Fisher's procedure of the least square difference was used to measure the statistical differences between treatments. Differences were considered significant at P value <0.05 and were considered a trend at the P value between 0.05 and 0.1.

## RESULTS AND DISCUSSIONS

***Chemical composition of rapeseed meal***

Rape seed meal is a rich source of protein and other nutrients such as unsaturated lipids, fiber and phytochemicals (e.g., polyphenols, organic acids, vitamin E, vitamin complex B, minerals (Chen et al., 2019; Wickramasuriya et al., 2015). It is considered as the second source of protein in the world after soybean meal (Xue et al., 2009). In our study, the chemical analysis detected a content of 33.51% crude protein in unfermented rapeseed meal. It contained also fiber (8.38%) and is rich in bioactive compounds: minerals (iron, 208.03 ppm, zinc, 495.91 ppm, manganese, 174.86 ppm), polyphenols (85.38 mg GAE/g total polyphenols), unsaturated fatty acids (oleic acid, 44.78 g/100g of FAME, linoleic acid, 31.37 g/100 g of FAME). The fermentation with *Saccharomyces cerevisiae* enriched the rapeseed meal in crude protein (35.08% fermented *vs* 33.51% unfermented, +4.7%), fiber (11.89% fermented vs 8.38% unfermented, +41.9%), minerals: iron (266.38 ppm fermented *vs* 208.03 ppm unfermented, +28.1%), zinc (536.52 ppm fermented *vs* 495.91 ppm unfermented, +8.2%) and manganese (226.42 ppm fermented *vs* 174.86 ppm unfermented, +29.5%) as described by (I. Taranu, Marin, D.E., Pistol, G.C., Untea, A., Vlassa, M., Filip, M., Gras, M., Rotar, C., Anghel, A.C., 2022). The most important improvement that yeast fermentation brought in the composition of rapeseed meal was the decrease level of anti-nutrients such as glucosinolates. Elevated level of glucosinolates was one of the reasons for which rapeseed meal was less used in animal feed in the past (Mejicanos et al., 2016). Many studies have shown a reduction in animals' performance, an impairment in the immune response and internal organ functionality because of their toxicity (Drazbo et al., 2018; Drażbo et al. 2020; Hu et al., 2016; Onarman Umu et al., 2018). The new varieties of rapeseed (e.g. canola) or various biotechnological treatments (e. g. fermentation) have led to a decrease in the concentration of glucosinolates and their negative effects on animal health and performance. Our results showed a significant reduction in glucosinolates concentration (1.70 mg *vs* 3.70 mg sinigrin/g) and one of their most abundant classes, 3-butenil- glucosinolate (4.10 mg *vs* 7.99 mg/g) in *S. cerevisiae* fermented rapeseed meal. Similarly, 50% reduction in glucosinolates content and one third in the content of 4-hydroxy-glucobrassicin was reported by (Maribo, 2012) after fermentation of rapeseed cake and canola with a mix of bran, soy molasses, lactic acid bacteria potato peel and water (Maribo, 2012) resulting in an increase of lactic acid by 5.6% compared to unfermented rapeseed. Moreover, Plaipetch & Yakupitiyage (2013) found 100% reduction of total glucosinolates and 17.5% of phytic acid after fermentation of canola meal with *Saccharomyces cerevisiae.* Yeast fermentation enhanced crude protein (+9.2%), fiber (+9.2%) and several minerals (iron, +26.2%, zinc, +32.1%, Copper, 25.4%).

***Effect of FRSM diet on immune response mediators***

Taken into consideration the changes produced by fermentation on rapeseed meal we further investigated the effect of the diets with or without rape seed meal on several mediators of immune response in spleen as one of the most secondary important immune organs where the immune response is built (Jhun et al., 2013). It is the site of lymphocytes and monocytes/ macrophages producing antibodies and cytokines (Jia & Pamer, 2009) which are the effectors and mediators of the immune response and inflammation. Thus, in the study herein we analysed the effect of dietary rapeseed meal fermented or not on several inflammatory mediators such as interleukins in spleen tissue. Our results revealed a significant ($p = 0.05$) reduction in the concentration of interleukine-1 beta (IL-1β) and a decreasing tendency ($p = 0.098$) for IL-6 in spleen of piglets receiving fermented rapeseed meal diet in comparison to control diet (Figure 1). IL-1β and IL-6 secreted mainly by macrophages in different organs are significant mediators of inflammation and of tissue injury (Arend, 2002; Braunstein et al., 2020). IL-1β is one of the most important members of the interleukins family also involved in the modulation of the immune response by activating T and B lymphocytes (Cavaillon, 1996).

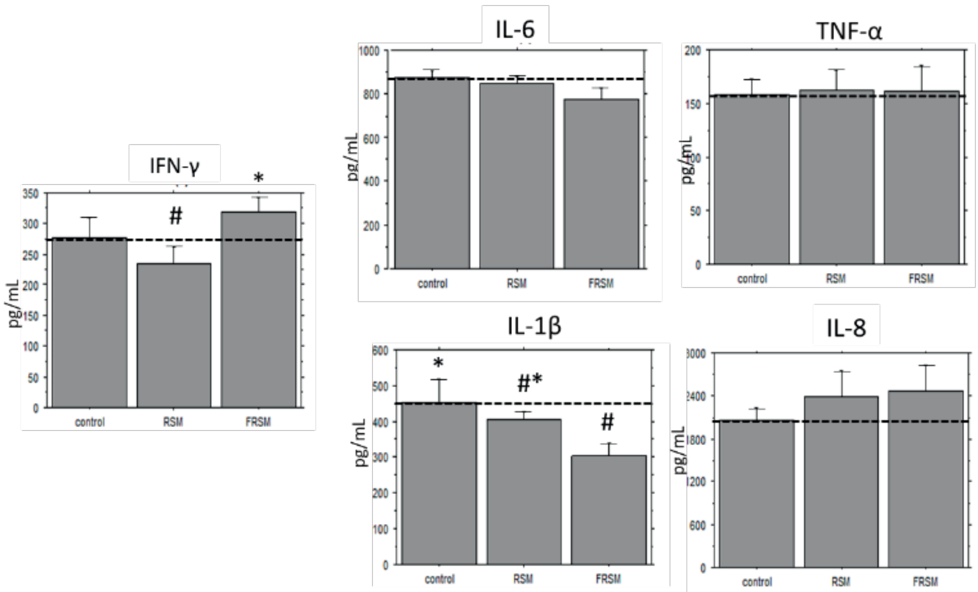


Figure 1. Effect of FRSM diet on inflammatory cytokines in spleen

*Pigs received three different dietary treatments: basal diet (control), RSM (unfermented rapeseed meal) and FRSM diet (fermented rapeseed meal). The means value ± SEM were calculated and presented as histogram (n = 8). Statistical analysis was performed using one-way ANOVA followed by Fisher test (*#P< 0.05)*

This result suggests that fermented rapeseed meal is able to counteract the transient inflamemation that might occur after weaning when piglets switch from the milk to solid feed and confront pathogenic infections. This might be due to the fact that fermentation modulated the level of different nutritional and anti-nutrients (decreased glucosinolates and increased minerals and n-6 and n-3 fatty acids, etc) with beneficial impact on animal health and could bring to the feed diet ß-glucans from the yeast wall. Municio et al. (2013) reported that differentiated macrophages form serum stimulated with ß-glucan produced low level of IL-1β and TNF-α (Municio et al., 2013). Also, an interesting study of (Liu et al., 2021) showed that phenethyl isothiocyanate, a degradation product of glucosinolates (GSLs) in a nontoxic dose (1.25-5 μM) was able to suppress the increase in pro-inflammatory cytokines (IL-1β, IL-6, IL-18, TNF-α) produced by 4 μM of deoxynivalenol (DON), a fusarium mycotoxin or by co-contamination with DON and *E. coli* -LPS. The study offered a basis for rational use of rapeseed meal in animal feed. Compared with unfermented rapeseed, FRSM diet significantly (p =

0.05) increased the concentration of IFN-γ, a cytokine with an essential role in antiviral and antibacterial defence being implicated in ROS production to respond in the case of infection (Awaad et al., 2011) as well as in the modulation of the immune response (Figure 1). An upward trend of IFN-γ level in FRSM group was also observed in comparison to the control, even if the difference was not significant. A slight non significantly increase in interleukin-8 (IL-8) concentration was noticed as well in both groups fed rapeseed meal fermented or not and no effect on TNF-α. Modulatory effects on immune mediators have been reported in other studies that have investigated the impact of feed sources rich in bioactive compounds. For example, active ingredients (e.g. proanthocyanidin) from grape seed and grape skin extract (GSSE) or cocoa extract diminished the Th17 cells and pro-inflammatory interleukin 17 (IL-17) in spleen (Bedhiafi et al., 2018) as well as the production of TNF-α, MCP-1, IL-6 and IL-8 in plasma and whole blood cells (Pérez-Cano et al., 2013). Moreover, grape proanthocyanidin significantly improved the weight and functions of important immune organs such as spleen and thymus and inhibited the growth of Sarcoma 180 tumour cells in mice (Tong et al., 2011).

***Effect of FRSM diet on oxidative and antioxidative response***

Beside the immune response, the antioxidant system is part of the body's defence which served to counteracts the harmful effect of excessive oxidants and include two main categories: enzymatic and non-enzymatic components (Birben et al., 2012). We further investigated the effect of dietary rapeseed meal either fermented or not on several antioxidant enzymes activity. Our results show a significant enhanced of superoxide dismutase and catalase activity in the spleen derived from piglets fed dietary fermented rapeseed meal compared to piglets fed control diet. The difference is also significant between fermented and unfermented groups (Figure 2). These results suggest that by increasing SOD and CAT activity the diet containing FRSM is able to counteract the accumulation of superoxide anion ($O^{2-\cdot}$ dismutation by SOD) and of hydrogen peroxide ($H_2O_2$ degradation by CAT), two major components of produced ROS (reactive oxygen species).

Although the activity of glutathione peroxidase, another antioxidant enzyme involved in the reduction of hydrogen peroxide and lipid peroxidation was not influenced by the FRSM diet, TBARS-MDA, a marker of lipid peroxidation decreased significantly in the spleen of piglets receiving FRSM diet. Compared to control this diet has a higher content of bioactive compounds (minerals, zinc and manganese, n-6, n-3 unsaturated fatty acids etc) that support the antioxidant system. Zinc for example is essential for the maintenance of redox homeostasis (Yi et al., 2022). Broiler chickens fed diet supplemented with 80mg/kg hot-melt extrusion (HME) processed zinc sulphate ($ZnSO_4$) had a higher SOD activity in serum and liver as well as reduced malonaldehyde (MDA) concentration compared to control (Lee et al., 2022). Unsaturated fatty acids, n-6 and n-3 are also recognised bioactive compounds with the capacity to modulate antioxidant system. (Avramovic et al., 2012; Lionetti et al., 2012; Taranu et al., 2014), demonstrated that n-3 PUFA supplements derived from camelina cakes, fish oil, or donkey's milk enhanced the activity of SOD, CAT, GPx, GST enzymes in spleen of pigs, brain tissue, and liver of rats and decreased MDA concentration. These studies indicated that the underlying molecular mechanism is based on the activation of nuclear factor 2 erythroid-related factor 2 (Nrf2) pathway and the inhibition of nuclear factor kappa B (La Marca et al., 2013; Lionetti et al., 2012), two important pathways which interfere in controlling the transcription of oxidative stress and inflammatory processes.

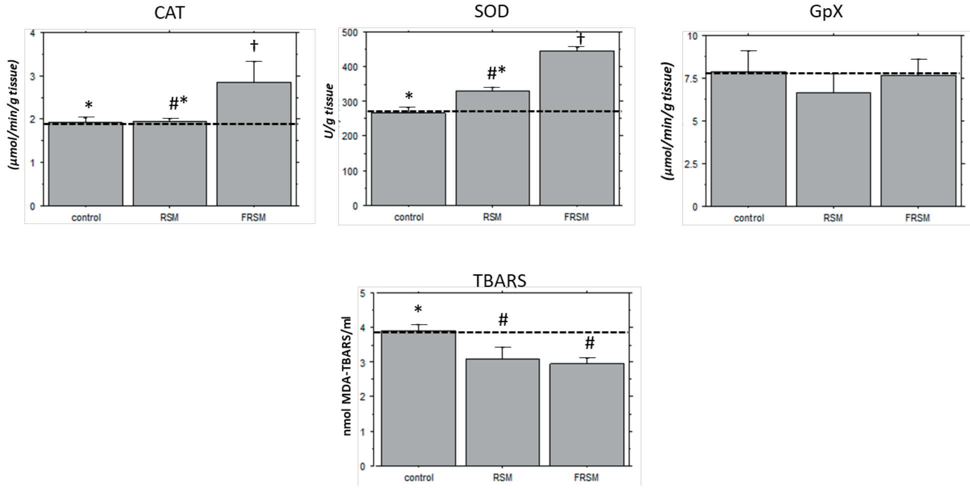


Figure 2. Effect of FRSM diet on oxidant and anti-oxidant markers

*Pigs received three different dietary treatments: basal diet (control), RSM (unfermented rapeseed meal) and FRSM diet (fermented rapeseed meal). The means value ± SEM were calculated and presented as histogram (n = 8). Statistical analysis was performed using one-way ANOVA followed by Fisher test (*#P< 0.05)*

## CONCLUSIONS

In conclusion, our results showed that fermentation is efficacy to reduce the level of anti-nutrients by decreasing the concentration of glucosinolates by half in fermented rapeseed meal compared to unfermented. It also enriched the rapeseed meal in several bioactive compounds such as minerals, (iron, zinc, manganese), unsaturated fatty acids (n-6 and n-3) and fiber. The presence of these compounds in the diet containing 10% rapeseed meal positively impacted the immune and antioxidant response in spleen of piglets after weaning. Our results revealed a significant reduction in the concentration of interleukine-1 beta and IL-6, important mediators of inflammation as well as an increase in the concentration of IFN-γ, a cytokine involved in the antiviral and antibacterial response whose strengthening is crucial during and after weaning when piglets are confronted with many challenges (feed and environmental change, pathogen infection). An increase in antioxidant SOD and CAT activity and a reduction in lipid peroxidation was found in spleen of piglets fed FRSM diet with suggest also the capacity of FRSM diet to counteract the oxidative stress. Further investigations are needed to see the effect of other dietary inclusion rates of fermented rapeseed meal on immune and antioxidant status.

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# THE FORAGE QUALITY OF TIMOTHY GRASS, *PHLEUM PRETENSE,* CULTIVAR 'TIROM' GROWN UNDER THE CONDITIONS OF THE REPUBLIC OF MOLDOVA

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***Abstract***

*Timothy grass, Phleum pratense, belongs to Poaceae family and is one of the most cultivated forage and pasture grasses in temperate regions. The aim of this study was to evaluate the forage quality of green mass and hay, silage and haylage prepared from timothy grass, Phleum pretense cv. 'Tirom', created at the Research-Development Institute for Grasslands, Braşov, and cultivated in the experimental plot of the "Alexandru Ciubotaru" National Botanical Garden (Institute), Chisinau. It has been determined that the dry matter of harvested timothy grass green mass contained 10.4-12.4% CP, 28.9-35.1% CF, 7.5-8.5 % ash, 31.4-36.8 % ADF, 49.5-58.9 % NDF, 3.6-4.1 % ADL, 27.8-37.4 % Cel, 18.1-27.7 % HC, 170-27.3 g/kg TSS, 56.9-61.4% DMD, 54.9-60.0% OMD, RFV=95-121, 11.91-12.60 MJ/kg DE, 9.78-10.38 MJ/kg ME, 5.81-6.42 MJ/kg NEl. The biochemical composition and nutritive value of prepared hay was: 9.3-12.2 % CP, 30.1-36.7% CF, 7.1-9.6 % ash, 33.6-38.4 % ADF, 54.1-62.1 % NDF, 3.7-4.3 % ADL, 29.9-38.3 % Cel, 20.5-26.3 % HC, 165-181 g/kg TSS, 52.8-56.9% DMD, 50.0-53.5% OMD, RFV=88-108, 11.69-12.36 MJ/kg DE, 9.60-10.18/kg ME, 5.62-61.7 MJ/kg NEl. The ensiled timothy grass fodder (silage, haylage) had pleasant color and smell, pH = 4.07-5.61, 1.6-6.9 g/kg acetic acid, 12.9-27.7g/kg lactic acid and free of butyric acid, 9.0-9.5 % CP, 6.7-8.4 % ash, 40.8-41.6 % ADF, 68.1-71.6 % NDF, 2.9-3.8 % ADL, 37.0-38.3 % Cel, 27.3-30.0 % HC, 65-131 g/kg TSS, 51.7-56.0% DMD, 46.7-46.9% OMD, 11.24-11.36 MJ/kg DE, 9.23-9.33 MJ/kg ME, 5.25-5.34 MJ/kg NEl.*

***Key words***: *biochemical composition, cv. 'Tirom', green mass, hay, haylage, nutritive value, Phleum pretense, silage, timothy grass.*

## INTRODUCTION

The human population on Earth is steadily growing, which leads to an increase in food and energy demands and aggravates the environmental challenges.

Grasslands have a wide range of ecological functions and are home to highly diverse, specialized ecosystems. *Poaceae* family has economic and ecologic importance. Grasses are represented in almost all ecosystems and are an important part of the natural food chain.

Grasslands are essential for feeding livestock, which then supply milk and meat to human populations. Meat and milk from domestic herbivores provide 16% and 8% of the global protein and kilocalorie consumption, respectively. They also provide a variety of essential micronutrients but can contribute to overweight and obesity when consumed in excess. Domestic herbivores also make significant contribution to food security through the production of manure, draught power and transport and the generation of income at household and national level. They have a key role to play in women's empowerment and gender equality, both in rural and urban areas (Mottet et al., 2018).

Livestock production has traditionally been based on forages as the primary feed, either as grazed grass or as conserved silage or hay. The forage quality is important for animal health, meat and milk production, its quality indices (Coșman et al., 2018).

The Plant List includes 154 scientific plant names of species rank for the genus *Phleum,* 18 of these are accepted species names, native to Europe, Asia, North Africa, North and South America. In the spontaneous flora of the Republic of Moldova, there are 4 species.

*Phleum pratense* L. (syn. *Phleum nodosum* L., *Phleum parnassicum* Boiss. & Heldr. ex Nyman*, Phleum praecox* Jord., *Plantinia pratensis* (L.) Bubani, *Stelephuros pratensis* (L.) Lunell) a cool-season hexaploid perennial, known as Timothy grass, is native to the Eurasian area. It is a plant of $C_3$ metabolic pathway for carbon fixation. It forms sperse tufts, grows 48-150 cm tall, the culms are erect and thicker at the base, the leaves are flat, 25-40 cm long and 6-10 mm wide, gradually narrowed, the ligule 3-7 mm long, slightly toothed, with three larger teeth; the sheaths – smooth, glabrous, with transverse striations. The inflorescence is a spike-like, cylindrical, dense panicle, 7-15 cm long, greenish in color, with spikelets containing a single flower, glumes 2-3 mm long, linearly elongated, truncated, with a long, rigidly ciliated keel, ending in an 1-2 mm long awn, the lower palea half as long as the glumes, translucent, truncated and slightly denticulated at the tip. It blooms in May-June, bears fruit in July-August. The seed – oval or convex caryopsis, covered by the lower palea, colorless, truncated at the tip and slightly dentate, finely porous on the veins, whitish-silver, the caryopsis is often glabrous. The seed is 1.5-2.0 mm long and 0.4-0.6 mm wide. The weight of 1000 seeds is 0.30-0.52 g. The seed yield is 600-800 kg/ha. The chromosome number 2n = 42. (Esser, 1993; Tran & Lebas, 2015; Țîței & Roșca, 2021).

It has been cultivated since the beginning of the 18th century and is one of the most important forage grasses in the temperate regions of the world. It is used in pasture mixtures on wetlands and for erosion control. *Phleum pratense* has the largest resistance to low temperatures compared to the other blades-grass. It has a high freezing resistance (Lemežine et al., 2004). It is sensitive on strong and prolonged drought and high temperatures, because of its root system's poor suction power.

It is well known that timothy (*Phleum pratense* L.), which is one of the most winter-hardy grasses (Nissinen, 1998), produces highly digestible forage with good conservation characteristics under cold and temperate conditions (Deinum et al., 1981). Under good management and soil conditions, the yield of timothy grass is comparable to other cool season grasses, although 70% of it is usually obtained in the first cutting (Lacefield et al., 1980).

In the Catalogue of Plant Varieties of the Republic of Moldova there are no registered varieties of *Timothy grass, Phleum pratense.*

The aim of this study was to evaluate the forage quality of green mass, prepared hay, silage and haylage from timothy grass, *Phleum pratense* cv. 'Tirom' grown under the conditions of the Republic of Moldova.

## MATERIALS AND METHODS

The cultivar 'Tirom' of timothy grass, *Phleum pratense* created in the Research-Development Institute for Grassland Brasov, Romania, 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 samples were collected in the second and third growing seasons, the first cut of timothy grass plants was done manually in May (flowering stage) and the second cut – in August. The prepared hay was dried directly in the field. The haylage was prepared from wilted biomass. For ensiling, the green and the wilted biomass was chopped into 1.5-2.0 cm pieces by using a forage chopping unit, shredded and compressed in well-sealed glass containers. The dry matter content was detected by drying samples up to constant weight at 105°C. After 45 days, the containers were opened, and the sensorial and fermented indices of conserved forage were determined in accordance with standard laboratory procedures, the Moldavian standard SM 108 for forage quality analysis. Some assessments of the main biochemical parameters: protein, 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 evaluated using the near infrared spectroscopy (NIRS) technique PERTEN DA 7200 at the Research-Development Institute for Grassland Brasov, Romania. The concentration of hemicellulose

(HC) and cellulose (Cel), relative feed value (RFV), digestible energy (DE), metabolizable energy (ME), net energy for lactation (NEl) were calculated according to standard procedures.

## RESULTS AND DISCUSSIONS

The seedlings of the cultivar 'Tirom' of timothy grass emerged at the soil surface 8-10 days after sowing, showing a high resistance to the frosts that occured in March and April. The young plants, during the period May-June, were characterized by optimal growth and development rates, they formed a tuft of vegetative shoots, and in the second half of July, in some plants, the appearance of generative shoots was also observed, and until the end of the growing season 10-15% of plants produced viable seeds. It was established that the fresh mass yield in the first season reached 2.88 kg/m$^2$, with a content of 16% dry matter and foliage of 64%.

In the second and following years, it starts growing in early spring, when the average temperature is above +5 °C. It is more vulnerable to soil moisture fluctuations, to conditions of atmospheric drought and prolonged heat as compared with other perennial grasses.

In the spring of the third year of growth, the weather conditions, characterized by high amount of rainfall and optimal air temperatures as compared with the previous year, helped the plants produce more shoots and were favorable for their growth, development and biomass production. We would like to mention that the cultivar 'Tirom' of timothy grass, in second growing season, at the first cut, reached 95.1 cm in height, but after regrowing, at the second cut – 54.3 cm in height. In the third growing season, the timothy grass plants at the first cut were taller – 108.9 cm. In the harvested biomass, in the second growing season, the leaf content was 47.3-66.7%, the amount of dry matter – 30.0-37.0%. In the third growing season, the first cut biomass contained 41.8% leaves and 35.3% dry matter. The green mass yield in the second growing season, reached 3.10 kg/m$^2$ at the first cut and 1.23 kg/m$^2$ at the second cut, but in the third growing season – 3.90 kg/m$^2$.

Several literature sources have described the productivity of timothy grass. According to Maruşca et al. (2011), the productivity of timothy grass cultivar 'Tirom' in Romania was 55-60 t/ha green mass or 14-15 t/ha dry matter and 600 kg/ha seeds. Esser (1993) mentioned that green mass of timothy grass, cut in the full bloom stage, was 4.0 tons/acre DM. Virkajärvi (2006) reported that, in Finland, the pastures of timothy grass yielded green mass containing 2000-3500 kg/ha dry mass and the proportion of leaves was 0.46-0.68. According to Berzins et al. (2015), under the agro-climatic conditions of Latvia, the annual dry matter yield of perennial grasses was: 6.72-8.07 t/ha *Phleum pratense,* 5.37-6.20 t/ha *Dactylis glomerata*, 4.86-5.98 t/ha *Lolium perenne*, 4.42-5.08 t/ha *Festuca pratensis* and 6.81-7.19 t/ha *Lolium*×*Festuca* hybrids.

The biochemical composition, nutritive and energy value of the green mass and hay from timothy grass*, Phleum pratense* cv. 'Tirom' in second growing season are presented in Table 1. Analysing the results of the biochemical composition of green mass, we found that the dry matter contained 113-124 g/kg CP, 289-315 g/kg CF, 75-85 g/kg ash, 314-338 g/kg ADF, 495-550 g/kg NDF, 36-39 g/kg ADL, 278-299 g/kg Cel, 181-212 g/kg HC. The dry matter obtained at the second cut had a high concentration of crude protein and ash, but a low concentration of cellulose, hemicellulose and lignin in green mass. Digestibility is the most important factor influencing nutritive and energy value, animal welfare and its productivity. The green mass of timothy grass harvested in the second growing season was characterized by 60.1-61.4% DMD, 59.3-60.0% DOM, RFV=106-121, 12.33-12.60 MJ/kg DE, 10.13-10.39 MJ/kg ME and 6.14-6.42 MJ/kg NEl. The nutritive and energy value were significantly higher in the green mass obtained at the second cut.

Analyzing the results regarding the quality of green mass from *Phleum pratense* cv. 'Tirom', in the third growing season, Table 2, we would like to mention that dry matter contained a low amount of crude protein and high amount of structural carbohydrates, lignin, which contributed to the reduction of digestibility, relative feed value and energy concentration as compared to the green mass harvested in the second growing season.

Table 1. The biochemical composition and nutritive value of green mass and hay from timothy grass, *Phleum pratense* cv. 'Tirom' in the second growing season

| Indices | First cut | | Second cut | |
|---|---|---|---|---|
| | green mass | hay | green mass | hay |
| Crude protein, g/kg DM | 113 | 98 | 124 | 122 |
| Crude fibre, g/kg DM | 315 | 354 | 289 | 301 |
| Ash, g/kg DM | 75 | 75 | 85 | 96 |
| Acid detergent fibre, g/kg DM | 338 | 376 | 314 | 336 |
| Neutral detergent fibre, g/kg DM | 550 | 612 | 495 | 541 |
| Acid detergent lignin, g/kg DM | 39 | 42 | 36 | 37 |
| Total soluble sugars, g/kg DM | - | - | 273 | 186 |
| Cellulose, g/kg DM | 299 | 334 | 278 | 299 |
| Hemicellulose, g/kg DM | 212 | 236 | 181 | 205 |
| Digestible dry matter, g/kg DM | 601 | 560 | 614 | 569 |
| Digestible organic matter, g/kg DM | 593 | 530 | 600 | 535 |
| Relative feed value | 106 | 91 | 121 | 108 |
| Digestible energy, MJ/kg | 12.33 | 11.80 | 12.66 | 12.36 |
| Metabolizable energy, MJ/kg | 10.13 | 9.69 | 10.39 | 10.18 |
| Net energy for lactation, MJ/kg | 6.14 | 5.71 | 6.42 | 6.17 |

Literature sources indicate considerable variation in the chemical composition and nutritional value of harvested *Phleum pratense* plants. Tingle& Elliott (1975) compared the yield and quality of several grass species and mentioned that *Phleum pratense* yielded 4.6 t/ha DM with 10.9% CP and 64.1% DMD, *Phleum bertolonii* 3.7 t/ha DM with 11.6% CP and 67.9% DMD, *Festuca rubra* 2.9 t/ha DM with 12.7% CP and 59.7% DMD, *Dactylis glomerata* 2.2 t/ha DM with 12.0 % CP and 65.3% DMD, *Phalaris arundinacea* 4.1 t/ha DM with 13.1 % CP and 61.8% DMD. Mason& Flipot (1988) mentioned that timothy grass cultivars harvested for the first time in the flowering stage contained 84-99 g/kg CP, 401-423 g/kg ADF, 662-695 g/kg NDF, 53-59 g/kg ADL with 50.8-53.2 % IVDMD, but the regrown forage – 125-184 g/kg CP, 319-351 g/kg ADF, 568-607 g/kg NDF, 38-45 g/kg ADL, 62.2-64.9 % IVDMD, respectively. According to Burlacu et al. (2002), the nutritive composition of first cut timothy grass was 200-260 g/kg DM, 7.0-7.8% ash, 10.5-11.3% CP, 3.0-3.5 % fats, 29.5-29.6% CF, 47.0-47.8% NFE, 3.0% ADL, 30.2% Cel, 20.2% HC, 18.4 MJ/kg GE, but second and third cut – 190-270 g/kg DM, 8.0-1.1% ash, 10.0-160% CP, 3.5-4.5 % fats, 24.5-31.5% CF, 44.0-47.0% NFE, 18.2-18.3 MJ/kg GE. Esser (1993) mentioned that the green mass of timothy grass contained 7.2% CP, 2.57% EE, 28.3% CF, 5.13% ash, 0.37% Ca, 0.20% P and 29.8 mg/kg carotene. Wang et al. (2014), reported that the dry matter content and the nutritive value of harvested mass of *Phleum pratense* were: 124-133 g/kg DM, 136-174 g/kg CP, 35-46 g/kg fat, 323-360 g/kg ADF, 581 g/kg NDF, 61-83 g/kg WSC, 20.2-20.3 MJ/kg GE. Hetta et al. (2003) remarked that the forage quality of pure timothy first-cut green mass was 182 g/kg DM, 12.4% CP, 54.5% NDF, 9.2% WSC and 11 MJ/kg ME, but – of second-cut green mass – 219 g/kg DM, 13.7% CP, 52.0% NDF, 9.2% WSC and 11 MJ/kg ME, respectively. Tran & Lebas (2015) revealed that *Phleum pratense* fresh aerial part contained 27 % DM, 13.89% CP, 2.2 % EE, 31.8 % CF, 62.2 % NDF, 34.2 % ADF, 4.6 % lignin, 8.0 % ash, 1.4 g/kg Ca and 2.1 g/kg P, 66.7% ODM, 18.2 MJ/kg GE, 11.6 MJ/kg DE, 9.6 MJ/kg ME. Janković et al. (2018) found that the tested timothy grass population grown under the climatic conditions of Serbia contained 13.20-14.52% CP and 24.30-26.98% CF, and are available to us for a successful selection process in order to obtain new varieties of *Phleum pratense*. Karbivska et al. (2020) reported that *Phleum pratense* plants without fertilizers contained 10.7% CP, 2.8 % fats, 28.8% CF, 51.35% NFE, 7.4 % ash, 0.40% Ca, 0.25% P, 58% DMD, 0.7 fodder units/kg DM, 8.1 MJ/kg ME and 109 g digestible protein/fodder unit, but *Phleum pratense* fodders when applying $N_{90}P_{60}K_{60}$ contained 14.2% CP, 2.9 % fats, 29.0% CF, 45.9% NFE, 7.4 % ash, 0.45% Ca, 0.27% P, 59% DMD, 0.71 fodder units/kg DM, 8.3 MJ/kg ME and 144 g digestible protein/fodder unit.

Hay represents a low-cost and abundant source of nutrients, remains one of the main fodders in the diets of animals, as it helps the normal functioning of the stomach and intestines. It is the only roughage containing vitamin D, which regulates mineral metabolism in animal organism, and is vital to keep animals healthy and performance. We would like to mention that the hay prepared from timothy grass, *Phleum pratense* cv. 'Tirom' (Tables 1, 2) contained 93-122 g/kg CP, 301-367 g/kg CF, 71-96 g/kg ash, 336-384 g/kg ADF, 541-621g/kg NDF, 37-43 g/kg ADL, 299-383 g/kg Cel, 205-263 g/kg HC and 165-181 g/kg TSS. The digestibility, nutritive value and the energy value of the timothy hay were 52.8-56.9% DMD, 50.0-53.5% DOM, RFV=88-108, 11.69-12.36 MJ/kg DE, 9.60-10.1 MJ/kg ME and 5.62-6.17 MJ/kg NEl. During the process of preparing hay, we observed an increase in the concentration of structural carbohydrates, lignin, ash and a decrease in the crude protein and total soluble sugar content, dry matter digestibility and relative feed value and energy concentration as compared to harvested green mass. The timothy hay prepared from second cut green mass is characterized by high content of crude protein and ash, but optimal cell wall concentration, nutritive and energy value. The amounts of crude fibre, cellulose, hemicellulose and lignin increased significantly in the hay obtained in the third year, which had a negative effect on relative feed value and energy concentration.

Some authors mentioned various findings about the quality of timothy hay. According to Udén & Soest (1982), the chemical composition of timothy (*Phleum pratense*) hay was: 12 g/kg nitrogen, 671 g/kg cell walls, 291 g/kg cellulose, 292 g/kg hemicellulose, 84 g/kg lignin. Petit et al. (1985) revealed that the biochemical composition of timothy hay prepared from herbage harvested during head emergence stage were 6.2% ash, 10.4% CP, 2.90% EE, 33.2% CF, 47.3% NFE, 62.7% NDF, 39.4% ADF, 4.4% ADL, 23.3% HC, 35.0% Cel. Maeta et al. (1992) mentioned that first cut timothy hays prepred in June contained 5.4-8.2% ash, 5.4-10.2% CP, 66.2-75.6 % NDF, 35.1-43.3% ADF, 4.40-4.50 kcal/g, but hays prepred in July contained 5.3-5.6 % ash, 5.4-5.9% CP, 69.4-75.6 % NDF, 39.1-44.2% ADF, 4.21-4.53 kcal/g. Burlacu et al. (2002) reported that first cut *Phleum pratense* hay contained 6.8-7.8% ash, 8.6-10.5% CP, 2.5-2.8% EE, 32.8-34.8% CF, 46.1-47.3% NFE, 32.9-42.1% ADF, 30.6-32.3% Cel, 3.6-8.1% ADL, 18.3-18.5 MJ/kg GE, but second and third cut hay – 6.8-7.8% ash, 7.5-10.5% CP, 2.5-3.5 % EE, 27.5-34.5% CF, 44.0-46.5% NFE, 40.2 % ADF, 33.9% Cel, 6.1% ADL, 18.2 MJ/kg GE.

Table 2. The biochemical composition and nutritive value of green mass and hay from timothy grass, *Phleum pratense* cv. 'Tirom' in the third growing season

| Indices | First cut | |
|---|---|---|
| | green mass | hay |
| Crude protein, g/kg DM | 104 | 93 |
| Crude fibre, g/kg DM | 351 | 367 |
| Ash, g/kg DM | 75 | 71 |
| Acid detergent fibre, g/kg DM | 368 | 384 |
| Neutral detergent fibre, g/kg DM | 589 | 621 |
| Acid detergent lignin, g/kg DM | 41 | 43 |
| Total soluble sugars, g/kg DM | 170 | 165 |
| Cellulose, g/kg DM | 374 | 383 |
| Hemicellulose, g/kg DM | 277 | 263 |
| Digestible dry matter, g/kg DM | 569 | 528 |
| Digestible organic matter, g/kg DM | 549 | 500 |
| Relative feed value | 95 | 88 |
| Digestible energy, MJ/kg | 11.91 | 11.69 |
| Metabolizable energy, MJ/kg | 9.78 | 9.60 |
| Net energy for lactation, MJ/kg | 5.81 | 5.62 |

Table 3. The fermentation quality, biochemical composition and nutritive value of silage and haylage from timothy grass*, Phleum pratense* cv. *'Tirom'* in the third growing season

| Indices | Silage | Haylage |
|---|---|---|
| pH index | 4.07 | 5.61 |
| Content of organic acids, g/kg | 42.5 | 17.7 |
| Free acetic acid, g/kg | 1.7 | 0 |
| Free butyric acid, g/kg | 0 | 0 |
| Free lactic acid, g/kg | 6.2 | 3.2 |
| Fixed acetic acid, g/kg | 6.9 | 1.6 |
| Fixed butyric acid, g/kg | 0 | 0 |
| Fixed lactic acid, g/kg | 27.7 | 12.9 |
| Crude protein, g/kg DM | 90 | 95 |
| Crude fibre, g/kg DM | 394 | 391 |
| Ash, g/kg DM | 84 | 67 |
| Acid detergent fibre, g/kg DM | 416 | 408 |
| Neutral detergent fibre, g/kg DM | 716 | 681 |
| Acid detergent lignin, g/kg DM | 29 | 38 |
| Total soluble sugars, g/kg DM | 65 | 131 |
| Cellulose, g/kg DM | 387 | 370 |
| Hemicellulose, g/kg DM | 300 | 273 |
| Digestible dry matter, g/kg DM Digestible | 560 | 517 |
| organic matter, g/kg DM | 469 | 467 |
| Relative feed value | 73 | 78 |
| Digestible energy, MJ/kg | 11.24 | 11.36 |
| Metabolizable energy, MJ/kg | 9.23 | 9.33 |
| Net energy for lactation, MJ/kg | 5.25 | 5.34 |

Muller & Uden (2007) mentioned that the herbage from a permanent grassland consisting of timothy grass, *Phleum pratense*, meadow fescue, *Festuca pratensis*, and a small proportion (0.1) of couch grass, *Agropyron repens* contained: 352 g/kg DM, 5.9% ash, 10.8% CP, 7.0% DP, 59.0% NDF, 12.4% WSC, 74.6 % DOM and 10.1 MJ/kg ME for horses, but the prepared hay – 884 g/kg DM, 6.4% ash, 10.8% CP, 7.0% DP, 60.5% NDF, 10.1% WSC, 77.0 % DOM and 9.8 MJ/kg ME for horses, respectively. Tran& Lebas (2015) revealed that *Phleum pratense* hay contained 88 % DM, 9.1% CP, 2.3 % EE, 35.6% CF, 65.4 % NDF, 37.8 % ADF, 4.4 % lignin, 6.6 % ash, 1.6% starch, 11.5% TS, 7.5%WSC, 58.5% ODM, 18.1 MJ/kg gross energy, 10.0 MJ/kg DE, 8.0 MJ/kg ME

The management of forage as silage provides the opportunity to harvest the crop at a desired level of digestibility for subsequent feeding. Silage production minimizes the risk associated with field losses, which can be incurred under rainy conditions during hay making. Wilting herbage prior to ensiling has many advantages including reducing effluent production and fuel consumption, improved characteristics of ensiling, reduced quantities of silage for transport during feed out and reduced straw requirement for bedding livestock. Grass, when harvested and stored as silage and haylage, is an important source of nutrients for livestock, is a great way of preserving nutrients for autumn - middle spring, a period when grasslands are less productive. When opening the glass vessels with fermented fodder, silage and haylages, prepared from first cut timothy grass*, Phleum pratense* cv. 'Tirom'*,* in the third growing season, there was no gas or juice leakage from the preserved mass. The ensiled fodder had pleasant colour and smell, the consistency was retained, in comparison with the initial green mass, without any mould and mucus. During the sensorial assessment, it was found that the prepared timothy silage had homogeneous yellow colour with pleasant smell, like pickled vegetables, but haylage – was light brown leaves and yellow stems with pleasant specific smell of pickled fruits.

The fermentation quality, biochemical composition and nutritive value of silage and haylage from timothy grass*, Phleum pratense* cv. 'Tirom' are shown in Table 3. It has been determined that the pH index was 4.07-5.61, the concentrations of organic acids reached 17.2-42.5 g/kg, and most amounts of organic acids were in fixed form. Butyric acid was not detected in the fermented fodder. The high content of organic acids, inclusive lactic acid was in silage. Analyzing the results regarding the quality of fermented timothy forage in the third growing season, we found that the dry matter was characterized by 90-95 g/kg CP, 391-394 g/kg CF, 67-84 g/kg ash, 408-416 g/kg ADF, 681-716 g/kg NDF, 29-38 g/kg ADL, 65-131 g/kg TSS, 370-387 g/kg Cel, 273-300 g/kg HC, with digestibility, nutritive and energy value 51.7-56.0% DMD, 46.7-46.9% DOM, RFV=73-78, 11.24-11.36 MJ/kg DE, 9.23-9.33 MJ/kg ME and 5.25-5.34 MJ/kg NEl. There was a significantly higher content of crude protein, total soluble sugars and low concentration of ash and structural carbohydrates in the prepared timothy haylage. According to Petit et al. (1985), the nutrient concentration and fermentation characteristics of timothy silage were 419 g/kg DM, 6.6% ash, 12.7% CP, 3.48% EE, 42.7% NFE, 59.8% NDF, 39.8% ADF, 3.9% ADL, 20.0% HC, 35.9 % Cel with pH=4.7, 1.8 % lactic acid, 0.36 % acetic acid, 0.18% butyric acid. Narasimalu et al. (1989) mentioned that the dry matter content and chemical composition of timothy first cut silage were 319-337 g/kg DM, 16-21 N g/kg, 599-629 g/kg NDF, 368-371g/kg ADF, 38-39 g/kg ADL. Burlacu et al. (2002), reported that timothy silages contained: 200-220 g/kg DM, 7.3-8.0% ash, 10.0-11.2% CP, 3.3-4.0 % EE, 25.4-32.4% CF, 47.0-51.4% NFE, 18.4-18.8 MJ/kg GE, but haylage (wilting silage) 35.0% DM, 7.5-8.6% ash, 10.1-11.5% CP, 3.2-3.3% EE, 26.0-31.7% CF, 47.5-49.2% NFE, 18.3-18.4 MJ/kg GE. Hetta et al. (2003) mentioned that ensiled timothy first-cut fresh mass contained 168 g/kg DM, 12.4% CP, 52.7% NDF, 0.5% WSC, pH=4.64, 3.17% lactic acid, 2.88% acetic acid, 2.34% butyric acid, but ensiled timothy with lactic acid bacteria and molasses contained 187 g/kg DM, 14.1% CP, 39.6% NDF, 2.8% WSC, pH=3.92, 12.8% lactic acid, 0.9% acetic acid, 0.1% butyric acid. Muller & Uden (2007) compared the quality of conserved grass from permanent grassland consisting of timothy grass, *Phleum pratense*, meadow fescue, *Festuca pratensis*, and a small proportion (0.1) of couch grass, *Agropyron repens*, and found that the prepared silage had pH=4.94, 31.8 g/kg lactic acid, 6.6 g/kg acetic acid, 1.1 g/kg butyric acid, 309 g/kg DM, 11.3% CP, 7.4%DP, 6.6% ash, 58.5% NDF, 2.6% WSC, 77% DOM, 9.7 MJ/kg ME for horses; the haylage had pH=5.63 and contained 2.6 g/kg lactic acid, 1.4 g/kg acetic acid, 0.4 g/kg butyric acid, 577 g/kg DM, 10.8% CP, 7.2%DP, 6.4% ash, 60.8% NDF, 6.9% WSC, 74% DOM, 9.4 MJ/kg ME for horses. Ragnarsson & Lindberg (2008) reported that the dry matter content and the chemical composition of early-cut timothy haylage (stem elongation to flowering) were 469 g/kg DM, pH=5.6, 42 g/kg lactic acid, 1.1 g/kg acetic acid, 0.1 g/kg butyric acid, 17.5% CP, 25.6% CF, 8.2% ash, 4.1% EE, 6.2% sugars, 50.3.8% NDF, 29.9% ADF, 2.0% ADL, 19.3 MJ/kg GE. Kuoppala et al. (2010) remarked that the chemical composition and the feed value of silage prepared from primary growth cut timothy grass (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) were: 283 g/kg DM, 6.8-8.2% ash, 12.7-15.5% CP, 49.8-58.9% NDF, 5.0-9.7% iNDF, 44.8-49.2 pdNDF, 2.3-2.7% lignin, 3.09-15.0% WSC, pH=3.97-4.22, 6.84% lactic acid, 1.31-1.74% acetic acid, 0.13-0.48% butyric acid with 64.4-70.4% DOM and 10.3-11.3 MJ/kg ME, but the silage prepared from regrown plants 227-334 g/kg DM, 9.0-9.82% ash, 11.6-15.7% CP, 51.3-53.9% NDF, 6.0-9.3% iNDF, 44.3-45.3 pdNDF, 2.2-2.8% lignin, 3.06-8.61% WSC, pH=3.92-4.30, 3.09-6.50% lactic acid, 1.12-1.55% acetic acid, 0.04-0.09% butyric acid with 60.9-66.4% DOM and 9.7-10.6 MJ/kg ME, respectively. Wang et al. (2011) revealed that the fermentation quality and chemical composition of timothy silages were: pH=4.46-4.75, 15.9-35.3 g/kg lactic acid, 24.7-36.9 g/kg acetic acid, 0.1-0.3 g/kg butyric acid 134-138 g/kg DM, 134-171 g/kg CP, 99.6-135.1 g/kg DCP, 54-57 g/kg fat, 359-384 g/kg ADF, 564-612 g/kg NDF, 16-18 g/kg WSC, 20.2-20.3 MJ/kg GE, 13.3-15.1 MJ/kg DE. Tahir et al. (2013) mentioned that timothy silage from early-cut mass contained: 238 g/kg DM, 58 g/kg ash, 167 g/kg CP, 27 g/kg EE, 482 g/kg NDF, 3 g/kg starch, 2 g/kg WSC, 204 g/kg NFC, pH=4.0, 55 g/kg lactic acid, 16 g/kg acetic acid, 80.5% OMD, 10.3-11.3 MJ/kg ME, but the silage prepared from late-cut mass – 286 g/kg DM, 62 g/kg ash, 129 g/kg CP, 18 g/kg EE, 587 g/kg NDF, 408-410 g/kg ADF, 3 g/kg starch, 3 g/kg WSC, 286 g/kg NFC, pH=3.7, 83g/kg lactic acid, 23 g/kg acetic acid, 69.9% OMD, 10.2 MJ/kg ME. Tran & Lebas (2015) revealed that *Phleum pratense* silage contained

30.8% DM, 13.9% CP, 34.6% CF, 56.2 % NDF, 36.9% ADF, 3.7 % lignin, 8.2 % ash, 7.9% TS, 6.2%WSC, 64.2% ODM, 18.2 MJ/kg gross energy, 11.1 MJ/kg DE, 8.9 MJ/kg ME. Huuskonen&Pesonen (2017) found that the first cut timothy silage contained 222 g/kg DM with 94.5% OM and 70% DOM, 15.2% CP, 3.5% EE, 59.2% NDF, 6.5% WSC, pH=3.90, 4.9% lactic+formic acids, 11.2 MJ/kg ME; the second cut silage contained 326 g/kg DM with 93.2% OM and 68.5% DOM, 14.7% CP, 3.45% EE, 53.3% NDF, 11.5% WSC, pH=4.26, 3.7% lactic+formic acids, 11.0 MJ/kg ME; the third cut silage contained 314 g/kg DM with 91.7% OM and 74% DOM, 18.6% CP, 3.20% EE, 44.0% NDF, 14.8% WSC, pH=4.56, 3.2% lactic+formic acids, 11.0 MJ/kg ME. Müller &. Johansen (2020) remarked that the quality of haylage consisting predominantly of *Phleum pratense* with the presence of *Lolium perenne* and *Festuca pratensis*, conserved in big round bales, was as follows: 556 g/kg DM, 8.9% CP, 60.0% aNDFom, 6.3% ash, 12.0% WSC, 73.5% IVDOM, pH=5.61, 2.4% lactic acid, 1.1 % acetic acid and 9.2 MJ/kg ME for horses. Richard et al. (2020) compared the feed quality and energy value of silage and mentioned that timothy silage contained 313 g/kg DM, 916 g/kg OM, 154 g/kg CP, 583 g/kg aNDF, 385 g/kg ADF, 86.3% IVTD, 1.23 Mcal/kg NEl, but tall fescue silage – 341 g/kg DM, 903 g/kg OM, 136 g/kg CP, 543 g/kg aNDF, 353 g/kg ADF, 86.13% IVTD, 1.26 Mcal/kg NEl.

## CONCLUSIONS

The harvested green mass from timothy grass, *Phleum pretense* cv. 'Tirom', contained 30.0-37.0% dry matter, with biochemical composition, nutritive and energy value: 10.4-12.4% CP, 28.9-35.1% CF, 7.5-8.5 % ash, 31.4-36.8 % ADF, 49.5-58.9 % NDF, 3.6-4.1 % ADL, 27.8-37.4 % Cel, 18.1-27.7 % HC, 170-27.3 g/kg TSS, 56.9-61.4% DMD, 54.9-60.0% OMD, RFV=95-121, 11.91-12.60 MJ/kg DE, 9.78-10.38 MJ/kg ME, 5.81-6.42 MJ/kg NEl.

The biochemical composition and the nutritive value of the prepared hay were: 9.3-12.2 % CP, 30.1-36.7% CF, 7.1-9.6 % ash, 33.6-38.4 % ADF, 54.1-62.1 % NDF, 3.7-4.3 % ADL, 29.9-38.3 % Cel, 20.5-26.3 % HC, 165-181 g/kg TSS, 52.8-56.9% DMD, 50.0-53.5% OMD, RFV=88-108, 11.69-12.36 MJ/kg DE, 9.60-10.18 kg ME, 5.62-61.7 MJ/kg NEl.

The ensiled fodder (silage, haylage) of timothy grass cv. 'Tirom' had pleasant color and smell, pH = 4.07-5.61, 1.6-6.9 g/kg acetic acid, 12.9-27.7 g/kg lactic acid and free of butyric acid, 9.0-9.5 % CP, 6.7-8.4 % ash, 40.8-41.6 % ADF, 68.1-71.6 % NDF, 2.9-3.8 % ADL, 37.0-38.3 % Cel, 27.3-30.0 % HC, 65-131 g/kg TSS, 51.7-56.0% DMD, 46.7-46.9% OMD, 11.24-11.36 MJ/kg DE, 9.23-9.33 MJ/kg ME, 5.25-5.34 MJ/kg NEl.

Under the climatic conditions of the Republic of Moldova, cv. 'Tirom' of timothy grass, *Phleum pratense,* has optimal productivity and biochemical composition, can be used to re-cultivate permanent grasslands and to establish temporary grasslands in order to help prevent soil erosion, in monoculture or associated with other grasses and forage legumes, and the harvested mass can be fed to the livestock as fresh green mass, hay, silage and haylage.

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# THE QUALITY OF GREEN MASS AND THE SILAGE FROM PEARL MILLET, *PENNISETUM GLAUCUM*, GROWING UNDER THE CONDITIONS OF THE REPUBLIC OF MOLDOVA

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***Abstract***

*Pearl millet, Pennisetum glaucum (L.) R. Br.] is a $C_4$ climate-resilient plant species, the sixth most important cereal crop of the world, has great potential as grain and multi-purpose forage for arid and semi-arid ecosystems. We studied some agrobiological peculiarities, the concentration of nutrients in green mass and silage prepared from pearl millet, Pennisetum glaucum, grown in an experimental field of the National Botanical Garden (Institute), Chişinău. It was established that the pearl millet plants harvested in the flowering period contained 200 g/kg dry matter, its biochemical composition was:10.19% crude protein, 3.11 % crude fats, 31.61% crude cellulose, 40.28% nitrogen free extract, 5.45% soluble sugars 1.79 g/kg starch, 14.80% ash, 6.0 g/kg calcium 3.9 g/kg phosphorus and 50.0 mg/kg carotene. The quality of the prepared silage was: pH= 4.08, 25.0 g/kg lactic acid, 7.3 g/kg acetic acid, butyric acid – not detected, 7.42 % crude protein, 3.87 % crude fats, 30.56% crude cellulose, 47.29% nitrogen free extract, 1.55% soluble sugars 1.19 g/kg starch, 10.86% ash, 4.2 g/kg calcium 2.7 g/kg phosphorus and 28.0 mg/kg carotene.*

***Key words**: biochemical composition, green mass, pearl millet, Pennisetum glaucum, silage.*

## INTRODUCTION

Demand for animal-source foods is increasing because of population growth, rising incomes and urbanization. The global human population is estimated to reach 9.6 billion in 2050, with about 70% living in urban areas, whereas incomes are expected to increase by 2% a year. As a consequence, the growth of the livestock sector is expected to continue in the coming decades. The global meat and milk production growth was possible due to significant increases in livestock numbers but also to productivity growths. Most of the meat and milk from domestic herbivores is produced by cattle, with 20% of meat and 83% of milk; buffaloes produce 1% of global meat production and 13% of milk; small ruminants have a smaller contribution, with 5% of meat and 4% of milk (Mottet et al., 2018). Forages are the major part of the diet of ruminant animals and provide energy, proteins, minerals, vitamins.

Climate change affects crop production by directly influencing biophysical factors such as plant and animal growth along with the various areas associated with food processing and distribution. Assessment of the effects of global climate changes on agriculture can be helpful to anticipate and adapt farming to maximize the agricultural production more effectively. The incorporation of neglected and underused crops, the domestication of new species would promote agricultural diversity and could provide a solution to many of the problems associated with food security, nutrition, healthcare, medicine and industrial needs. It has been well established that the plant species with $C_4$ photosynthesis type can easier face the adverse effects of high temperature, water insufficiency and salinity stress, besides such crops have the potential to maintain productivity, increase income and food security of farming communities in semiarid and arid regions. Millets are especially gaining popularity due to their high resilience to climate change effects and acceptable productivity and nutritional value (Jukanti et al., 2016).

Pearl millet, *Pennisetum glaucum* (L.) R. Br., *Poaceae* family (syn. *Pennisetum americanum, Pennisetum typhoides, Pennisetum typhoideum, Pennisetum spicatum, Setaria glauca*) is cultivated extensively in the Indian subcontinent and African semiarid regions since prehistoric times. Currently, Pearl millet is the sixth most important cereal crop after rice, wheat, maize, barley and sorghum in the world with over 33 million hectares, accounts for approximately 50 % of the total world production of millets and it is a crop of major importance in arid and semi-arid regions. Pearl millet or cattail millet is a robust, strongly tillering, annual, herbaceous, grass plant, usually 1-4 m tall, with basal and nodal tillering, producing an extensive and dense root system, which may reach a depth of 1.2-1.6 m, sometimes even of 3.5 m; sometimes the nodes near ground level produce thick, strong prop roots. The stem is slender, 1-3 cm in diameter, solid, often densely villous below the panicle, with prominent nodes. The leaf sheath is open and often hairy; the ligule is short, membranous, with a fringe of hairs; the leaf blade is linear to linear-lanceolate, up to 1.5 m × 5-8 cm, and has margins with small teeth, scaberulous and often pubescent. The inflorescence is cylindrical or ellipsoidal, contracted, with a stiff and compact panicle, similar to a spike, 15-200 cm long. The spikelet is 3-7 mm long, consisting of 2 glumes and usually 2 florets. The caryopsis is globose, subcylindrical or conical, 2.5-6.5 mm long, the colour varies from white, pearl, or yellow to grey-blueish and brown, occasionally purple (Oyen & Andrews, 1996; Marsalis et al., 2012; Sharma et al., 2021). Pearl millet is drought- and heat-tolerant and has a considerable ability to grow and yield in poor, sandy and saline soils under arid, hot, and dry climates; this is an advantage over other popular forage grasses in the region, such as fodder maize. It is also a hydrocyanic and prussic acid-free crop, which gives it nutritional superiority over sorghum species (Hassan et al., 2014; Jukanti et al., 2016).

The aim of this study was to evaluate some biological peculiarities, the biochemical composition and the fodder value of green mass and silage from pearl millet, *Pennisetum glaucum.*

## MATERIALS AND METHODS

The introduced ecotype of pearl millet, *Pennisetum glaucum*, which was cultivated in the experimental plot of the National Botanical Garden (Institute) Chişinău, N 46°58′25.7″ latitude and E 28°52′57.8″ longitude, served as subject of the research, and the traditional fodder crops – corn, *Zea mays* and sudangrass, *Sorghum sudanense* were used as control variants. The pearl millet and sudangrass green mass samples were collected in full flowering period, while the corn – in the kernel milk-wax stage. The leaf/stem ratio was determined by separating leaves and flowers from the stem, weighing them separately and establishing the ratios for these quantities. For this purpose, samples of 1.0 kg harvested plants were taken.

The dry matter content was detected by drying samples up to constant weight at 105 °C. For chemical analyses, the samples were dried at 65 ± 5 °C. For ensiling, the green mass was shredded and compressed in well-sealed containers. After 45 days, the containers were opened, the organoleptic assessment was carried out. The content of crude protein (CP), crude fats (EE), crude cellulose (CF), nitrogen free extract (NFE), soluble sugars (TSS), starch, ash, calcium (Ca), phosphorus (P), carotene, lactic, acetic and butyric acids, silage pH index, nutritive units and metabolizable energy were appreciated in accordance with standard laboratory procedures at the Institute of Biotechnology in Animal Husbandry and Veterinary Medicine, Maximovca.

## RESULTS AND DISCUSSIONS

Analyzing the results of the assessment of agro-biological peculiarities, it can be noted that pearl millet, *Pennisetum glaucum* seedlings emerged on the soil surface 3-9 days after sowing. For uniform germination, pearl millet needs higher soil temperature, 14-15°C, than traditional fodder crops: corn and sudangrass. Compared to sudangrass, pearl millet has the potential to produce many effective tillers that may expand on large areas. The colour of the investigated pearl millet plants is deep purple. In the full flowering period, the *Pennisetum glaucum* plants were shorter than *Sorghum sudanense* plants, but the stems were four times thicker and thus had a positive impact on tiller mass. The yield of *Pennisetum glaucum* plants harvested in full flowering period reached 5.65 kg/m$^2$ green mass or 1.17 kg/m$^2$ dry matter, with 25.2 % leaves, 58.1 % stems and 16.7 % panicles, but the yield of *Sorghum sudanense* was 4.58 kg/m$^2$ green mass or 0.83 kg/m$^2$ dry matter with 26.3 % leaves, 64.4 % stems and 9.3 % panicles. The biomass productivity of *Zea mays* harvested in kernel milk-wax period was 5.88 kg/m$^2$ green mass or 1.81 kg/m$^2$ dry matter.

Several literature sources have described the productivity of *Pennisetum glaucum* plants. According to Medvedev& Smetannikova (1981), in the Kuban region of Russia, the green mass productivity of *Pennisetum glaucum* var. *aristatum* was 40.5-51.0 t/ha, but *Pennisetum glaucum* var, *inermis* yielded 34.0-43.0 t/ha. Shashikala et al. (2016) found that fodder yield potential of multicut pearl millet genotypes were 55.2-81.1 t/ha green mass, 13.3-27.7 t/ha dry matter and 1.39-3.04 t/ha crude protein. Toderich et al. (2016) reported that, in some marginal lands of Central Asia, the productivity of pearl millet ranged from 42.23 to 45.12 t/ha green mass at the first cut and 27.18-31.23 t/ha green mass at the second cut, respectively, the total annual aboveground dry matter varied from 27.18 to 31.23 t/ha. As a result of a research conducted by Gurinovich et al. (2020) in the Oryol region of Russia, it has been revealed that the three years' period average yield of pearl millet cultivar *'Gurso'* was 65.4 t/ha green mass and cultivar *'Sogur'* 62.4 t/ha green mass.

The bio-morphological characteristics of the whole plant have a significant impact on the biochemical composition and feed value of the green mass. The quality of the harvested green mass of studied *Poaceae* species, is presented in Table 1. It was found that the dry matter content of the whole pearl millet plant is 220.00 g/kg, but in the harvested mass from traditional fodder crops, it varied from 182.20 g/kg in sudangrass to 307.70 g/kg in corn. Analysing the results of the biochemical composition of dry matter, we would like to mention that pearl millet fodder was characterised by a significantly higher content of proteins (10.19 %), as compared with sudangrass (8.91 %) and corn green mass (6.61 %). The concentrations of crude fats in pearl millet fodder also were high. The level of crude cellulose in pearl millet fodder was low as compared with sudangrass and higher as compared with corn green mass. The nitrogen free extract content in pearl millet fodder reached 40.28%, which was lower than in corn green mass and optimal as compared with Sudan grass. The dry matter in pearl millet fodder contained a low amount of starch and soluble sugars as compared with corn green mass. The concentrations of minerals, including calcium and phosphorus were very high in the pearl millet green mass. It was found that the concentrations of carotene in pearl millet fodder also were significantly high. Therefore, 100 kg of pearl millet green mass contained 19.3 nutritive units, 1.57 kg digestible protein and 196 MJ metabolizable energy; 100 kg of Sudan grass green mass – 17.8 nutritive units, 1.14 kg digestible protein and 188 MJ metabolizable energy; 100 kg of corn green mass – 30.0 nutritive units, 1.07 kg digestible protein and 319 MJ metabolizable energy. Different results regarding the biochemical composition and the nutritive value of the green mass from pearl millet, *Pennisetum glaucum*, whole plants are given in the specialized literature. Sheta et al. (2010) reported that "forage pearl millet contained 8.08-11.95 % CP, 71.38-77.49 % NDF, 40.07-45.45 % ADF; the application of higher nitrogen doses increased protein yields, but decreased ADF and NDF contents, while potassium application increased protein yields and decreased NDF contents". According to Heuze et al. (2015), "the average feed value of fresh pearl millet aerial part was: 19.4 % dry matter, 12.4 % CP, 2.0 % EE, 29.2 % CF, 64.8 % NDF, 34.5 % ADF, 4.2 % lignin, 2.7 % WSC, 12.3 % ash, 5.5 g/kg Ca and 2.8 g/kg P, 63.8 % DOM, 17.6 MJ/kg GE, 10.8 MJ/kg DE and 8.7 MJ/kg ME". Babiker et al., (2015) mentioned that "pearl millet contained 8.8-16.2 % CP, 29.2-43.9 % CF, 32.8-50.5 % NFE, and crude protein yield varied from 560 to 1717 kg/ha". Anjum & Cheema (2016) remarked that "the harvested fresh millet forage contained 32.15% DM, 7.12% CP, 21.82% CF, 69.81% NDF, 42.93% ADF and 52.55% TDN". Toderich et al. (2016) found that "the concentrations of nutrients in the pearl millet dry matter of the tested ecotype at the first cut were 7.31-14.88 % CP, 2.65-3.80 % EE, 24.72-30.90 % CF, 32.62-50.31 % NFE, 5.29-11.02 % minerals, 0.82% Ca and 0.32% P, but in the dry matter at the second cut: 7.81-15.34 % CP, 1.11-4.59 % EE, 24.30 - 29.77 % CF, 36.07-50.77 % NFE 6.80-12.13 % minerals, 0.83% Ca and 0.30% P, respectively, and in third cut dry matter: 7.22-15.37 % CP, 1.07-2.04 % EE, 24.32 -30.49 % CF, 42.69-

51.47 % NFE, 6.96-11.51% minerals, 0.80% Ca and 0.33% P". Other researchers, such as Costa et al., (2018) found that "the chemical-bromatological composition of pearl millet was 314 g/kg DM, 149 g/kg CP, 545 g/kg NDF, 308 g/kg ADF, 48 g/kg EE, 20 g/kg ash, 695 g/kg TDN with 692 g/kg IVDMD". Machicek et al., (2019) compared the forage production and the feed quality of green mass from pearl millet and sorghum-sudangrass and found that "pearl millet produced 6.29 - 9.87 t/ha DM with 4.3-5.1 % CP, 58.9-64.5 % NDF, 38.0-39.3 % ADF, 58.6-59.9 % TDN, RFV 85.5-90.8, while sorghum-Sudan grass hybrid – 11.05-15.51 t/ha DM with 4.2-4.4 % CP, 58.3-62.0 % NDF, 38.60-39.9 % ADF, 57.9-59.5 % TDN, RFV 88.5-92.5". Muhanov (2019) revealed that "*Pennisetum glaucum* green mass contained 233-253 g/kg dry matter with 11.7-12.4 % CP, 1.9 % EE, 32.6-32.8 % CF, 2.2-2.3 % TSS, 9.0 % ash, 20.1-20.5 mg/kg carotene, 0.20-0.22 nutritive units/kg green mass and metabolizable energy 2.14-2.33 MJ/kg green mass, but *Sorghum sudanense* green mass contained 217-233 g/kg dry matter with 10.7-11.4% CP, 2.2-2.3 % EE, 33.6-34.7 % CF, 1.1-1.2 % TSS, 19.8-20.1 mg/kg carotene, 7.8-8.2 % ash, 0.20-0.21 nutritive units/kg green mass and metabolizable energy 2.17-2.33 MJ/kg".

Table 1. The biochemical composition and the fodder value of the green mass from the studied *Poaceae* species

| Indices | *Pennisetum glaucum* | *Zea mays* | *Sorghum sudanense* |
|---|---|---|---|
| Dry matter content, g/kg | 220.00 | 307.70 | 182.20 |
| Crude protein, % DM | 10.19 | 6.61 | 8.91 |
| Crude fats, % DM | 3.11 | 2.85 | 2.56 |
| Crude cellulose, % DM | 31.61 | 19.19 | 43.52 |
| Nitrogen free extract, % DM | 40.28 | 67.44 | 34.99 |
| Soluble sugars, % DM | 5.45 | 9.65 | - |
| Starch, % DM | 1.79 | 23.42 | - |
| Ash, % DM | 14.80 | 3.91 | 10.02 |
| Nutritive units/ kg GM | 0.19 | 0.30 | 0.18 |
| Metabolizable energy, MJ/kg GM | 1.96 | 3.19 | 1.88 |
| Calcium, % | 0.60 | 0.24 | 0.49 |
| Phosphorus, % | 0.39 | 0.19 | 0.23 |
| Carotene, mg/ kg GM | 50.75 | 15.83 | 42.00 |

Table 2. The biochemical composition and the fodder value of the silage from studied *Poaceae* species

| Indices | *Pennisetum glaucum* | *Zea mays* | *Sorghum sudanense* |
|---|---|---|---|
| Dry matter content, g/kg | 205.9 | 312.9 | 200.0 |
| pH index | 4.08 | 3.88 | 3.82 |
| Content of organic acids, g/kg | 32.3 | 32.2 | 33.6 |
| Free acetic acid, g/kg | 3.3 | 3.0 | 2.5 |
| Free butyric acid, g/kg | 0 | 0 | 0 |
| Free lactic acid, g/kg | 10.3 | 9.1 | 12.3 |
| Fixed acetic acid, g/kg | 4.0 | 3.4 | 2.4 |
| Fixed butyric acid, g/kg | 0 | 0 | 0.1 |
| Fixed lactic acid, g/kg | 14.7 | 16.7 | 16.3 |
| Total acetic acid, g/kg | 7.3 | 6.4 | 4.9 |
| Total butyric acid, g/kg | 0 | 0 | 0.1 |
| Total lactic acid, g/kg | 25.0 | 25.8 | 28.6 |
| Acetic acid, % of organic acids | 22.6 | 19.9 | 14.6 |
| Butyric acid, % of organic acids | 0 | 0 | 0.3 |
| Lactic acid, % of organic acids | 77.4 | 80.2 | 85.1 |
| Crude protein, % DM | 7.42 | 6.68 | 5.38 |
| Crude fats, % DM | 3.87 | 4.10 | 2.51 |
| Crude cellulose, % DM | 30.56 | 18.16 | 41.32 |
| Nitrogen free extract, % DM | 47.29 | 67.33 | 43.49 |
| Soluble sugars, % DM | 1.55 | 2.30 | - |
| Starch, % DM | 1.19 | 24.77 | - |
| Ash, % DM | 10.86 | 3.19 | 7.30 |
| Nutritive units/ kg GM | 0.19 | 0.30 | 0.19 |
| Metabolizable energy, MJ/kg GM | 1.93 | 3.19 | 2.03 |
| Calcium, % DM | 0.42 | 0.28 | - |
| Phosphorus, % DM | 0.27 | 0.21 | - |
| Carotene, mg/ kg GM | 28.00 | 24.77 | 38.5 |

According to Gurinovich et al. (2020), in the harvested green mass of the new '*Gurso*' cultivar of pearl millet, the content of dry matter ranged from 15.1 to 18.0%, the dry matter contained 14.6% CP, 9.5% DP, 27.3% CF, 10.5% sugar with nutritive value 0.69 feed units/kg and 9.25 MJ/kg metabolizable energy. Salama et al. (2020) found that "the concentrations of nutrients and energy in the dry matter of the tested genotype of pearl millet at the first harvest, were 6.5-7.07 % CP, 64.47-68.22 % NDF, 33.36-35.89 % ADF, 2.94-3.75 % ADL, 49.52-54.38 % NFE, 40.35-42.17 % TDN.

Silage is the main conserved green succulent roughage fodder for domestic herbivores, important way for reducing feed costs and increasing profitability. During the sensorial assessment, it was found that, in terms of colour, the silage from pearl millet had specific dark green leaves and red-maroon stems and panicles, with pleasant smell, specific to pickled apples, while the silage made from corn and sudangrass were homogeneous green-yellow with pleasant smell, specific to pickled vegetables. The results regarding the quality of the prepared silage are shown in Table 2. It has been determined that the pH values of the prepared silage depended on the species, thus, *Pennisetum glaucum* silage had higher pH value than *Sorghum sudanense* and *Zea mays* silages. The content of organic acids in the silages prepared from the studied *Poaceae* species did not vary essentially, most organic acids were in fixed form. Butyric acid was not detected in pearl millet silage, but acetic acid level reached 7.3 g/kg, which was higher in comparison with corn and sudangrass silages. It was found that during the process of ensiling, the concentrations of crude protein and soluble sugars decreased, but the level of crude cellulose did not modify essentially in comparison with the green mass. In pearl millet and sudangrass silages, the amount of nitrogen free extract was high, but lower as compared with corn silage.

Several studies have evaluated the potential of pearl millet as silage for ruminants. According to Hernández et al. (2013), the chemical composition of silage was: 10.26-10.98 % CP, 8.68-9.31 % DP, 57.80-61.87% NDF, 35.05-37.12% ADF, 5.24-6.01% EE, 12.82-13.04% ash, 0.48% Ca, 0.17-0.18%P. Anjum & Cheema (2016) reported that "the silage was characterized by 31.97 % DM, pH 4.12, 6.18% lactic acid, 7.02% CP, 22.15% CF, 71.82% NDF, 44.15% ADF and 55.18% TDN. Costa et al. (2018) found that "the monocropped pearl millet silage was characterized by pH 3.75, 47.3 g/kg lactic acid, 6.7 g/kg acetic acid, 0.1 g/kg butyric acid, 148.1 g/kg CP, 573.2 g/kg NDF, 337.1 g/kg ADF, 47.3 g/kg EE, 16.7 g/kg ash, 689 g/kg TDN with 683.5 g/kg IVDMD". Alix et al. (2019) remarked that "after 90 ensiling days, the pearl millet silage had pH 3.8, 55-60 g/kg lactic acid, 10-12 g/kg acetic acid, 0.33-0.46 g/kg propionic acid; the silage corn had pH 3.7-3.8, 34 g/kg lactic acid, 8-12 g/kg acetic acid, 0.07-0.17 g/kg propionic acid; sweet sorghum silage had pH 3.8, 49-73 g/kg lactic acid, 14-17 g/kg acetic acid, 0.17-0.43 g/kg propionic acid and 0.02-0.43 g/kg butyric acid".

## CONCLUSIONS

The introduced ecotype of pearl millet, *Pennisetum glaucum*, under the climatic conditions of the Republic of Moldova, was characterized by optimal growth rate and productivity.

The green mass and silage prepared from pearl millet contain a lot of nutrients, which make them suitable to be used as a part of diverse livestock diets.

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# BASIL, THYME AND SAGE HERBAL PLANTS AND THEIR ASSOCIATED ESSENTIAL OILS AS FEED ADDITIVES IN CHICKEN BROILERS. A LITERATURE REVIEW

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***Abstract***

*The use of different herbal plants and their associate essential oils as feed additives is of great importance for various purposes in poultry production. This trend started since 2006 due to ban on use of certain antibiotics in poultry diets, in the European Union, because they are suspected of contributing substantially to increasing resistance among human pathogens. Some investigations have shown that a number of plant feed additives and their essential oils, have shown significant beneficial effects on animal production, health status and meat quality. These natural feed additives not only act as antibiotic replacements for the animals, but also exert beneficial properties in the poultry products, especially, antioxidant properties in meat. However, the overall efficacy of herbal plants and their associate essential oils, together with their nutritive value with impact on the health status of animals and humans (via the food chain), requires constant research on standardization of correct dosages for particular functions to be studied.*

***Key words***: *broiler chickens, essential oils, feed additives, herbal plants.*

## INTRODUCTION

For more than seven decades, antibiotics have been applied at low levels in monogastric feed to promote growth performance. Indeed, the antibiotics growth promoters have proved that they are capable to improve performance and decrease mortality in monogastric animals, but due to continue and excess uses of antibiotics in food for animal production has developed bacterial resistance to antibiotic growth promoters and created public health threats. Compared with synthetic antibiotics or inorganic chemicals, plant-derived products (phytobiotics of phytogenic feed additives) are natural, less toxic than antibiotics, and typically residue free. Phytobiotics includes a wide range of plant-derived products such as spices, and botanical products such as aromatic, medicinal plants, their extracts and mainly their essential oils and oleoresins. They can be added to the diet of commercial animals to improve their productivity through enhancing feed properties, promoting animals' production performance, and improving the quality of products derived from these animals (Vlaicu et al., 2022). In addition to the above-mentioned definition, Windisch et al. (2008) have recommended some other commonly used terms to classify different phytogenic compounds based on their origin and processing, including herbs (flowering, non-woody and non-persistent plants), spices (herbs with an intensive smell or taste commonly added to human food), essential oils (volatile lipophilic compounds) and oleoresins (extracts derived by non-aqueous solvents). After many studies have been conducted the results still have inconsistent effects on poultry performance, mainly due to differences in their botanical origin, processing, and composition or their huge variety of chemical substances (Reisinger et al., 2011). These plant-based feed additives may improve the palatability of feed which may lead to advanced performance, have verified potent antioxidative and antimicrobial efficacy in broilers. A number of *in vivo* studies show that phytogenic feed additives may specifically enhance activities of digestive enzymes and nutrient absorption. Also, numerous experimental studies provide further comparisons of phytogenic feed additives with antibiotics and their analogous actions in the intestine, thus reflecting an overall improved animal health (Riyazi et al., 2015a; Sheoran et al., 2017; Nouri, 2019). The efficacy on feed conversion ratio, daily weight gain, feed consumption, higher apparent ileal digestibility of nutrients was also reported (Windisch et al., 2008, Hong et al., 2012). In addition, specific compounds of some plants and their extracts have the potential to enhance products quality through different mechanism, due to their antioxidant potential (Untea et al., 2020; Vlaicu et al., 2022; Saracila et al., 2022). Among potential candidates, basil, thyme and sage and their essential oils represent an exciting group of feed additives, originating from *Lamiaceae* family. However, the future of using herbal plants in animal feeding will be in great measure depend on the knowledge of chemical structure, their value and characteristics of practical herbs or their extract physiological needs and well-being of animal, and, above all on consumer's preferences and expectations. In this context, this paper reviews the different aspects of application of basil, thyme and sage plants and their essential oils in diets of broiler chickens and their impact as phytogenic feed additives.

## MATERIALS AND METHODS

### Methodology of searching and selecting relevant articles

The present search was limited to article having full text available in English. Databases used included Web of Science, PubMed, Google Scholar, Springer Link, Springer Nature, MDPI and Elsevier. Primary search keywords used were basil, thyme, sage, essential oils, herbal plants, herbs, herbal formulations, chicken broilers, antioxidant effect, performances, etc. Searches were also done using the taxonomic names of the plants. Bibliographies of included studies were also searched for additional references. Book chapters, proceedings of conferences, if available online have been also incorporated wherever possible to best extent of their availability and access. From literature survey we found substantial number of papers which described mostly the chemical composition of the plants and their associate essential oils with antioxidant, antimicrobial, anticoccidial and growth promoting effects, used in different combinations. Based on the extensive search performed, the results are presented in divided sections classified based on the most effective effects described found. From over 150 research articles and review papers found, we included only those from 2006 till present, the rest of them being excluded, because the ban of antibiotics started from that year.

## RESULTS AND DISCUSSIONS

### Description of the reviewed herbal plants

Basil is the common name for the culinary herb *Ocimum basilicum* of the family *Lamiaceae* (Table 1). It has been widely used in traditional medicine in the treatment of headaches, coughs, constipation, diarrhoea, warts, worms, and kidney problems (Falowo et al., 2019). Also, various pharmacological actions have been described, such as stomachic, antioxidant, antiviral, antimicrobial, analgesic, anti-inflammatory, antidiabetic, and anti-stress activities, and emmenagogue properties, among others (Bilal et al., 2012). It is widely cultivated for the production of essential oils and also marketed fresh, dried, or frozen. Essential oils extracted from fresh leaves and flowers can be used as aroma additives in food, pharmaceuticals, and cosmetics and has been shown to possess antifungal, insect-repelling and toxic activities (Nadeem et al., 2020).

Table 1. Classification according to the Integrated Taxonomic Information System - Report

| Item | Basil | Thyme | Sage |
|---|---|---|---|
| **Kingdom** | Plantae | Plantae | Plantae |
| **Subkingdom** | Viridiplantae | Tracheobionta | Viridiplantae |
| **Superdivision** | Embryophyta | Spermatophyta | Embryophyta |
| **Division** | Magnoliophyta | Magnoliophyta | Tracheophyta |
| **Class** | Magnoliopsida | Magnoliopsida | Magnoliopsida |
| **Subclass** | Asteranae | Asteridae | Asteranae |
| **Order** | Lamiales | Lamiales | Lamiales |
| **Family** | Lamiaceae | Lamiaceae | Lamiaceae |
| **Genus** | Ocimum L. | Thymus L. | Salvia L |
| **Species** | Ocimum basilicum L. | Thymus vulgaris L | Salvia officinalis L |

Chemical investigations have shown that basil contains various active compounds, such as flavonoids, tannins, saponin, glycosides, terpenes and steroids (Lee et al., 2005), antioxidant capacity, polyphenols, vitamin E, luthein and zexanthin, minerals, especially zinc (Vlaicu et al., 2022).

Thyme (*Thymus vulgaris*) is a medicinal plant of the family *Lamiaceae* and has anticough, antibloating, antimicrobial, antifungal, and antispasmodic properties. Thyme is rich in carotenoids, fatty substances, bitter compounds, and a high level of manganese. Thyme essence contains pinne, deptante, carvacrol, and thymol, but its disinfecting property contains thymol and timic acid (Miraj & Kiani, 2016). Its oil also has antimicrobial activity against gram-positive and gram-negative bacteria (*Salmonella typhimurium, Clostridium perfringens, and hilcopilari*). Thyme was identified as useful herbal plant as natural additives in poultry diets. The studies in which thyme was tested, claims that compared with synthetic antibiotics, thyme may be used without any adverse effects (Bampidis et al., 2005). However, due to its compounds such as carvacrol, parasymon, and thymol, could present some side effects, which restrict its utilization in high dosages. Thymol is a phenol used as antiseptic and as stabilizing agent in drug products. Carvacrol is the main ingredient in the essential oil, insoluble in water but soluble in alcohol and ether (Seidavi et al., 2021).

Sage *(Salvia officinalis)* is a perennial green shrub with woody stems, greyish leaves, and blue to purplish flowers in the family of *Lamiaceae*. It grows worldwide and in traditional medicine, it has been used for the treatment of seizure, ulcers, gout, rheumatism, inflammation, dizziness, tremor, paralysis, diarrhea, mild dyspsia, and hyperglycemia (Garcia et al., 2016). Many researchers have investigated the common uses of sage and found different pharmacological functions such as anticancer, anti-inflammatory, antioxidant, antimicrobial, antimutagenic, hypolipidemic and hypoglycemic (Ghorbani & Esmaeilizadeh, 2017). It has been reported that the extract of sage exerted significant antibacterial activity against different bacterial species (*Bacillus mycoides, Bacillus subtilis, Enterobacter cloacae,* and *Proteus* spp.) (Stanojevic et al., 2010). Moreover, Kermanshah et al. (2009) found that the hydroalcoholic extract of sage has strong inhibitory effect on *Streptococcus mutans*, *Lactobacillus rhamnosus*, and *Actinomyces viscosus*. These demonstrated effects make sage as a valid alternative source to the traditional antibiotics (Khalil & Li, 2011; Hamidpour et al., 2014). As presented in Figure 1, there are many reasons for which we should use plants or essential oils as new generation of feed additives.

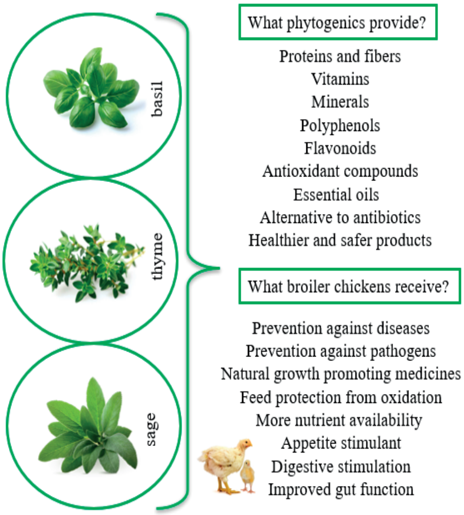


Figure 1. Schematic representation of benefits from using plants as feed additives in broiler diets

## Chemical composition of basil, thyme and sage plants

Literature data revealed that the chemical composition of these herbs is very variable. Gurbuz& Ismael (2015) reported that crude protein in basil was 22.08% and 25.52% crude fiber content. Thyme is scarce in crude protein (5.23%) but with higher crude fiber (18.10%) content (Gerencsér et al., 2014). A very low concentration of crude protein in sage (1.3%) was reported by Khalil et al., (2012) but the content of crude fiber (31%), was the highest compared with basil and thyme.

Recently, Turcu et al. (2020) reported that thyme and sage have similar crude protein content (14.67% and 14.19%) but lower than basil (18.06%). Same authors, showed that the crude fiber content in these plants in relatively high (10.88% in basil, 20.24% in sage and 24.63% in thyme), which could be a limiting factor for their usage in broiler diets. Besides its high nutritional value, basil is also rich in vitamins, (C, E, K, A, β- carotene, B complex), minerals (Fe, Ca, Mg, P, Mn, Na, K, and Zn), and it also contains many secondary metabolites, (essential oils, phenols, flavonoids, anthocyanins, tannins, and steroids) (Filip, 2017). Similarly, it was reported that thyme contains minerals such as Zn, Ca, K, Na, Fe, P. The plant is also a rich source of many important vitamins such as B-complex, folic acid, beta carotene, vitamin A, K, E and C. Thyme is capable to provide about 0.35 mg of vitamin B6 which represents about 27% of daily recommended intake (Komaki et al., 2016).

Sage is also rich in minerals (P, Na, Fe, Ca, Cu, N, K, Mg, Zn) with significant contents of vitamins from which Vitamin C was the most abundant and variable (0.24 to 615.8 µg/mL) as reported by Yaman (2020).

**Chemical composition of their essential oils**

Essential oils are composed of a complex mixture of active substances extracted from plants through a steam distillation process or generated via chemical synthesis.

The concentration of the biologically active components in essential oils is variable and dependent on the species, the part of the plant used, soil, environmental conditions, and time of harvest (Lee et al., 2004). These substances can produce several beneficial effects, but the most important is to increase animal performance (Zhang et al. 2005).

The chemical composition of basil leaves examined by gas chromatography showed that estragole (52.60%) and limonene (13.64%) are the dominants components followed by p-cymene (2.32%) and exo-fenchyle acetate, as reported by Chalchat & Özcan (2008).

The thyme extraction revealed that the two major components comprise of thymol (60.18%) and p-cymene (15.44%) and small quantities of other constituents including carvacrol (Ahmad et al., 2014).

The composition of sage essential oil is composed by cis-thujone (18–43%), trans-thujone (3–8.5%), camphor (4.5–24.5%), 1,8-cineole (5.5–13%), α-humulene (0–12%), α-pinene (1–6.5%), camphene (1.5–7%), limonene (0.5–3%), linalool, and bornyl acetate (2.5% maximum) as reported by Bettaieb et al., (2009).

**Main activities and uses of basil, thyme and sage plants and their essential oils**

The *Lamiaceae* family contains important aromatic plants used in traditional and modern medicine, in the food and pharmaceutical industries (Figure 2). Rosemary, basil, thyme, oregano, and sage are the most popular plants in Eastern European countries for traditional remedies and are often used for the treatment of gastritis, infections, dermatitis, bronchitis, and inflammation. Some of these plants have been extensively studied for their antioxidative and antimicrobial activity.

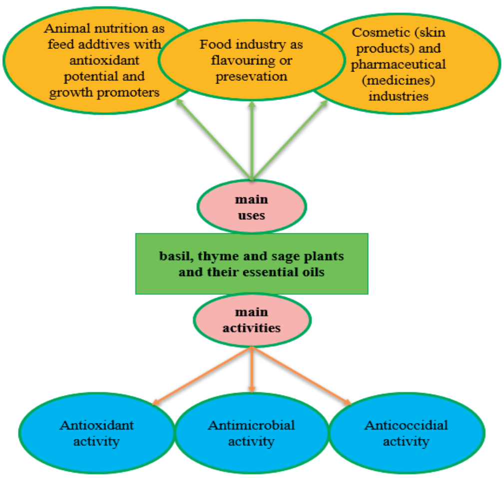


Figure 2. Overview of the main uses and activities of basil, thyme and plants and their essential oils

The vegetation of many European countries is rich in aromatic plants, for this reason makes these countries the major producers and processors of medicinal plants. Sage is mostly exploited for the extraction of essential oils of importance for the pharmaceutical, agronomic, and food industries. Basil and thyme are aromatic plants that are used extensively to add a distinctive aroma and flavour to food. The leaves can be used fresh or dried for use as a spice. Essential oils extracted from fresh leaves and flowers can be used as aroma additives in food, pharmaceuticals, and cosmetics (Javanmardi et al., 2002; Shahrajabian et al., 2020). The extracted essential oils are of scientific and popular interest because they may act synergistically with other techniques of preservation. Some studies reported that basil (Riyazi et al., 2015b), thyme (Saracila et al., 2021), sage (Traesel et al., 2011), rosehip (Vlaicu et al., 2017) oregano and rosemary (Mathlouthi et al., 2012; Turcu et al., 2018) oil extracts present antidiarrheal and anti-inflammatory properties and antimicrobial activity against different harmful bacteria and other microorganisms. They are generally recognized as safe (FAO, 2010), and they show antioxidant, antibacterial, antidiabetic, antimutagenic, non-toxigenic, and antimycotic properties which are promising for their use as bioactive compounds in different foods and feeds (Saad et al., 2013). However, even if FAO recently recognised essential oils as safe, they are used since 16 th century, when Paracelsus used the term essential oil to name the effective component of each drug as *"quinta essentia"* (Freires et al., 2015). Nevertheless, care must be taken because essential oils can also produce toxic effects in chickens when used in high doses and it was reported that they may be dose dependent (Zhang et al., 2005).

**Antioxidant activity of the reviewed plants and their essential oils**

The antioxidant properties of many plants have been investigated, in the light of recent scientific developments, throughout the world due to their potent antioxidant activities. Due to carcinogenic effects of synthetic antioxidants, such as butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT) and tert-butyl hydroquinone (TBHQ) which are banned in Japan and certain European countries, research for a safer and effective natural antioxidant is underway and several natural feed additive sources are being examined. The basil plants are among the most important crops which essential oils stand out for the quality, quantity and chemical diversity. As reported by Turcu et al. (2020) basil plant presented high antioxidant capacity and moderate concentration of polyphenols when compared with other plants. Also, basil contains a wide range of essential oils rich in phenolic compounds and a wide array of other natural products including polyphenols such as total phenolics, phenolic acids, flavonoids and anthocyanins (Flanigan & Niemeyer, 2014). Of the phenolics, rosmarinic, cichoric caftaric and caffeic acids, have been characterized with the highest antioxidant activities, in basil (Baâtour et al., 2012). However, the composition of these compounds determined in basil varies among species, cultivars, plant part, and origin.

Another natural feed additive with high antioxidant capacity, polyphenols content and vitamin E is thyme (Turcu et al., 2020). Thyme contains high concentrations of phenolic components which are primarily responsible for its antioxidative activity. The most dominant phenols in thyme are thymol (12–61%) and carvacrol (0.4–20.6%), followed by 1,8-cineole, ρ-cymene, linalool, borneol, α-pinene and camphor (Dogu-Baykut et al., 2014). However, according to the World Health Organization, thymol residues in food are without danger to the consumer as long as they do not exceed 50 mg/kg (FAO/WHO, 2008).

Alongside with rosemary and oregano, sage has been showed to have the strongest antioxidant activity among the reviewed plants. The antioxidant activity in sage plant given by the total phenolics varied from 50.3 mg GAE/g to 167.1 mg GAE/g (Tosun et al., 2009; Turcu et al., 2020). The main effective phenols have been shown to be phenolic acids, carnosol derivatives, and flavonoids, namely, rosmarinic acid, carnosic acid, and carnosol followed by caffeic acid, rosmanol, rosmadial, genkwanin, and cirsimaritin (Farhat et al., 2014). Some of these phenolic compounds presented excellent scavenging activity of active oxygens such as superoxide anion radicals, hydroxyl radicals, and singlet oxygen, which further inhibit lipid peroxidation (Shivakumar and Yogendra, 2018) and increase the antioxidant capacity in meat (Vlaicu et al., 2021). For this reason, their corresponding extracts have been used to stabilize the fat content and shelf-life in products. However, the use of herbal plants and their essential oils as antioxidants in food industry still faces the problems of the interactions with food matrix components (fat, protein and starch), alteration of sensorial characteristics of foodstuff at high concentration, sensitivity to heat, light and oxygen during processing, utilization and storage, given by their high volatile character.

**Antimicrobial activity of the plants and their essential oils**

It is known that multidrug-resistant bacterial pathogens have raised a growing public health threat. For this reason, antibiotic-resistant microorganisms have been an ever-growing concern over the past years. Therefore, there is a continuous need for effective natural compounds to be used instead of synthetic ones. The investigations of herbal plants for their biologically active constituents have gained the attention of research communities as new sources of feed additives with antimicrobial activities. The medicinal plants contain various metabolites that demonstrate antimicrobial activity in vitro and vivo against harmful pathogens. The most concerning microorganisms are *Escherichia* coli, *Pseudomonas aeruginosa, Klebsiella pneumoniae, Acinetobacter baumanii, Helicobacter pylori, Mycobacterium tuberculosis*, penicillin-resistant *Streptococcus pneumoniae, Shigella, and Salmonella* (Abreu et al., 2012). Previously, many plants, essential oils and secondary metabolites derived from herbal plants from multiple countries/regions were tested against microbes that cause various infections (Verma et al., 2012; Gnat et al. 2017; Vlaicu et al., 2017; Turcu et al., 2018; Saracila et al., 2018). It has been also proven that plants with antimicrobial activity support more antagonistic endophytic bacteria against human pathogenic microbes due to their useful essential oils with antimicrobial properties (Nikolic et al., 2014). The natural antimicrobial agents present in thyme (thymol) have proven its effectiveness in medical, food, agricultural and veterinarian (Glenn et al., 2010) applications. Thymol is able to inhibit development of some bacteria, including the potential pathogenic strains of *Escherichia* coli, *Bacillus subtilis*, and *Staphylococcus aureus* (Qader et al., 2013). It was reported that beside thymol, its isomer carvacrol, as well as citronellal, eugenol from essential oil demonstrate antibacterial activity (Guimarães et al., 2019). The antimicrobial activity of thymol and carvacrol is much more effective due to their synergistic effect, compared with pure thymol (Burt et al., 2013). The aqueous extract of sage exerted significant antibacterial activity against different bacterial species such as, *Bacillus mycoides, Bacillus subtilis, Enterobacter cloacae*, and *Proteus* spp., making it as a valid alternative source to the traditional antibiotics (Stanojevic et al., 2010). These beneficial results are given by the plant extracts which exhibit the beneficial action by disrupting microbes' cell membrane; by stimulating and replicating the beneficial bacteria growth in gut, defending intestine from microbial attack; by stimulating the proliferation and growth of absorptive cells in gut; and by enhancing the production of digestive enzymes.

**Anticoccidial activity of the plants and their essential oils**

Coccidiosis represents a serious threat to the poultry industry, affecting production and causing high morbidity, mortality and significant costs resulting from treatment and prophylaxis. Coccidiosis is a parasitic disease caused by seven species of the genus *Eimeria* with different localizations within the intestinal tract of chickens. *Eimeria acervulina*, *Eimeria maxima* and *Eimeria tenella* are the most prevalent species in broilers in the intensive poultry management system (Haug et al., 2008). In-feed anticoccidials have been used for decades for managing avian coccidiosis and were very effective until drug resistance emerged. The use of natural remedies has become a promising alternative in combating coccidiosis in chickens. Among the most important areas of performance improvement for broiler chickens has been the advance in the use of feed additives with beneficial bioactive compounds that can protect against bacteria and parasites. Among other, the reviewed plants and their essential oils represent potential source of bioactive compounds that could be used as a natural growth enhancer with potent anticoccidial properties. As mentioned before, basil and thyme are naturally rich in thymol and carvacrol as major constituents. They are promising bioactive compounds because they can interfere with the membrane permeability of pathogens, causing a cascade of reactions that involve the entire cell and eventually leads to its death, as it was mentioned before (Nazzaro et al., 2013). Previously it was reported that they exerted an antiparasitic activity on *Eimeria* spp. and disrupted the invasion of *Eimeria tenella* (Burt et al., 2013; Jitviriyanon et al., 2016). Besides that, numerous other plant-based products have been found to be effective at treating chicken coccidiosis and intestinal pathogens. Some examples are thyme plant (Lahlou et al., 2021), sage extract (Arczewska-Wlosek & Swiatkiewicz, 2012), artemisia annua

(Saracila et a., 2017; Jiao et al., 2018), oregano (Vlaicu et al., 2018; Turcu et al., 2018; Sidiropoulou et al., 2020), garlic (Pourali et al., 2014), turmeric (Kim et al., 2013) and many others essential oils or plants mixtures. However, no information found regarding basil effect on anticoccidial activity. Nevertheless, the majority of these natural compounds do not always aim directly at the parasites but have immunomodulatory effects and antioxidative or anti-inflammatory properties which act on the intestinal tract, thus helping the host organism to fight against the coccidia and pathogens infection (Wunderlich et al., 2014; Vlaicu et al., 2020b). Beside the above mentioned main biological activities, these feed additives exhibit also antiviral, antibacterial, antifungal, anti-inflammatory, immunostimulatory effects and enhance the enzymatic activity in the intestinal tract when used in broilers diets. Moreover, these feed additives are excellent natural sources of growth and health promoting activities as presented in Figure 3.

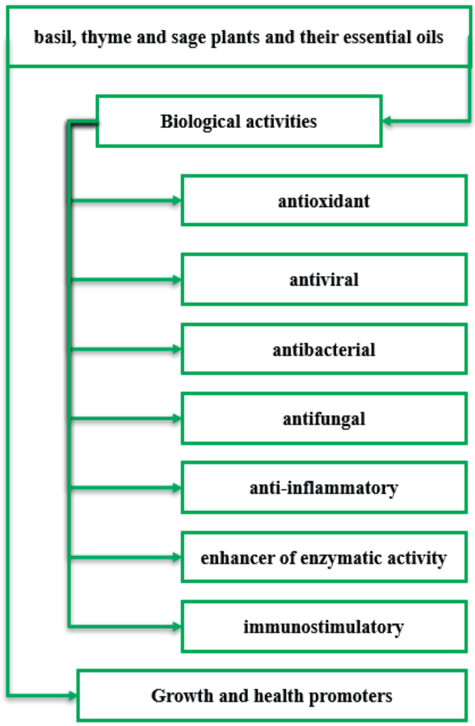


Figure 3. Major biological activities exhibited by herbal plants and essential oils used as feed additives in poultry

**Growth and health promoting effect of reviewed plants and their essential oils in broiler chickens' diets**

It has been mostly reported that addition of herbal plants and their associate essential oils to diets has growth promoting effect on broilers (Table 2). Gurbuz et al. (2016) compared the effect of 1% versus 1.5% of basil on broilers performance and obtained significantly ($P<0.05$) higher feed conversion ratio compared with control treatment. They suggested that 1% basil significantly increased the final body weight of chicken broilers compared with 1.5% basil or peppermint plant. Abbas (2010) has conducted an experiment feeding broilers with 3 g/kg basil and observed significantly ($P<0.05$) improved body weight. Higher quantity of basil (3%, 4% or 5%) showed that body weight, body weight gain and feed conversion ratio were significantly ($P<0.05$) increased as compared with the control, and significantly ($P<0.05$) decrease feed intake compared with the control group (Al-Kelabi, 2013).

The essential oils derived from basil plant (200, 400 and 600 ppm) in combination with probiotic (150 ppm) and antibiotic (150 ppm) had no effect on broilers performances in Arian broilers hybrids, but significantly decreased the total bacteria counts ($P<0.05$), without affecting the colony-forming units of lactobacilli (Riyazi et al., 2015a, b). Contrary, Elnaggar & El-Tahawy (2018), reported that 10 and 20 g/kg of basil powder and 0.5 and 1 g/kg basil essential oil on broilers diets significantly ($P<0.05$) improved body weight, with significant improvements in blood parameters and meat edible parts. The use of thyme at 5g/kg or 10 g/kg thyme powder and 0.5g/kg or 1g/kg thyme essential oil on broilers diets significantly ($P<0.05$) improved body weight as reported by Elnaggar & El-Tahawy (2018) and enhanced the health status by significantly decreased serum triglycerides and cholesterol. Significant body weight on broilers was also noted at 2% thyme with decreased feed intake and mortality (El-Ghousein & Al-Beitawi, 2009). Contrary, 100 or 200 ppm thyme essential oil, was reported to increase feed intake (Al-Kassie, 2009) but with increase final body weight. In addition, 0.2%, 0.5% or 0.8% of thyme plant produced beneficial effects on health status by increasing the immune status of birds (Hassan, & Awad, 2017; Ahmadian et al., 2020). The essential oil of thyme extract (thymol) at 0.04% inclusion level was reported to increased intestinal populations of beneficial bacteria such as *Lactobacillus* in broilers (Nouri, 2019). However, some reports showed no effects of thyme on broilers diet (Demir et al., 2008; Dahal & Farran et al., 2011). Limited studies have been documented to identify the effects of sage plant or essential oil in broilers feed, which makes sage a new unexploited source of feed additive. Hernandez et al. (2004) reported that a mixture of essential oils which contained sage, had no differences in feed intake, feed conversion or organs development, however improved apparent fecal digestibility of dry matter and ether extract. Also, 50, 100, and 150 mg/kg of essential oils of oregano, sage, rosemary, and pepper increase the health status of broilers, as reported by Traesel et al. (2011).

Table 2. Effect of application of plants and their essential oils in chicken broiler nutrition

| Item | Obtained results | Reference |
|---|---|---|
| Basil | 1% and 1.5% basil showed significantly ($P<0.05$) higher feed conversion ratio as compared with peppermint and control treatments; 1% basil significantly increased the final body weight of chicken broilers | Gurbuz et al., 2016 |
| | 3 g/kg basil significantly ($P<0.05$) improved body weight and reduced serum cholesterol compared with the control and fenugreek diets, but with no effect on carcass quality | Abbas, 2010 |
| | 3%, 4%, 5% basil revealed that significantly ($P<0.05$) increased body weight, body weight gain and feed conversion ratio in Hubbard chickens as compared with the control, and significantly ($P<0.05$) decrease feed intake compared with the control group. | Al-Kelabi, 2013 |
| | 200, 400 and 600 ppm basil essential oil with 150 ppm probiotic and 150 ppm antibiotic had no effect of broilers performances, but 200 ppm basil essential oil supplementation significantly ($P<0.05$) decreased abdominal fat | Riyazi et al., 2015a |
| | 200, 400 and 600 ppm basil essential oil with 150 ppm probiotic (Protexin) and 150 ppm antibiotic (Avilamycin); used on Arian broilers significantly decreased the total bacteria counts ($P<0.05$), without affecting the colony-forming units of Lactobacilli | Riyazi et al., 2015b |
| | 10 and 20 g/kg of basil powder and 0.5 and 1 g/kg basil essential oil on broilers diets significantly ($P<0.05$) improved body weight, economic efficiency and production index compared to control; also, significantly decreased serum triglycerides and cholesterol. All supplementations increased percentage of dressing and total edible parts compared with control. | Elnaggar & El-Tahawy, 2018. |
| | 1% of basil in Cobb 500 broiler chickens increased ($P<0.05$) gizzard weight, total polyphenols content and the antioxidant capacity; lowered cholesterol content in breast meat muscles and altered the instrumental color and textural parameters. | Vlaicu et al., 2021 |
| | 0.5% basil in Cobb 500 chickens significantly ($P<0.05$) increased the antioxidant parameters and in MDA level with a significant controlling and prevention effect on Escherichia coli infection. | Kilany et al., 2018 |
| | 0.5% and 1.0% of basil powder in Ven Cobb broiler diets, improved ($P<0.05$) body weight gain, feed conversion efficiency and immune status by augmenting the T-cell mediated immune response. | Sheoran et al., 2017 |
| Thyme | 100 and 200 ppm essential oil, increased feed intake, body weight and FCR, dressing percentage, organs and decreased abdominal fat | Al-Kassie, 2009 |
| | 5 and 10 g/kg thyme powder and 0.5 and 1g/kg thyme essential oil on broilers diets significantly ($P<0.05$) improved body weight, economic efficiency and production index compared to control; also, significantly decreased serum triglycerides and cholesterol. All supplementations increased percentage of dressing and total edible parts compared with control. | Elnaggar & El-Tahawy, 2018 |
| | 300 mg oil increased only body weight in broilers diets | Al-Mashadani et al. 2011 |
| | 2% thyme increased body weight, organs development, decreased feed intake and mortality | El-Ghousein and Al-Beitawi 2009 |
| | 5g/kg increased body weight gain compared with antibiotic supplemented diet | Toghyani et al. 2010 |
| | the addition of 0.5% of thyme powder to 1-day-old Cobb chicks for 35 days improved the immune status and antioxidant activities in broilers while the lipid peroxidation of meat was reduced | Ahmadian et al. 2020 |

| | | |
|---|---|---|
| | supplementation at lower levels, 0.2–0.8%, produced benefits in weight gain and immune status | Hassan, & Awad 2017 |
| | 0.04% thymol increased intestinal populations of Lactobacillus and Escherichia coli have increased in broilers | Nouri, 2019 |
| | 1% of thyme in Cobb 500 broiler chickens increased ($P<0.05$) gizzard weight, total polyphenols content and the antioxidant capacity; lowered cholesterol content in breast meat muscles and altered the instrumental color and textural parameters. | Vlaicu et al. 2021 |
| | No effect on performances | Dahal and Farran et al. 2011. |
| | 70 mg/kg diet of thyme in Ross-308 broilers increased abdominal fat pad | Ocak et al., 2008 |
| **Sage** | 2.5 or 5.0- and 7.5-mL sage extract / kg were included in the diets of Alectoris chukar partridges, no effect was found in performances, carcass development or blood parameters. | Yurtseven et al., 2008 |
| | 5.000 ppm extract from sage, thyme, and rosemary in broilers diet had no differences in feed intake, feed conversion or organs development, however improved apparent fecal digestibility of dry matter and ether extract | Hernandez et al., 2004 |
| | 1% of sage in Cobb 500 broiler chickens increased ($P<0.05$) gizzard weight, total polyphenols content and the antioxidant capacity; lowered cholesterol content in breast and thigh meat muscles and altered the instrumental color and textural parameters. | Vlaicu et al., 2021 Vlaicu et al., 2022 |
| | 50, 100, and 150 mg/kg of essential oils of oregano, sage, rosemary, and pepper increase in serum levels of lipase, uric acid, urea, and aspartate aminotransferase however it is suggested that they may cause kidney and liver impairment, with no effect on performances. | Traesel et al., 2011 |
| | 0.05% or 0.1% sage oil improved weight gain, feed conversion ratio and immunity status, while decreased the serum cholesterol, triacylglycerol and abdominal fat | Kishawy et al., 2016 |
| | 100, 200, 300 and 400 ppm of essential oil in Ross 308 broiler diets resulted in no differences among treatments for body weight gain, feed intake and feed conversion ratio; decreased serum cholesterol, triglycerides and low-density lipoprotein; significantly ($P < 0.05$) enhanced the immunity response with additional significant ($P < 0.05$) bactericidal effect for Escherichia coli. | Rasouli et al., 2020 |
| | 0.5% of sage powder used in Ross 308 broilers significantly ($P \leq 0.05$) increased body weight and reduced feed conversion ratio; 0.2% of sage powder increased the immunity titers against Newcastle disease and avian influenza viruses; plasma cholesterol, triglyceride, and low-density lipoprotein (LDL) concentration were reduced and high-density lipoprotein (HDL) concentration was increased significantly | Farhadi et al., 2020 |

**Basil, thyme and sage plants and their essential oils as antioxidants in broiler meat**

The antioxidant activity of herbal plants and their essential oils is another biological property of great interest, due to their ability of scavenging free radicals which may play an important role in preventing some diseases (i.e., cancer and heart diseases) caused by free radicals. The content of active substances and the chemical composition of herbal plants and their extracts in the final products may vary widely depending on the plant parts used, geographical origins, and harvesting season as mentioned early. It has also been suggested that the benefits of the use of essential oils of plants is often variable because it depends on all the constituents working together, however, plenty of studies have indicated the antioxidant activity of their usage (Miguel, 2010). The potential effect of herbal plants from the Labiatae family containing phenolic compounds on improving the oxidative stability of poultry meat (Kilany et al., 2018; Ahmadian et al., 2020; Vlaicu et al., 2021) was previously investigated. The use of 1% of basil, thyme or sage in broilers diet was very effective on improving the antioxidant activity in breast meat of broilers (Vlaicu et al., 2021). Significant improvements were also reported on meat quality at 0.5% or 1% levels of basil inclusion (Kilany et al., 2018). The addition of 0.5% of thyme powder to broilers for 35 days improved the antioxidant activities in meat by reducing the lipid peroxidation of meat (Ahmadian et al., 2020). Gheisar et al. (2015) reported that the thiobarbituric acid reactive substances (TBARS) value of breast meat was significantly reduced by the herbal plants blend containing thyme, oregano and rosemary. It was also reported that thymol reduced the oxidation of fatty acids indicated by the lower malondialdehyde level in meat (Placha et al., 2014). It can be concluded that these significant improvements regarding the antioxidant activity of *Labiatae* family plants on broilers meat may be due to their contents of phenolic compounds.

## CONCLUSIONS

This review concludes the fact that plant cultivars vary in their nutrient concentrations. This variability in chemical composition, minerals, antioxidant compounds and fatty acids can be attributed to the moment of harvest, climate genotype and storage conditions, temperature, light, soil type and other conditions, which further could lead to different results when tested on chickens' meat quality. There are many advantages of using plants than antibiotics. The future of using plants and essential oils in animal feeding will in great measure depend on the knowledge of chemical structure, their value and characteristics of herbal plants or their extracts physiological needs and well-being of animal, and, above all on consumer's preferences and expectations. As a consequence of the reduced number of cows but an increased milk yield, milk production has continuously increased, except the year 1995 when it recorded the lowest level.

The North Eastern region is traditionally suitable for cow rearing, due to its pastures and meadows, the important number of cow livestock and possibilities to produce ecological milk.

## ACKNOWLEDGEMENTS

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# RESISTANCE PROPERTIES OF THE ORGANISM UNDER THE INFLUENCE OF THE MINERAL PREMIX "PMVS" AND THERMAL FACTORS

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***Abstract***

*This paper presents the results of the study of low temperature effects of moderate stress intensity and the mineral premix "PMVS" on some indicators of calf defense abilities in postnatal ontogenesis to determine the parameters that can be used as a way of increasing the resistance and the defense abilities of animals to adverse environmental influences. The values obtained from the studied parameters show the change in the resistance of calves in the postnatal period. Thus, the increase of the biological value of the ration, by including the mineral premix "PMVS" and the application of the low temperature of a moderate stress intensity have led to an increased serum phagocytic activity, bactericidal activity and lysozyme activity throughout the experimental period. During the whole research period, the diurnal weight gain in the experimental group was 766 g. Maintenance of the organism's homeostasis within optimal physiological limits is the main task of the animals physiology. Determination of the parameters of influence of the food and heat factors on homeostasis and calf development in postnatal ontogenesis allows to create favorable conditions that can facilitate the acceleration of the functional maturation of vital organs and systems, increase the organism's resistance and adaptive abilities of the organism to the influence of stress factors and the realization of the genetic potential of animals.*

***Key words**: bactericidal activity, calves, mineral premix, phagocytic activity, temperature.*

## INTRODUCTION

There is a very complex correlation between the organism and the conditions of the environment because the environmental factors act on the organism in most cases combined and the effect of the action depends on their nature and intensity (Чегина, 1993; Hoteteu, 2004; Курденко et al., 2017; Кляпнев, 2019). Moreover, the effects that appear after their separate or combined action are diverse and are manifested by the depth of changes in various physiological processes that determine the development and growth of the organism (Фурдуй, 1986; USDA, 1997; Jegou et al., 2006; González & Partida, 2011; Brouček, 2014; Roland et al., 2016). Since the temperature (the most abiotic physical factor of the environment with the greatest impact) acts in combination with other environmental factors, it is currently of interest to carry out the research in order to study the action of low temperature of moderate stress intensity and food factor (the second most important abiotic factor) on the functional state, endurance and adaptive capacity of the organism in postnatal ontogenesis. Also of great importance for maintaining homeostasis is the determination of parameters that can be used as a way of increasing the resistance and adaptability of the animal's organisms to the unfavorable effects of environment (Lorenz et al., 2011; Бочаров, 2015; Das et al., 2016; Monteiro et al., 2016; Pusta, 2006; Курденко et al., 2017).

In the carried out research, the effects of influence of the temperature of a moderate stress intensity and the dietary factor on calves in postnatal ontogenesis have been studied according to the periodization developed by the Institute of Physiology and Sanocreatology of the Republic of Moldova in critical periods of development: imprinting, depression of the stress response, immunodeficiency, as well as the beginning of periods of domination and retardation of the growth, functioning and development of organ systems. According to the proposed scheme of the experiments, the organism resistance indices have been investigated: phagocytic activity, bactericidal activity and lysozyme. The body weight of the calves throughout the experiment was also recorded as an integral index of the action of the studied factors on the animal productivity.

## MATERIALS AND METHODS

The scientific investigations have been carried out on a herd of black-spotted calves, selected according to the analogy principle, taking into account breed, age, sex and body weight.

The experiments have been performed according to the calves' growth periodization, developed by the Institute of Physiology and Sanocreatology, which covered the time span from the 3rd to the 90th day of postnatal ontogenesis, the period in which the adaptive capacity of the calves is expected to increase.

The animals in both groups included in the experiment during the research period were in similar conditions of maintenance and consumed the same basic ration, which consisted of hay, straw, silage and concentrate fodder according to the existing norms. The calves access to food has been unlimited. At the same time, each calf in both groups consumed 300 liters of whole milk during the research period. Calves from the additional experimental group received the mineral premix "PMVS" to the main ration from the 7th day in the amount of 2.0 g per 1 liter of the consumed milk. The mineral premix "PMVS" was developed in the Institute of Physiology and Sanocreatology and represents an optimal mixture of micro- and macroelements in different concentrations and excipients. The quantity of mineral substances in the premix was calculated on the basis of the existing norms, the content of the mineral substances in the blood of the animals and their estimated value in fodder. This concentration, depending on the active properties of the integrated compounds, constituted various values in the range from 0.1 mg to 100.0 g.

At the same time, low temperatures of moderate stress intensity were applied to the calves in the experimental group for increasing the organism's adaptive capacity and resistance to environmental factors. The temperature of +5ºC has been applied as a stress factor. The application of the temperature of moderate stress intensity on the calves was performed during the early postnatal ontogenesis at the age of 3, 7, 15, 20, 25, and 30 days. After placing the animals into the „Zootron" climate chamber and adapting them to the new conditions within 1 hour, the temperature has gradually been decreased from the temperature recorded in the „Zootron" (which corresponds to the temperature in the stall for keeping calves) to +5ºC. A gradual decrease in temperature is carried out for 30 minutes. At the age of 3, 7 and 15 days the exposure to "low temperatures" lasts 1 hour, and at the age of 20, 25 and 30 days - 2 hours. Blood samples were collected at 3, 7, 30, 60, and 90 days of postnatal ontogenesis.

The following indices of natural resistance were investigated in order to determine the effect of low temperature of moderate stress intensity and the mineral premix "PMVS" on the resistance of the calves organisms according to the scheme of the proposed experiment: phagocytic activity (after Gostev), bactericidal activity (after Matusevici) and lysozyme (by photoelectrocolorimetric method). The statistical processing of the results has been performed according to the laws of variable statistics and probability theory. All biological indices investigated have been distributed according to the legitimacy of the normal distribution. In the statistical processing of the obtained data, special attention was paid to determining the reliability of the difference between the comparative values. The main conclusions in the paper are based on the statistically authentic differences between the control and experimental groups. The results are expressed as Mean ± standard deviation. The significance threshold shown: $P<0.05$ (Ивантер, 2010).

## RESULTS AND DISCUSSIONS

The resistance of the calves' organisms to the application of moderate intensity stress temperature and the mineral premix "PMVS" was assessed by the level of phagocytic activity, bactericidal activity and lysozyme in the blood serum. The data showing the values of the cellular factor (phagocytic activity) of the natural resistance are presented in table 1.

Table 1. Indices of phagocytic activity in calves subjected to the effect of low temperature of moderate stress intensity and the mineral premix "PMVS" in postnatal ontogenesis

| Age of calves (days) | Phagocyte activity (%), M±m | |
|---|---|---|
| | Control Group (CG) (10 heads) | Experimental Group (EG) (10 heads) |
| 3 | 63.41±1.93 | 63.97±1.84 |
| 7 | 62.00±2.00 | 64.67±3.72 |
| 30 | 54.67±1.34 | 60.67±4.67 |
| 60 | 35.33±1.77 | 42.00±1.16* |
| 90 | 55.33±1.91 | 62.67±1.77* |

Note: * - the differences are statistically truthful between the experimental and the control groups (P<0,05).
Here and further: CG - Control Group; EG - Experimental Group.

The level of phagocytic activity in 3-day-old calves in both groups was sufficiently high and amounted to 63.41±1.93%, and 63,97±1,84%. This fact shows that at the beginning of the experiments the colostrum immunity was quite high in both groups of animals. At the age of 30 days, phagocytic activity in the control and experimental group decreased in comparison with the initial level to 54.67±1.34% and 60.67±4.67%. The most obvious influence of the combination of the studied factors on phagocytic activity was recorded in 60-day-old calves. In the experimental group this amounted to 42.00±1.16%, and in the control group to 35.33±1.77% (P<0.05).

A similar tendency was observed in more mature calves. At the age of 90 days, the calves in the experimental group surpassed the calves of the control group in phagocytic activity. At the same time, the level of phagocytic activity in the experimental group (62.67±1.77%) is significantly higher in comparison with its level in the control group (55.33±1.91%) (P<0.05).

At the same time, a decrease in phagocytic activity was observed from the 3rd to the 60th day in both groups. In the control group it decreased from 63.41±1.93% to 35.33±1.77% (1,79 times), and in the experimental group from 63.97±1.84% to 42.00±1.16% (1.52 times). The obtained data correlate with those of the specialized literature, which show that in the early postnatal period cellular factors of immunity predominate and they compensate for the deficiency of humoral factors, which are formed during the growth and development of calves in different periods. Later, with age, the phagocytic activity of calves decreases slightly and the activity of humoral factors increases significantly. As early as 1987, Плященко observed an increase in the level of phagocytosis in calves at the age of up to five days, and in the following days, starting from the age of ten, a decrease in phagocytes was observed. In the course of these processes, the bactericidal activity of the blood serum gradually and continuously increased. Therefore, up to the age of ten days of calves, the high level of phagocytic activity compensates for the low level of bactericidal activity. At the age of two months, the level of bactericidal activity in calves is close to the level recorded in adult animals. In this context, Фурдуй (1986, 1994), as well as Петрянкин (2014) points out that the calves defense system begins to stabilize only at the age of 1.5-2 months.

Blood serum has pronounced bacteriostatic properties against many infectious agents. Therefore, the bactericidal activity of blood serum is an integral expression of antimicrobial properties, which are part of the humoral factors of non-specific defense. The bactericidal activity has also been studied as a humoral index of defense which characterizes stress resistance and adaptive capacities of the organism to the effects of stressors (table 2).

Table 2. Indices of bactericidal activity in calves subjected to the effects of low temperature of moderate stress intensity and the mineral premix "PMVS" in postnatal ontogenesis

| Age of calves (days) | Group of animals (CG - 10 heads) (EG - 10 heads) | Bactericidal activity (%), M±m | | |
|---|---|---|---|---|
| | | 1 hour | 3 hours | 6 hours |
| 3 | CG | 78.12±1.84 | 79.34±2.76 | 80.87±2.41 |
| | EG | 78.53±1.12 | 79.94±2.03 | 81.78±1.97 |
| 7 | CG | 80.00±3.00 | 79.33±4.06 | 81.33±1.34 |
| | EG | 74.33±2.61 | 82.33±3.53 | 90.33±2.70* |
| 30 | CG | 83.00±1.00 | 88.00±2.65 | 92.00±2.74 |
| | EG | 88.67±2.97 | 90.33±1.20 | 94.00±1.16 |
| 60 | CG | 89.33±4.67 | 87.67±1.45 | 89.33±4.67 |
| | EG | 91.00±1.16 | 88.33±3.93 | 91.00±1.16 |
| 90 | CG | 66.00±3.22 | 76.67±3.34 | 80.33±2.61 |
| | EG | 71.33±3.34 | 82.33±3.39 | 88.33±2.19* |

Note: * - the differences are statistically truthful between the experimental and the control groups (P<0,05).

The data presented in table 2 show that the application of low temperature of moderate stress intensity and the mineral premix "PMVS" in postnatal ontogenesis had a beneficial effect on bactericidal activity. At almost all studied ages, when exposed to 1, 3 and 6 hours, the bactericidal activity was higher in the experimental group. At the age of 7 days in the experimental group there was observed a significant increase in bactericidal activity, which was higher in comparison with the control group at the 6-hour exposure and amounted to 90.33±2.70% in comparison with 81.33±1.34% (P<0.05). At the age of 90 days when exposed to 1, 3 and 6 hours there was an obvious tendency and a significant increase in bactericidal activity in the experimental group, where it amounted, accordingly, to 71.33±3.34%. 82.33±3.39% and 88.33±2.19% (P<0.05) in comparison with 66.00±3.22%, 76.67±3.34% and 80.33±2.61% in the control group.

Further, according to the experimental scheme, the amount of lysozyme in the blood serum was studied as a humoral factor of the nonspecific defense of the organism (table 3).

Table 3. Indices of lysozyme in calves subjected to the action of low temperature of moderate stress intensity and the mineral premix "PMVS" in postnatal ontogenesis

| Age of calves (days) | Lysozyme (%), M±m | |
|---|---|---|
| | CG (10 heads) | EG (10 heads) |
| 3 | 26.09±0.33 | 26.18±0.38 |
| 7 | 27.16±0.07 | 26.29±0.05 |
| 30 | 22.08±0.17 | 26.02±0.28* |
| 60 | 29.84±0.62 | 33.76±0.71* |
| 90 | 31.43±0.27 | 34.06±0.68* |

Note: * - the differences are statistically truthful between the experimental and the control groups (P<0.05).

The data in table 3 show that throughout the experiment the lysozyme in the experimental group was higher than in the control group except on the 7th day when its content decreased. At later ages of calves, at 30, 60 and 90 days, the lysozyme content increased accordingly from 22.08±0.17%, 29.84±0.62% and 31.43±0.27% to 26.02±0.28%, 33.76±0.71% and 34.06±0.68% (P<0.05).

The results obtained are in a reciprocal link with the data of the specialized literature, which show that at the beginning of the postnatal period, the values of lysozyme, and bactericidal activity of the blood serum are low. In the next 2-3 weeks of calves life, a rapid increase in humoral factors was established, which reached a relative stability at the age of 6 months, and their final formation at the age of 11-12 months (Фурдуй, 1986, 1994; Плященко, 1987; Петрянкин, 2014).

The experimental data obtained indicate that raising the biological value of the diet by supplementing with the developed mineral premix and the simultaneous action of the thermal factor had a positive effect on the cellular and humoral link of the natural resistance of animals in postnatal ontogenesis.

Another important indicator of the effect of the mineral premix "PMVS" and of the studied temperature variation on the functional state, adaptive capacities and resistance of the organism is the productivity of the animals expressed in terms of body weight. The dynamics of the body mass of the experimental animals in the studied periods of age are presented in table 4.

Table 4. The dynamics of weight in calves subjected to the effects of low temperature of moderate stress intensity and the mineral premix "PMVS" in postnatal ontogenesis

| Age of calves (days) | Weight (kg), M±m | |
|---|---|---|
| | CG (10 heads) | EG (10 heads) |
| 3 | 29.7±0.76 | 29.2±0.84 |
| 7 | 32.77±0.86 | 32.55±1.08 |
| 30 | 47.78±1.37 | 47.34±2.09 |
| 60 | 67.82±1.99 | 69.50±1.88 |
| 90 | 90.90±1.11 | 95.80±1.21* |

Note: * - the differences are statistically truthful between the experimental and the control groups (P<0.05).

The data in table 4 show that the studied factors applied in postnatal ontogenesis have influenced the body productivity of calves. At the beginning of the experiments, when the groups of calves were formed according to the “analogy” principle, the average weight of the calves was 29.7±0.76 kg in the control group and 29.2±0.84 kg in the experimental group. Despite the fact that at the age of 7 and 30 days the action of low temperature of moderate stress intensity and the mineral premix "PMVS" had a positive influence on phagocytic activity, bactericidal activity and lysozyme, however, there were not significant changes in body weight. The last one constituted at the age of 7 days in the control group 32.77±0.86 kg and in

the experimental group 32.55±1.08 kg, at the age of 30 days respectively, it was 47.78±1.37 kg in the control group and 47.34±2.09 kg in the experimental group.
The recorded positive deviations of the animals defense capacity indices (phagocytic activity, bactericidal activity and lysozyme) show that the effect of low temperature of moderate stress intensity and the mineral premix "PMVS" was sufficient only for the increase of defense capabilities and maintenance of the functional stability of the body, but it was not enough for increasing the organism's productivity and, in particular, for increasing the body weight.
At the age of 60 days, the weight of the calves in the experimental group was 69.50±1.88 kg compared to 67.82±1.99 kg in the control group and it was 1.02 times higher. The data presented show that at the age of 60 days the studied factors had a positive effect on animals' productivity. This tendency was maintained at the age of 90 days. The weight of the calves during this period was 90.90±1.11 kg in the control group and 95.80±3.21 kg in the experimental group ($P<0.05$). On the whole, during the whole research period, the diurnal weight increase of the calves in the experimental group was 766 g compared to 703 g and was 1.1 time higher than in the control group.

## CONCLUSIONS

Based on the results obtained, it was found that the effect of low temperature of moderate stress intensity and the mineral premix "PMVS" on the body of calves in postnatal ontogenesis causes changes in phagocytic activity, bactericidal activity and lysozyme. The tendency to an increase in phagocytic activity throughout the entire study period in comparison with this activity in the control group has been experimentally determined. Changes in all studied periods of calves age were also observed in the dynamics of bactericidal activity and lysozyme. The recorded growth denotes an increase of the organism's defense capabilities and optimal maintenance of homeostasis.
The increase of the biological value of the food ration, by including the mineral premix "PMVS" and the simultaneous use of the low temperature of a moderate stress intensity also positively influenced the body growth of the calves in the experimental group.
Maintenance of the organism's homeostasis within optimal physiological limits is the main task of animal physiology. Determining the parameters of the influence of food and heat factors on homeostasis and calves development in postnatal ontogenesis allows to create favorable conditions that can facilitate the acceleration of functional maturation of vital organs and systems, increase the organism's resistance and adaptive capacity to the influence of stressors and realize the genetic potential of animals.

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# IMPACT OF VARIOUS FACTORS ON LIVE BIRTH WEIGHT LAMBS - REVIEW

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***Abstract***

*The survival of the newborn in the first days is directly dependent on live birth weight. The indicator is related to the vitality and mortality of lambs, and also plays an important role in the later development of the young organism. Factors influencing live birth weight are genetic (breed, the effect of heterosis) and non-genetic (age, weight, body condition of the sheep, diet, year, season, month of birth, type of birth, sex, etc.). In all mammals, there is an "optimal" birth weight, as a result of which the birth process proceeds naturally and without complications. The objective of this survey is to investigate and summarize the factors that affect live birth weight of lambs.*

***Key words:*** *birth weight, factors, lambs*

## INTRODUCTION

Sheep are widespread throughout the world, they are a major sub-sector of animal husbandry in different countries.

Live weight at birth is of interest because of its positive genetic correlation with the further live weight of the animals (Mellado et al., 2016), and also plays a key role in achieving better economic results on the farm. Live birth weight affects the vitality, mortality and growth of lambs (Cloete et al., 2001; Zapasnikiene, 2002; Berhan & Arendonk, 2006; Petrovic et al., 2009; Vatankhah & Talebi, 2009), which defines it as the initial factor influencing the later development of the young organism (Riggio et al., 2008).

The characteristic varies among breeds in different regions of the world because of the impact of genetic (breed, the effect of heterosis) and non-genetic factors (age, weight, body condition of the sheep, diet, year, season, month of birth, type of birth, et al.) (Kafi et al., 2004; Mandal et al., 2006; Zhang et al., 2009; Hussain et al., 2013; Karmakar et al., 2018).

Knowledge of these factors is particularly important given the association of this characteristic with the health of newborns and adults (Gardner et al., 2007; Chniter et al., 2009).

The most common method for increasing lamb production is the industrial crossbreeding (heterosis effect) with meat-producing breeds, as it directly affects the increase in live weight at birth of crossbred lambs (Petrović et al., 2011; Ivanov et al., 2015).

Alsheikh (2005) reported that the high level of inbreeding in Barki lambs had a negative effect on their birth weight.

The ewe's age had a significant effect ($P<0.01$) on the birth weight of the lamb. Younger ewes use some of the energy for their own growth and development, and the rest for the fetus, which leads to lower birth weight lambs.

While older ewes have already completed their growth and can direct all their energy to productivity and the birth of heavier lambs (Babar et al., 2004).

According to Wu et al. (2006) with increasing age of ewes, the size of the uterus, placenta and nutrient transfer from mother to fetus increases, leading to higher birth weight.

The weight of ewes during the mating season was positively associated with increased ovulation, leading to improved reproductive performance (Scaramuzzi et al., 2006).

Also, heavier ewes are usually well fed and tend to give birth to heavier lambs (Koritiaki et al., 2013).

Feeding of ewes is essential for the supply of nutrients necessary for fetus development (Robinson et al., 2002

Insufficient or excessive feeding of the mother can significantly affect prenatal and postnatal growth and development of the lamb (Barker, 2004; Caton & Hess, 2010).

The marketing year affects live birth weight through climatic characteristics for different geographical regions (Mellado et al., 2016), through farm management and disease outbreaks (Gardner et al., 2007).

The level of management depends on the abilities of the farm manager, his efficiency in the supervision of the staff, the care of the staff for the ewes and lambs, the financial resources, the availability of fodder and others.

Numerous studies have shown the strong impact of the season (rainfall rate, wind speed, humidity, temperature, day length, vegetation growth on pastures) on the birth weight of lambs in different breeds (Yilmaz et al., 2007, Rosov & Gootwine, 2013; Petrović et al., 2015).

The studied trait was also significantly influenced ($P<0.01$) by the type of birth and sex of the newborns.

Single lambs are born with a higher live weight than twins, triplets, as they have no competition for nutrients in the mother's womb (Petrović et al., 2011; Momoh et al., 2013). Male lambs are also born heavier than females due to the anabolic action of male sex hormones (Babar et al., 2004; Rashidi et al., 2008).

Saghi et al. (2006) reported that in the Iranian Baluchi breed, male lambs and females had higher birth weights than twins.

Survival of up to 48 hours is most affected by lamb birth weight (Oldham et al., 2011).

## MATERIALS AND METHODS

The study was based on the analysis of current bibliographic sources with the theme in the factors that affect live birth weight of lambs.

## RESULTS AND DISCUSSIONS

In this survey, the genetic and non-genetic factors influencing the live weight at the birth of lambs is observed.

**Breed** – The sheep breed is one of the genetic factors influencing the live weight at birth of lambs. There is a significant variation of the trait between different breeds.

Siddalingamurthy et al. (2017) received an average live birth weight of 2.07±0.01 kg for the Indian breed Mandya, while Achkakanova and Staykova (2021) reported an average of 5.193 kg for the Ile de France breed. In Nigerian breeds, the trait varies from 3.11±0.04 kg in Uda to 3.55±0.04 in Balami (Momoh et al., 2013). The birth weight of Dorper lambs reported by various authors is 3.3-3.9 kg (Hinojosa-Cuéllar et al., 2013; Mellado et al., 2016). And in Santa Inês is in the range of 3 to 4 kg, (Peruzzi et al., 2015; Torres et al., 2021). The values of the studied trait in Lacon female and male lambs were 3.90±0.7 and 4.6±0.2 kg, respectively (Barillet et al., 2002; Thomas et al., 2014). The average live birth weight of the Rambouillet breed was 3.17±0.04 kg (Mika et al., 2018), and the average live weight of Thalli was 4.11±0.82 kg (Hussain et al., 2013). The lowest average values of live weight were measured in lambs of Karakachan breed (3.40 kg), while in Teteven and Srednostaroplaninska offspring they were 4.50 kg and 4.10, respectively (Genkovski, 2006). In Tsigai, bred in Romania, the trait varies from 4.1 to 4.3 kg (Ilişiu et al., 2013).

Another genetic factor influencing birth weight in lambs is the effect of heterosis.

**Heterosis effect (from genotype)** – Heterosis (heterosis effect) occurs when crossing two or more breeds of sheep and leads to a much larger number of combinations of genes and thus is more likely to express important and favorable economic traits (Petrovic et al., 2013).

In the resulting crossings (F1) heterosis is expressed in increased live weight at birth, viability, higher growth capacity, higher productivity, better resilience and adaptability to environmental conditions, better utilization of feed compared to parental forms (Leymaster, 2002; Petrović et al., 2011, 2019). The most widely heterosis effect is used through the so-called industrial (user) crossbreeding. The purpose of this method is to obtain animals for commodity production with higher growth capacity, better quality meat, lower fodder consumption per 1 kg of growth, which leads to early reaching the desired market weight and brings faster farmer income (Hussain et al., 2013). A number of authors have found that the growth rates of crossbred lambs are better than those of local lambs, as well as the growth rates before and after weaning (Dawson & Carson, 2002; Momani et al., 2010). Lakew et al. (2014) reported an average birth weight of lambs of local Ethiopian breeds and crossings between local with Dorper, 2.36±0.05 and 3.24±0.04 kg, respectively. The indicator varies from 3.56 kg in Pirot x Württemberg crosses to 3.69 kg in Sjenica x Württemberg (Petrović et al., 2015). Crossbred lambs obtained between 'Bulgarian Dairy Synthetic Population' (BDSP) and Mouton Charollais have the highest average live weight at birth with 4.18 kg, while BDSP weigh an average of 2.83 kg, and crossings between BDSP x Ile de France with 3.978 kg (Ivanov et al., 2015).

**Age of ewes** – The weight of the ewes and the order of birth have an effect on the birth weight of the lambs. Older ewes give birth to heavier lambs than two-year-old lambs, but this effect decreases when ewes reach the age of 8 (Amores et al., 1998; Babar et al., 2004; Pettigrew et al., 2019). These results are in line with the findings of Gama et al. (1991) in several sheep breeds Finnsheep, Dorset, Rambouillet, Suffolk and Targhee. Depending on the age of the ewe, the variation in birth weight is from 3.38 to 3.82 kg in crossbred lambs from Pirot x Württemberg, while in Sjenica x Württemberg is from 3.43 to 3.95 kg (Petrović et al., 2015). In local breeds of sheep, such as Pirot and Svrljig, Petrovic et al. (2011) found that young and old ewes gave birth to lighter lambs, while middle-aged sheep gave birth to heavier lambs, with statistically significant differences ($P<0.01$). Aljubouri et al. (2021) reported that Avasi and Karakul sheep over 4 years of age gave birth to lambs with a higher live weight (4.45±0.08 kg). In a study by Mellado et al. (2016) young Dorper ewes (aged <20 months, 3.6 kg) gave birth to lambs that were 300 g lighter ($P<0.05$) at birth than ewes aged > 20 months (3.9 kg). Koritiaki et al. (2013) reported a higher birth weight of Santa Inês lambs born to older ewes (4.23±0.19 kg) than those born to younger ewes (2.93±0.27 kg). Eight-year-old ewes of the Ascanian fine fleece breed gave birth to lambs with a live weight higher than the average for the population (101 g), while the youngest ewes had lambs with significantly lower than the average for the population (155 g) (Ktamarenko et al., 2020).

**Weight and body condition of ewes** – The body weight of ewes at the end of pregnancy is important because a sheep with a higher body weight gives birth to heavier lambs with better survival (Koritiaki et al., 2013). Lambs with higher live weight at birth have more intensive growth and they produce heavier carcasses (Cemal et al., 2005).

Petrovic et al. (2015) report that lambs are heavier in both genotypes if their mother is heavier. The differences were 0.14 kg for Pirot x Württemberg lambs ($P<0.05$) and 0.26 kg for Sjenica x Württemberg lambs ($P<0.05$). Identical results were obtained by Mahala et al. (2019) in lambs of the Avikalin breed. In two local Serbian sheep breeds, Pirot and Svrljig, the effect of maternal weight on live weight at birth was found to be statistically significant ($P<0.05$). Heavier lambs (3.41 kg for Pirot and 3.50 kg for Svrljig) were born from sheep with higher live weight, and lighter lambs were obtained from mothers with lower live weight (3.33 kg for Pirot and 3.37 kg for Svrljig) (Petrović et al., 2011). The birth weight of the lambs was not affected by the body condition score (BCS) of the ewes in mid-pregnancy, but was affected by BCS at the end of pregnancy ($P<0.0001$). Pesántez-Pacheco et al. (2019) reported that Lacon lambs born to sheep with high BCS ($BCS\geq 3$) were heavier and larger than those born to sheep with low BCS ($BCS \leq 2$). Sheep of the Norduz breed with BCS 2.5 gave birth to lambs with a live weight of 4.77±0.11 kg, those with BCS 3-4.92±0.14 33 kg, and with BCS 3.5-5.18±0.29 kg (Karakus & Atmaca, 2016). Live weight and body condition score of ewes are related to nutrition.

**Ewes' feeding** – The ewes' feeding during pregnancy is a major factor in fetus growth (Caton & Hess, 2010). It must be adapted to the physiological condition of the animals in order to prevent the use of their own body reserves (Robinson et al., 2002). The combination of higher nutrient requirements and low intake, during the beginning of lactation and at the end of pregnancy, in high-yielding animals, can lead to a negative energy balance. This significantly increases the risk of metabolic diseases, especially ketosis, which contributes to significant production losses (Chilliard et al., 2000). According to Reed et al. (2007) and Swanson et al. (2008) decreased nutrient intake by the ewes during the last two thirds of pregnancy leads to weight loss at birth. The level of maternal feeding between the 30th and 80th day affects birth weight ($P<0.01$), placental weight, placental activity and the average surface area of cotyledon ($P<0.01$) (Aysondu & Ozyurek, 2020).

Placental growth in sheep begins approximately on the 30th day of pregnancy (Symonds et al., 2007) and ends by the 100th day (Redmer et al., 2004). Poor feeding from the 28th to the 78th-80th day of pregnancy, when maximum placental growth occurs, reduces placental mass (Symonds et al., 2007) and placental size (Clarke et al., 1998). Changes in placental growth can lead to low birth weight due to the high correlation between placental weight and fetal weight (Mellor & Murray, 1982). It has been found that overfeeding of young ewes during pregnancy leads to rapid growth of the ewe and especially maternal adipose tissue, but at the expense of the nutritional needs of the pregnant uterus. As a result, the rapid growth of the mother leads to limited placental growth, premature birth of low-weight lambs (Wallace et al., 1996, 1999, 2001). In merino sheep Oldham et al. (2011) reported that the effect of poor feeding up to the 100th day of pregnancy can be completely overcome by improving it at the end of pregnancy.Which will lead to weight gain at birth and increase the survival of offspring.

**Year, season and month of lam**bing – Differences in feeding (especially during pregnancy), farm management, availability of fodder, diseases and others in different years are the reasons for the effect of the marketing year on the birth weight of lambs (Hassan & Seyed, 2009). In lambs of Staroplaninski Tsigai and Karakachan breeds, Ivanova et al. (2021) found a highly reliable effect of the economic year on live weight at birth in both sexes ($P<0.001$). The results obtained for live weight at birth in female lambs of Staroplaninski Tsigai vary between 2.916 kg and 3.706 kg, and of males – between 3.109 kg and 4.271 kg. In both sexes, the highest live weight was at birth in 2019 ($P<0.001$), and the lowest in 2021. The female Karakachan lambs born in 2019 has the lowest weight (2.822 kg) ($P<0.001$), and the heaviest are those born in 2020 (3.110 kg). The results are identical for male lambs of the same breed in 2019 (3.211 kg) and in 2020 (3.718 kg). Mean birth weights showed wide variations over the years, ranging from 3.13±0.07 kg (1984) to 4.77±0.07 kg (1995) for the Thalli breed (Hussain et al., 2013). Assan & Makuza (2005) reported that the marketing year had a significant effect ($P<0.05$) on birth weight in Sabi, Mutton Merino and Dorper sheep. The birth weight observed over three years ranged from 3.35 to 3.87 kg in Pirot x Württemberg lambs and from 3.40 to 3.93 kg in Sjenica x Württemberg lambs (Petrović et al., 2015). Lambs born during the rainy season were heavier at birth than those born during the dry season (Momoh et al., 2013). Higher birth weights were reported in Avikalin lambs born in the spring, probably due to the presence of pastures for ewes before the lambing season (Mahala et al., 2019). Avasi and Karakul lambs born in January showed significantly higher birth weight values (4.65±0.04 kg) than those born in November (3.95±0.08 kg) and December (4.34±0.11 kg) (Aljubouri et al., 2021). The highest ($P<0.05$) average birth weight was recorded in summer and the lowest in winter and spring in Dorper lambs reared in the intensive care system in Mexico. The lower body weight of lambs born in winter and spring underscores the need to provide additional food to ewes in late autumn to increase fetus growth rates (Mellado et al., 2016).

**Type of birth** – The type of birth (singles or twins, triplets, etc.) has a significant effect on birth weight and can be explained by the limited uterine space and feeding of lambs during pregnancy (Gamasaee et al., 2010; Momoh et al., 2013). Baneh & Hafezian (2009) report that the live weight of single lambs of all ages and their average daily gain is higher than that of twins due to competition between twins for breast milk, which leads to breastfeeding less milk. The reduction in birth weight compared to the type of birth is greater in females. The most important factor influencing the birth weight of lambs is the number of offspring. The uterine space of the ewe has a limited capacity and with increasing number of offspring the weight at birth of the individual decreases. Lambs born as singles were 0.6 kg heavier ($P<0.05$) than twins, 1.6 kg heavier than triplets and 1.9 kg heavier than quadruplets (Gluckman & Hanson, 2004; Mellado et al., 2016). The type of birth affects the body weight of Pirot and Svrljig lambs. Changes in birth weight ranged from 3.27

(twins) to 3.48 kg (singles) in the Pirot breed and from 3.36 (twins) to 3.53 kg (singles) in the Svrljig breed (Petrović et al., 2011). Identical results were obtained by Mirderikvandi et al. (2016) in lambs of the Iranian breed Lori Bakhtiari.

In the Ile de France breed, the average live weight at birth of single male lambs (5.1 kg) was 4.36 kg for twins and 3.7 kg for triplets. In female singles the indicator is 4.65 kg, in twins it is 4.23 kg and in triplets – 3.32 kg (Ivanova, 2021).

**Lamb's sex** – The effect of sex on live weight at birth can be explained by differences in the number of cotyledons (higher in ewes carrying male lambs) and the weight of the placenta (heavier in ewes carrying male lambs) (Jawasreh et al., 2009). The anabolic effect of male sex hormones may also be the reason for the higher birth weight of male lambs (Hafex, 1962). Male lambs are likely to start releasing androgens earlier, growing and developing faster than females (Ebangi et al., 1996). Estrogen hormone has a limited effect on the growth of long bones in females. This may be one of the reasons why female lambs have less body and less weight than males (Rashidi et al., 2008). In the Thalli breed, male lambs showed a higher birth weight (4.21±0.10) than females (3.85±0.08) (Hussain et al., 2013). Similar results were obtained by Babar et al. (2004) in the Indian breed Lohi, in male lambs the average live weight at birth was 3.69±0.02 kg, and in females 3.48±0.02 kg. Mellado et al. (2016) found that male Dorper lambs were on average 200 g heavier at birth than females (P<0.01). The average birth weight of both sexes of lambs is almost the same (3.39 and 3.36 kg in Pirot and 3.48 and 3.43 kg in Svrljig) (Petrovic et al., 2011).

## CONCLUSIONS

Establishing the links between all these factors in different sheep breeds raised in different climatic zones is necessary to obtain high live weight lambs at birth, alive and healthy, and hence to achieve better economic results on the farm.

It has been found that older and heavier ewes give birth to heavier lambs, and male lambs compared to female, and singles compared to twins have higher birth weight.

Genetic factors, nutrition and farm management have a major impact on live birth weight.

There is an "optimal" birth weight, as a result of which the birth process proceeds naturally and without complications.

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# SHEEP GENERAL ANESTHESIA FOR EXPERIMENTAL RESEARCH PROCEDURES

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***Abstract***

*We evaluated an anesthesia protocol for sheep as an experimental animal for surgical procedures. The entire group (10 sheep, breed Tsigai, 4 years old, 60.91 kg mean body weight) underwent experimental dentistry surgery and received a complete anesthetic protocol: intramuscular premedication (midazolam 0.18 mg/kg, ketamine 4.6 mg/kg, butorphanol 0.1 mg/kg), intravenous induction (propofol 4.45 mg/kg), intubation (endo tracheal cuffed tubes, size 7.5 mm and 8 mm), gas maintenance (isoflurane minimum alveolar concentration of 1.5-2%, a standard small animal circle circuit and spontaneous respirations during the procedures). The protocol was completed with analgesia (meloxicam 1 mg/kg) and clinical monitoring during the entire surgical procedure and in the recovery phase. Sheep were infused at a rate of 5 ml/kg/h with Ringer's Lactate solution, during anesthesia. The anesthesia duration varied between 32 -215 minutes, with a mean of 83.7 minutes. No incidents or complications were recorded during anesthesia. One sheep presented myopathy and lameness (right forelimb) in the first 72 hours after anesthesia, possibly associated with the positioning and the length of the procedure (135 minutes).*

***Key words****: anesthesia, intubation, isoflurane, protocol, sheep.*

## INTRODUCTION

The scientific progress made in the recent years have enabled the research of new medical technologies, bringing together human and animal specialists in complex projects. In this context, it is important to design and conduct experimental studies with reliable animal models. While sheep (*Ovis aries*) are widely used as large animal models, current literature generally focuses on anesthesia and analgesia protocols used for the farm level (Stillman & Whittaker, 2019). The sheep is an excellent animal model for the evaluation of materials for bone regeneration and osteointegration of dental implant system in dentistry (Sartoretto et al., 2016). Adult sheep offer the advantage of a body weight, bone mineral composition and metabolic and remodelling rates similar to human patients (Pearce et al., 2007).
Due to the lack of literature comprehensive data regarding anesthesia for sheep animal models, it is necessary to contribute to the standardisation of the techniques and protocols, according to different types of experimental procedures, covering.
The choice of medication and the administration route used for anesthesia and analgesia during the procedures, need to be appropriate for the animal as well as compatible with the project.

## MATERIALS AND METHODS

The researches described in this study were favourably approved by the ethics commission Cantacuzino National Military-Medical Institute for Research and Development, Bucharest, Romania, decision 43/13.04.2021, project authorization 625/17.05.2021.
All researches were performed in accordance with the rules for the care and use of animals used for scientific purposes established by national and European regulations and the application of the 3R principles of replacement, reduction and refinement.
The studies were performed in Băneasa location, in the Preclinical Testing Unit, part of Cantacuzino National Military-Medical Institute

for Research and Development and authorized as a user unit by the competent authority.

Ten adult sheep (*Ovis aries*), breed Tsigai, 4 years old, weighing between 53-69 kg (60.91 kg mean body weight) underwent general anesthesia for experimental dentistry surgery. The complete anesthetic protocol consisted in premedication with midazolam (0.18 mg/kg, i.m., Midazolam®, Baxter Holding B.V., Olanda), ketamine (4.6 mg/kg, i.m., Ketamidor®, Richter Pharma, Austria) and butorphanol (0.1 mg/kg, i.m., Butomidor® Richter Pharma, Austria), induction with propofol (4.45 mg/kg, i.v., Proposure® Richter Pharma, Austria), followed by endotracheal intubation and maintenance with isoflurane (Vetflurane®, France), started with 3-4% for 5 minutes and maintained at 1.5-2 % during the procedure.

In order to ensure a multimodal approach and analgesia, mandatory during long and complex procedures (Valverde & Doherty, 2009), the protocol was completed with ketamine infusion (0.5 mg/kg/h, i.v., Ketamidor®, Richter Pharma, Austria) during maintenance phase and one dose of meloxicam (1 mg/kg, i.m., Meloxidolor®, Le Vet Beheer B.V., Olanda) in the early recovery.

During anesthesia sheep were infused at a rate of 5 ml/kg/h with Ringer's Lactate solution (Soluție Ringer Lactat®, B Braun, Germany), thus ensuring the maintenance of homeostasis.

## RESULTS AND DISCUSSIONS

The entire group was prepared for the surgical dentistry procedure, under general anesthesia. The choice of anesthesia and analgesia protocols was decided based on species particularities, type of surgery, possible pain and equipment avaible.

In order to minimize regurgitation and the development of ruminal tympany in the recumbent sheep, food was restricted for 24 h before anesthesia (Drake et al., 2021).

Free access to water was allowed until the premedication protocol was administered. Food and water withholding before anesthesia and endotracheal intubation with cuffed tubes after induction are both decreasing the risk for regurgitation and were included in our protocol for safety reasons.

Preanesthetic evaluation (physical condition, physical examination), was performed under no stress conditions, avoiding brutal contention methods or loud noises, in order to reduce stress factors (Costea, 2017). After the evaluation the entire group was assigned to ASA 1 risk group classification adapted for veterinary medicine, that allowed us to design an anesthetic protocol for healthy animals, with a low anesthesia risk compared to other patient's risk categories (Table 1).

Table 1. American Society of Anesthesiologists-ASA classification (Costea, 2017)

| | |
|---|---|
| **ASA 1** | A normal healthy patient, with no organic disease |
| **ASA 2** | A patient with mild systemic disease |
| **ASA 3** | A patient with severe systemic disease that limits activity but it's not incapacitating |
| **ASA 4** | A patient with sever systemic disease that is a constant threat to life |
| **ASA 5** | A moribund patient who is not expected to survive 24 hours without intervention |

Weight was measured with an electronic scale weighing between (60.91 kg mean body weight). For an accurate estimation of weight, in order to improve the safety of anesthesia, we decided to exclude the wool weight (57.89 kg mean body weight without wool).

Taking in to consideration the breed particularities, regarding wool production and data form the literature (Hutu et al., 2020; Pascal et al., 2014), we decided to use for the entire group a "wool free weight", by reducing the total body weight measured, with 5%. The results were recorded on individual anesthesia sheet.

The anesthetic protocol began with the administration of premedication by intramuscular injection in the triceps muscle. Sheep were premedicated with a combination of midazolam (0.18 mg/kg, i.m., Midazolam®, Baxter Holding B.V., Olanda), ketamine (4.6 mg/kg, i.m., Ketamidor®, Richter Pharma, Austria) and butorphanol (0.1 mg/kg, i.m., Butomidor® Richter Pharma, Austria).

Ketamine in combination with a benzodiazepine (midazolam) and an opioid (butorphanol) provides moderate relaxation, good analgesia and minimal cardiovascular depression (White & Taylor, 2000).

An intravenous catheter (20 gauge) was placed in the cephalic vein after premedication, prior to induction. The catheters were easily placed, the animals premedicated and under sedation.

Mean time between premedication and prior to the induction of anesthesia was 17 minutes (11–20 min).

Anesthesia was induced with propofol (4.45 mg/kg, i.v., Proposure® Richter Pharma, Austria) by slow intravenous injection until effect and after the intubation procedure was started.

With sheep in sternal recumbency and the neck in hyperextension, the trachea was intubated. A long laryngoscope blade was necessary to visualize the laryngeal opening. The thick of the base of the tongue increased the difficulty of intubation.

The intubation was done carefully, under direct visual control, without forcing the insertion of the tube into the trachea. During the intubation, especially if the procedure lasted longer, we tried to additionally provide oxygen to the patient through a flow by technique.

Tracheal palpation and direct visualisation during intubation, determined us to select the appropriate tube size. Sheep required an endotracheal tube with an internal diameter of 7.5 or 8 mm. After intubation the endotracheal tube was cuffed and secured to the mandible with textile ropes, leaving the tongue free.

Eyes were lubricated with a mild ophthalmic ointment (OptixCare® Plus Eye Lube; CLC MEDICA), in order to reduce the risk of corneal lesions during anesthesia (Riebold, 2007).

A support was placed in the submandibular region (textile roll), to raise the cephalic extremity and to avoid saliva regurgitation (Figure 1).

Figure 1. Sheep in sternal position, intubated and connected to the anesthesia machine, with a support placed in the cervical region

Particular care was taken to ensure adequate padding in order to prevent excessive pressure against nerves and major muscle groups.

Anesthesia was maintained with isoflurane 1.5-2% (Vetflurane®, France),) in 100% oxygen delivered through a standard small animal circle circuit and supplemented with ketamine by continuous rate infusion (0.5 mg/kg/h, i.v., Ketamidor®, Richter Pharma, Austria).

Sheep were positioned in sternal recumbency allowing a good surgical access for the oral cavity and increasing the chances to maintain a stable ventilation, cardiac output and arterial blood pressure during anesthesia (Desmecht et al, 1995).

The anesthesia machine with ventilator was prepared with settings for 10 breaths per minute, inspiratory: expiratory ratio to 1:2, positive end expiratory pressure to 10 cm $H_20$, tidal volume 10 mL/kg, PIP 15 cm $H_2O$.

The mean duration of the maintenance phase (from induction to extubating time, in the recovery phase), was 66.7 minutes, times varying from 32 minutes to 215 minutes.

During the procedures, the entire group, all 10 sheep were breathing spontaneously, none requiring assisted ventilation, even during deep anesthesia.

Intravenous fluid therapy during anesthesia was considered necessary to prevent hypotension and hypoperfusion, with a maintenance rate of 5 ml/kg/h with Ringer's Lactate solution (Soluție Ringer Lactat®, B Braun, Germany).

Monitoring the anesthetic plane was continuous throughout the procedures (absence of the palpebral reflex, mandibular tonus, cardiopulmonary stability, any movements or swallowing).

Unlike for other species, rotation of the eye cannot be considered a reliable indicator of the depth on anesthesia (White & Taylor, 2000).

Advanced clinical monitoring was used while maintaining anesthesia: pulse-oximetry (probe positioned on tongue or ear pinna), capnography, rectal temperature measuring (rectal probe), blood pressure measurement (indirectly by using a pressure cuff) and continuous electrocardiography (Figure 2).

A mean arterial blood pressure of at least 75 mmHg (systolic 100 mmHg, diastolic 60 mmHg) was maintained. Values were recorded in the anesthesia sheet, every 5 minutes.

Saliva production during anesthesia was not problematic, since the patient was positioned with the cervical area elevated, allowing secretions to drain.

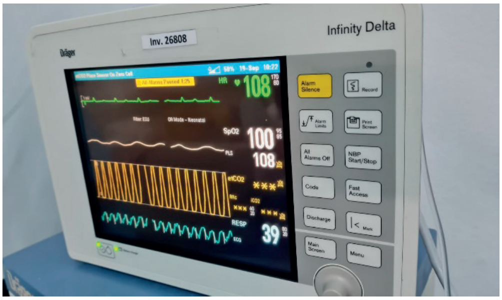


Figure 2. Monitoring anesthesia, during maintenance phase

We did not used anticholinergic drugs, since salivation is not markedly reduced by anticholinergics and may become more viscid and therefore more liable to produce airway obstruction (White & Taylor, 2000), we preferred to assure an optimal positioning.
In order to prevent excessive gas production during anesthesia a stomach tube was placed during the entire procedure.
Given the need for continuous monitoring as well as ensuring the possibility of the gastric tube maneuver to eliminate gas at any time, the animal was positioned with its head elevated, in a lateral position, thus facilitating direct and unrestricted access to the surgical procedure (Figure 3).

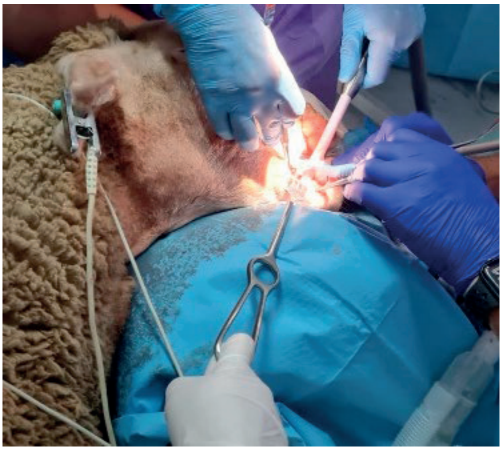

Figure 3. Surgical field access

Cuff deflation and extubating were delayed until the patient was clinically light enough and palpebral reflex, coughing, swallowing and movements of the limbs or head were regained. The mentioned reflexes reappeared extremely quickly after the cessation of the administration of isoflurane, at about 2-3 minutes the animals can be extubated.
Certainly, the fact that the animals were spontaneous throughout the maintenance of anesthesia represented an advantage for the recovery phase.
After extubating, the careful monitoring of the vital functions was continued, as well as the administration of the infusion solution, for another 10 minutes. During this phase animals were calm and cooperative. Efforts have been made to reduce any additional stressors, such as loud noises, excessive light or sudden movements.
After this interval, the animals were moved from the operating room to the wake-up area where their clinical monitoring continued. Animals were placed in a warm, sheltered space, to avoid hypothermia that often results in a prolonged recovery (Galato, 2011). No incidents were recorded in the early recovering phase.
Recovery from anesthesia was uneventful, sheep recovering completely after extubating in 15-22 minutes (mean 19 minutes), with no corelation regarding the duration of anesthesia.
Despite the duration of the procedures and the anesthesia protocols used, which involved a deep anesthesia with a high degree of analgesia, all the animals recovered very quickly.
When animals were able to adopt the four-legged position and to move without hesitation, they were transferred to their accommodation where they had access to food and water.
Animals resumed their routine of free movement, drinking and feeding at about 40- 45 minutes (mean 44 minutes) after completion of anesthesia (Figure 4).

Figure 4. Feeding after the recovery phase

One incident was noted in the late recovery phase. It was noticed 1 hour after anesthesia and it lasted for 72 hours. A sheep presented acute lameness (right forelimb). Myopathy was suspected. Muscle perfusion may be affected during recumbency due to arterial hypotension, pressure on muscles due poor positioning, noncompliant surfaces or prolonged periods of recumbency, especially for large animals (Seddighi & Doherty, 2016). In this case myopathy was possibly associated with the positioning and the length of the procedure (135 minutes for this case), despite padding, the careful positioning and the attention for maintaining adequate arterial blood pressure.

This sheep received anti-inflammatory therapy (meloxicam- Meloxidolor®, 1 mg/kg, i.m., for 3 consecutive days), during which time lameness gradually subsided.

## CONCLUSIONS

The anesthetic protocols developed for experimental research procedures, should provide quick and deep anesthesia and analgesia to suit the type and duration of the surgical procedure and the individual characteristics of the patient.

Choosing the dosages for the protocol and the right equipment for anesthesia should take into account the weight of the patient, ideally without the wool weight, which can vary according to age, breed or raising system and requires experience for a correct estimation.

The premedication protocol used provided sedation and analgesia enabling the subsequent procedures, calming the patients and assuring a smooth recovery.

Orotracheal intubation can be performed quickly and safely, after positioning the sheep in sternal recumbency with the cervical region in hyperextension and using a long blade laryngoscope.

Gas maintenance should be considered mandatory for all prolonged surgical procedures and included, if necessary, in multimodal protocols.

Spontaneous breathing during maintenance phase proved to fulfil a good state of anesthesia and analgesia, while no respiratory depression was noted.

Recovery should be rapid and uneventful, while the patient must be exposed to a minimum number of stress factors.

Monitoring vital functions as well as animal behaviour assessment have to continue throughout the entire recovery phase in order to assure a good evolution and to correct any possible problems that may occur during this phase.

Assuring adequate arterial blood pressure and providing a comfortable padding during anesthesia are important factors in order to decrease the risk of myopathy.

Sheep tolerate general anesthesia with isoflurane very well, the absence of any anesthetic incidents as well as their rapid and complete recovery are important benefits for their successful use in various research procedures.

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# RESEARCH REGARDING THE RAMS INFLUENCE IN TRIGGERING OFF-SEASON SEXUAL CYCLES

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***Abstract***

*In order to achieve the fundamental objective, an experimental protocol was developed which was applied at the same level and respecting all the experimental variables for three consecutive seasons, the period of each season being the same, respectively May-June. The experimental batch consisted of 30 adult females of the Karakul de Botoșani breed, aged between three and seven years. In each experimental season the batch of selected adult sheep had a good body condition and was maintained in a separate compartment. In order to study the effect due to the presence of rams on females and on the possibility of initiating the sexual cycle during periods outside the natural breeding season, an adult breeding ram was introduced inside the batch. In order to avoid physical exhaustion, the ram was changed at certain intervals. Adult sheep exhibiting sexual cycles were mounted immediately after detection, with breeding repeated after 10 hours. The data obtained were statistically processed, and analysing the average values shows that the proportion of sheep that manifested heat and were mounted was higher than 56% in each of the three seasons.*

***Key words**: birth rate, ewe, ram, reproduction season, sheep Karakul of Botoșani.*

## INTRODUCTION

The seasonal manifestation of sexual cycles in breeding sheep is a characteristic of the species inherited from wild forms, and is therefore of genetic origin. This aspect demonstrates the lack of completing taming process in a direction useful for the breeder (Barid et al., 1981; Thwaites, 1982). The biological and economic efficiency of sheep farming depends mainly on the way in which it is coordinated and the way in which the breeding activity is applied on the farm.

In order to perform in the management activity of the farm, it is necessary to intensify the breeding function in sheep, developed and applied according to the particularities of each existing population because the results in this field are directly influenced by the value of breeding characters (Pascal et al., 2000; Stoica et al., 2003).

Among the ways and practical solutions that do not involve allocation of large material and financial resources, which can intensify reproduction in sheep, we list: inducing puberty to use lambs for breeding in first autumn and using the influence of biological and natural factors to trigger heat in adult sheep in periods considered to be in the off-season.

There is a lot of scientific information in the specialized literature that sustain that breeding rams, in certain situations and under certain conditions, can stimulate oestrus and ovulation in females, this phenomenon being called the "ram effect". This effect, if properly managed and exploited in the management of small ruminants, can have a favourable effect in that it can stimulate the onset of both puberty and oestrus. Another positive effect due to rams' influence also refers to the fact that when used at the beginning of breeding season it can have a positive influence in both oestrus and ovulation synchronization. Although the degree of response obtained varies by genotype and latitude, it is a valuable management tool in most circumstances (Delgadillo et al., 2009).

## MATERIALS AND METHODS

Research regarding the breeding rams influence on the onset of sexual cycles in the off-season was an important objective and aimed to assess the possibility of increasing the total number of

lambs obtained from adult females belonging to the Karakul de Botoșani breed.
In order to investigate how the presence of breeding rams influences the onset of sexual cycles during periods outside the natural breeding season, a batch of 30 adult females aged between three and six years, was set up.
The research was carried out for three consecutive seasons, being organized in calendar periods placed outside the natural breeding season, respectively in May-June. In order to obtain relevant results, the same protocol was observed in each experimental period, the batch was maintained in a separate compartment, having maintenance and feeding conditions adequate for the established objectives.
In each experimental season, in order to obtain the goal of the experiment, the selected sheep were isolated from the breeding rams for 40 days. During this period, none of the 30 females didn't came into contact with the rams (i.e. visually, by sound or smell), being completely separated and kept at a considerable distance. Through this technique, we aimed to achieve a synchronization of oestrus activity throughout the batch.
Also, in order to study the breeding rams influence on sexual cycles onset in off-season, in each experimental period in the batch of adult sheep, a breeding ram was introduced that had an apron attached to the abdominal area that prevented the possibility of mounting. To avoid exhaustion, it was changed every 5 days. Every day, when one of the females showed heat, she was taken out of the lot and subjected to the natural mating process. For the statistical processing of the data but also for testing the statistical significance of the differences between the average value specific to the studied parameters, the algorithm Variable Analysis (ANOVA Single Factor) and the Pearson Correlation were used, both included in the MsExcel 2007 software package.

## RESULTS AND DISCUSSIONS

Applying off-season breeding has high economic advantages because in a period of two years we could organize three or more breeding seasons.
Taking into account the geo-climatic conditions and the biological peculiarities of the local breeds, the efficient use of the mounts in off-season would consist in carrying out activities that would trigger heat and implicitly breeding females during the spring months (April-May) or in early summer (June-July) and lambing should take place in early autumn (September-October) or late autumn and early winter (November-December).
The organization of two lambing per year is financially justified on large numbers. In holdings with a large number of females intended for breeding, separate herds may be set up, which may undergo certain techniques to stimulate the onset of heat in other seasons of the year. In this way, we create the possibility that basic production (milk or meat) has a permanent character and the revenues obtained from their constant capitalization generate the improvement of management in the respective farm (Tăpăloagă et al., 2018).
Also, in order for the activity carried out to induce heat in the off-season to be efficient, methods that do not generate the commitment of high financial and material resources must be applied, with an effect in increasing the costs per productive cycle. Therefore, the main conditions for frequent or multiple lambing are the most efficient methods of off-season head induction and refers to:
- photojournalism;
- reduction of postpartum anoestrus;
- use of natural factors;
- use of supplementary feeding;
- use of stimulating rams.

The effect due to the presence of rams is part of the technique based on bio stimulation because it has been found that when sheep that do not have a sexual cycle are stimulated to ovulate by the presence of ram. The explanation for this is that rams produce chemicals called pheromones, which are perceived by sheep and can stimulate the appearance of oestrus in peripubertal sheep or in sheep approaching the beginning of the breeding season.
Applying adequate techniques is necessary because local breeds, including the Karakul de Botoșani, are characterized by a high degree of lateness and the proportion of females that naturally show heat in the off-season is less than 5% (Pascal et al., 2009).
Studies regarding the possibilities of subjecting sheep to techniques and procedures to manifest

heat in atypical seasons has been intensively researched in several countries around the world. Based on the results and analysis of the biochemical changes that occur in sheep in the presence of the male, it is assumed that their maintenance, during periods considered to be in the off-season, with breeding rams would alter the level of cortisol concentration which also attracts increases in LH concentrations there four accelerating occurrences of ovulation (McCosh et al., 2010).
In a more unique experiment, conducted over a two-year period, in which the aim was to study whether the isolation of ram from sheep is necessary to obtain a more favourable response to ram's effect and whether they react in May as well as in natural breeding season in early October, it was found that 86% of eligible sheep responded positively to the ram's effect and had sexual cycles. The response was higher ($P<0.05$) in June in the second year too ($P=0.05$) (Cushwa et al., 1992).
Other research has been done to highlight the presence of ram effect. Thus, in a 3-year study of a total of 331 Nungua black-headed and 104 African dwarf sheep in West Africa, it was found that sheep of these breeds reacted strongly to the stimulation caused by sudden introduction of rams after a 10 months' total separation. The sudden presence of ram obviously changed the proportion of sheep that showed oestrus with typical manifestations of behaviour immediately after contact with a ram. More than 25% of sheep have reacted to the effect of ram from day one, but studies have also confirmed the existence of racial differences in the response of sheep to the sudden presence of males (Ngere et al., 1975).
To investigate how the presence of breeding rams influences the onset of sexual cycles in breeding females during periods atypical of the normal breeding season, but also for economic reasons, each year 30 adult females were selected. Only adults in very good physical condition were chosen and they were all maintained separately from the basic herd. A breeding ram was introduced inside this batch, but to avoid exhaustion it was changed every 5 days.
The research was carried out in calendar months outside the natural breeding season, respectively in May-June, and was repeated for three consecutive seasons. That flock of sheep was kept in a stable and separate compartment, and the feed was based on the same categories of feed used during the cold season (Figure 1).


Figure 1. Batch of ewes used for off-season mating

In each of the three seasons, the batches were set up in the first three days of May and at the beginning of July all the sheep were transferred to the basic herd. A ram chosen from the batch of testers was present throughout this period in the batch.
In the spring concerning the first season of 2018, it was found that first females that had sexual cycles were in third decade of May, about 22 days after the start of research. During the whole research period of the same year, it was found that a total number of 11 females showed sexual cycles, representing 36.66% of the total population (Table 1). In the following season, under the same conditions and under the influence of the same experimental treatment, the proportion of sheep that showed heat was only 30% and, in the season, placed in 2020 it increases to 43.33%.

Table 1. Statistic of ewes that manifested heat cycle in off-season due to ram's influence

| Specification | n | Sheep with no heat triggered | | Sheep that triggered heat | | Pregnant sheep | | Twin lambing sheep | |
|---|---|---|---|---|---|---|---|---|---|
| | | n | % of the total batch | n | % of the total batch | n | % of mounted sheep | n | % of pregnant sheep |
| Season I (2018) | 30 | 19 | 63.34 | 11 | $36.66^{bc}$ | 10 | 90.90 | 1 | $10.00^{ns}$ |
| Season II (2019) | 30 | 20 | 70 | 10 | $30.00^{ab}$ | 10 | 100.0 | 2 | 20.00* |
| Season III (2020) | 30 | 17 | 56.67 | 13 | $43.33^{cd}$ | 13 | 100.0 | 1 | $7.69^{ns}$ |

Note: $^{a, b, c, d}$ – environments with different symbols show significantly different values ($P<0.05$);
* statistically significant differences ($P<0.05$); *ns* – non-statistically significant differences ($P>0.05$);

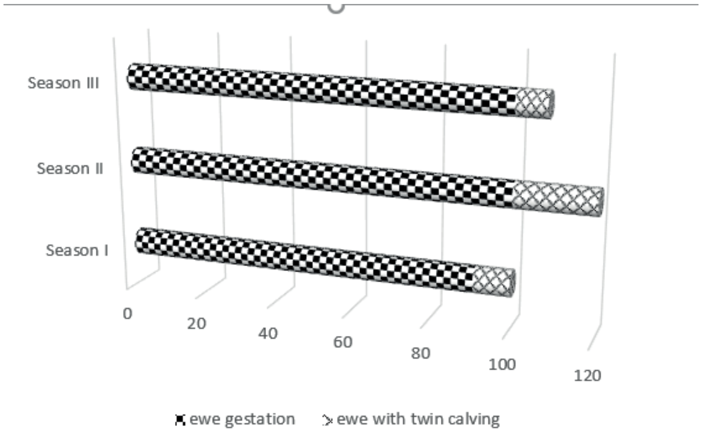

Figure 2. Sheep that showed sexual cycle under the influence of the experimental factor

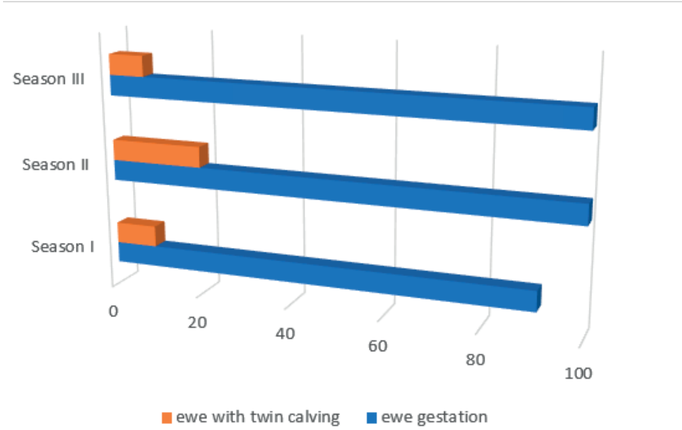

Figure 3. Ewes that became pregnant and lambed twins out of the total number of those that showed sexual cycle

All adult sheep that were found having sexual cycles were mounted with the nursery rams included in the mating list. The mating was performed immediately after detection and repeated after an interval of 10 hours. The statistical processing of obtained data confirms that the proportion of sheep responded positively to the experimental factor, and showed sexual cycles, has different degrees of statistical significance for $P<0.05$.

Tracking the number of adult sheep that showed heat and were mounted in each of the three seasons indicates that most remained fertile and completed the gestation, with the exception of one female, which during the first season showed a repeat of the sexual cycle and she did not become pregnant. It is interesting that in each breeding season in which the research was carried out, within the batch of pregnant adult sheep a proportion between 7 and 20% had twin lambs (Table 1 and Figure 3).

However, their comparative proportion has no significance for the statistical threshold taken into account, except for the proportion of ewes with twin lambs from season II which was statistically significant for $P<0.05$.

From the average values but also from the degree of statistical significance, it can be concluded that the presence of rams has a favourable bio stimulating effect and this procedure can be successfully applied in other sheep farms, the major disadvantage being the labour involved in implementing such a technological flow.

## CONCLUSIONS

In order to correctly highlight the bio stimulating ram's effect, the research was carried out in calendar months outside the natural breeding season, respectively in May-June and was repeated for three consecutive seasons.

In the batch consisting of adult females that were to be put in contact with male breeders during off-season, maintained in the stable in common compartment, the feed was based on the same categories of feed used during the cold season.

In the spring of the first season, it was found that the first females had sexual cycles in the third decade of May, about 22 days after the start of the research, and during the entire duration of the research it was found that a total 11 females had sexual cycle, representing 36.66% of the total batch.

In the following seasons, under the same conditions and under the influence of the same experimental treatment, the proportion of sheep that showed heat after ram introduction in compartment, was 30% and in the third season it increases again to 43.33%.

Statistical processing of the data obtained confirms that the proportion of sheep that responded positively to the experimental factor and showed sexual cycles has different degrees of statistical significance for $P<0.05$.

The results obtained in the research carried out have the role of improving the management activity and the statistical data obtained are correlated and converge as meaning and with the effect generated by the use of a vasectomized ram. In both circumstances the effect of the ram is similar because its presence allows the sheep to perform several cycles of oestrus before the desired date of reproduction, which will increase fertility (American Sheep Industry Association, 2015; Schoenian, 2018).

From the average values but also from the degree of statistical significance of the differences, it can be concluded that the presence of rams has a favourable bio stimulating effect and this procedure can be successfully applied in other sheep breeding units.

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# RESEARCH ON THE IMMUNOMODULATORY EFFECT OF LEVAMISOLE IN SWINE

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***Abstract***

*Immunomodulation is an important alternative in combating many diseases, being considered a potential weapon in the fight against pathological entities that cause major economic losses in pig herds. Levamisole, in addition to its anthelmintic effect, has also an immunostimulatory effect, for which it has also been used as a vaccine adjuvant. The aim of the research was to evaluate the non-specific immune response in modern techniques in pigs given levamisole. The results showed significant differences in the case of the ratio of lymphocyte subpopulations, there was an increasing trend in favor of T lymphocytes (with 10.44%), and the % of T lymphocytes blastic transformation (with 56%). Also, we observed a significant increase of the LTh/LTs ratio (with 65.92%) which supports the immunomodulatory potential of the levamisole and its involvement on the coordination of immune processes.*

***Key words***: *immunomodulation, levamisole, lymphocytes, pigs.*

## INTRODUCTION

Immunomodulation is an important alternative in combating many diseases and in the control of many infectious diseases in animals, the importance of the application of immunomodulatory therapies affecting the health of food, public health according to the common goal formulated based on the principles of "One Health" (Savu & Petcu, 2002; Goncearov et al., 2004; Petcu, 2006; Petcu et al., 2007).

The use of immunomodulatory preparations and microelements in pig herds, both in professional farming and in households, can increase the resistance of subjects to the action of pathogens, by positively regulating the duration and intensity of the immune response. Through the development and application of vaccines throughout history, many biological threats have been defeated, but techniques for enhancing the non-specific immune response have not received the same attention in the field of research. Non-specific immunomodulation becomes fundamental along with the principles of biosecurity in the case of the evolution of infectious pathologies that do not yet know a vaccine (Thacker, 2010; Marin et al., 2013).

Immunity, defined as resistance to disease, has as its main element a set of tissues, cells and molecules called the immune system, the corroborated action of its constituents giving rise to the reaction called the immune response (Fairbairn & Kapetanovic, 2011).

Immunomodulation is still an area of interest for veterinary medical research, being considered a potential weapon in the fight against pathological entities that cause major economic losses in pig herds. If immunosuppressive therapies are aimed at those situations in which it is necessary to resolve a harmful immune response. Immune stimulation may be a prophylactic or therapeutic alternative in case of infectious or parasitic pathologies.

Pharmaceutical products used in veterinary medicine for their immunostimulatory properties are either synthetic products or phytotherapeutic extracts or principles obtained from various microorganisms. The most common substances in this category are vaccine adjuvants intended to increase the effect of immunoprophylactic preparations in which they are included (Ioniță et al., 2014).

Levamisole, an imidazothiazole derivative, used primarily for its anthelmintic properties, has also an immunostimulatory effect, for which it has

also been used as a vaccine adjuvant (Galtier et al., 1983). After the administration, it resulted in better activation of regulatory T lymphocytes and intensified antibody production (Charerntantanakul & Roth, 2006). Moreover, the action of this molecule was correlated with a better maturation of dendritic cells, being stimulated the phagocytic functions of neutrophils and monocytes (Sajid et al., 2005).
The aim of the research was to evaluate the non-specific immune response with modern techniques in pigs given levamisole.

## MATERIALS AND METHODS

In order to follow the objectives formulated in the paper, two groups of 10 animals each (control group and experimental group), of similar ages (6-7 months), belonging to a non-professional farm, were made. The selection of individuals was aimed at forming groups characterized by homogeneity in genetic characteristics, those related to age and maintenance conditions with full compliance with biosecurity conditions. Particular attention was paid to limiting the action of stressors, because stress hormones influence the dynamics of the leukocyte population (Ghiță et al., 2015). Additionally, the groups were homogeneous in terms of body mass because an excess of adipose tissue (by producing leptin) can change the ratio of lymphocyte populations (Ghiță et al., 2021).
The control group was subjected to treatment with saline, administered i.m., in a dose of 5 ml, 3 consecutive days.
The experimental group was treated with a product based on levamisole, respectively Levamisole 7.5%, solution for injection. It was administered i.m. at a dose of 2.5 mg/kg for 3 consecutive days.
Seven days after the last administration, blood samples were taken to check the immunomodulatory effect. Blood samples were collected on vacutainer devices by puncturing the auricular vein.
In order to make an objective assessment of the change in immune status following the administration of levamisole, a range of techniques characterized by high applicability were selected.
The methods used were: determination of WBC and Granulocytes/agranulocytes ratio (with IDEXX analyser), separation of total lymphocyte populations (using the separation technique with Ficoll medium), determination of LT and LB percentage (by EA rosetting technique), determination of ratio between LTh/LTs (by E rosetting technique), separation of populations from LT and LB (by separation technique on nylon fiber), determination of lymphoblastic transformation percentage of LT and LB (by lymphoblastic transformation technique (TTL) with mitogen Concanavalin A (Con A), variant based on the determination of the glucose consumption index in the medium of reaction), separation of the population from neutrophil polymorphonuclear (by the separation technique with the Dextran medium), determination of the locomotor capacity of the separated neutrophils (by the Boyden filter technique - directed migration density). It is noted that that the collected T and B lymphocyte layer isn't pure, containing 5% monocytes. The white layer that is formed on top of the erythrocyte layer contains most of the granulocytes. In case of separation of a bigger volume of blood (25 ml) the layer is denser, and it can be collected easier after 24 hours. In case that the entire deposit is collected, erythrocytes can be lysed by an osmotic shock with ammonium chloride. If the lysis is complete, a white deposit will form composed of polymorphonuclear granulocytes, and a red supernatant (haemoglobin and lysed erythrocyte membranes). The supernatant is removed, the remaining cell are washed three times in Hanks media, then the granulocytes are numbered, and they are resuspended with the desired concentration. The lymphocytes which form rosettes at higher temperatures and in the presence of lower erythrocyte concentration are called ***high affinity lymphocytes*** and ***high affinity E rosettes***, respectively. They represent 55-65% of the lymphocyte population, and apparently, they are part of the T helper lymphocyte subpopulation. If you subtract the number of high affinity E rosettes out of the total number of rosettes, you get the number of ***low affinity rosettes***, which apparently, are part of the T suppressor lymphocytes subpopulation.
The evaluation of blastization capacity by quantifying glucose consumption can be done by using multiple techniques. By heating glucose in an acidic solution, glucose forms with

ortho-toluidine a green compound with different colour intensity. With the help of a spectrophotometer you can measure the amount of used glucose after blastization. The technique is based on the phagocyte's capacity to cross a filtration system which contains chemoattractant substances. If the filtration system is transparent, the phagocytes which cross it will change the optical density of the filter, directly proportional with the number of migrating cells. First, FMLP solution is filtered through the Boyden filtration chamber. After filtration, the chemoattractant solution sticks to the filter. The phagocyte solution is inserted on the filter, after which the filter is incubated a few hours at 37°C. Phagocytes will cross the filter in the direction of the chemoattractant. The most widely used method consists on using transparent filters. Phagocytes get coloured while passing the filter, thus the filter will get coloured as well. The evaluation principle is based on quantifying the optical density of the filter (based on standard solution) which is proportional with the number of migrated cells.
Statistical analysis was performed using the t test (Student test).

## RESULTS AND DISCUSSIONS

The obtained results will be presented in the form of a summary table and graphs, accompanied by comments.
Table 1 shows the analysed parameters for the two groups of animals.

Table 1. Values obtained for the two groups of animals (*p<0.01)

| Analyzed parameter | Control group | Experimental group |
|---|---|---|
| WBC ($10^9$/l) | 13.1 | 13.9 |
| Granulocytes % | 46 | 41.5 |
| Agranulocytes% | 54 | 58.5 |
| T lymphocytes % | 77.6 | 85.7* |
| B lymphocytes % | 22.4 | 14.3 |
| LTh/LTs ratio | 2.5 | 3.9* |
| Lymphoblastic transformation LT% | 40.2 | 66.7* |
| Lymphoblastic transformation LB % | 44.1 | 44.9 |
| Directed migration density (μ) | 1674 | 1725 |

The results of the total leukocyte count (WBC) are shown in Table 1 and Figure 1.

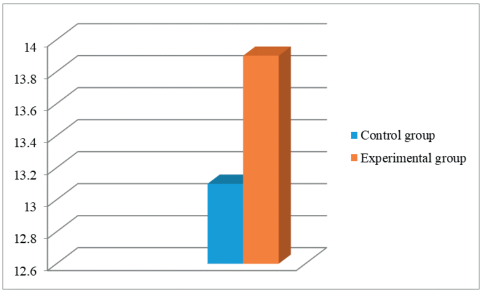


Figure 1. WBC values for both groups

Following the leukocyte count, an increase of 6.11% was observed in the experimental group, which reveals a small stimulatory effect of levamisole. This observation corresponds to data reported by other authors (Krakowski et al., 1999). Following the statistical analysis, it is observed that this increase is insignificant (p <0.05). We mention that the values obtained fall within the physiological limits, in the case of both groups.
The results obtained regarding the granulocyte-agranulocyte ratio are presented in Table 1 and Figure 2.

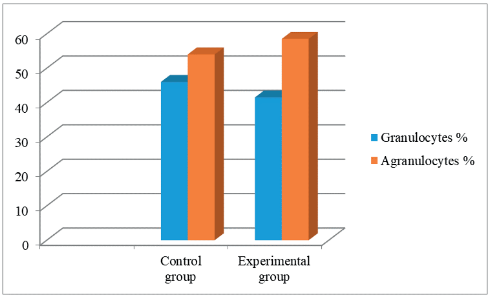


Figure 2. Granulocytes/agranulocytes ratio for both groups

Analysing the results presented in table 1 and figure 2, there is an insignificant increase (p<0.05) with 8.34% of the percentage of agranulocytes in the case of the experimental group. Corroborating with WBC, it can be concluded that the increase of this parameter is made on account of agranulocytes, respectively of lymphocytes, knowing that these are the most numerous agranulocytes. This observation can be explained by a mobilization of lymphocyte populations and subpopulations, in response to the stimulating effect of the administered substance (Valpotić et al., 2014).

Corroborating the data presented above, it is observed that 7 days after the end of treatment with Levamisole, the values regarding the number of total leukocytes and the granulocyte / agranulocyte ratio were kept within the physiological limits.
The T lymphocytes/B lymphocytes ratio results are shown in Table 1 and Figure 3.

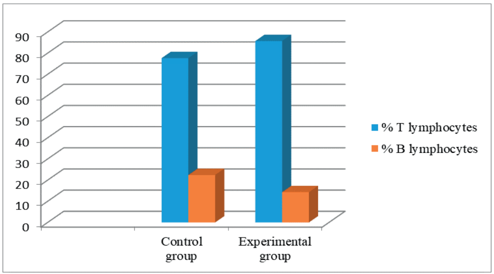


Figure 3. T lymphocytes/B lymphocytes ratio for both groups

Regarding the T lymphocytes / B lymphocytes ratio, resulting from the use of the EA rosetting technique, there is a 10.44% increase in the percentage of T lymphocytes. This significant increase ($p<0.01$) is due to the immunostimulatory effect of the administered product which stimulates the production of T lymphocytes, as also reported in the literature (Valpotić et al., 2009).
Regarding the calculation of lymphocyte subpopulation ratios, it showed an increasing trend in favor of T lymphocytes.
The obtained results regarding the LTh/LTs ratio using the EA rosetting technique are shown in Table 1 and Figure 4.

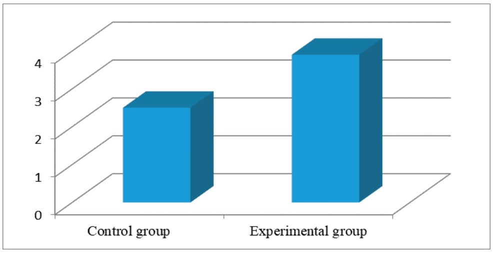


Figure 4. LTh/LTs ratio for both groups

Analysing the data presented above, a 56% increase in the LTh/LTs ratio is observed. This increase is significant ($p<0.01$) and is explained by the increase in LTh production following levamisole administration (Valpotić et al., 2009). In the case of our experiment, the hypothesis of an immunostimulatory effect obtained by administering levamisole is supported by the increase in the ratio of LTh/LTs. These cells represent in pigs over 90% of the total subpopulation of T lymphocytes.
The LTh/LTs ratio calculated 7 days after the last levamisole administration showed an increase in all 10 subjects in the experimental group. Thus, the dynamics of T lymphocyte populations with the increase of LTh weight, can support the immunomodulatory potential of the substance used and its effect on the coordination of immune processes (Suran et al., 2013, McHugh & Shevach, 2002).
The obtained results regarding the lymphoblastic transformation of T and B lymphocytes are presented in Table 1 and Figure 5.

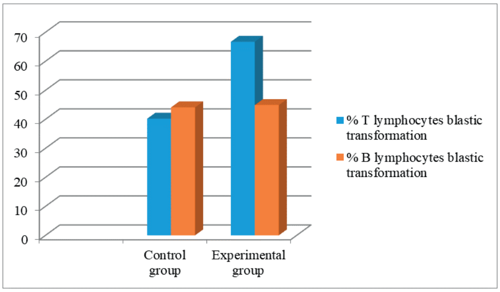


Figure 5. Percentage of T and B lymphocytes blastic transformation for both groups

Regarding the blastic transformation of T and B lymphocytes, there are increases in the percentage of blastic transformed lymphocytes for both categories of lymphocytes. But these increases are different. In the case of T lymphocytes, the increase has a value of 65.92% and is significant ($p<0.01$), while for B lymphocytes the increase is 1.82%, being insignificant ($p<0.05$). The mitogen used in the test was vegetal lectin recognized in the literature for having a specific receptor in both LT and LB. The difference observed in the case of lymphoblastic transformation of T lymphocytes is the notable change following the administration of levamisole.
The hypothesis of an immunostimulatory effect obtained by administering levamisole is supported by a significant increase in the lymphoblastic transformation index of T lymphocytes.

The blast index after stimulation with Con A changed very little in the case of B lymphocyte, the values obtained being close to the physiological ones. For T lymphocyte, however, there was an increase in this parameter in all pigs in the experimental group, with values between 62 and 68% (reference range after stimulation with Con A being 42-50%). These data may support the hypothesis that Levamisole has the ability to stimulate the cell-mediated immune response, the effect on the molecular component of immune reactions being uncertain.

The results for determining the migration capacity of neutrophils by determining the optical migration density directed by the Boyden filter are shown in Table 1 and Figure 6.

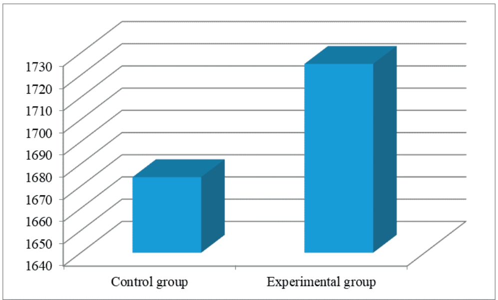


Figure 6. Directed migration density for both groups

From the presented data there is an insignificant increase ($p < 0.05$), with 3.05% in the case of the experimental group. This difference occurs when assessing the locomotion capacity of circulating phagocytes by the migration test under the agarose layer. The density of directed migration increases slightly, which shows that, in this case, levamisole may have caused an increase in phagocyte chemotaxis. Similar results were communicated by other authors (Gâjâilă et al., 2016).

When evaluating the locomotor functions of circulating phagocytes, results have been reported that cannot fully support the stimulatory effect on the chemotaxis of these cells. The optical density of directed migration increased significantly (by 54.4%) only in the case of a single individual, which even decreased by 1.6% according to data obtained from another subject in the same group. A possible bacterial infection without clinical expression in the animal in which the increase of the migration index was found could be a plausible explanation (Gâjâilă et al., 2016). The optical density values, regardless of the percentage change suffered, were kept within the reference range: 1200-1900µ. These aspects do not allow us to establish the effect of levamisole on phagocytic functions.

## CONCLUSIONS

Levamisole altered the ratio between T lymphocytes and B lymphocytes in favor of T lymphocytes, which demonstrates an activation of the cell-mediated immune response.

Levamisole has been implicated in the mechanisms of coordination of immune processes, increasing the ratio of LTh/LTs to values exceeding the physiological threshold.

The action of levamisole on B lymphocytes is insignificant because the population of these lymphocytes has not increased in number and percentage.

The test used to evaluate the phagocytic functions demonstrated its limitations and did not allow the signaling of a direct way to stimulate the chemotaxis of the tested phagocytes.

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# EXTERIOR EXAMINATION OF 'LIMOUSIN' COWS REARED IN THE CENTRAL GEOGRAPHICAL REGION OF BULGARIA

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***Abstract***

*A characteristic of the external features of 'Limousin', reared in the Central Geographical Region of Bulgaria is presented. The breed is specialized in beef production. External measurements and body mass indices of 'Limousin' cows reared in herds in the area of vicinity the town of Troyan, Central Bulgaria were made. External dimensions of offspring of cows born in Bulgaria and Hungary were taken. The study was conducted on 50 first-calf heifers raised on 3 different farms for the period 2019-2021. The removal of the external dimensions took place during spring calving, 100-150 days after birth. The country of birth of cows had a significant impact on the rump height at the sacrum and sciatic bones and chest depth of their offspring (P <0.001). Differences in wither height, athwart body length, and cannon circumference were demonstrated in (P <0.05). The cows of offspring born in Hungary were 2.5 cm higher at the withers than those born in Bulgaria, and the difference in chest depth reached 8.3 cm. Pronounced body superstructure was observed in cows, descendants of cows born in Hungary.*

***Key words:*** *development, exterior measurements, index, 'Limousin', origin.*

## INTRODUCTION

The new economic and dietary requirements for the quality of beef cattle breeds predispose to the breeding of large-sized animals, with high live weight and intensive growth of muscle tissue, and low fat deposition. 'Limousin' breed is characterized by good meat productivity and adaptation indicators (Baharev et al., 2017; Caranfilov et al., 2019).

In the selection of animals, the exterior selection is especially important, the so called phenotypic expression selection. Only the proper physiological development of cattle allows a representative, under good feeding and breeding conditions, to show its best genetic potential for productivity (Soldatov et al., 1988; Kayumov, 2006; Chikalev and Yuldbasheva, 2012; Ivanov and Volkina, 2017).

Cattle of beef breeds are distinguished by their compact constitution, wide back, wide and deep chest, thick neck and small, harmoniously located head. Withers are low and wide. The linear evaluation by external forms is directly related to the realized productivity (Panayotova, 2011; Gergovska and Panayotova, 2016; Alekseeva et al., 2017).

The rate of genetic improvement in cattle breeds is closely dependent on the hereditary value of individuals (Byrne et al., 2015, Gorinov and Lidji, 2016). Linear measurements and the exterior have been shown to be related to the reproductive abilities and longevity of cattle species (Usenko et al., 2004; Zink et al., 2019; Karamfilov, 2020).

The objective of the present study is the exterior features of first-calf cows of 'Limousin' breed, which are offspring of animals born in Bulgaria and Hungary, raised in the Central Geographical Region, on farms near the town of Troyan.

## MATERIALS AND METHODS

The study was conducted on 50 first-calf cows raised on three different farms located in the Central Geographical Region, located in the vicinity of the town of Troyan, in the period 2019-2021. The technological conditions in these farms are similar. During the period April-November pasture cultivation was applied, and rearing in a cowshed during the rest of the year. The study included young female animals, offspring of cows born in Bulgaria and Hungary.

The following zootechnical instruments were used: Lidtin's stick, Wilkens' compasses, a measuring tape, a retractable tape measure and forms for writing.
The exterior measurements were taken during the spring calving, 100-150 days after delivery. During the measurement, the cows were placed (fixed) in a metal frame, observing all zootechnical requirements for taking measurements. To increase the objectivity of the external assessment, according to the methodology of Nozhchev et al. (1983), the following measurements were taken**:** height at the withers, height at the sacrum, chest depth, chest width, chest girth, body length, cannon bone girth, rump width behind hips, rump width at ischial tuberosities. The live weight of the animals was weighed by an electronic scale. The measurements were compared with some of the standard indicators published in the selection program of the breed for the studied age and category.
The data were entered, coded and analyzed, biometrically by the methods of variation statistics in MS Excel with the help of R statistical software, version 3.5.2, and presented in tables.

## RESULTS AND DISCUSSIONS

The assessment of the external characteristics of the studied animals is based on the measurement of the respective external indicators according to the generally accepted methodology. The results are presented in Table 1.
The exterior characteristics of first-calf cows for several generations show the dynamics of changes in the general proportions of their physical development during the change of generations (Bakharev and Sheveleva, 2017).

Table 1. Measured body indicators of 'Limousin' first-calf cows in cm (x±Sx, Cv%)

| Measurement | (I group) n=25 | (II group) n=25 | Standard (according to Nikolov and Karamfilov, 2021) |
|---|---|---|---|
| | x±Sx | x±Sx | x±Sx |
| Withers height | 129.5±0.82* | 132.0±1.33* | 132.7±2.23 |
| Sacrum height, cm | 134.9±0.32*** | 137.6±0.58*** | 137.5±5.63 |
| Chest depth, cm | 77.3± 2.78 | 85.6±0.52 | 83.7±3.49 |
| Chest width, cm | 43.1±0.24*** | 46.7±0.51*** | 46.3±1.82 |
| Chest girth, cm | 184.3±1.22 | 188.9±1.11 | 189.2±4.71 |
| Athwart body length, cm | 153.7±0.29* | 154.3±0.32* | 154.7±4.18 |
| Cannon bone girth, cm | 18.9±0.23* | 19.1±0.27* | 19.2±0.49 |
| Rump width behind hips, cm | 46.9±0.30 | 49.4±0.25* | 49.6±3.41 |
| Rump width at the ischial tuberosities, cm | 43.8±0.19 | 45.2±0.38 | 45.7±2.58 |
| Live weight, kg | 594.7±3.46 | 612.4±2.03 | 610 |

P<0,05*, P<0,001**, P<0,0001***

First-calf cows, which are offspring of cows born in Hungary (group II), were 2.5 cm or 2.45% higher at the withers than those which were offspring of cows born in Bulgaria (group I), with 2.7 cm or 2.65 % higher at the sacrum, and in terms of chest depth, this difference reached 8.3 cm or 7.49%. Body height as well as body length are directly related to the possibility of achieving higher live weight in beef cattle (Lukuju et al., 2016). The country of delivery in cows had a significant effect on their offspring height on the rump at the sacrum and chest depth (P<0.001). Differences in wither height, athwart body length, and cannon bone girth were demonstrated at (P <0.05).
When comparing the measurements with the standard indicators of the breed we can note that the average indicators of both studied groups correspond to the set standard, and closer to it are the animals, which are offspring of those born in Hungary (group II).
Based on the obtained measurements for the evaluation of individual parts of the body, we calculated the body constitution indices of both comparable groups, which are presented in Table 2.

Figure 1. Descendants of cows from Hungary

Table 2. Body constitution indices of first-calf cows of 'Limousin' breed in %

| Body constitution indices | 'Limousin' first-calf cows | |
|---|---|---|
| | (I group) n=25 | (II group) n=25 |
| Body overbuilding index, % | 40.31 | 35.15 |
| Extension index, % | 118.67 | 116.89 |
| Pelvis-chest index, % | 91.90 | 93.73 |
| Chest index, % | 55.76 | 55.32 |
| Compactness index, % | 119.90 | 122.42 |
| Bone growth index, % | 14.59 | 14.46 |
| Overgrowth index, % | 104.17 | 104.24 |
| Pelvic width index, % | 107.08 | 109.29 |
| Meat index, % | 172.8 | 175.2 |

Body constitution index is the ratio of anatomically related measurements, expressed as a percentage. It is calculated according to previously developed formulas (Soldatov et al., 1983, Usenko et al., 2004).

The body overbuilding index shows the relative development of the limbs compared to the body. Significant body overbuilding was observed in cows, which are offspring of cows born in Hungary, with 35.15%. Higher overbuilding, was found in cows, offspring of cows born in Bulgaria, with 40.31%, or a difference of 5.16%.

In the exterior assessment it is important to get an idea of the proportional structure of cattle as a whole, as well as the growth and development of individual parts. The size of the measurement allows to compare individual parts of the animal's body, but does not give a complete description of the proportions of the body constitution (Belenkaya, 2016; Gorinov & Lidji, 2016). Extension index characterizes the ratio of the body to its height. In the present case, both values are within the standard of the breed, as higher were the values of the offspring of cows born in Bulgaria (group I) with 118.67%.

Pelvis-chest index shows the development of the front and back of the body. The values shown are higher in cows which are offspring of Hungarian-born cows (group II), by 1.83%.

Chest index gives information about the development of the chest. Here the advantage, although minimal by 0.44%, was for the offspring of cows born in Bulgaria (group I).
The compactness index shows the muscle development. It changes little over time and is an indicator of body development during the postnatal period. The values shown are higher for the cows, which were offspring of first-calf cows born in Hungary (group II), by 2.52%.
The selected indicators for analysis allow to assess the degree of development of cattle, its body proportions and its general constitutional type (Chikalev et al., 2012).
Bone growth index gives information about the development of bone tissue. With increasing age, the value of this index increases. That difference is small and in favor of first-calf cows, offspring of cows born in Bulgaria (group I), in the order of 0.13%.
For the other three calculated indices: the overgrowth index, the pelvic width index and the meat index, again the superiority was for first-calf cows, offspring of cows born in Hungary (group II), by 0.07%, 0.21% and by 2.4%. These differences are relatively minimal and do not show large deviations from the breed standard.
The body constitution indices of both groups of 'Limousin' first-calf cows are generally characteristic of female carnivores.

Figure 2. Descendants of cows from Bulgaria

These data are close, and in some cases are supplemented by those obtained by Gorinov and Lidji (2016), Baharev and Sheveleva (2017) and Caranfilov et al. (2019).

## CONCLUSIONS

The adaptation to the climatic conditions of the Central geographical region of Bulgaria, the surroundings of the town of Troyan confirms the development of the indices for typical body constitution and type, which when changing the generation, clearly show the dynamics of growth. The cows, which are offspring of cows born in Hungary, were 2.5 cm higher at the withers than those born in Bulgaria, and the difference in chest depth reached 8.3 cm. Significant body overbuilding was observed in both groups. The calculated indices of body constitution in both studied groups of first-calf cows of 'Limousin' breed are generally characteristic of carnivorous animals.

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# HEMATOLOGICAL AND BIOCHEMICAL BLOOD INDICATORS OF YOUNG GILTS AFTER ESTRUS SYNCHRONIZATION

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***Abstract***

*Synchronization and stimulation are used to obtain large numbers of embryos. Therefore, the studies carried out made it possible to assess homeostasis in the dynamics of metabolism in different phases of regulation of the reproductive cycle when using the proposed drug "Estrosynchron". The drug blocks the secretion of pituitary gonadotropins, which inhibits the growth of follicles and the ovulation process and, accordingly, the manifestation of the phenomena of the sexual cycle. It was found that over the period (18-20 days) of feeding Estrosynchron in gilts after treatment, blood parameters decreased: total protein, globulin fraction, α1-globulin, α2-globulin fraction compared with the indicators before treatment. After treatment, there was an increase in the number of erythrocytes, hematocrit, platelet count and thrombocyte, as well as a significant increase in the average hemoglobin content in an erythrocyte and a decrease in the rate of erythrocyte coagulation. At the same time, studies of blood parameters provide additional information about the characteristics of breeding pigs, depending on the age and physiological state during reproductive use.*

***Key words***: *estrosynchron, estrus synchronization, gonadotropins, homeostasis, leukogram, stimulation.*

## INTRODUCTION

Modern pig breeding requires intensive reproduction, stimulation and synchronization of the sexual function of gilts to replenish the main herd and planned predictable artificial insemination. In breeding farms, successful stimulation and estrus synchronization are produced when gilts reach a weight of 120-130 kg and have 2-3 sexual cycles before the start of treatment (Melnik et al., 2017; Melnik, 2018).

Fundamental and applied research in the field of physiology, nutrition, genetics, animal behavior, environment and management over the past 20 years has laid the foundation for the development of highly productive females and various management methods and technologies that have significantly increased the efficiency of reproduction in the breeding herd (Christiansen, 2005; Kraeling, & Webel, 2015; Kramarenko, et al., 2020).

Optimum reproductive performance are achieved through a variety of strategies to meet the high productivity expectations of modern farms. Hormones have been used to control the reproductive function of sows and gilts in various protocols in order to optimize management practices and increase the overall efficiency of pork production (Ziecik et al., 2021).

Also, synchronization and superovulation are used to obtain a large number of embryos for experimental and practical purposes, but with mixed results. In their studies Ziecik et al. (2005) compared the quantity, quality and in vitro development of embryos obtained from pigs after synchronization of single or double estrus and superovulation. The single and double hormonal stimulation regimens resulted in an increased number of embryos with a higher percentage of embryos classified as degenerated compared to the control group without stimulation. The number of embryos to be cultured did not differ between single and double synchronized groups. The highest percentage of embryos obtained was observed in the control group, where no stimulation was performed. Scientists have confirmed that synchronization and superovulation is sufficient to obtain a large number of embryos, however, both methods of synchronization resulted in a significant number of degenerated embryos (Ziecik et al., 2005).

Pharmaceuticals such as progesterone analogs and gonadotropins are commonly used in practice in a number of countries to improve the reproductive function of gilts and sows. Data from studies by Ziecik et al. (2021) show that gonadotropins used to control the reproductive function of sows and gilts, which depends on sexual maturity, affect endocrine and molecular environment of preovulatory follicles.

Various exogenous hormones can be used to control follicular development and synchronize ovulation in sows and gilts. Estrus can be induced by ECG administration with or without concomitant HCG, the latter being analogous to LH. The use of HCG is likely to be relatively more effective in conditions of limited endogenous LH, for example, in young females, because in pigs it is LH that is the main driver of follicular development from the middle follicle stage to the follicle stage ovulation point (Abbara, et al., 2018; Caárdenas & Pope, 2002; De Rensis & Kirkwood, 2016).

De Rensis & Kirkwood (2016) dealt with the problems of estrus control and estrus synchronization in gilts and sows. Studies have shown that in females with a regular cycle, estrus can be controlled and synchronized by blocking follicular growth at the mid-follicle stage by suppressing endogenous LH achieved by feeding the progestogen altrenogest.

This effectively stops the follicular phase and when altrenogest is withdrawn, the follicular phase begins.

The use of gonadotropins to synchronize the reproductive cycle in pigs was used in the studies by Day et al. (1965). The fertility rate of the first estrus after gonadotropin treatment was higher than the fertility rate of comparable untreated animals in terms of fertilization rates, the mean number of embryos at 25 days of gestation, and litter size at farrowing.

The procedure for using gonadotropin injections to synchronize reproductive cycles in pigs provides an effective and reliable means of reducing reproductive timing variability without compromising subsequent reproductive capacity (Day et al., 1965).

Quirino et al. (2020) also confirm that the estrous cycle can be controlled by hormones such as progestogens to suppress the follicular phase, allowing follicular growth to resume after cancellation hormones.

Altrenogest is currently the only commercial progestogen available in pigs and its efficacy in controlling the estrous cycle in pigs is well documented, resulting in over 79% estrous expression within 3-8 days after hormone withdrawal.

## MATERIALS AND METHODS

Experimental studies were carried out in the conditions of pedigree farms in Nikolaev, Kherson, Odessa regions of Ukraine.

To fulfill the assigned tasks, scientific and scientific-economic experiments were carried out, in which 1468 pigs were used, including 16 young pigs.

For the experiment, according to the principle of analogues, groups of sows of different breeds and combinations bred in Ukraine were formed: the large white pig breed (LW), which is universal and the most numerous, was taken for control. The following breeds were used for research: Landrace (L), Duroc (D), Red White-belted (RWB), Pietrain (P), Ukrainian Meat (UM) and F1 local pigs (LW × L) and other combinations.

Hematological studies were carried out on a complex of blood morphological composition, biochemical and hormonal parameters (Kondrakhin et al, 1985; Vlizlo, et al, 2012).

Pig blood samples were taken from the jugular vein using five- or ten-gram special disposable syringes of the "Monovet" type, which is a closed system consisting of a syringe-test tube and an injection needle. In the white "Monovet" there are plastic balls with a blood coagulation activator, due to which the blood quickly coagulates and a clear border is formed between the clot and the serum, in the red one there is heparin, which prevents blood clotting. Monovet is used for transportation, as a container and as a test tube for centrifugation. The animals were fixed in a standing position behind the upper jaw with a loop of a flexible steel cable fixed in a metal casing (Levchenko et al., 2004). The site for puncture of the jugular vein was determined in the groove formed by the long muscles of the neck, to the right or to the left, five centimeters cranial from the sternum. The Monovet needle was inserted from the bottom up and with an inclination in the caudomedial direction to the appropriate depth depending on the age, weight and breed of animals. The penetration of the needle into the lumen of the vein allows the syringe to be quickly filled with blood (Rybalko et al., 2005). Blood tests were performed on an automatic hematology analyzer BC-3000 pluse (Mindrey) for counting and measuring the sizes of WBC, RBC, PLT and measuring HGB by the electrical impedance method, based on measuring changes in electrical resistance generated by a particle passing through the aperture. Since blood cells are not light conductive, the moment they pass through the aperture, they cause an increase in impedance, which is in direct proportion to the size of the cells (Levchenko et al., 2004).

The determination of hormones and enzymes in pig blood serum was carried out on an automatic analyzer ChemWell (2in1) Awareness technology 1nc (USA), which is used to determine 200 biochemical and 170 enzyme-linked immunesorbent tests, provides simultaneous use of 40 reagents with the support of Windows software (Kondrakhin et al., 1985; Vlizlo et al., 2012).

An automatic universal analyzer Vitalab was used to determine total protein using a Liguick Cor-Total PROTEIN diagnostic kit for determining cholesterol using reagents Bio Systems S.A. (Spain), for measuring the concentration of CPK using the reagent Creatine Kinase (CK) Bio Systems S.A. (Spine) (Rybalko, et al, 2005).

Protein and lipid electrophoresis was performed using a UEF-01 "Astra" instrument system. A set of reagents was used to determine the protein fractions of blood serum by electrophoresis on cellulose acetate membranes, followed by densitometric determination of protein fractions (Christiansen, 2005).

## RESULTS AND DISCUSSIONS

In sexually mature gilts, the lability of homeostasis is aimed at maintaining the rhythmic stages of the estrous cycle to create optimal conditions for the fertilization of eggs. Monitoring data on the homeostasis of the body of pigs after synchronization of sexual estrus showed insufficient research in the field of the relationship between blood parameters and the administration of gonadotropins. Therefore, the studies carried out made it possible to assess homeostasis in the dynamics of metabolism in different phases of regulation of the reproductive cycle when using the proposed drug "Estrosynchron". The drug blocks the secretion of pituitary gonadotropins, which inhibits the growth of follicles and the ovulation process and, accordingly, the manifestation of the phenomena of the sexual cycle (Melnik et al., 2017; Melnik, 2018).

The results of the study of hematological and biochemical parameters of the blood of pigs subject to stimulation and synchronization of sexual estrus are shown in Tables 1-3. The data obtained indicate that for the period (18-20 days) of feeding the drug in gilts after treatment, blood parameters decreased compared to the period before treatment: total protein by 3.7 g / l, globulin fraction by 1.9%, α1- globulin - 0.5%, which is significant ($p < 0.05$) compared to the indicators before treatment, and the α2-globulin fraction – by 1.7% ($p < 0.001$). A slight decrease in the observed β-globulin fraction – 0.2%, as well as cholesterol – by 0.13 mmol / L. Analysis of the data showed an increase in the albumin fraction by 1.9% at $p < 0.05$ and γ-globulin - 1.0%, respectively, the A / G ratio increased by 0.06, and the amount of β-lipoproteins increased by 1.2 at. units (Table 1).

This difference can be explained by a slight change in homeostasis under the influence of the drug. As a result of observing the behavior of the pigs while feeding the drug, its calming effect was established, the pigs rested more, during this period the increase in live weight increased and they did not show signs of estrus.

Earlier studies Burton & Westphal (1972) also revealed a relationship between blood biochemical parameters, before and after estrus synchronization, in rats and their behavior. Scientists have identified proteins that form dissociative complexes with circulating steroid hormones in the serum: albumin, the most abundant plasma protein, and highly specific glycoproteins found in low concentrations: corticosteroid-binding globulin (CBG or transcortin), the sex steroid binding protein (SB) and progesterone-binding globulin (PBG)

Table 1. Blood biochemical parameters of gilts, subject to estrus synchronization by "Estrosynchron", n = 16, $\overline{X} \pm S\,\overline{x}$

| Index | Before treatment | After treatment | Difference (±) |
|---|---|---|---|
| Total protein, g/l | 65.1±0.81 | 61.4±1.07* | -3.7 |
| Albumin, % | 40.2±0.59 | 42.1±0.71* | 1.9 |
| Globulins, % | 59.8±0.59 | 57.9±0.71* | -1.9 |
| $\alpha_1$-globulins, % | 5.3±0.15 | 4.8±0.14* | -0.5 |
| $\alpha_2$-globulins, % | 15.2±0.29 | 13.5±0.26*** | -1.7 |
| β-globulins, % | 17.5±0.30 | 17.3±0.54 | -0.2 |
| γ-globulins, % | 21.7±0.68 | 22.7±0.78 | 1.0 |
| Coefficient A / G | 0.68±0.031 | 0.74±0.033 | 0.06 |
| Cholesterol, mmol / l | 2.59±0.07 | 2.46±0.09 | -0.13 |
| β-lipoproteins, u.u. | 17.9±0.71 | 19.1±0.73 | 1.2 |

*Marks:* * P≤0,05, ** P<0,01, *** P<0,001

After treatment, starting from day 16, the rats were more mobile and active. Albumin interacts primarily through hydrophobic bonds; the constants of steroid complexes with specific globulins are several orders of magnitude higher. Estrogenic hormones increase CBG and SBP levels; androgenic hormones have the opposite effect. In rats, the inhibitory effect of corticosteroid hormones on CBG was observed; adrenalectomized rats have increased CBG activity (Burton & Westphal, 1972).

The hematological blood parameters of gilts subject to estrosynchron synchronization (Table 2) also had some difference, but in most cases it was not reliable.

Table 2. Hematological parameters of the blood of gilts subject to estrus synchronization by "Estrosynchron", n = 16, $\overline{X} \pm S\,\overline{x}$

| Index | Before treatment | After treatment | Difference (±) |
|---|---|---|---|
| Erythrocytes, $10^{12}$/l | 6.3±0.11 | 6.6±0.15 | 0.3 |
| Hemoglobin, g/% | 12.2±0.18 | 12.1±0.26 | -0.1 |
| Hematocrit, % | 35.5±0.84 | 37.4±0.93 | 1.9 |
| Average erythrocyte volume, fl | 58.0±0.63 | 56.4±0.72 | -1.6 |
| Average hemoglobin content in erythrocyte, pg | 15.9±0.14 | 17.8±0.11*** | 1.9 |
| Average concentration of hemoglobin in erythrocytes, g/l | 329±3.01 | 325±2.39 | -4 |
| The width of distribution of erythrocytes by volume, % | 17.8±0.27 | 17.6±0.22 | -0.2 |
| Erythrocyte sedimentation rate, mm / year. | 3.8±0.42 | 1.6±0.31*** | -2.2 |
| Platelets, $10^9$/l | 251±19.5 | 267±27.7 | 16 |
| Average platelet volume, fl | 9.7±0.12 | 9.2±0.15 | -0.5 |
| Platelet distribution width by volume, % | 15.1±0.11 | 15.0±0.12 | -0.1 |
| Thrombokrit, % | 0.238±0.029 | 0.247±0.031 | 0.009 |

*Marks:* * P≤0,05, ** P<0,01, *** P<0,001

An increase after the treatment of erythrocytes by 0.3 × 1012 / l, hematocrit by 1.9%, the number of platelets – 16.0 × 109 / l and thrombocrit by 0.09% was found. We have established a significant increase ($p < 0.001$) in the average hemoglobin content in an erythrocyte by 1.9 pg. For other indicators, there was a slight decrease, but it is necessary to note a significant ($p < 0.001$) decrease in the rate of erythrocyte coagulation by 2.2 mm / h. With a normal platelet count, this phenomenon can be explained by medication, i.e. using Estrosynchron.

Also in the studies of Engovatov et al. (2011) it was proved that stimulation of estrus in gilts increases the content of erythrocytes and hemoglobin in the blood of piglets, which leads to a higher preservation of the litter and their increased viability. At the same time, there was no difference in the content of leukocytes in the blood, but there was a certain tendency to an increase in the amount of total protein in the blood serum, which occurred mainly due to globulin fractions, namely, due to the content of the fraction of γ-globulins characterizing the protective functions of the body. Consequently, the use of biostimulants for sexual heat in gilts leads to more intense redox processes in their body.

As a result of our studies, a comparison of the effect of the drug on the natural resistance of gilts before treatment and after feeding it was calculated a blood leukogram, presented in Table 3. Blood leukocytes are responsible for protective processes in the body of pigs, the production of antibodies, phagocytosis, and neutralization of toxins

Table 3. Leukogram of the blood of gilts subject to estrus synchronization by "Estrosynchron", n = 16, $\overline{X} \pm S_{\overline{x}}$

| Index | Before treatment | After treatment | Difference (±) |
|---|---|---|---|
| Leukocytes, $10^9$ | 14.9±0.68 | 15.0±0.72 | 0.1 |
| Eosinophils, % | 3.3±0.61 | 3.3±0.52 | - |
| Neutrophils, % | | | |
| - stab | 5.1±0.49 | 3.7±0.40* | -1.4 |
| - segmented | 37.4±1.96 | 36.7±2.38 | -0.7 |
| - juvenile | - | - | - |
| Lymphocytes, % | 50.8±2.34 | 53.5±2.68 | 2.7 |
| Monocytes, % | 3.6±0.27 | 2.7±0.38 | -0.9 |
| Basophils, % | - | - | - |
| Lymphocyte/neutrophil ratio | 1.20 | 1.32 | 0.12 |

*Marks:* * P≤0,05, ** P<0,01, *** P<0,001

The data in Table 3 indicate that a significant difference in blood leukogram indicators of gilts before and after treatment has not been established, except for a decrease in the number of stab neutrophils by 1.4% ($p < 0.05$).

An increase in the number of lymphocytes by 2.7% was found, but the difference was not significant. Using the leukocyte ratio of lymphocytes and neutrophils as a test, an increase of 0.12 was found, indicating a slight stress response in the gilts.

Similar data were obtained by Romanenko, (2015), for example, the marked insignificant lymphocytosis (by 3.6%), possibly indicates a slight decrease in the microphagocytic function of the body.

And an increase in the number of segmented neutrophils by 27.3% helps to increase the body's defenses to intoxication.

Kmieć & Terman (2006), Kondruchina et al. (2021) in their studies also confirm that the adverse effect on the animal's body is reflected in the hematological profile, and its indicators indicate the state of nonspecific resistance of the organism, which leads to reduced reproductive function. And they recommend the synchronization of sexual activity with biological products, as one of the ways to prevent the negative impact of stress factors on the body.

## CONCLUSIONS

Thus, it was found that for the period (18-20 days) of feeding Estrosynchron in gilts after treatment, blood parameters decreased: total protein by 3.7 g / l, globulin fraction by 1.9%, α1-globulin - 0.5 %, which is significant ($p < 0.05$), α2-globulin fraction - by 1.7% (p

<0.001) in comparison with the indices for treatment. An increase after the treatment of erythrocytes by 0.3 × 1012/l, hematocrit by 1.9%, the number of platelets - 16.0 × 109/l and thrombocyte by 0.09%, as well as a significant (p <0.001) increase in the average the hemoglobin content in the erythrocyte by 1.9 pg and a decrease in the rate of erythrocyte coagulation by 2.2 mm/h (p <0.001). And studies of blood parameters provide additional information about the characteristics of breeding pigs, depending on age and physiological state during reproductive use.

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# REPRODUCTIVE PERFORMANCE IN ALPINE GOATS ACCORDING TO THE APPLICATION OF A SIMPLIFIED PROTOCOL FOR INDUCING ESTROUS INTO THE REPRODUCTIVE OUT OF SEASON

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***Abstract***

*Studies in the field of goat breeding have shown that the most widely used and effective way to induce and synchronize estrus in the breeding season is the hormonal method with Chronogest sponges and the administration of Folligon and a prostaglandin. In the present study, we looked at the reproductive performance of a batch of 148 Alpina goats synchronized in April with Chronogest sponges maintained intravaginally for 11 days and the injection of 400 IU Folligon without prostaglandin administration. Artificial insemination with frozen semen was performed 43 +/- 2 hours after the removal of the sponges and the following reproductive aspects were established: the degree of the cervix opening at the time of artificial insemination, ultrasound diagnosis of 50 days gestation after artificial insemination with the identification of cases of pseud-gestation (4.05%), the rate of calving (56.76%) and prolificacy (310.71%). In farm conditions, specific to our country, the exclusion of prostaglandin administration decreases the risk of abortions by lysis of luteal bodies in possibly pregnant goats at the time of treatment and insemination.*

***Key words****: estrous, goats, reproduction indices, synchronization.*

## INTRODUCTION

The trend of the last decades has been to increase the goat herd throughout Europe and to establish development strategies in order to make this species profitable. In our country, too, the development of the goat sector has taken on a special scale in accordance with the requirements of the internal and external market. Thus, the studies published by ANICAP France show that at the end of 2017 Romania ranks among the top three countries in Europe in terms of goat herds, along with Greece and Spain (https://agriculture.gouv.fr). According to Eurostat, Romania ranks 3rd in Europe in terms of goats in 2021 (https://ec.europa.eu/eurostat/databrowser/view/apro_mt_lsgoat/default/table?lang=en).

The subsequent evolution was not characterized by a numerical increase but by the improvement of the present herds in relation to the direction of exploitation imposed by the internal market, respectively in the direction of milk production. Goats of specialized breeds were imported for milk production and breeding programs were developed for purebreds for both imported and domestic breeds. (https://www.caprirom.ro/rg).

Taking into account the seasonal reproduction of goats and the need to improve the purebred herds, in order to ensure genetic progress in the shortest possible time, it was necessary to resort to reproductive biotechnologies (Arredondo et al., 2015). Thus, the collection of semen from males with high genetic value and its preservation in refrigerated or frozen form have ensured the artificial insemination of a large number of females, from several farms, at a distance. The estrous induction and synchronization programs made it possible to remove the reproductive seasonality and perform artificial inseminations at any time of the year and also, by staggering calvings according to a commercial plan allowed continuity in the consumer market of dairy and goat meat products. The use of artificial inseminations has as its main purpose the improvement of goat herds by infusing valuable genes from tested males and the rapid diffusion of genetic progress (Leboeuf et al., 1998, 2008).

In the case of specialized goat breeds imported for the milk production, a number of bucks needed for natural breeding have been purchased, but in order to rotate the pairs and avoid inbreeding, it is more efficient to import frozen semen produced in the bucks' testing centers. In the economic conditions of our country, of the semi-intensive system of exploitation of goats, in the absence of capital infusion and conservatism specific to goat owners, it is necessary to adopt the most efficient and economical measures in order to apply scientific methods of genetic progress. The method of artificial inseminations with frozen semen is a viable solution, reducing the costs of acquiring and maintaining males throughout the year, avoiding the spread of disease and reducing the stress of adaptation. Moreover, by applying the programs of induction and synchronization of estrous, artificial inseminations can be made in the non-breeding season, each farm managing to constitute two lots of goats with autumn and spring calving and ensuring a continuity of milk and meat production during the whole year, which will allow them a better sale on the internal and external market (Fatet et al., 2011). The aim of this study is to reduce the costs of estrous synchronization and artificial insemination by reducing labor on females and avoiding possible abortions by suppressing prostaglandin F2α, provided that local goat owners practice is grazing the entire herd, including males, for about 300 days / year, and unplanned and unobserved pregnancies may occur. In the case of owners of imported specialized goat breeds, they have adopted the specific operating conditions, respectively the intensive system with permanent stabulation but with the maintenance in the same shelter, in separate boxes for females and males, which can lead to unplanned pregnancies.

## MATERIALS AND METHODS

The research was carried out on 200 Alpine goats with normal reproductive activity. Estrous synchronization and induction were performed in April 2021, at least 90 days after the previous calving date, all goats having a single calving registered in November-December 2020. Of the 200 goats, 52 represented the reference batch 1 and 148 goats constituted experimental batch 2. In the control batch 1, was applied the hormonal method of induction and synchronization of estrous recommended by INRA - Capgenes (France) and the most internationally used. The hormonal protocol INRA - Capgenes consists in the intravaginal application of Chronogest sponges, impregnated with 20 mg of fluorogeston acetate and maintained for 11 days. On day 9, 400 IU PMSG (Folligon commercial product) and 0.2 ml Roflavol (0.05 mg prostaglandin F2α) were administered. The role of Chronogest sponges is to block the estrous cycle for 11 days, through the action of progestogen, and at the time of their withdrawal the sexual activity resumes synchronously for all goats that have undergone treatment. The Folligon, that has as active substance Equine chorionic gonadotrophin, has a synergistic action of FSH type but also LH, achieving both the growth and maturation of ovarian follicles and follicular dehiscence by reaching the ovulatory peak of LH. The dose of Folligon can range between 400 and 700 IU being determined by the level of milk production. The goats monitorized in the experiment, being at the first unfinished lactation, registered milk productions from 2,200 kg up to 3,300 kg milk / day, which is why the minimum recommended dose of 400 IU / goat was used. Goats often have persistent luteal bodies that prevent the onset of pregnancy, which is why the prostaglandin F2α is used in order to perform their lysis.

At an interval of 12, 24, 36 hours after the removal of the sponges, the estrous was detected with test bucks, provided with an apron, to determine the goats that responded to the hormonal treatment and manifested estrous. In the present experiment, we performed artificial insemination with frozen semen at 43 hours after the sponges were removed from all goats, regardless of the clinical manifestation of estrous. In experimental batch 2 consisting of 148 Alpine goats, the protocol for inducing and synchronizing estrous was simplified by giving up the administration of prostaglandin F2α, and the injection of Folligon was done at the same time with the withdrawal of sponges and we gave up detecting the goats in estrous. In this case, the insemination was performed at a fixed point, respectively at 43 +/- 1 hour after the sponges were removed. Artificial insemination of goats was performed with frozen semen (MSC) imported from France.

Thus, we reduced the interventions performed on animals and the trips of the insemination team to 3, with a significant reduction of the workforce but also of the stress on the animals.
In order to perform the insemination of the goats from the experimental batch 2 in an optimal time, it was divided into 3 batches of females of 48/50/50 females that entered the experiment at an interval of 3 days, so that the actions performed at one batch should not overlap with other batches. All actions were performed by the same team of researchers and artificial insemination was done by a single operator, to remove as much as possible the factor of manipulation and execution.
Artificial insemination was performed with the vaginal speculum provided with its own light that allowed the visualization of the cervical ostium and the placement of the insemination pipette in the cervix. Depending on the appearance of the involted flora and the degree of penetration of the insemination pipette, the degree of opening of the cervix and implicitly the level at which the semen was deposited was assessed. When the cervix is closed the semen is deposited intravaginally, at the level of the involted flora, when the pipette penetrates 1-2 cm into the cervix, the insemination is intracervical and when the cervix is open, the pipette crosses the entire cervix and the artificial insemination is done intrauterine, similar to laparoscopic insemination.
Artificial insemination was performed with frozen semen imported from CAPGENE-France, kept in containers with liquid nitrogen at -196$^0$C. The preparation of the semen for insemination went through the following stages: 3 sequins of 0.25 ml were thawed, by immersion for 1 minute, in the thermostat defroster, the water having a temperature of + 38$^0$C, then the sequin was wiped, the insemination pipette was loaded, the laboratory stopper was cut, a drop was placed on a heated slide for microscopic examination and it was kept at a temperature of about 36-37$^0$C for 2-3 minutes, until the moment of insemination.
50 days after the artificial insemination, the diagnosis of ultrasonographic gestation was established by using the portable ultrasound WED 3100 with the multifrequency convex probe 2.5 / 3.5 / 5 MHz. Transabdominal ultrasound was performed on the animal in a four-legged position, with a slight neck restraint. The ultrasound probe was set to 3.5 MHz and placed in the groin, at the base of the breast, the area with reduced hair, and oriented to the area of the flank opposite the place of election. Ultrasound images showed the three physiological states in which the goats were, respectively non-gestation, gestation with highlighting the chains of uterine caruncles and the embryo or the state of pseudo-gestation with highlighting the accumulation of fluid in the embryonic vesicle and lysis of the embryo and placentomes. At 50 days after artificial insemination, the sensitivity of the transabdominal ultrasonographic diagnosis is estimated to be 99-100% (Traore et al., 2019a, 2019b, Gonzales et al., 2004), confirmed in the present experiment, when no abortion was recorded and the calving rate coincided with the ultrasound result.
Based on the observations from the artificial insemination, respectively from the level of the vaginal ostium and on the ultrasound data, we established the efficiency of applying the simplified protocol of induction and synchronization of estrous following the gestation rate recorded in the two groups of Alpine goats.
During October, the calvings were registered and the prolificacy was established following the artificial insemination in the counter-season of reproduction, on hormone-induced estrous.

## RESULTS AND DISCUSSIONS

Of the 52 goats in the 1st control batch, only one did not show estrous, respectively did not accept the male tester within 12-36 hours after the withdrawal of the Chronogest sponges, and at the time of insemination it was found that the cervix was closed. According to studies publicshed by Camacho (2020), the state of estrous manifests itself in the range from 16 up to 72 hours, with an average of 33 hours after the withdrawal of sponges. All the goats were artificially inseminated, and when the semen was deposited, the degree of penetration of the insemination pipette and the opening of the uterine cervix were observed. These degrees of cervical opening and artificial insemination, respectively, were correlated with the physiological condition of the goats assessed by ultrasound (Table 1).

Table 1. The result of the ultrasound gestation diagnosis compared to the degree of opening of the cervix in goats in batch control 1

| | CI | | CD1 | | CD2 | | IU | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|
| | N | % | N | % | N | % | N | % | N | % |
| P | 2 | 50 | 9 | 56.25 | 16 | 64 | 7 | 100 | 34 | 65.38 |
| PP | 1 | 25 | 0 | 0 | 0 | 0. | 0 | 0 | 1 | 1.92 |
| NP | 1 | 25 | 7 | 43.75 | 9 | 36 | 0 | 0 | 17 | 32.69 |
| Total | 4 | | 16 | | 25 | | 7 | | 52 | |

P- pregnant, PP- pseudo-pregnant, NP- non pregnant
CI- closed cervix, CD1- open cervix 1 degree,
CD2- open cervix 2-degree, IU- intra-uterine

The goats in experimental batch 2 (n = 148 goats) were artificially inseminated without detecting estrous, only with the assessment of the degree of opening of the cervix and its correlation with the reproductive status of goats determined by ultrasound, data presented in table 2.

Table 2. The result of the ultrasound gestation diagnosis related to the degree of opening of the cervix in goats in experimental batch 2

| | CI | | CD1 | | CD2 | | IU | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|
| | N | % | N | % | N | % | N | % | N | % |
| P | 2 | 40 | 20 | 45,45 | 48 | 57,83 | 14 | 87,50 | 84 | 56,76 |
| PP | 1 | 20 | 2 | 4,55 | 3 | 3,61 | 0 | 0,00 | 6 | 4,05 |
| NP | 2 | 40 | 22 | 50,00 | 32 | 38,55 | 2 | 12,50 | 58 | 39,19 |
| Total | 5 | | 44 | | 83 | | 16 | | 148 | |

P- pregnant, PP- pseudo-pregnant, NP- non pregnant
CI- closed cervix, CD1- open cervix 1 degree,
CD2- open cervix 2-degree, IU- intra-uterine

Figure 1 shows that 7.69% of the control goats had a closed cervix with the deposition of intravaginal semen, at the level of the involved flora (intravaginal insemination), 30.77% had an open cervix 1 degree, a situation in which the tip of the pipette penetrates and the seminal material is partially repressed intravaginally.

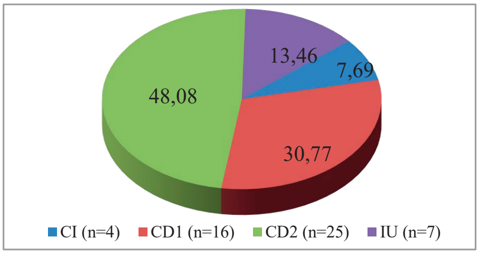


Figure. 1. The percentage representation of the cervical modification response to hormone treatment in batch witness 1

In 48.08% of goats the cervix was opened 1 degree, the insemination pipette penetrating the cervix path, without intravaginal discharge (cervical insemination) and in 13.46% of goats the pipette slips very easily, it completely crosses the cervical opening and the administration of the seminal material was done intrauterine.

In experimental batch 2, the assessment of the degree of opening of the cervix (Figure 2) showed that the percentage of goats with open cervix (intrauterine insemination) was lower than in control batch 1, respectively 10.81%, while the percentage of goats with closed cervix (intravaginal insemination) was 3.38% compared to 7.69% in control batch 1. Intracervical insemination was performed in 85.81% of goats in experimental batch 2 (29.73% - open cervix 1 degree and 56.08% open cervix 2 degree) compared to 78.85% in control batch 1.

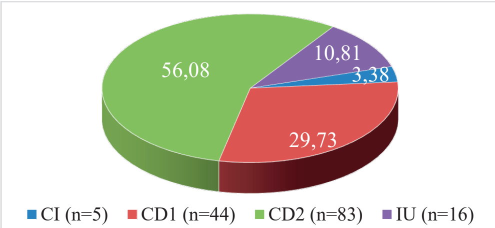


Figure 2. The percentage representation of the cervical modification response to hormone treatment in experimental batch 2

The estrous response expressed by the stages of cervical opening (CD1, CD2, IU) at 43+/-1 hours after the interruption of hormone treatment of 92.31% in control batch 1 and 96.62% in experimental batch 2 is similar to data reported by Hashemi & Sofdarian (2017), respectively 94.7% and is lower than the estrous response expressed by the 100% clinical manifestation reported by Dogan et al. (2004), 98.2% reported by Freitas et al (1997) and respectively 97% reported by Motlomelo et al. (2002).

The ultrasound examination established the gestation rate on each batch and calculated the fertility distribution according to the degree of opening of the cervix at the time of insemination. In the case of intravaginal insemination, the fertility was 40% in the experimental batch and 50% in the control batch, including the goat that did not show estrous and had a closed cervix at the time of insemination. The incidence of pseudo-gestation was 25% in the control batch and 20% in the experimental batch. Among the goats with closed cervix at the time of insemination, 25% of the control batch and 40% of the experimental batch were pregnant, which indicates the presence of estrous at intervals of more than 36-48 hours, knowing that ovulation occurs at the end of the heat (Figure 3).

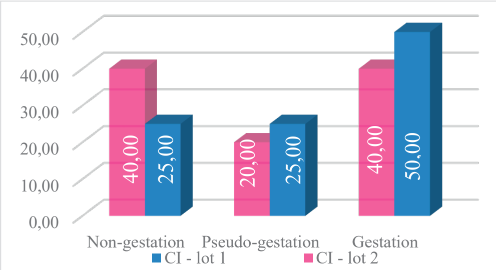


Figure 3. The representation of the gestational condition of artificially intravaginally inseminated goats

In the case of the open cervix (Figure 4), the gestation rate was 60.98% in intracervical insemination in control batch 1 and 53.54% in experimental batch 2, from which it is observed that in case of discharge the gestation rate is lower, respectively 56.25% compared to 64% when the insemination is intracervical in the 1st control batch and 45.45% (intracervical insemination with vaginal discharge) compared to 57.83% (without vaginal discharge) in the experimental one.

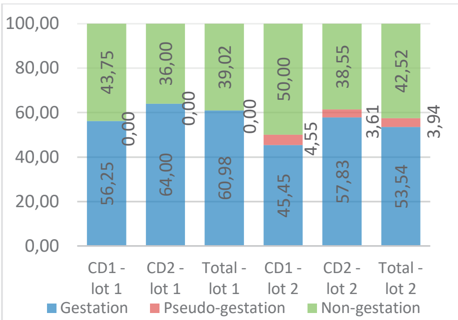


Figure 4. The representation of the gestational status of artificially intracervical inseminated goats

In the control batch to which the classic hormonal treatment of estrous induction was applied, with the use of prostaglandin no false pregnancies were found, while in the experimental batch 2 the pseudo-gestation rate is 3.94% with a higher incidence in case of intracervical insemination with seminal material, of 4.25% compared to 3.61% in the case of open cervix 1degree.

The highest percentage of non-gestation was obtained in experimental batch 2 in which the insemination was performed intracervical with vaginal discharge of the seminal material, of 54.55% of which 4.55% are represented by pseudo-gestations.

The picture of the physiological condition of the goats in the two groups confirms the incidence of pseudo-gestations in the absence of Prostaglandin F2α administration between 0.6% and 4.6 ^ in the Alpine breed following artificial insemination (Duquesnel et al., 1992). Studies conducted by Bousquet (2005) highlighted the higher incidence of pseudo-gestation as a result of the application of artificial insemination, reporting over 10% goats with pseudo-gestation in 16.7% of the farms studied.

At intrauterine insemination, a gestation rate of 100% was obtained in control batch 1 and experimental batch 2 registered 87.5% pregnant and 12.5% non-pregnant females (Figure 5).

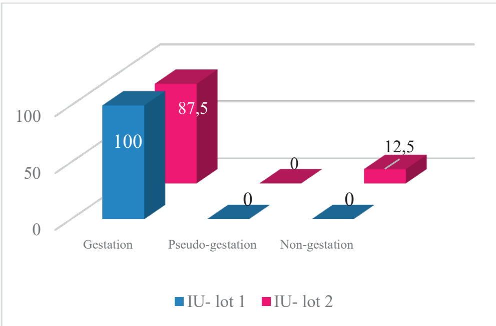


Figure 5. The representation of the gestational status of artificially intrauterine inseminated goats

If we consider the artificial insemination with frozen semen, on estrous induced by the two hormonal methods, in the counter-season of reproduction, regardless of the observations found in the vagina and cervix, it is observed that the gestation rate is 65.38% in the control batch 1 and 56.76% in experimental batch 2 (Figure 5). The results obtained in both groups are superior to those obtained by Dogan et al. (2004) which in the Saanen breed obtained a fertility of 50% or by Motlomelo et al. (2002) which registered a fertility of 47%. In 1997 Freitas reported a fertility rate of 75% after artificial insemination with frozen semen on synchronized estrous of goats that showed clinical estrous.

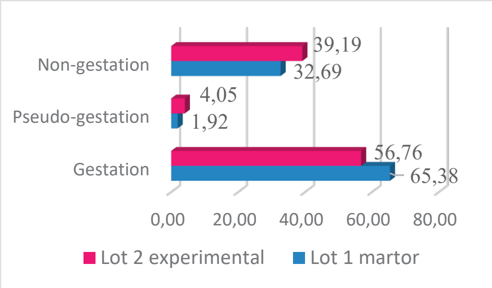


Figure 6. Representation of the state of gestation of artificially inseminated goats

Following the registration of calvings, all the goats diagnosed by ultrasound as pregnant gave birth, without any abortion. In experimental batch 2, out of the 84 goats, 261 kids were obtained, respectively a prolificacy of 310.71% and of the 34 goats in the 1st control batch, 107 kids were obtained, obtaining a prolificacy of 114.71%, values that fall specific to the breed described by CAPRIA-CAPGENE.

## CONCLUSIONS

Artificial insemination with frozen semen using the simplified protocol, respectively excluding the administration of prostaglandin to protect against accidental gestations and gonadotropin administration with the interruption of progestogen treatment has recorded lower fertility values than the control batch but is included in international research reports. This estrous induction and synchronization protocol can be extended under our country specific conditions, in which goat owners have adopted a semi-intensive breeding and reproduction technology.

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# TECHNOLOGIES OF ANIMAL HUSBANDRY

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# COMPARATIVE STUDY ON HOLSTEIN CALVES FEEDING TECHNOLOGY

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***Abstract***

*The Holstein-Friesian breed is the best known and most representative breed that produces large quantities of the best quality milk. A healthy Holstein calf weighs 40 kg at birth. Holstein bulls can weigh up to 1180 kg. The growth and development of calves are influenced by environmental conditions, but also by feed. For this study, the calf breeding group from the 0-3 months category was used. Calves were tested from a nutritional point of view, both in terms of the lactating diet and the concentrate mixture at different protein levels. The consumption of the mixture of concentrates per animal was measured, following the development of calves in this category and metabolic problems. The rations were differentiated by protein level. It was found that there are statistically significant differences in feed consumption due to the different ratios applied and the type of milk administered according to the feeding schedule.*

***Key words****: calf, cow, diet, feed ingredients, milk, substituent.*

## INTRODUCTION

Raising animals has been one of the most important activities of man, from ancient times to the present day. Among them, raising dairy cows is one of the most important sectors in raising and exploiting farm animals.

The Holstein breed is a breed of cattle, exploited for milk production, bearing different names depending on the country in which it is raised (for example: Holstein (Dutch Frieze), Canadian Friesian cattle, Israeli Holstein, American Friesian, Romanian Black Spotted). In Romania, the breed was imported and participated in the formation of the Romanian Black Spotted breed. It is a breed that is suitable for both the intensive exploitation system (Figure 1), and the extensive exploitation system and has a good resistance to leucosis disease.

Successful calves production, especially the management of dairy calves, is a key point for the profitability and sustainability of the dairy industry (Khan et al., 2016).

Calves feeding also has a key role. In the first months of life, the calf has to deal with three challenges: extra-uterine life, maintaining the prolonged pre-ruminant stage and weaning (Ignatescu (Timpau) et al., 2018).

It is important that new-born calves receive adequate colostrum intake as soon as possible after calving. Immunoglobulin concentration and intestinal permeability decrease rapidly in the first 24 hours after calving (Moore et al., 2005). The colostrum, the neonates first milk, is rich in nutrient and biologically active elements. Colostrum feeding has a major impact on post-natal development (Blum & Hammon, 2000).

After colostrum, milk or milk replacer containing high-protein should be preferred in feeding of Holstein calves, during the suckling period (Bayril et al., 2016).

The amount of milk and the feeding regime are different depending on the size of dairy cattle farms (Irimia et al., 2021).

Adherence to feeding procedures in the first part of life leads to obtaining healthy animals that are able to produce viable offspring and milk production (Dorobat et al., 2018).

Two to three weeks can make all the difference when it comes to developing the rumen and calf to be physically able to cope with weaning. Regardless of the nutrition received by a calf, when weaning is done early (at the age of 6 weeks), it struggles to cope with new nutritional changes, compared to a calf that is weaned at 8 or 9 weeks.

Figure 1. Feeding front of dairy cows (Original photo)

Mainly, young calves metabolize carbohydrates in the lower intestine, a function that decreases naturally at weaning. But the transition to carbohydrate metabolism in the rumen begins when the calf is 8 or 9 weeks old, regardless of previous diet. The starter consumed before this date results in the filling of the intestine compared to the real growth, because the rumen is not prepared to deal with high levels of cereals or fiber. Cows at the age of 8 or 9 weeks have a greater ability to consume and properly metabolize the initiator needed to meet nutritional needs. The physical size of the rumen is larger, the composition of the rumen microbiome is more diverse and mature, and there are several rumen tissues available for the absorption and metabolism of early feed.

When calves are weaned too early, the result is often a gap in the gut's ability to absorb and metabolize the original diet, and calves often experience a post-weaning increase. The microbiome of a 6-week-old calf is also very different from that of an 8- or 9-week-old calf. Research has shown that delaying the transition from a dairy diet to an exclusively solid diet (weaning) has reduced the severity of the microbiome change.

Calves weaned at eight weeks experienced a more gradual change in the microbiota than calves weaned at six weeks.

The transition from milk to solid feed can be a shock to a calf, so the slower the transition, the smaller the shock. A gradual transition allows the calf to slowly increase its initial intake of solid feed as milk intake decreases, maintaining an optimal nutrient range (Sharma et al., 2019).

## MATERIALS AND METHODS

For this study, the growth group from the 0-3 months category was used, a group that was nutritionally tested both from the point of view of the lactating diet and the concentrate mixture at different protein levels.

Following the development of calves in this category and metabolic problems, the rations were differentiated by protein level (Figure 2).

The biological material consisted of 90 bulls aged between 3 and 6 weeks. The subdivision was made according to age, as follows:

- Lot 1 was composed of calves aged between 3 and 4 weeks, and within the group 3 groups were formed, 10 calves each;
- Lot 2 was composed of calves aged between 4 and 5 weeks and 3 groups within the lot;
- Lot 3 was composed of calves over 6 weeks old and the same as in the case of the first and second lots; 3 groups were formed within the lot.

Regarding the feed, the experimental plan considered the use of 3 types of rations, as follows:

- Group 1 in each lot received the A1 ratio (23.96% protein level in the concentrated mixture);
- Group 2 in each lot received the A2 ratio (17.90% protein level in the concentrated mixture);
- Group 3 in each lot received the A3 ratio (15.36% protein level in the concentrated mixture).

Figure 2. Overview of the calf rearing area (Original photo)

In order to have a statistical validation and to be able to say exactly whether or not there are significant differences in the intake of bulls from the three groups that received the 3 ratios, two statistical tests were used, Student and Fisher Tests.

The Student test was calculated according to the following formula (Sandu, 1995):

$$\hat{t} = \frac{\bar{X}_1 \cdot \bar{X}_2}{\sqrt{\frac{(\sum X_1^2 + \sum X_1^2)\cdot(n_1+n_2)}{(n_1+n_2-2)\cdot(n_1 \cdot n_2)}}} \qquad (1)$$

The Fisher test was calculated by ANOVA (Analysis of Variance) (Sandu, 1995) (Table 1).

Table 1. Analysis of Variance (ANOVA) parameters

| Source of Variation | DF | SS | MS | F |
|---|---|---|---|---|
| Between Groups (I) | $DF_I = n-1$ | $SS_I = \sum C - \sum TC$ | $MS_I = SS_I / DF_I$ | |
| Within Groups (i) | $DF_i = p-1$ | $SS_i = \sum X^2 - \sum TC$ | $MS_i = SS_i / DF_i$ | $F = MS_I / MS_i$ |
| Total | $DF_T = N - 1$ | $SS_T = \sum X^2 - \sum C$ | | |

NB: n - the total number of individuals in a group, p-the number of groups; N - the number of individuals; DF – degrees of freedom; SS – sum of square; MS – mean of squares; ∑C – sum of corrections; ∑TC – sum of total corrections; $\sum X^2$ – the sum of the values squared.

Nutrition is, according to Georgescu (1998), the most important technological link in calf rearing.

The calves were analysed over a period of three weeks, each mixture of concentrate being administered for one week. The differences consist in the protein level of the concentrate mixture on feeding schedule with a colostrum administration and a staged passage in 3 days:

- 2/3 colostrum milk 1/3 milk substitute day 1;
- 1/2 colostrum and 1/2 milk substitute day 2;
- 1/3 colostrum and 2/3 milk substitute day 3.

From the fourth day, only the milk substitute was administered (Table 2).

Powdered milk has been used as a substitute to avoid fluctuations in whole milk fat and calves metabolic problems. Whole milk fat is a limiting factor for ingestion and can cause gastrointestinal upset. The colostrum and milk substitute feeding schedule of calves was used for a period of 70 days (Table 3).

Table 2. Milk substitute composition

| Analytical components | Value | Unit |
|---|---|---|
| Skimmed milk powder | 50 | % |
| Start+ safety concept | included | |
| Crude protein | 22 | % |
| Crude fat | 17 | % |
| Crude Ash | 7.7 | % |
| Crude fiber | 0 | % |
| Calcium | 1 | % |
| Phosphorus | 0.8 | % |
| Sodium | 0.7 | % |
| Vitamin A | 25000 | IU/kg |
| Vitamin D3 | 5000 | IU/kg |
| Vitamin E | 150 | mg/kg |
| Iron sulphate | 100 | mg/kg |
| Copper sulfate | 9 | mg/kg |
| Zinc sulfate | 100 | mg/kg |
| Manganous sulphate | 30 | mg/kg |
| Calcium iodate | 1 | mg/kg |
| Sodium selenite | 0.2 | mg/kg |
| Zinc-L-selenomethionine | 0.1 | mg/kg |
| Bacillus subtilis DSM 5750 | $1.3 \times 10^9$ | CG+FU/kg |

Table 3. Colostrum and milk substitute feeding schedule (quantity and meals number/day)

| Age | Colostrum | | Milk substitute | |
|---|---|---|---|---|
| | Quantity (l) | Meals (no.) | Quantity (l) | Meals (no.) |
| 0-3 days | 3.5-4 | 1 | 6 | 3 |
| 4-18 days | - | - | 6 | 2 |
| 19-53 days | - | - | 8 | 2 |
| 54-60 days | - | - | 6 | 2 |
| 61-65 days | - | - | 3 | 1 |
| 66-70 days | - | - | 1.5 | 1 |

In the experimental period, in each lot, the first group of calves received a mixture of concentrate with a protein content of 23.96% (A1); the second group received a mixture of concentrate with a protein content of 17.90% (A2), and the third group received a mixture of concentrate with a protein content of 15.36% (A3). The mixture of concentrates was distributed *ad libitum*, and the consistency and size of the particles were similar.

The raw materials used and their percentages in composing of the experimental recipes A1, A2 and A3, are presented in Tables 4-6.

Ratio A2 is characterized by a mixture of medium protein concentrate from both high-quality protein sources such as soybean meal and lower protein such as soybean peel (which has a lower protein content and digestibility than soybean meal).

Table 4. Structure of A1 ratio

| Raw materials | Quantity (kg) | % |
|---|---|---|
| Maize | 370.00 | 37.00 |
| Soybean meal | 158.00 | 15.80 |
| Lucerne hay | 200.00 | 20.00 |
| Rapeseed meal | 240.00 | 24.00 |
| Premix starter | 20.00 | 2.00 |
| Calcium carbonate | 10.00 | 1.0 |
| Salt | 2.00 | 0.2 |
| Total | 1000.00 | 100.00 |

Table 5. Structure of A2 ratio

| Raw materials | Quantity (kg) | % |
|---|---|---|
| Maize | 300.00 | 30.00 |
| Soybean peel | 250.00 | 25.00 |
| Soybean meal | 200.00 | 20.00 |
| Lucerne hay | 80.00 | 8.00 |
| Rapeseed meal | 80.00 | 8.00 |
| Triticale | 60.00 | 6.00 |
| Premix starter | 20.00 | 2.00 |
| Calcium carbonate | 6.00 | 0.60 |
| Salt | 4.00 | 0.40 |
| Total | 1000.00 | 100.00 |

Table 6. Structure of A3 ratio

| Raw materials | Quantity (kg) | % |
|---|---|---|
| Maize | 270.00 | 27.00 |
| Soybean peel | 220.00 | 22.00 |
| Triticale | 200.00 | 20.00 |
| Soybean meal | 120.00 | 12.00 |
| Lucerne hay | 80.00 | 8.00 |
| Rapeseed meal | 80.00 | 8.00 |
| Premix starter | 20.00 | 2.00 |
| Calcium carbonate | 6.00 | 0.60 |
| Salt | 4.00 | 0.40% |
| Total | 1000.00 | 100.00 |

Ratio A3 is characterized by a mixture of low protein concentrate from both high-quality protein sources such as soybean meal, and lower protein such as soybean peel (which has a lower protein content and digestibility than soybean meal) with a higher weight of soybean peel versus mixed soybean meal.

## RESULTS AND DISCUSSIONS

Three ratios (A1, A2, A3) were administered for each group of calves, and was followed the individual ingesta during experimental period. For a good statistical analysis, in the first stage it was verified the homogeneity of the intakes for the 30 calves from the 3 lots based on the primary statistics (mean, variant, standard deviation, coefficient of variability).

Based on the average intake per batch, it can be seen that there are quite large differences between the 3 groups of lot 1 (Table 7).

Table 7. Average ingesta of calves in the 3-4 weeks age group

| Statistics parameters | A1 ratio | A2 ratio | A3 ratio |
|---|---|---|---|
| 1 | 604 | 252 | 126 |
| 2 | 162 | 648 | 312 |
| 3 | 432 | 214 | 50 |
| 4 | 478 | 272 | 60 |
| 5 | 508 | 178 | 84 |
| 6 | 446 | 150 | 484 |
| 7 | 484 | 292 | 436 |
| 8 | 342 | 624 | 456 |
| 9 | 466 | 160 | 434 |
| 10 | 580 | 264 | 350 |
| n | 10 | 10 | 10 |
| $\bar{X}$ | 450.2 | 305.4 | 279.2 |
| $s^2$ | 15680.4 | 32735.2 | 32221.5 |
| s | 125.2 | 180.9 | 179.5 |
| CV% | 27.8 | 59.2 | 64.3 |

A1 ratio had the best ingesta (450.2 g/day) compared to A2 ratio (305.4 g/day), and A3 ratio (279.2 g/day). This is also supported by the coefficient of variability which shows that lot 1, the one that received the ratio A1, has the lowest coefficient of variability (27.8 %). As a result, the A1 ratio was adapted to the needs of the calves.

Table 8. Average ingesta of calves in the 5-6 weeks age group

| Statistics parameters | A1 ratio | A2 ratio | A3 ratio |
|---|---|---|---|
| 1 | 222 | 556 | 338 |
| 2 | 800 | 350 | 191 |
| 3 | 560 | 710 | 530 |
| 4 | 394 | 476 | 536 |
| 5 | 520 | 524 | 504 |
| 6 | 576 | 461 | 386 |
| 7 | 1358 | 1020 | 356 |
| 8 | 824 | 814 | 442 |
| 9 | 1002 | 740 | 504 |
| 10 | 1312 | 482 | 486 |
| n | 10 | 10 | 10 |
| $\bar{X}$ | 756.8 | 613.3 | 427.3 |
| $s^2$ | 142369.1 | 41217.8 | 12043.6 |
| s | 377.3 | 203.0 | 109.7 |
| CV% | 49.9 | 33.1 | 25.7 |

According to the variability coefficient, in the calves groups of 5-6 weeks age, the most homogeneous group is the one that received the A3 ratio (CV - 27.8%) (Table 8). As in the case

of the 3-4 weeks old calf group, the group that received the A1 ratio recorded the highest ingesta (756.8 g/day) compared to A2 ratio (613.3 g/day), and A 3 ratio (427.3 g/day).
The results of the primary statistical analysis for calves in the age group over 6 weeks are similar to those in calves with age between 4 and 5 weeks (Table 9).

Table 9. Average ingesta of calves over 6 weeks age group

| Statistics parameters | A1 ratio | A2 ratio | A3 ratio |
|---|---|---|---|
| 1 | 832 | 964 | 366 |
| 2 | 824 | 1110 | 512 |
| 3 | 1142 | 718 | 304 |
| 4 | 1116 | 850 | 352 |
| 5 | 2468 | 396 | 442 |
| 6 | 2646 | 496 | 184 |
| 7 | 642 | 468 | 258 |
| 8 | 558 | 458 | 462 |
| 9 | 708 | 460 | 518 |
| 10 | 372 | 592 | 578 |
| n | 10 | 10 | 10 |
| $\bar{X}$ | 1130.8 | 651.2 | 397.6 |
| $s^2$ | 621027.7 | 61387.7 | 15910.9 |
| s | 832 | 964 | 366 |
| CV% | 824 | 1110 | 512 |

The Student's test was used to determine if there were statistical differences in the homogeneity of the ingesta calf average. Three possible combinations between the three groups were analysed, for each lot of animals (Tables 10-12).

Table 10. Student test applied to Lot 1 of calves

| Lot 1 | Group1 / Group2 | Group1 / Group3 | Group2 / Group3 |
|---|---|---|---|
| t critical | 2.11 | 2.11 | 2.1 |
| t calculated | 2.08 | 2.47 | 0.32 |
| p - value | 0.05 | 0.02 | 0.74 |

Analysing the data from Table 10 it can conclude that the biggest differences are between group 1 and group 3 (t calculated - 2.47). This fact shows that the protein level and its quality positively influence the ingesta.

Table 11. Student test applied to Lot 2 of calves

| Lot 2 | Group1 / Group2 | Group1 / Group3 | Group2 / Group3 |
|---|---|---|---|
| t critical | 2.14 | 2.20 | 2.14 |
| t calculated | 1.05 | 2.65 | 2.54 |
| p - value | 0.3 | 0.02 | 0.02 |

Similar to Lot 1 is the case of Lot 2 of calves (Table 11). On the value of *p* (0.02) for the comparative analysis between group 1 and group 3 shows again the superiority of the ratio with a higher percentage of protein (t calculated - 2.65).

Table 12. Student test applied to Lot 3 of calves

| Lot 3 | Group1 / Group2 | Group1 / Group3 | Group2 / Group3 |
|---|---|---|---|
| t critical | 2.26 | 2.26 | 2.16 |
| t calculated | 2.79 | 2.90 | 2.88 |
| p - value | 0.02 | 0.01 | 0.01 |

In Lot 3, consisting of calves over 6 weeks of age, the differences in the homogeneity of the average ingesta are large (CV – 512%). Statistically, there are significant differences in all three combinations of Lot 3 of calves. There is a significant difference $p = 0.02$ between groups 1 and 2 and another highlighted by the value of $p = 0.01$ when comparing groups 1 with 3 and 2 with 3 (Table 12).
From the point of view of the homogeneity of the variants, in Table 13 it can be seen that there are no significant statistic differences in Lot 1 of calves.

Table 13. ANOVA for Lot 1 of calves (Fisher test)

| Source of variation | DF | SS | MS | F calculate | P value | F critical |
|---|---|---|---|---|---|---|
| Between groups | 2 | 169648.3 | 84824.13 | 3.16 | 0.058 | 3.35 |
| Within groups | 27 | 725733.6 | 26879.02 | | | |
| Total | 29 | 895381.9 | | | | |

For Lot 2 of calves, ANOVA test shows that there are statistically significant differences ($p = 0.026$) in terms of homogeneity of variants. This confirms that there are significant differences between the three rations administered and influences the ingesta of calves between 5- and 6-weeks age old (Table 14).

Table 14. ANOVA for Lot 2 of calves (Fisher test)

| Source of variation | DF | SS | MS | F calculate | P value | F critical |
|---|---|---|---|---|---|---|
| Between groups | 2 | 545861.7 | 272930.8 | 4.185 | 0.026 | 3.354 |
| Within groups | 27 | 1760674 | 65210.14 | | | |
| Total | 29 | 2306535 | | | | |

As can be seen when the data were analysed with the Student's test, the biggest differences between calf intakes were when they were older than 6 weeks. Also, in the case of the analysis of the homogeneity of the variants, it shows that the biggest differences from the statistical point of view are between the calf groups from Lot 3 ($p$ = 0.007) (Table 15).

Table 15. ANOVA for Lot 3 of calves (Fisher test)

| Source of variation | DF | SS | MS | F calculate | P value | F critical |
|---|---|---|---|---|---|---|
| Between groups | 2 | 2773038 | 1386519 | 5.956 | 0.007 | 3.354 |
| Within groups | 27 | 6284938 | 232775.5 | | | |
| Total | 29 | 9057975 | | | | |

Overall, there is a significantly increased ingesta of concentrate mixture in calves fed with 23.96% protein in ratio. There were also no metabolic problems.

## CONCLUSIONS

The study concludes that a controlled and optimized feeding serves the morpho – physio-logical needs of Holstein calves in order to obtain healthy and productive animals for the future production.

When it is desired for calves to have a good ingesta feed, it is important that the percentage of protein to be about 23%, and that this protein should come from good quality feed.

Only by ensuring a balanced feed with a good palatability will it be possible to wean the calves in the best conditions and with excellent results.

The nutrition in the case of calves during weaning has a critical role in their future development.

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# THE INFLUENCE OF PROTEIN LEVEL IN DAIRY COW FEED ON THE PRODUCTION

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***Abstract***

*Cattle are the most widespread category of domestic animals, with a special importance for the economy and agriculture of any country. Cattle produces 96% of the world's milk consumption, over 30% of meat and 90% of leather production. An important category in cattle is the "dairy cow", considered a living plant that transforms feed into animal products with a special nutritional value for human consumption. It is also an increasingly powerful "animal machinery", whose efficiency and productivity depend on its genetic background, diet and management. The present study showed that the level of protein in food can influence milk production.*

***Key words***: *cow, feed ingredients, fresh category, milk.*

## INTRODUCTION

Cattle farming is a major branch of world agriculture due to the volume, diversity and value of products and products obtained from this activity. In the EU agricultural sector, the productivity of the sector is very heterogeneous. In the next period, a further increase in the supply of milk and beef is expected (European Parliament's Committee on Agriculture and Rural Development, 2017). In the EU countries, most farms specializing in the production of milk and beef are located in Austria and Romania, in the mountainous areas of the Alps and the Carpathians (Figure 1).

In the food of the population of our country, food products of animal origin provide 25% of energy consumption, about 45% of daily consumption of protein and 50-56% of fat. Protein from beef products represents about 55-57% of the animal protein consumed by humans. The biological value varies depending on the nature of the product, meat or milk, between 74-82%, the digestibility coefficient is about 97%, the net use of protein is 70-77%, the protein having special qualities due to the balance in essential amino acids. The complex economic function of cattle also stems from the fact that they give very high yields per animal. In one year, milk is provided by a cow for lactation, a consumption for 12-14 inhabitants and an optimal meat consumption for 6-8 inhabitants (www.fao.org).

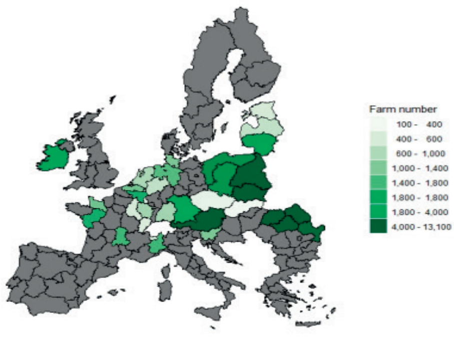


Figure 1. EU regional distribution of dairying and meat farms (European Parliament's Committee on Agriculture and Rural Development, 2017)

In Romania, cattle own about 41% of the herd expressed in UVM and share 50% in the value of global animal production, provide over 80% of total milk production, about 25% of meat production, 90% of total skins processed in the industry profile and about 70% of organic fertilizer (Georgescu coord., 2007; Maciuc et al., 2015). An important category in cattle is the "dairy cow", considered a living plant that transforms feed into animal products with a special nutritional value for human consumption (Georgescu coord., 2007; Fardet et al., 2019). Of the total production of cow's milk held in our country, 12% is technological consumption, 42% is consumed in the family, 25% is delivered directly to the market and only 21% is delivered to processing units (www.madr.ro).

## MATERIALS AND METHODS

Ensuring the right protein quantity for high milk production dairy cows is a constant challenge for farmers (Dragomir et al., 2010; Arghiriade et al., 2013).

The aim of this study is to highlight the role of feed protein in dairy cows in the "fresh" category, which has the greatest impact on milk production and the reproductive cycle (Beever and Doyle, 2007; Salo, 2018).

The animals were followed from the moment of calving until the formation of the production batch. To carry out the experiment, two batches of Holstein dairy cows (Lot 1 and Lot 2) were made, each batch and which 21 animals. The 42 animals were chosen based on their physiological condition. Thus, it was intended that all cows be at a similar stage in terms of lactation. The analysed character was the daily milk production. The animals also benefited from the same accommodation conditions and maintenance throughout the experiment, the technological flow being the same for both batches of animals that were the subject of the experiment. The measurement of the quantity of milk obtained during the differentiated feeding was done in 3 moments of lactation: at 14 days of lactation, at 21 days of lactation and at 45 days of lactation.

In the first 21 days after calving, for the duration of the “fresh” category, Lot 1 of animals was administered a ration with a protein content of 15.938% (A1 ratio) (Tables 1, 2), and Lot 2 of animals received a ratio of 17.019% (Ratio 2) protein content (Tables 3, 4).

Table 1. A1 ratio characteristics

| Indicator | UM | Calculated value | Indicator | UM | Calculated value |
|---|---|---|---|---|---|
| Dry substance | g | 18855.325 | UDP/Crude protein | % | 36.522 |
| Crude fiber | g | 3278.656 | Energy metabolizable protein | Kcal | 1837.275 |
| Crude fat | g | 814.209 | Metabolizable energy based on Nitrogen | Kcal | 1946.316 |
| Ash | g | 1376.997 | Magnesium | g | 61.9 |
| Net Energy Lactation | MJ | 123.667 | Lysine | g | 10.412 |
| Crude protein | g | 3005.124 | Methionine | g | 3.529 |
| Ca | g | 140.939 | Zinc | mg | 2550 |
| Phosphorus | g | 84.445 | Copper | mg | 550 |
| Ca/P | % | 1.669 | Manganese | mg | 2350 |
| NaCl | g | 62.6 | Iron | mg | 625 |
| Fiber/dry substance | % | 17.388 | Cobalt | mg | 14 |
| Crude fat | g | 814.209 | Molybdenum | mg | 187.5 |
| Non-nitrogenous extractive substances | g | 10380 | Selenium | mg | 12 |
| Ash | g | 1376.997 | Iodine | mg | 21 |
| Energy concentration | MJ/kg | 6.559 | A-vitamin | NE | 277500 |
| Protein concentration | % | 15.938 | Energy for maintenance | MJ/kg | 125.668 |
| Protein balance | - | 109.041 | Energy for fattening | MJ/kg | 79.513 |

Table 2. A1 ratio - Ingredients quantity

| Crt. No. | Ingredients | Quantity (Kg) |
|---|---|---|
| 1 | Basic-Energy | 1 |
| 2 | Premix | 0.25 |
| 3 | wheat | 1.4 |
| 4 | Corn grain | 3 |
| 5 | Soybean meal.46% | 1.3 |
| 6 | Rapeseed meal | 1.2 |
| 7 | Alfalfa hay | 2.5 |
| 8 | Corn silage | 20.4 |
| 9 | Alfalfa semi-hay | 7 |
| 10 | Brewers grains | 4 |
| 11 | Urea | 0.035 |
| 12 | Sodium bicarbonate | 0.1 |
| 13 | Max-Fat-HP protected fat 99% | 0.15 |
| 14 | Salt | 0.06 |

Table 3. A2 ratio characteristics

| Indicator | UM | Calculated value | Indicator | UM | Calculated value |
|---|---|---|---|---|---|
| Dry substance | g | 19820.275 | UDP/Crude Protein | % | 37.058 |
| Crude fiber | g | 3329.435 | Energy metabolizable protein | Kcal | 2017.349 |
| Crude fat | g | 1076.832 | Metabolizable energy based on Nitrogen | Kcal | 2208.006 |
| Ash | g | 1453.492 | Magnesium | g | 63.33 |
| Net energy lactation | MJ | 135.9 | Lysine | g | 14.767 |
| Crude protein | g | 3373.124 | Methionine | g | 4.524 |
| Ca | g | 143.179 | Zinc | mg | 2550 |
| Phosphorus | g | 89.565 | Copper | mg | 550 |
| Ca/P | % | 1.599 | Manganese | mg | 2350 |
| NaCl | g | 62.6 | Iron | mg | 625 |
| Fiber/dry substance | % | 16.798 | Cobalt | mg | 14 |
| Crude fat | g | 1076.832 | Molybdenum | mg | 187.5 |
| Non-nitrogenous extractive substances | g | 10587.391 | Selenium | mg | 12 |
| Ash | g | 1453.492 | Iodine | mg | 21 |
| Energy concentration | MJ/kg | 6.857 | A-vitamin | NE | 277500 |
| Protein concentration | % | 17.019 | Energy for maintenance | MJ/kg | 131.945 |
| Protein balance | - | 190.657 | Energy for fattening | MJ/kg | 83.773 |

Table 4. A2 ratio - Ingredients quantity

| Crt. No. | Ingredients | Quantity (Kg) |
|---|---|---|
| 1 | Basic-Energy | 1 |
| 2 | Premix | 0.25 |
| 3 | Wheat | 1.4 |
| 4 | Corn grain | 3 |
| 5 | Soybean meal 46% | 2.1 |
| 6 | Rapeseed meal | 1.2 |
| 7 | Alfalfa hay | 2.5 |
| 8 | Corn silage | 20.4 |
| 9 | Alfalfa semi-hay | 7 |
| 10 | Brewers grains | 6 |
| 11 | Urea | 0.035 |
| 12 | Sodium bicarbonate | 0.1 |
| 13 | Max-Fat-HP protected fat 99% | 0.4 |
| 14 | Salt | 0.06 |

After 21 days to 45 days of calving, a high production ratio with 15.983 % protein (A3 ratio) was administered to both groups (Tables 5, 6).

Table 5. A3 ratio characteristics

| Indicator | U.M. | Calculated value | Indicator | UM | Calculated value |
|---|---|---|---|---|---|
| Dry subtance | g | 24515.384 | UDP/Crude protein | % | 36.096 |
| Crude fiber | g | 4436.182 | Energy metabolizable protein | Kcal | 2437.654 |
| Crude fat | g | 1234.621 | Metabolizable energy based on nitrogen | Kcal | 2528.523 |
| Ash | g | 1612.378 | Magnesium | g | 77.74 |
| Net lactation energy | MJ | 167.282 | Lysine | g | 15.754 |
| Crude protein | g | 3918.208 | Methionine | g | 5.655 |
| Ca | g | 186.41 | Zinc | mg | 2550 |
| Phosphorus | g | 103.145 | Copper | mg | 550 |
| Ca/P | % | 1.807 | Manganese | mg | 2350 |
| (NaCl) | g | 81.8 | Iron | mg | 625 |
| Fiber/dry subtance | % | 18.096 | Cobalt | mg | 14 |
| Crude fat | g | 1234.621 | Molybdenum | mg | 187.5 |
| Non-nitrogenous extractive substances | g | 13313.995 | Selenium | mg | 12 |
| Ash | g | 1612.378 | Iodine | mg | 21 |
| Energy concentration | MJ/kg | 6.824 | A-vitamin | NE | 277500 |
| Protein concentration | % | 15.983 | Energy for maintenance | mj/kg | 162.771 |
| Protein balance | g | 90.869 | Energy for fattening | MJ/kg | 102.334 |

Table 6. A3 ratio - Ingredients quantity

| Crt. No. | Ingredients | Quantity (Kg) |
|---|---|---|
| 1 | Premix | 0.25 |
| 2 | Wheat | 2.5 |
| 3 | Corn grain | 4.4 |
| 4 | Soybean meal.46% | 1.8 |
| 5 | Rapeseed meal | 1.35 |
| 6 | Alfalfa hay | 3.6 |
| 7 | Corn silage | 23.5 |
| 8 | Alfalfa semi-hay | 9.5 |
| 9 | Brewers grains | 10 |
| 10 | Urea | 0.07 |
| 11 | Sodium bicarbonate | 0.15 |
| 12 | Max-Fat-CS protected fat 84% | 0.35 |
| 13 | Max-Fat-HP protected fat 99% | 0.25 |
| 14 | Salt | 0.08 |

The statistical analysis of data recorded during the experimental period highlighted the primary statistical parameters, as well as the significance tests of the obtained results (Fisher Test and Student Test).

The Student test was calculated according to the following formula (Sandu, 1995):

$$\hat{t} = \frac{\bar{X}_1 \cdot \bar{X}_2}{\sqrt{\frac{(\sum X_1^2 + \sum X_1^2)\cdot(n_1+n_2)}{(n_1+n_2-2)\cdot(n_1 \cdot n_2)}}} \qquad (1)$$

The Fisher test was calculated by Analysis of Variance (Sandu, 1995) (Table 7).

Table 7. Analysis of Variance (ANOVA) parameters

| Source of Variation | DF | SS | MS | F |
|---|---|---|---|---|
| Between Groups (I) | $DF_I = n-1$ | $SS_I = \sum C - \sum TC$ | $MS_I = SS_I / DF_I$ | $F = MS_I / MS_i$ |
| Within Groups (i) | $DF_i = p-1$ | $SS_i = \sum X^2 - \sum TC$ | $MS_i = SS_i / DF_i$ | |
| Total | $DF_T = N - 1$ | $SS_T = \sum X^2 - \sum C$ | - | |

NB: n - the total number of individuals in a group, p-the number of groups; N - the number of individuals; DF – degrees of freedom; SS – sum of square; MS – mean of squares; ∑C – sum of corrections; ∑TC – sum of total corrections; ∑X² – the sum of the values squared.

## RESULTS AND DISCUSSIONS

The results obtained from the differentiated feeding of the two groups of dairy cows are shown in Tables 8 and 9. Regarding the calving situation, in Group 1, it was found that 85.71% of the animals gave birth normally and in group 2, the percentage was 95.24%.

Table 8. Evolution of milk production in Lot 1 of dairy cows

| Crt. No. | Cow No. | Birth date | Calving situation | Milk production (l) | | |
|---|---|---|---|---|---|---|
| | | | | at 14 days | at 21 days | at 45 days |
| 1 | 263 | 05.02. | Twin calving | 19 | 24 | 27 |
| 2 | 583 | 10.02. | Normal | 31 | 36 | 39 |
| 3 | 618 | 15.02. | Normal | 29.9 | 34.9 | 37.9 |
| 4 | 789 | 16.02. | Normal | 21.7 | 26.7 | 29.7 |
| 5 | 586 | 14.02. | Placental retention | 2. 3 | 28 | 31 |
| 6 | 623 | 20.02. | Normal | 19.6 | 24.6 | 27.6 |
| 7 | 77 | 20.02. | Normal | 15.4 | 20.4 | 23.4 |
| 8 | 324 | 25.01. | Normal | 24.6 | 29.6 | 32.6 |
| 9 | 266 | 01.01. | Normal | 19.9 | 24.9 | 27.9 |
| 10 | 779 | 18.01. | Normal | 28 | 33 | 36 |
| 11 | 791 | 18.02. | Placental retention | 23.3 | 28.3 | 31.3 |
| 12 | 598 | 23.02. | Normal | 27.2 | 32.2 | 35.2 |
| 13 | 408 | 24.02. | Normal | 31.1 | 36.1 | 39.1 |
| 14 | 132 | 24.02. | Normal | 22.8 | 27.8 | 30.8 |
| 15 | 796 | 25.02. | Normal | 16.9 | 21.9 | 24.9 |
| 16 | 665 | 26.02. | Normal | 23.1 | 28.1 | 31.1 |
| 17 | 797 | 28.02. | Normal | 24.6 | 29.6 | 32.6 |
| 18 | 365 | 04.03. | Normal | 25.3 | 30.3 | 33.3 |
| 19 | 799 | 05.03. | Normal | 28.2 | 33.2 | 36.2 |
| 20 | 800 | 06.03. | Dead foetus | 23.6 | 28.6 | 31.6 |
| 21 | 801 | 07.03. | Normal | 31.4 | 36.4 | 39.4 |

Table 9. Evolution of milk production in Lot 1 of dairy cows

| Crt. No. | Cow No. | Birth date | Calving situation | Milk production (l) | | |
|---|---|---|---|---|---|---|
| | | | | at 14 days | at 21 days | at 45 days |
| 1 | 788 | 05.02. | Normal | 24.8 | 29.8 | 32.8 |
| 2 | 612 | 05.02. | Normal | 25 | 30 | 33 |
| 3 | 649 | 07.02. | Normal | 26.1 | 31.1 | 34.1 |
| 4 | 382 | 06.02. | Normal | 28.7 | 33.7 | 36.7 |
| 5 | 309 | 08.02. | Normal | 23.6 | 28.6 | 31.6 |
| 6 | 211 | 10.02. | Normal | 31.1 | 36.1 | 38.1 |
| 7 | 184 | 16.02. | Normal | 40.1 | 45.1 | 48.1 |
| 8 | 790 | 17.02. | Normal | 29.2 | 34.2 | 37.2 |
| 9 | 625 | 20.02. | Normal | 23.4 | 28.4 | 31.4 |
| 10 | 792 | 22.02. | Normal | 25.6 | 30.6 | 33.6 |
| 11 | 793 | 22.02. | Normal | 30.7 | 35.7 | 38.7 |
| 12 | 659 | 07.03. | Normal | 33.2 | 38.2 | 41.2 |
| 13 | 802 | 08.03. | Normal | 35 | 40 | 43 |
| 14 | 632 | 09.03. | Normal | 34 | 39 | 42 |
| 15 | 794 | 23.02. | Normal | 33.6 | 38.6 | 41.6 |
| 16 | 795 | 23.02. | Normal | 30 | 35 | 38 |
| 17 | 542 | 27.02. | Normal | 43.9 | 48.9 | 51.9 |
| 18 | 420 | 28.02. | Normal | 32.2 | 37.2 | 40.2 |
| 19 | 798 | 29.02. | Normal | 32.1 | 37.1 | 40.1 |
| 20 | 804 | 15.03. | Normal | 30 | 35 | 38 |
| 21 | 805 | 15.03. | Dead foetus | 31.1 | 36.1 | 39.1 |

For a first comparison of the two groups of animals fed with rations with two different protein levels (15.983% and 17.019%), in the first 21 days after calving, it is necessary to know the statistics of the groups. Thus, the average milk production shows that there are

quantitative differences between the two batches.
Depending on the number of days for which the quantity of milk obtained was measured, Lot 1 of dairy cows had an average quantity of milk of 24.25 l (at 14 days), 29.27 l (at 31 days) and 32.22 l (at 45 days). Lot 2 of dairy cows had an average amount of milk of 30.64 l (at 14 days), 35.62 l (at 31 days) and 38.59 l (at 45 days) (Table 10).
This difference can most probably be explained by the fact that the ration for Lot 2 was richer in protein when the cows were in the "fresh" category. Thus, the high level of protein provides the cow with the necessary to support a higher milk production.
The coefficient of variability decreases with the duration of lactation, which shows that the groups become more homogeneous after 45 days of lactation (Table 10). This is explained by the fact that the cow goes through the critical postpartum period.
Regarding the Analysis of Variance (ANOVA), concluded with the calculated Fisher value, it can be said that those between the two groups are very significant differences in terms of homogeneity of variants (Table 11).

Table 10. Primary statistical analysis of the two groups of animals

| Lot 1 | | | |
|---|---|---|---|
| Specification | Milk production at: | | |
| days | 14 | 21 | 45 |
| The amount | 509.6 | 614.6 | 677.6 |
| Mediate | 24.25 | 29.27 | 32.22 |
| Standard deviation | 4.63 | 4.60 | 4.57 |
| Coefficient of variability | 19.07% | 15.82% | 14.35% |
| Lot 2 | | | |
| Specification | Milk production at: | | |
| days | 14 | 21 | 45 |
| The amount | 643.4 | 748.4 | 810.4 |
| Mediate | 30.64 | 35.62 | 38.59 |
| Standard deviation | 5.18 | 5.14 | 5.08 |
| Coefficient of variability | 16.90% | 14.53% | 13.42% |

The significant difference noticed by the Fisher test shows that the different protein levels in the rations received by the two batches influence the milk production.

Table 11. Fisher's test to test the homogeneity of the variance for the two batches of dairy cows

| Source | Degrees of freedom | The sum of the squares | Average squares | F-calculated | p-value | F-critical |
|---|---|---|---|---|---|---|
| Between groups | 2 | 686 | 343 | 16.00909 | 2.68E-06 *** | 3.150411 |
| Enter groups | 60 | 1285.52 | 21.42533 | - | - | - |
| Total | 62 | 1971.52 | - | - | - | - |

* - Significant; ** - Distinctly significant; *** -Very significant.

When the batches were compared in terms of the homogeneity of the media, it was observed that in all 3 combinations between the moments of lactation there are significant differences (Table 12).
The analysis of the homogeneity of group 2 shows that there are significant differences between the 3 moments of lactation in which the amount of milk was measured (Table 13).
Similar to batch 1, and in this case, it can be said that when the duration of lactation increases and the homogeneity of the variants between successive measurements increases.

Table 12. Student (t) test for homogeneity testing in Lot 1

| Comparison | Number of days | | |
|---|---|---|---|
| | 14/21 | 14/45 | 21/45 |
| t critical | 2.02 | 2.02 | 2.02 |
| t calculated | 3.5 | 5.6 | 2.1 |
| p - value | 0.0011*** | 0.00000017 *** | 0.042* |

* - Significant; ** - Distinctly significant; *** - Very significant.

Table 13. Fisher's test for testing the homogeneity of the variance for Lot 2

| Source | Degrees of freedom | The sum of the squares | Average squares | F-calculated | p-value | F-critical |
|---|---|---|---|---|---|---|
| Between groups | 2 | 678.698413 | 339.3492 | 12.65051 | 2.61E-05 *** | 3.150411 |
| Enter groups | 60 | 1609.49714 | 26.82495 | - | - | - |
| Total | 62 | 2288.19556 | - | - | - | - |

* - Significant; ** - Distinctly significant; *** - Very significant.

For Lot 2, the Student's test shows that there are significant differences in the comparison of milk production on day 14 with production on days 21 and 45, but there are no significant differences in the homogeneity of the averages when comparing milk production on day 14. 21 with milk production from day 45 (Table 14).

Table 14. Student (t) test for homogeneity testing in Lot 2

| Comparison | Number of days | | |
|---|---|---|---|
| | 14/21 | 14/45 | 21/45 |
| t critical | 2.02 | 2.02 | 2.02 |
| t calculated | 3.12 | 4.97 | 1.84 |
| p-value | 0.003** | 0.00000128 *** | 0.072 |

* - Significant; ** - Distinctly significant; *** - Very significant.

## CONCLUSIONS

Following this study and statistical analysis, the impact of protein levels in rations administered during the onset of lactation on milk production is highlighted, with higher milk production observed in cows given a higher protein ratio than those which had a lower protein ratio.

The differences in the quantities of milk obtained were given by the differences in protein in the two rations from the "fresh" period. These differences between milk production were maintained during lactation in high production.

The percentage of protein in the lactation onset ration is a determining factor in the amount of milk obtained later, provided that the rate is in an energy-protein balance.

The level of protein in rations given to lactating cows positively influences milk production by achieving high milk production.

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# ALBANIAN AGRICULTURAL ADVISORS AND FARMERS' PREFERENCES ON EXTENSION SERVICE ACTIVITIES

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***Abstract***

*A survey was carried out during the lockdown period (April-May 2020), to assess agricultural advisors' preferences on extension service activities. A questionnaire was mailed to the 66 advisors of the Regional Agencies of Extension Service of Tirana and Korça and the descriptive statistic method was used to analyse the data collected. Before disseminating the innovations, the advisors need more trainings on extension methods & activities, and agricultural practices. "Trainings combined with field visits for the practical side" is their first choice to get new knowledge on topics related to their work, while as a second choice they have preferred "open field days". Extensionists perception is that that "Demonstrations" and "Trainings combined with on-farm visits" are the two main activities that the extensionists think of as the most suitable activities for the farmers and for the realization of their plan of extension activities. As less important activities extensionists listed: "In-country trainings", "Brochure/Leaflet", "Study tours" and "Open field days".*

**Key words:** *agricultural advisors, extension service activities, farmers, preferences, survey.*

## INTRODUCTION

Agriculture in Albania contributes around 18% of the economy's GDP (World Bank, 2020) and it employs 37.4% of the country workforce (INSTAT, 2019). In addition, agriculture is the main sources of income for rural households and it is also important in terms of alleviating poverty and improving the standard of living, (Rama et al., 2018; Bicoku & Subashi, 2020).

Agriculture in the last 30 years has signed a positive development of production and the sector is undergoing a transition from subsistence sector to a commercial one (Gjeci et al., 2018).

Although the positive trends the sector is facing several challenges starting from the small farm size (1.26 ha), and fragmentation of land (about 4 plots per farm)[1]; lack and when it is in place weakness of farmers' organizations; limited access to agricultural credit; limited access to markets and low standards of products; inefficient farm management practices; and all these weaknesses lead to low level of competitiveness of agriculture (Gjeçi et al., 2018). Several of these weaknesses are continued over the last 20 years, such as the low technology level of farmers, or the public and private advisory services not at the level required by farmers.

The public advisory system (PAS), which was recently organized in four Regional Agencies of Agricultural Extension (RAAE)[2], consists of about 260 agronomist and livestock specialist. The extensionists are working in district and village level, as well in 120 Agricultural Information Centres. Except the public sector the farmers receive advice from different sources such as: agro-input dealers, Agriculture Technology Transfer Centres (ATTCs), the Agriculture University of Tirana, donor supported projects and private sector organizations (Bicoku & Subashi, 2020).

The PAS started its operation in 1992, and for several years was supported with technical assistance by donors financed projects.

Skreli et al. (2014) and IPESA (2020) emphasis that the impact of government/public extension

[1] The land reform implemented after August 1991, in which the state agricultural land was equally distributed to the rural population, resulted in small and fragmented farms that hamper the growth and competitiveness of agriculture.

[2] Since March 2018, by the Government Decision no. 147, date 12.03.2018

service on farm performance is limited, and also the coverage of public extension services is limited, specially to the contact farms[3], most of them categorized as medium farms while the private advisory services are the main source of advice for largest farms. However, for the preparation of extension work plans the main opinion is of contact farmers, and large farms compared to small farms (89.4 versus 40.9%) at a time when in the vision of extension organizations is the support of small and medium farmers (Bicoku & Subashi, 2020).

In the advisory process one of the key factors is the education of extensionists and through it the farmers receive better technical knowledge and information, which help them to make decisions about the future of the farm. However, before starting any advisory program it is important to evaluate the knowledge of extension agents toward the innovations that need to be disseminated (Al-Shayaa et al., 2012). Because as it mentioned by Van den Ban & Hawkins (1996) the goals of agricultural extension include the transfer of information from the international knowledge base and from country research to farmers, enabling them: (i) to clarify their goals and opportunities, (ii) educating them on how to make better decisions, and (iii) stimulate desired agricultural development. To accomplish their job, extensionists need to be trained in various aspects of the extension process, which usually is done during the professional training of the extensionists (Oakley & Garforth, 1985).

Extensionists need to understand the communities they work for and be accountable for serving them. In addition to good technical knowledge, they must possess knowledge and skills for planning, implementing and evaluating extension programs, because the lack of training, after being employed, by the agricultural extension organization, affects negatively the success of their work. Good communication skills are much better in all aspects of their job (Suvedi & Kaplowitz, 2016; Man et al., 2016; Khan et al., 2011; Erbaugh et al., 2007).

According to Davis & Sulaiman (2016), the extensionists must have knowledge of agronomy, plants, livestock and natural resources. They also need to understand human nature and how people make their choices. Thus, the discipline of extension includes elements of the natural sciences, as well as education, sociology, anthropology, communication, and much more.

Nevertheless, Suvedi & Kaplowitz (2016) are emphasising that, extensionist should not be judged only on how much knowledge they have in their area of technical expertise, but how skilled they are at providing services to their clients. It should also be noted that the basic needs of competencies depend on the circumstances in which the extensionists work, and they affect both the competency requirements and the level of competencies. Therefore, competency improvement is essential for all extension staff training. In addition, the level of skills and competencies required for extensionists can vary depending on the circumstances.

While Salah et al. (2016) says that one of the most important steps of training needs is the performance development and the factors that motivate employees for continuity and stability of their employment. Training removes dust from acquired knowledge. It is therefore important that all employees update their knowledge periodically and become aware of the constant changes of science or technology. Because, training is the process of acquiring specific skills to perform the job better. It includes the processes of teaching, informing and educating people.

Training is not a luxury but something necessary for extensionists, it is a kind of investment for them (Ahmed & Khalid, 2013).

## MATERIALS AND METHODS

The purpose of the survey was to identify the preferences of the extensionists, of the Regional Agencies of Agricultural Extension (RAAE) - Tirana and Korça[4], for their extension activities. The realization of the survey has been made possible by the use of primary, secondary sources and literature data related to extension service in the field of agriculture.

The survey was conducted with 66 extensionists (all of them who have a computer, about 90% of the staff)) whom are staff of RAAE of Tirana and Korça.

[3] Farmers with whom the advisers have regular contacts.

[4] There are four RAAE in Albania (subordinate to Ministry of Agriculture and Rural Development) with centres in Shkoder, Tirane, Korçe and Lushnje.

For the purpose of this survey, a questionnaire is designed for interviewing extension agents and collecting the data needed. The questionnaire consists of a series of questions. There are questions about the extensionists personal background, such as age, gender, year of graduation, working experience, education background. Other variables in the dataset relate to extensionists' preferences for the extension activities they conduct.

The questionnaire was subject to review by a panel of three experts, which was conducted via Google Meet[5], in the case of inconsistent questions, it was modified accordingly.

The survey was administered in early May 2020, and the questionnaires were filled electronically by the extensionists, as Covid-19 protocols didn't allow the direct interviews. The extensionists were clarified about the purpose of the interview and the survey, as well that the data would be confidential.

The questionnaire contained open-ended and closed-ended questions. Open-ended questions allow for a greater variety of responses from participants, but are difficult to analyse statistically because data has to be coded or reduced in some way. While, closed-ended questions are easy to analyse statistically, but they seriously limit the answers that participants can provide (Jackson, 2009). Also is used a Likert-type scale (1932), because it is very easy to analyse statistically and it is very used in agricultural research (Clason & Dormody, 1994).

The data obtained were entered in Microsoft Excel and transferred into SPSS. The analysis is based on descriptive statistics, namely frequencies.

## RESULTS AND DISCUSSIONS

### 1. General data on the interviewed agricultural advisors

In this study, we aimed to identify the preferences of AREB-Tirana and Korça extension employees for extension activities.

As can be seen from the data in Table 1, 62% of the interviewees interviewed are male and 38% female (63% and 37% of RAAE Tirana and 61.5% and 38.5% of RAAE Korça).

The extensionists have a long working experience in agriculture (23 years as average; 26.5 years of RAAE Tirana and 20.7 of RAAE Korça), but only 11.3 years in extension (11.7 years in Tirana and 11.0 in Korça); difference which is explained by the frequent movements made in the direction of the Ministry of Agriculture and the Governments.

In terms of education, 62.1% of extensionists have a degree in agronomy, 22.7% in animal science, 6% in plant protection and by 3% in fruit growing, in agrarian economics, and in agro-environment (Figure 1).

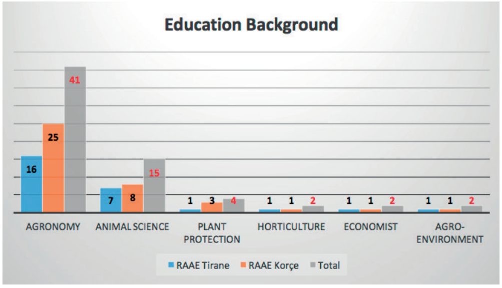


Figure 1. Extensionists education background

It should be noted that 36.4% of extension workers were employed without interviews and only 48.5% were interviewed by DPA, which is the only institution to conduct interviews for employees working in the public administration (Table 2). In addition, the cost of transport from home to workplace should be covered by the extensionists and therefore about 25% of them express it as unsatisfactory part of their work (Table 4).

Extensionists respond that getting to know farmers' problems is the most satisfying part of their job. The same is reported from the study of Agunga (1995). What stands out is that only two extensionists place demonstrations as a satisfactory part of their work, at a time when demonstrations are the main activities they perform, during each year (Table 3).

The least satisfactory part are the expenses they make for transport, bureaucracies (frequent requests from MARD[6] for filling in various forms, or operational data), as well as the lack of support of the farms with subsidies from ARDA[7].

[5] The Covid-19 protocols didn't allow the direct interviews and meetings.

[6] Ministry of Agriculture and Rural Development

[7] Agriculture and Rural Development Agency (Payment Agency).

Table 1. Main sample socio-demographic

| RAAE | Extensionists | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Number of Interviews | Gender | | Age (years) | Experience (years) | | Education | | | | | |
| | | M | F | | Total | In extension | Agronomy | Zootechnic | Plant protection | Horticulture | Economist | Agro-environment |
| Tiranë | 27 | 17 | 10 | 53.6 | 26.5 | 11.7 | 16 | 7 | 1 | 1 | 1 | 1 |
| Korçë | 39 | 24 | 15 | 51.7 | 20.7 | 11.0 | 25 | 8 | 3 | 1 | 1 | 1 |
| **Total** | **66** | **41** | **25** | **52.5** | **23.1** | **11.3** | **41** | **15** | **4** | **2** | **2** | **2** |

Table 2. Hiring and distance of work

| RAAE | Hired | | | Transport used to reach the workplace | | Distance from home to work place or clients | | |
|---|---|---|---|---|---|---|---|---|
| | Without interview | Interviewed by | | | | Average | minimum | maximum |
| | | DPA[8] | RAD[9] | Public | Private | | | |
| Tiranë | 4 | 16 | 7 | 15 | 12 | 16.2 | 13 | 27.7 |
| Korçë | 20 | 16 | 3 | 30 | 9 | 14.5 | 9.3 | 27.8 |
| Total | **24** | **32** | **10** | 45 | 21 | 15.2 | 10.8 | 27.76 |
| Percentage | **36.4** | **48.5** | **15.1** | **68.2** | **31.8** | | | |

Table 3. The most satisfying part of the work of extensionists

| RAAE | Opportunity to know the problems of farmers | When farmers are satisfied with the advice received | Meeting with farmers in Extension Office as they believe in the advice received | Field demonstrations where knowledge is confronted with practice | When farmers apply the advice received | TOTAL |
|---|---|---|---|---|---|---|
| **Tiranë** | 14 | 7 | 3 | 1 | 2 | 27 |
| **Korçë** | 38 | | | 1 | | 39 |
| **Total** | 52 | 7 | 3 | 2 | 2 | 66 |
| **Percentage** | 78.8 | 10.6 | 4.6 | 3.0 | 3.0 | 100% |

Table 4. The least satisfactory part of the work of extensionists

| RAAE | Transport cost | Bureaucratic requirements for issues that do not pertain to extension | When the farmer fails to receive the subsidy and he start to loses the trust to RAAE | Non-implementation of advice by the farmer | Distance of farms from each other | I do not have | TOTAL |
|---|---|---|---|---|---|---|---|
| **Tiranë** | 8 | 6 | 6 | 1 | | 6 | 27 |
| **Korçë** | 9 | 4 | 2 | 6 | 2 | 16 | 39 |
| **Total** | 17 | 10 | 8 | 7 | 2 | 22 | 66 |
| **Percentage** | 25.8 | 15.2 | 12.1 | 10.6 | 3.0 | 33.3 | 100 |

Extensionists help farmers complete the documentation required by ARDA for their support of national schemes. At the end of the process there is no information from ARDA to the extensionists whom from the farmers won and also to know the reasons why some of the farmers are not qualified for the support schemes. Since years the extension service is facing difficulties for the dissemination of information, as in the work territory of one advisor are performing their activity approximately 2400 farms and only 26.5% of them are visited annually by the extensionists, but a good part of them is visited only once a year (IPESA, 2017; Hoxha, 2021).

The number of farmers who have received information during the year is only 31% higher

[8] Department of Public Adminstration

[9] Regional Agricultural Directory

than the farms visited. Contact[10] farms constitute 10% of farms who have received information during the year. This should be a problem that: (i) farmers do not visit the extensionists offices to obtain information, (ii) a part of farm visits are not for information, but for data collection required by MARD.

Extensionists answer that about 15% of the working time they are not at their place of work, meaning that they are in the RAAE offices, for meetings (with limits of 1 to 6 days); while farm visits occupy about 55% of their time (with limits of 2 to 16 days), and the rest of the time (30%) is spent in the office where farmers come for information (Table 5). Only 89.4% of farmers seek advice from extensionists after receiving financial support from ARDA, and in 89.8% of cases is related to the implementation of the technology for which the grant was received, while the rest ask for information to receive a grant in the following year.

Most extension activities (Table 6) take place for crop production and fruit growing (80.5-83.8%) and the rest for livestock (17.5-19.5%). Demonstrations are the main activities, where on average each extension holds one demonstration per month. After the demonstrations are trainings and open field days. While leaflets are prepared by the subject matter specialists of RAAE or ATTCs, and extensionists only make multiplications of them, according to the farmers' requests. A survey conducted by Franz et al. (2010) with Virginia-US extensionists, reports that the most common methods used by extension service for new technologies and information were: (i) demonstrations, (ii) trainings, (iii) visits to other farms, (iv) experiments and, (v) on-farm problem-solving visits.

## 2. The best way of acquiring new knowledge for extensionists

Table 7 summarizes the forms that extensionists like to receive new information on topics related to their work. The results are interesting as extensionists as a first choice have preferred "Trainings combined with on-farm visits", the same is reported by Lakai et al. (2012). The other alternatives are on the same level, but "Open field days" are the second form, which is also reported by Andrango & Bergtold (2015). The three lowest rated formats were videos (2.5%), brochures / leaflets (9.9%) and study tours (11.2%).

These findings contradict those of some authors who report travel abroad (Chizari et al., 1999; Al-Rimawi et al., 2017) or university publications, internet, and newsletters (Andrango & Bergtold, 2015) as the most effective forms to get new information. Whereas, Khan (2011) reports demonstrations as the best form to train extensionists.

From the eight forms of knowledge acquisition we see that there are some differences between agronomists and zootechnics (Table 8). In four of them, zootechnics consider them more important than agronomists (Open field days, Training abroad, Brochure / leaflet, and Video), as agronomists consider more important than zootechnics only one of them (In-country training). In three of them they answer in the same way (Trainings combined with on farm visits, Demonstrations, Study tours).

Extensionists of both groups (1-10 years of experience and over 11 years) consider "Trainings combined with on-farm visits" as the best form for acquiring new knowledge.

Extensionists who have fewer years in extension give a more positive answer in terms of Field days, demonstrations, in-country training, training abroad, compared to extensionists with more years of extension experience.

Regarding the institutions for conducting trainings, the extensionists evaluate five of them for different topics as given in table 9. Draws attention to the fact that extensionists choose Agricultural University of Tirana (AUT) for topics related to plant protection. This is due to the fact that the Plant Protection Laboratory - Durres is under the auspices of the Department of Plant Protection of AUT.

## 3. The best form of acquiring new knowledge for farmers and implementing the extension plan

Extensionists perception is that that "Demonstrations" and "Trainings combined with on-farm visits" (Table 10) are the two main activities that those who are suitable for farmers think about and for the realization of their plan of extension activities.

[10] As it is impossible to cover many farmers the extensionists has selected few small and medium farms that are consulted and informed regularly during the year.

Table 5. Data on daily work of the extensionists

| RAAE | Farms in your area | | Livestock farms in your area | | Number of farmers getting information during the year | | Days per month | |
|---|---|---|---|---|---|---|---|---|
| | Total | Farms visited per year | Total | Farms visited per year | Total | Contact farmers | You are not in the working place | Farm visits |
| Tiranë | 2447 | 371 | 774 | 69 | 726 | 65 | 3 | 9 |
| Korçë | 2363 | 821 | 602 | 181 | 914 | 98 | 3 | 21 |
| Average | 2398 | 637 | 672 | 135 | 837 | 85 | 3 | 10.8 |

Table 6. Activities perform as average in the last three years

| RAAE | Activities | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Demonstrations on | | | Open Field Days | | | Trainings | | | Leaflets | | |
| | Agriculture | Livestock | Total | Agr. | Liv. | Total | Agr. | Liv. | Total | Agr. | Liv. | Total |
| Tiranë | 25.3 | 4.3 | 29.6 | 11.2 | 1.9 | 13.1 | 19.8 | 3.9 | 23.7 | 33.0 | 12.7 | 45.7 |
| Korçë | 29 | 8.3 | 37.3 | 19.6 | 4.1 | 23.7 | 12.7 | 2.9 | 15.6 | 18.1 | 5.8 | 23.9 |
| Average | 27.6 | 6.7 | 34.3 | 16.0 | 3.1 | 19.1 | 15.5 | 3.3 | 18.8 | 24.0 | 8.5 | 32.5 |
| Percentage | 80.5 | 19.5 | 100 | 83.8 | 16.2 | 100 | 82.5 | 17.5 | 100 | 73.8 | 26.2 | 100 |

Table 7. Most appropriate way to get new knowledge for extensionists [11]

| Activities | RAAE | | | | Total | |
|---|---|---|---|---|---|---|
| | Tiranë | | Korçë | | | |
| | persons | % | persons | % | persons | % |
| Trainings combined with on-farm visits | 26 | 17.1 | 35 | 20.6 | 61 | 18.9 |
| Open field days | 18 | 11.8 | 30 | 17.7 | 48 | 14.9 |
| Demonstrations | 18 | 11.8 | 28 | 16.5 | 46 | 14.3 |
| Trainings abroad | 22 | 14.5 | 24 | 14.1 | 46 | 14.3 |
| In-country trainings | 25 | 16.5 | 20 | 11.8 | 45 | 14.0 |
| Study tours | 20 | 13.2 | 16 | 9.4 | 36 | 11.2 |
| Brochures/Leaflets | 18 | 11.8 | 14 | 8.2 | 32 | 9.9 |
| Video | 5 | 3.3 | 3 | 1.7 | 8 | 2.5 |
| Total | 152 | 100 | 170 | 100 | 322 | 100 |

Table 8. Extensionists most appropriate way to acquire new knowledge according to the professions[12]

| Activities | Profession | | | | | |
|---|---|---|---|---|---|---|
| | Agronomist | | Zootechnics | | Other | |
| | persons | % to the number of agronomist | persons | % to the number of zootechnics | persons | % |
| Trainings combined Witt on-farm visits | 38 | 92.7 | 14 | 93.3 | 9 | 90.0 |
| Open field days | 28 | 68.3 | 12 | 80.0 | 8 | 80.0 |
| Demonstrations | 30 | 73.2 | 11 | 73.3 | 5 | 50.0 |
| Trainings abroad | 27 | 65.8 | 13 | 86.7 | 6 | 60.0 |
| In-country trainings | 29 | 70.7 | 9 | 60.0 | 7 | 70.0 |
| Study tours | 21 | 51.2 | 8 | 53.3 | 7 | 70.0 |
| Brochures/ leaflets | 17 | 41.5 | 10 | 66.7 | 5 | 50.0 |
| Video | 3 | 7.3 | 5 | 33.3 | 0 | 0 |

Table 9. Institutions offering trainings

| Institution | Respondent | | Percentage | Topic |
|---|---|---|---|---|
| | Total | For main topics | | |
| ATTC | 44 | 28 | 63.6 | New technologies |
| AUT | 35 | 15 | 42.9 | Plant protection |
| MARD | 34 | 9 | 26.5 | Agricultural strategies approx. with those of the EU |
| Donors' projects | 32 | 12 | 37.5 | Adaptation of cultivars and indigenous breeds |
| NGO | 14 | 11 | 78.6 | Establishment of agricultural associations/cooperatives |

[11] Total answer is bigger than the number of the extensionists because were multiple choices.
[12] Total answer is bigger than the number of the extensionists because were multiple choices.

Table 10. The most appropriate activities to implement the extension program for farmers

| Activities | RAAE | | | | | |
|---|---|---|---|---|---|---|
| | Tirane | | Korçe | | Total | |
| | Persons | % to the number of extensionists | Persons | % to the number of extensionists | Persons | % to the number of extensionists |
| Demonstration | 24 | 88.9 | 37 | 94.5 | 61 | 92.4 |
| Trainings combined with on-farm visits | 22 | 81.5 | 36 | 92.3 | 58 | 87.9 |
| In- country trainings | 15 | 55.5 | 31 | 79.5 | 46 | 69.7 |
| Brochures/Leaflets | 12 | 44.4 | 32 | 82.1 | 44 | 66.7 |
| Study tours | 13 | 48.1 | 29 | 74.3 | 42 | 63.6 |
| Open field days | 14 | 51.8 | 28 | 71.8 | 42 | 63.6 |
| Video | 5 | 18.5 | 6 | 15.4 | 11 | 16.7 |
| Trainings abroad | 1 | 3.7 | 0 | 0 | 1 | 1.5 |
| Visits to the fruit and vegetable markets | 1 | 3.7 | 0 | 0 | 1 | 1.5 |

Table 11. Information channels liked by farmers according to extensionists opinion

| Activities | RAAE | | | | | |
|---|---|---|---|---|---|---|
| | Tirane | | Korçe | | Total | |
| | Persons | % to the number of extensionists | Persons | % to the number of extensionists | Persons | % to the number of extensionists |
| Individual discussions (extensionists-farmer) | 27 | 100.0 | 38 | 97.4 | 65 | 98.5 |
| Leaflets | 27 | 100.0 | 31 | 79.5 | 58 | 87.9 |
| Group discussions | 26 | 96.3 | 30 | 76.9 | 56 | 84.8 |
| Demonstrations | 3 | 11.1 | 32 | 82.0 | 35 | 53.0 |
| Visits to other farms (exchange of experience) | 1 | 3.7 | 32 | 82.0 | 33 | 50.0 |
| Open field days | 1 | 3.7 | 29 | 74.4 | 30 | 45.5 |
| TV | 3 | 11.1 | 14 | 35.9 | 17 | 25.8 |
| Internet | 6 | 22.2 | 10 | 25.6 | 16 | 24.2 |
| Radio | 0 | 0.0 | 1 | 2.6 | 1 | 1.5 |

After these two activities, on the same level the extensionists list are “In- country trainings”, “Brochure/Leaflet”, “Study tours” and “Open field days”.

Andrango & Bergtold (2015), in their study, conducted with extensionists of some US states, emphasize that extensionists ranked field days as the main activity to inform farmers, while radio/TV programs as the least used. While in the study conducted by Franz et al. (2010), the most preferred extension activity by farmers are demonstrations, field days, farm visits, while when extensionists are asked which activities farmers like the most they list farm visits, demonstrations and open field days. Whereas, Declaro-Ruedas (2019), in a study conducted in the Philippines reports that the most preferred activities by extension to introduce new technologies to farmers are group discussions/ meetings of farmers, demonstrations and farmers field schools. Radhakrishna et al. (2003) in the study conducted in South Carolina as a preferred method by farmers mentioned the agricultural magazines, and field days.

In the opinion of extensionists, farmers prefer individual discussions with extensionists, leaflets and group discussions more than all information channels (Table 11). The same is reported by Chaudhry et al. (2006) and Siddiqui & Mirani (2012) in their studies.

According to a study conducted with farmers in the area of Vora-Tirana (Bicoku & Subashi, 2020) farmers thought that the main methods that were most valuable to them were: (i) demonstrations, (ii) meetings with other farmers, (iii) open field days, as well as (iv) discussions with advisors, other farmers and dealers. The same is mentioned by Luukkainen (2012), who states that farmers are keen to see how a new idea works and how it can affect their farm production and these can be done with a demonstration. Explaining why farmers say demonstrations are an effective method may be that they are able to see a particular technique or technology in practice. It also states that the farmer-to-farmer method is the most productive for farmers.

## CONCLUSIONS

Our survey shows that we have a gender disproportion in the ranks of extensionists, where 62% are male and 38% female, at a time when female extensionists had to be at least 50%, since most farm work is done by women.
Extensionists have a long work experience of 23 years but only 11.3 years in extension, which tells us about frequent movements of extension staff, which negatively affects not only the work of the extensionists but also farmers.
Disproportion is also observed in employment by profession where 62.1% of extensionists are agronomists, and only 22.7% zootechnical, when livestock production provides about 52% of total agricultural production
About 1/3 of the extensionists have started working without interviews, which should be improved in the future, through the interview we understand the level of their knowledge and their training needs in the future.
The distances from home to work place are considerable and the extensions have to bear the cost of transportation themselves and therefore about ¼ of them express it as an unsatisfactory part of their work. Whereas, as the most satisfactory part of their work, ¾ the extensionists mention ¨the acquaintance with the farmers' problems¨.
The least satisfactory part are the expenses they make for transport, bureaucracies (frequent requests from MARD for filling in various forms, or operational data), as well as the lack of support of the farms with subsidies from ARDA.
About 4/5 of the extension activities take place for crop production and fruit growing and the rest for livestock. Demonstrations are the main activities, where on average each extension holds one demonstration per month. After the demonstrations are trainings and open field days. It should be noted that the number of participants in each extension activity is low: only 6.3 participants for demonstration and trainings, and seven participants for open field days.
In terms of the appropriate form to receive new information, on topics related to their work, extensionists as a first choice have preferred "Training combined with on-farm visits", while as a second choice have preferred "Open field days¨, forms which are also mentioned by other foreign authors. The three lowest rated formats were videos, brochures/leaflets and study tours. However, there are some differences between agronomists and zootechnics in terms of forms suitable for acquiring knowledge. The zootechnics consider more important than agronomists, the "Open field days, Training abroad, Brochure/leaflet, and Videos, while agronomists consider more important than zootechnics only the In-country training. While for activities such as Trainings combined Witt on-farm visits, Demonstrations, and Study tours, the agronomist and zootechnics have the same opinion.
Extensionists who have fewer years experience in extension service give a more positive answer in terms of ¨Open field days, demonstrations, in-country training, training abroad", compared to extensionists with more years of extension service experience. Extensionists of both groups (1-10 years of experience and over 11 years) consider "Trainings combined with on-farm visit" as the best form for acquiring new knowledge.
According to extensionists opinion "Demonstrations" and "Trainings combined with on-farm visits" are the two most suitable activities for farmers and for the implementation of their plan of extension activities. After these two activities, the extensionists list "Training", "Brochure/Leaflet", "Study tours" and "Open field days".
Farmers prefer individual discussions with extensionists, leaflets and group discussions, according to the extensionists opinion.

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# THE USE OF THE ROMANOV BREED IN DIFFERENT CROSSBREEDING PROGRAMS

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***Abstract***

*Ewe reproductive rates are under 100 percent in most sheep breeding countries. The efficiency of the sheep industry together with the production of lambs needs to be improved. A great potential exists to increase sheep productivity and efficiency by increasing reproductive rate, largely through the exploitation of genetic variation among breeds. Profitability mainly depends on lamb production and various genetic and management methods exist to increase lamb output which depends on fecundity, prolificacy, lamb survival, and the number of lambings per lifetime. There are a few highly prolific sheep breeds available in the present and the challenge is to exploit this potential commercially. The aim of this paper is to review the Romanov prolific sheep breed and its use in different crossbreeding programs. The fastest way to improve prolificacy in local sheep breeds is by crossing them with rams from prolific breeds, like Romanov breed. Internally, within the I.C.D.C.O.C - Palas Constanta, the Prolific Line - Palas was created, following the crossing of Merinos de Palas sheep with rams from Romanov, Friesian, and Finnish Landrace breeds, which are characterized by an average prolificacy of 160-180%. The Romanov breed can be used in practice differently depending on the purpose pursued, namely use in purebred, use for the creation of new populations or lines with high prolificacy, use in simple industrial crosses to increase meat production (Romanov females x males meat), or use in double or triple industrial crosses (obtaining prolific hybrid females F1 in the year I - females of local breed x male of Romanov breed, which in the second year are crossed with males of specialized breeds of meat).*

***Key words***: *crossbreeding, litter size, prolific breed, reproduction rates, Romanov breed.*

## INTRODUCTION

Sheep breeding and exploitation is an ancient activity, with great traditions in Romania, it represents a basic branch of animal husbandry that has developed in different pedoclimatic areas, depending on the biological particularities of the exploited breeds and market requirements (Ștefănescu et al., 1973).

At the national level, the reconsideration of the directions of sheep exploitation and the orientation of the breeding activity of this species on the principles of the market economy have stimulated the concerns for the increase of milk and meat production (Pascal, 2015).

At present, sheep farming is increasingly geared towards meat production, which will become the main production in some areas of the country in the near future. Increasing the production of sheep meat and increasing the economic efficiency of this activity are largely conditioned by the intensification of the breeding process.

The orientation of the breeding and exploitation of sheep for meat production worldwide, imposed a basic technological element, namely the intensification of the breeding process, as obtaining as many lambs as possible, is the most important goal in increasing this production (Taftă, 1983; Pădeanu & Voia, 2010).

Reproduction intensification includes a series of measures and methods whose main purpose is to transform seasonal polyestricity into annual polyestricity, facilitating the installation of gestation, including in the anestrus phase, as well as advancing the age of the entry of reproduction from 18 months to approx. 8-10 months, which will increase the prolificacy, the possibility of organizing births throughout the year, and obtaining 1-2 lambs every 7-8 months (Răducuță, 2000).

Assisted reproduction, shortening of the birth-breeding period, deseasonalization of heat and birth as well as accelerating calving by non-hormonal and hormonal methods, can successfully contribute to two births per year or three birth in two years and increasing the prolificity of sheep (Răducuță, 2000).

The increase of the reproduction indices creates the premise of the profitability of the sheep regardless of the exploitation system practiced. The interest is channeled especially for the increase of the fertility, fertility, and especially of prolificacy indices.

The intensification of prolificacy is a major objective in the exploitation of all breeds of sheep because it leads to an increase in the number of livestock and implicitly of meat production. Twin lambs have an intense growth energy, which allows the weight of the simple ones to be equalized until the age of the first haircut, and the expenses occasioned by the maintenance of the second lamb are generally reduced (Taftă et al., 1997).

Most sheep breeds that are bred in our country are characterized by relatively low values of prolificacy (105-110%). Prolificity is largely influenced by genetic factors and especially race. There are only a few breeds worldwide that have a prolific value of over 200%, such as Romanov, Finish Landrace, Frieze, Booroola, Han Yang, Hu Yank.

The main method of increasing the prolificacy, however, remains the crossing of local sheep with rams of the prolific breeds, the obtained mestizos being characterized by a prolificacy much superior to the local breeds.

This paper aims to analyze the use of the Romanov breed in various breeding programs, both globally and nationally.

## MATERIALS AND METHODS

To make this material, the following bibliographic materials have been studied: specialized books on domestic animal breeding or sheep breeding, represented either by unique textbooks specific to the profile faculties in our country, or by specialized textbooks, specialized brochures, specialized courses, papers presented at various national and international symposium.

The methodology of the work consisted in presenting a history of the emergence, development and spread of the Romanov breed, the presentation of the evolution of the average production characteristics and current ones, as well as the presentation of how to use the breed in different breeding programs, both nationally and worldwide, and finally the presentation of the conclusions arising from the researched material.

## RESULTS AND DISCUSSIONS

**The emergence, development and spread of the Romanov breed**. The first official records of the Romanov race date back to 1802, in the town of Romanov (now Tutayev), Yaroslavl region. Breeders were looking for a fast and easy breed of sheep, even in the absence of special conditions. The selection was made on the basis of local sheep breeds, by simple breeders, without special scientific methods. But the result was exceptional. Romanov sheep are currently among the top meat breeds in Russia but also in other parts of the world. According to the official standard, Romanov sheep have the following characteristics:

- Average height - 70 cm;
- Robust constitution;
- Strong skeleton and muscles;
- Wide chest;
- Straight back;
- The sacrum slightly lowered;
- Strong and straight limbs.

The presence of horns in both sexes is not allowed, as this is an indication that the breed is not pure. The tail of the Romanov sheep is short. At birth, the lambs are black, with white spots on the head and sometimes on the limbs or tail, which depigment after the age of 1-3 months, becoming gray in various shades, but the embers remain black throughout life.

**Presentation of the evolution of the average and current production characteristics.** The most important characteristic of Romanov sheep is their prolificacy. A female gives birth to 2-3 viable lambs. Cases of 7 or even 9 lambs per calving were also recorded. In addition, sheep have 2 calves per year, or 3 times in 2 years without special stimulation, which would easily mean 4-6 lambs per year in a female (Răducuță & Tăpăloagă, 2010).

***Productivity indices:***

- Morpho-productive type - meat prolificacy;
- Average weight of 50-60 kg in sheep and 75-90 kg in rams;
- Birth weight: 2.5-3.0 kg;

- Weight at 90 days - 17-20 kg;
- Weight at 180 days: lamb - 30-35 kg;
- Prolificacy 250-300%;
- The age of introduction of females at reproduction - 7 months (or reaching a body weight of 40 kg);
- Duration of gestation - 145 days (less than in other breeds);
- Slaughter yield - 50%;
- Weight of the wool coat (at 3 haircuts per year): 1.4-1.8 kg for sheep and 2.5-3.5 kg for rams;
- Milk production is 80-150 liters in 90-120 days of lactation.

The breed is also characterized by a highly developed maternal instinct, very good fertility, hardiness, high vigor of lambs at birth, entry into breeding from the first year of operation, very long duration of the breeding season, respect-tively almost all year round. calendar, being able to successfully organize two calvings per year, or three calvings in 2 years (Răducuță & Tapaloaga, 2010). In addition, 30-40 days after calving, the sheep return to the heat, thus being able to organize a new reproductive cycle.

**Presentation of the ways of using the Romanov breed in different crossing programs at national level.**

The hybridization of local sheep with prolific Romanov and Finnish Landrace breeds to increase prolificacy was tested in our country at the National Research Institute for sheep and goat breeding (ICPCOC Palas Constanța), the mestizos obtained being characterized by a prolificity far superior to local breeds. Ionescu et al., 1985). The data obtained for the use of the Romanov breed show us that the highest value of prolificacy was the F1 Romanov x Merinos de Palas (196.9%), and the lowest was recorded at the F1 Romanov x Țurcană breed (164.7%) (Table 1).

Table 1. The effect of hybridization of local sheep with the Romanov breed to increase prolificacy (Ionescu et al., 1985)

| Sheep breeds | Average prolificacy (%) | |
|---|---|---|
| | Maternal breed | Half-breed F1 |
| Romanov x Merinos de Palas | 127.3 | 196.9 |
| Romanov x Spancă | 135.7 | 170.3 |
| Romanov x Țurcană | 102.9 | 164.7 |

The purpose of crossbreeding sheep of less prolific breeds with rams of breeds with this pronounced trait is to obtain F1 females with hereditary substrate enriched with genes for prolificacy. Mixed females are then crossed with rams of the meat breeds to obtain an increased number of lambs for meat (Taftă et al., 1997).

In the 1990s, the Prolific Line - Palas was created within the ICDCOC Palas, following the crossing of Merino de Palas sheep with Romanov and Finish Landrace rams, which is characterized by an average prolificacy of 175% (Vicovan et al., 1995, quoted by Taftă, 1998).

This Prolific Line started from the native Merinos de Palas breed, within the ICDOC Palas, through a complex program of crosses during the years 1973-1988, when a sufficient number of combinations between this breed and the prolific breeds were applied and tested: Romanov (200-250%), Finnish Landrace (230-250%), Border Leicester (190-210%), Friesland (180-225%) and Ile de France (150-180%).

Based on a large number of cross-breeding variants and applying the selection, mainly after prolificacy and adaptation to local conditions, in 1989 the animals of the desired type were obtained.

The number of sheep of this type, since 1990, has been reproductively isolated for 7 genera-tions (the interval between generations of 4.4 years), having as main objectives the improvement by selection of prolificacy (over 150%), breastfeeding capacity and limitation inbreeding below 1% per generation. The genetic similarity with the founding breeds is 39.7% with the Romanov breed, 28.13% with the Merinos de Palas breed, 15.63% with the Friesian breed, 9.36% with the Border Leicester breed, 6.25% with the Ile de France breed and only with 1.56% with the breed Finnish Landrace.

The purpose of forming this line was to obtain a new type of sheep with high prolificacy. The current herd is characterized by several productive and reproductive characters and traits, namely:

- prolificacy - 160-180%;
- lambs weaned at 100 sheep - 140-160 heads;
- long reproductive season, with maximum peaks placed in the spring and early autumn months;
- total milk production - 160-180 liters.

This type of sheep can be used in various cross-breeding schemes, especially those aimed at the production of lambs. The practice of crossbreeding with local breeds shows a good degree of transmission of prolificacy.
The Palas Prolific Breed, approved as a purebred local breed in 2020, gives breeders a solution to increase meat and milk production, depending on the direction of exploitation they want (Pădeanu, 2021).
In 2015, within the ADER 5.1.2 Project - Efficiency of family farms with Merino sheep by increasing the prolificacy and increasing the quality of products according to the require-ments of the EU market - within ICDCOC Palas, experiments were carried out on 60 sheep hybrid F1 Romanov x Merinos de Palas and 60 sheep Merinos de Palas control group), all at calving I, and at the Project Partner the works were carried out on 62 sheep F1 Prolific Breed Palas x Merinos and 64 sheep Merinos (control group) (MADR, 2018). These herds were registered at calving and their products were registered and weighed individually at calving, at one month, at two months and at weaning, the average age being between 75-80 days.
For each population of sheep studied, the lactation capacity, the average gestation time, the reproduction indices and the average number of lambs weaned per calved sheep (the most important reproduction index) were established.
The F1 Romanov x Merinos de Palas hybrid sheep achieved a prolificacy of 169.62% at the first calving compared to the Merinos de Palas sheep in which the prolificacy was 105.0% (Table 2).

Table 2. Reproduction indices at first birth

| Specification | Fecundity (%) | Prolificacy (%) |
|---|---|---|
| Sheep F1 Romanov x Merinos de Palas | 91.23 | 169.62 |
| Sheep Merinos de Palas | 96.77 | 105.00 |

The gestation period was 147.23 days for hybrids and 148.79 days for Merinos, in accordance with the provisions of O.M.1045 / 2018 (Table 3).

Table 3. Pregnancy duration (days)

| | |
|---|---|
| Sheep F1 Romanov x Merinos de Palas | 147.23 |
| Sheep Merinos de Palas | 148.79 |

The average body weight at weaning in hybrids (males + females) was 19.51 kg / head and in Merinos de Palas lambs it was 20.68 kg / head, the difference of approx. - 6% being statistically insignificant. F1 hybrid sheep had a lactation capacity of 122.66 liters of milk / head, which was higher by approx. 42% compared to Merinos de Palas sheep that had a lactation capacity of 86.58 liters of milk / head, the difference being very significant. F1 hybrid ewes at first calving weaned 1.52 lambs per calved sheep compared to Merinos de Palas sheep weaning only 1.05 lambs per calved sheep, the difference being significant
The aim of the project was to streamline family farms with Merinos sheep by increasing the prolificacy and increasing the quality of pro-ducts according to the requirements of the EU market.
In conclusion, research has shown that hybrid sheep of Romanov blood in different propor-tions had much higher reproductive perfor-mance (prolificacy, earliness and survival rate of lambs) compared to domestic breeds.

**Presentation of the ways of using the Romanov breed in various cross-breeding programs worldwide.** Romanov breed has 1.85-2.90 lambs obtained per birth, depending on location and nutrition, as well as other influencing factors (Table 4 and Table 5). The average birth weight is between 2.5 and 3.0 kg. Meat production capacity differs place to place. From the data presented, it appears that the use of this breed significantly increases the proli-ficacy of other breeds. In the USSR, Shatskii et al. (1976; 1978), cited by Kukovisc (1984), stated in their experiments that the use of the Romanov breed in crosses increases the prolifi-cacy and fattening percentage. The production of meat of three cross breeds has been studied by Sallam (1978), Antonova (1979) and Erokhin et al. (1981), cited by Kukovisc (1984), and reported fairly good results (Table 4).
In Russia, the crossing of Romanov sheep with rams resulting from the crossing of Romanov x Argali breeds (3/4 R x 1/4 A) had positive influences in increasing the performance and musculature of newborn lambs (7/8 R: 1 / 8 A). Therefore, the male newborn lambs had a high survival rate, growth performance and a high digestibility of nutrients, the males thus obtained

being 4.8 kg heavier at the age of 8 months (Kukovisc, 1984).

Czechoslovak researchers (Jakubec 1975, Jakubec et al. 1978, 1979; Machacek and Jakubec, 1981), cited by Kukovisc (1984), also improved the prolificacy of their breeds (Czigaja, Valashka and Sumava), but reported some disadvantages in terms of concerns wool production.

Many cross-breeding tests have been carried out in Spain to improve the breeding rate of local breeds. Sierra (1978; 1980) reported several experiments. According to these data, Romanov × Aragon F1 sheep produced 25-70% more lambs than pure Aragon sheep, depending on the time of the first mating. The number of products obtained by calving F1 sheep after the autumn mating was 2.12, after the spring mating only 1.65, while the purebred had 1.39 and 1.17, respectively.

Sierra (1983), cited by Kukovisc (1984), reported on a new Spanish breed of synthetic sheep, which has 50% of the blood of the Romanov and Aragon breeds. Its fertility is 86%, and the litter size is 1.87 and 2.13 after the spring and autumn mating. Espejo et al. (1977; 1982), reported reports on acceptable meat production of the Spanish crosses Merinos and Romanov (Table 5). In Spain, there was a hybrid program in which the first Aragon × Romanov crossbred sheep were mated with Ile de France rams to obtain first-class lambs for slaughter.

In France, Romanov is preferred as a prolific breed due to its high prolificacy (250%), long breeding season (8 months / year), excellent fertility, strong maternal behavior and good product viability (Răducuță & Tăpăloagă, 2010).

According to Ricordeau et al. (1976), Romanov purebred lambs produced 2.88 lambs per sheep and the best combination, Romanov × Berrichon du Cher, had a number of 2.05 calving products (Cotentin breeds were used in the study, Border Leicester, Berrichon du Cher and Romanov. Ricordeau et al. (1977) reported that the mixed F1 and F2 sheep of the Romanov x Berrichon du Cher combination had a number of products obtained at calving 0.66% higher than that of purebred Berrichon du Cher sheep. The growth rate of the Berrichon du Cher × Romanov, 1/2 Berrichon du Cher × 1/4 Cotentin × 1/4 Romanov and 1/2 Berrichon du Cher × 1/4 Border Leicester × 1/4 Romanov combinations exceeded o that of purebred Berrichon du Cher sheep with 0.57, 0.30 and 0.36 respectively (Tchamitchian et al., 1976).

The design rates for F1, F2, F3 and F4, Berrichon du Cher × Romanov crosses were 86%, 82%, 97% and 99%, respectively, while the corresponding farrowing rates were 1.67, 1.87, 1.98, and 2.01 (Ricordeau et al., 1982).

The INRA 401 maternal line was produced by the crossing of the Romanov and Berrichon du Cher breeds, which was done by Tchamitchian et al. (1979) for INRA cross-cutting programs.

In France, on the basis of the Romanov breed and following the successive crosses between Romanov x Berrichon du Cher, the Romane breed was formed, which arose as a result of the desire to develop a new breed, the result being a breed of sheep with white and short wool, which has a good prolificacy and a good conformation to produce quality lambs.

The Romane breed is characterized by the following parameters (Răducuță & Tăpăloagă, 2010):

- Morpho-productive type – meat and prolificacy;
- Average weight of 60-70 kg in sheep and 90-100 kg in rams;
- Prolificacy - 200-230%.

In a comparison of Limousine and Limousine × Romanov F1 sheep, Marzin & Brelurut (1979) found that the number of products obtained during calving was 1.63 and 2.25, the birth weight was 3.56 and 3.40 kg, and the mortality was 10.4 and 12, respectively, 8% for the two genotypes. Although the sheep had lambs three times in two years, the lambs born per sheep per year were 2.15 and 3.06, and the weaning rate per sheep per year was 1.92 and 2.66, respectively.

In South Africa, Faure et al. (1983) cited by Kukovisc (1984) tried to improve the prolificacy of Karakul sheep. They produced crossbred sheep with 25, 50 and 75 percent Romanov blood, and the number of products obtained by calving these genotypes was 1.17 and 1.74, respectively. The first genotype does not differ significantly from the purebred Karakul.

In Hungary, a research program developed the so-called "Fertile Merinos" by crossing the Romanov breed with the Hungarian Merinos. The purpose of this program was to produce a genotype of 1/4 Romanov × 3/4 Merinos (Veress and Lovas, 1978, cited by Kukovisc 1984; Veress, 1982). This sheep population produced Merinos wool 20% longer than the original Merinos wool, without colored fibers. These sheep had 20–30 percent more lambs per year (1.3 - 1.4) and a 20–30% higher number of products obtained at calving (1.50–1.60) than Merinos. The wool production data of this breed are as follows: thigh length 9–11 cm, wool weight 4.8 - 5.2 kg, yield 49%, fiber diameter 23–24 microns (only wool weight data are less favorable than those of Merinos).

Table 4. Number of products obtained at calving of the Romanov breed and of the half-breed resulting from crossbreeding with the Romanov breed (Kukovics, 1984)

| Source | Breed or cross | Litter size (prolificacy) |
|---|---|---|
| Jakubec, 1975 | Romanov | 1.86 – 2.34 |
| | Romanov × German Merinos | 1.29 |
| Shatskii et al., 1976 | Precoce | 1.1 |
| | Romanov | 2.3 |
| | Precoce × Romanov | 2.36 |
| | Romanov × Precoce | 1.1 |
| Veress, 1976 | Romanov | 2.42 |
| Ricordeau et al., 1976 | Romanov | 2.88 |
| Sierra, 1977–78 | Aragon | 1.17 – 1.39 |
| | Aragon × Romanov | 1.65 – 2.12 |
| Sierra, 1978 | Romanov × Aragon | 1.29 – 1.72 |
| Shatskii et al., 1978a | Romanov | 2.52 |
| | Romanov × Precoce | 1.27 |
| | Precoce × Romanov | 1.93 |
| Shatskii et al., 1978b | Precoce | 1.06 |
| | Romanov × Precoce | 1.26 |
| | Romanov | 2.52 |
| | Precoce × Romanov | 1.93 |
| | Finn × Romanov | 2 |
| Jakubec et al., 1978 | Tzigaja | 1.11 |
| | Romanov × Tzigaja | 1.72 |
| Jakubec et al., 1979 | Romanov × Improved Valashka | 1.74 |
| Flamant et al., 1979 | Romanov | 2.16 |
| Marzin et al., 1979 | Limousin | 1.63 |
| | Romanov × Limousin | 2.25 |
| Antonova, 1979 | Romanov × Russian Merinos | 1.57 – 2.00 |
| | Romanov × Tzigaja | 1.10 – 1.71 |
| Sierra, 1980 | Romanov | 1.96 – 2.96 |
| | Aragon | 1.07 |
| | Romanov × Aragon | 1.96 |
| Faure et al., 1983 | 1/4 Romanov - 3/4 Karakul | 1.17 |
| | 1/2 Romanov - 1/2 Karakul | 1.74 |
| | 3/4 Romanov - 1/4 Karakul | 1.74 |
| Machacek and Jakubec, 1981 | Improved Sumava | 1.05 – 1.29 |
| | Romanov × I. Sumava | 1.50 – 1.56 |

Table 5. The influence of the Romanov breed on meat production in half-breed resulting from different breeds (Kukovics 1984)

| Source | Breeds or cross | Body weight (kg) | | | Average daily weight gain | Carcass weight | Slaughter yield |
|---|---|---|---|---|---|---|---|
| | | Birth | 3 months | 8 months | (g) | (kg) | (% ) |
| Shatskii et al., 1976 | Precoce | 4.3 | | | | 17.8 | 42.7 |
| | Romanov | 2.5 | | | | 13.9 | 45.9 |
| | Precoce × Romanov | 2.8 | | | | 17.1 | 43.3 |
| | Romanov × Precoce | 3.9 | | | | 16.4 | 42.8 |
| Espejo et al., 1977 | Romanov × Spanish Merinos | | 19.7 | | | | |
| | Spanish Merinos | | 18.6 | | | | |
| Shatskii et al., 1978 | Precoce | 4 | | | 231 | | 45.8 |
| | Romanov | 2.6 | | | 180 | | 49.2 |
| | Romanov × Precoce | 4 | | | 263 | | 45.2 |
| | Precoce × Romanov | 3.2 | | | 245 | | 44.8 |
| | Finn × Romanov | 2.7 | | | 241 | | 47.8 |
| Sallam, 1978 | Romanov × Tzigaja/x Suffolk | 3.8 | | 37.3 | | | 46.4 |
| | Romanov × Tzigaja/x Romanov | 3.6 | | 33.8 | | | 45 |
| | Romanov × Tzigaja/x lle de France | 3.7 | | 35.9 | | | 48.2 |

In a study conducted in South Africa regarding puberty and ovulation rate of Romanov, Dorper, and their crosses during the first breeding season, Greeff et al. (1993), found that genotype had a significant ($P < 0.01$) effect on age and mass at first oestrus and also on ovulation rate. Thus, an increase in the percentage Romanov genes resulted in a decrease in ewe mass and an increase in ovulation rate.

The performance of purebred Romanov (R) lambs and crossbred lambs of Romanov and Morkaraman breed were compared under a semi-intensive production system in Turkey (Korkmaz & Emsen, 2015). The results showed a significant improvement in early reproductive traits (age at first presence of sperm, percentage of ewe lambs showing the first sign of estrus, age at first estrus) can be obtained from crossbreeding the fat-tailed Morkaraman breed with the Romanov breed.

Recently, in Russia, Dvalishvili et al. (2015), performed a study to investigate growth performance, nutrient digestibility, carcass quality measures and some serum blood parameters of purebred and crossbred Romanov male lambs (7/8 Romanov:1/8 Argali). The results of this study revealed that, the crossbred Romanov male lambs (7/8 Romanov:1/8 Argali) had 4.80 kg more in their body weight as well as 19 g of average daily gain by the age of 8 months. Also, they had 2.8 kg more in their hot carcass weight over the purebred one, a higher lamb keeping index, growth performance and nutrient digestibility than purebred ones and a better metabolic blood profile.

Starting from the idea that the incorporation of super-prolific and less seasonal breeds of sheep such as the Romanov into domestic flocks could improve ewe productivity during suboptimal spring mating seasons was the objective of a recent experiment conducted in the United States (Freking & Murphy, 2021). Thus, crossbred ewes were generated by mating Romanov ewes to 1 of 5 ram breeds (Dorset, Rambouillet, Katahdin, Dorper, and White Dorper) and, for this experiment, were evaluated in a spring mating/fall lambing system at 4, 5, and 6 years of age. The results showed that on average, ewe fertility in spring mating (81 to 7%) and prolificacy (1.46 to 1.71 lambs/ewe/year) was greatly improved with the addition of 50% Romanov germplasm to common domestic sheep breeds.

## CONCLUSIONS

Increasing prolificacy is a major goal in the exploitation of all breeds of sheep because it leads to an increase in the number of livestock and thus the production of meat.

From the presented material, it appears that the prolificacy of the local breeds of sheep is still at a very low level, being necessary its rapid increase.

The main and rapid method of increasing prolificacy is the crossing of local breeds of sheep with rams of the prolific breeds and especially with the Romanov breed.

The Romanov breed is characterized by a remarkable prolificacy (250%), long reproductive season (12 months), excellent fertility, strong maternal behavior and high viability of the products, characteristics that recommend it for its use for increasing the prolificacy of different local or foreign breeds.

The Romanov breed can be used in practice differently depending on the purpose pursued, namely use in purebred, use for the creation of new populations or lines with high prolificacy, use in simple industrial crosses to increase meat production (Romanov females x males meat), or use in double or triple industrial crosses (obtaining prolific hybrid females F1 in the year I - females of local breed x male of Romanov breed, which in the second year are crossed with males of specialized breeds of meat).

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# IMPROVING THE FORMIC ACID-BASED FORMULAS USED IN VARROOSIS CONTROL BY BROOD BRUSHING PROCEDURE

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***Abstract***

*The paper aims to present some preliminary results regarding the effectiveness of different lower concentration of formic acid formulas, used by brushing procedure, in killing the varroa mites (Varroa destructor) which are found in the reproductive phase in the capped brood. To perform the experiments, honeybee capped brood combs from untreated colonies were collected and treated in two experimental groups with different dilutions (20%, 30%, 40%, 50%) of concentrated formic acid (85%) used in water-based and alcohol-based dilutions. The measurements were focused on the evaluation the varroa mite mortality, as response variable, at 72 hours from the treatment application. Out of the obtained results one could remark that the mortality of mites increased as the concentration of formic acid increased in different formulas. Highly significant differences were established between the two experimental groups as well as between the mortality of different categories of varroa. The results clearly show that the use of formic acid is very effective in varroosis control also when used in lower concentrations (30-40%) by brushing procedure. The results also show that new formulas could be further optimised by setting up a standard protocol to evaluate the critical stages of mites inside brood and their vitality, which are affected following the application of treatments.*

***Key words****: brushing procedure, capped brood, formic acid, honeybee, varroa, varroosis.*

## INTRODUCTION

The recent efforts to control varroosis (*Varroa destructor*) are more and more focused on different alternative approaches (Van der Steen & Vejsnæs, 2021; Vilarem et al., 2021; Roth et al., 2020), taking into account the need to practice a sustainable beekeeping which protect honeybees as well as their environment and products. In the frame of these approaches, the biotechnological methods combined with the application of treatments based on soft, organic acaricides, have a special place (Büchler et al., 2020; Lodesani et al., 2019; Siceanu et al., 2019; Gregorc et al., 2017; VanEngelsdorp et al. 2008; Amrine & Noel, 2007; Fries, 1991). Generally, all these treatments aim to lower the infestation level before critical moments in the active season in parallel with taking the measures to avoid the hive contamination and the resistance phenomenon in the mite population (Büchler et al., 2019; Lodesani et al., 2019; Sara Hernández-Rodríguez, et al. 2021). In the last years, it was developed an alternative strategy to control varroa mite infestation by using a biotechnical procedure of brushing the capped brood with concentrated organic volatile acids (Siceanu et al., 2021). This procedure proved to be very effective in killing the mites found in the reproductive phase. In a pilot study (Căuia & Căuia, 2022), using the formic acid of 65% concentration by brood brushing in spring applications, the infestation level in late summer was drastically reduced. This is the period of the year in temperate climate when, naturally, the varroa mite population increases and poses serious risks for honeybee colony health for the next beekeeping season. Taking into account some practical observations on the immediate effectiveness of these targeted treatments on varroa mite mortality an important question arose on the effectiveness of organic volatile acids when used in lower concentration. The use of lower concentrations of formic acid could also offer a better protection and manipulation of the brood combs, but the whole colony too, allowing the reintroduction of the treated combs in the colony just after the treatment application. No less important is the fact that a softer formula could offer a better protection for beekeepers, diminishing thus the potential hazardous risks of treatment product manipulation. Thus, the aim of this research work was to preliminary test the effectiveness of different lower concentration of formic acid formulas, used by brushing procedure, in killing the varroa mites which are found in the reproductive phase in the capped brood

## MATERIALS AND METHODS

**Experiment design.**

1. ***Biologic material.*** To perform the experiments, there were used honeybee capped brood combs collected in the active seasons of the 2020-2021 years. The combs originated from untreated colonies (*Apis mellifera carpatica*), belonging to an experimental apiary of the Honeybee Genetics and Breeding laboratory in the frame of the Institute for Beekeeping Research and Development located in Bucharest. The honey bee colonies that provided varroa infested brood were managed in Dadant hives on 10 frames. These colonies were not treated one season before (in the end of summer and in the autumn of the previous year) in order to have a higher level of infestation with varroa mite in the experimental units, so to increase the probability to easily find infested cells to facilitate the measurements.

2. ***Experimental groups.*** For the purpose of this study there were established two experimental groups (EG1 and EG2) and different dilutions (20%, 30%, 40%, 50%) of the concentrated formic acid (85%). The two experimental groups were set up to test two types of dilutions, based on distilled water (EG1), respectively on concentrated alcohol (ethanol) of 97% (EG2). The main information regarding the experimental design is presented in the table 1. A total number of 25 capped brood combs were treated and evaluated, with 2-4 combs on each experimental variant. This biologic material was collected out of 10 colonies, at different time intervals in July-August period when level of infestation naturally increases. The combs were selected to have brood in the pupae stage so that to identify the state of varroa mites (live or dead) in different developmental stages in the treated brood. The individuals were identified based on their morphologic characteristics (Mondet et al., 2020; Rosenkranz et. al 2010).

Table 1. The experimental design to test the impact of different concentrations of the formic acid formulations on varroa mite in the capped brood, following the treatments by brushing procedure application.

| Experimental groups | Active substance | Solvent | Concentration of formic acid in the treatment formulas (%) | No of treated combs by brushing procedure |
|---|---|---|---|---|
| Variants of EG1 | | | | |
| 1.1 | Formic acid 85% | Distilled water | 50 | 2 |
| 1.2 | Formic acid 85% | Distilled water | 40 | 4 |
| 1.3 | Formic acid 85% | Distilled water | 30 | 4 |
| 1.4 | Formic acid 85% | Distilled water | 20 | 4 |
| Variants of EG2 | | | | |
| 2.1 | Formic acid 85% | Ethanol 97% | 50 | 2 |
| 2.2 | Formic acid 85% | Ethanol 97% | 40 | 2 |
| 2.3 | Formic acid 85% | Ethanol 97% | 30 | 3 |
| 2.4 | Formic acid 85% | Ethanol 97% | 20 | 4 |

3. ***Treatments.*** The treatments were applied on capped brood combs, using the brushing procedure as explained in the literature (Siceanu et al., 2021).

Immediately after treatments application, the treated combs were returned into the origin colonies for 72 hours, in order to better highlight the effect of treatments on the varroa mite mortality. This time interval was chosen to better evaluate the varroa mortality, as by previous observations (Siceanu et al., 2021) the level of mortality can be influenced by time, concentration in volatile substances or the degree of exoskeleton sclerotization of the mite, as for example, the adult mites are the most resistant to this type of treatment. The chosen time interval could offer also supplementary information about the status of each individual, their vitality and other information regarding the reproduction, e.g., the presence of eggs or protonymphs which can reflect an interruption of reproductive process.

4. ***Measurements***. After 72 h since the treatment application, the combs were collected again and examined under stereomicroscope (Olympus SZ61), at 6.7X-45X magnifications, in order to easily identify the infested cells and different categories of varroa mites.

The capped cells were opened with tweezers, cell by cell, in more rows, to increase the probability to identify the infested cells. Generally, at least 200 cells were checked out on each treated comb (Dietemann et al., 2013).

The measurements were focused on the evaluation the mite mortality as response variable, by counting the total number of found mites which was split into live individuals (including those with a low vitality) and dead individuals for every developmental stage. The statistical analysis was performed using the NCSS 2021 v21.0.2 software, following the literature recommendations (Sandu, 1995).

## RESULTS AND DISCUSSIONS

By previous researches (Siceanu et al., 2021) it was shown that the capped brood treatment with formic acid (65%, respectively 85%) by using the brushing procedure has a high effectiveness in killing both, the adult and juvenile stages of varroa mite (Ave.= 90.48%, respectively 92.64%) found inside cells, at 24 hours evaluations after treatment application. By lowering the concentration of formic acid in different formulation using various dilutions and solvents there were obtained different results in terms of the effectiveness on varroa mite mortality evaluated at 72 hours after treatments application (Table 2).

Table 2. The results regarding the effectiveness of different concentrations of formic acid formulations on the mortality of varroa in capped brood using the brushing procedure

| Experimental groups | Total mites (no.) | The number of dead mites/The number of total mites (% of mortality) | | | | | Mortality of mites (%) |
|---|---|---|---|---|---|---|---|
| | | *Foundress female* | *Adult male* | *Protonymph* | *Deutonymph* | *Adult daughter* | |
| **EG 1. Variants of water-based formulas** | | | | | | | |
| 1.1. **50% FA** | 210 | 71/86 (82.5) | 6/7 (85.7) | 83/85 (97.6) | 26/31(83.9) | 0/1 (0) | 88.6 |
| 1.2. **40% FA** | 172 | 65/80 (81.3) | 3/ 4 (75) | 42/44 (95.5) | 26/28 (92.9) | 7/16 (43.8) | 83.1 |
| 1.3. **30% FA** | 214 | 27/85 (31.8) | 1/12 (8.3) | 46/48 (95.8) | 32/46 (69.5) | 8/23 (34.8) | 53.3 |
| 1.4. **20% FA** | 207 | 23/113 (20.4) | 0/9 (0) | 37/50 (74) | 15/26 (57.7) | 1/9 (11.1) | 36.5 |
| **Average** | **200.1** | **186/364 (51.1)** | **10/32 (31.2)** | **208/227 (91.6)** | **99/131 (75.5)** | **16/49(32.6)** | **64.6** |
| **EG2. Variants of ethanol-based formulas** | | | | | | | |
| **2.1. 50% FA** | 121 | 66/71 (93) | 1/1 (100) | 40/40 (100) | 5/5 (100) | 4/4 (100) | 95.9 |
| **2.2. 40% FA** | 56 | 35/38 (92.1) | 1/1 (100) | 10/10 (100) | 7/7 (100) | 0/0 (-) | 94.6 |
| **2.3. 30% FA** | 198 | 75/85 (88.2) | 5/9 (55.6) | 54/56 (96.4) | 30/30 (100) | 13/18 (72.2) | 89.4 |
| **2.4. 20% FA** | 201 | 62/104 (59.6) | 12/18 (66.7) | 35/38 (92.1) | 19/21 (90.5) | 10/20 (50) | 68.7 |
| **Average** | **144** | **238/298 (79.8)** | **19/29 (65.5)** | **139/144 (96.5)** | **61/63 (96.8)** | **27/42 (64.3)** | **84.0** |

Out of these results one can notice that the mortality of mites increases as the concentration of formic acid increases in different formulas (Figure 1). Additionally, in the alcohol-based dilution formulas a higher mortality of varroa mite was registered as compared with water-based dilution ones, in all experimental variants. One explanation could be the fact that alcohol amplify the vaporisation process of the formula and/or together with formic acid could have cumulative effects on varroa mite mortality.

Applying the MANOVA statistical analysis highly significant differences were established between the two experimental groups (F ratio=20.9, P= 0.010, α=0.05), illustrated in the figure 2. Highly significant differences were also established between the mortality of different categories of mites (F ratio=12.74, P= 0.015, α=0.05) as a result of different treatments, the differences being well illustrated in the Figures 2 and 3.

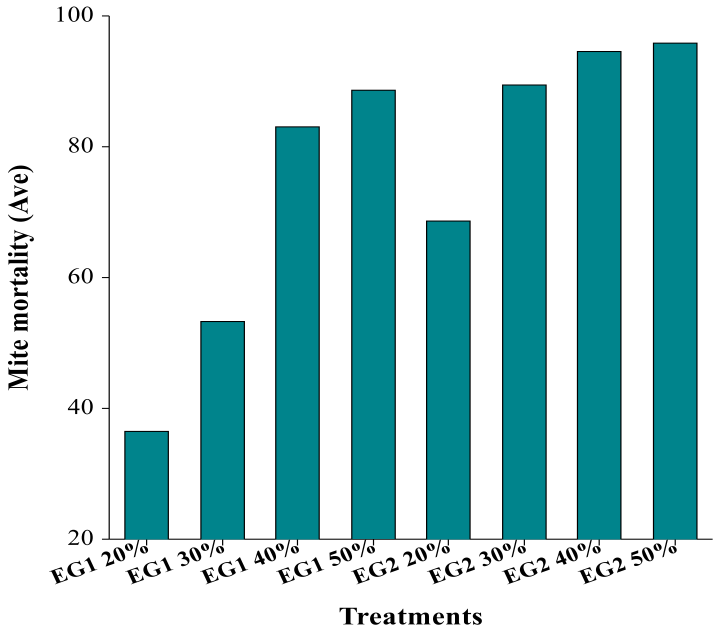


Figure 1. The effect of different treatments in the experimental variants on total varroa mite mortality.

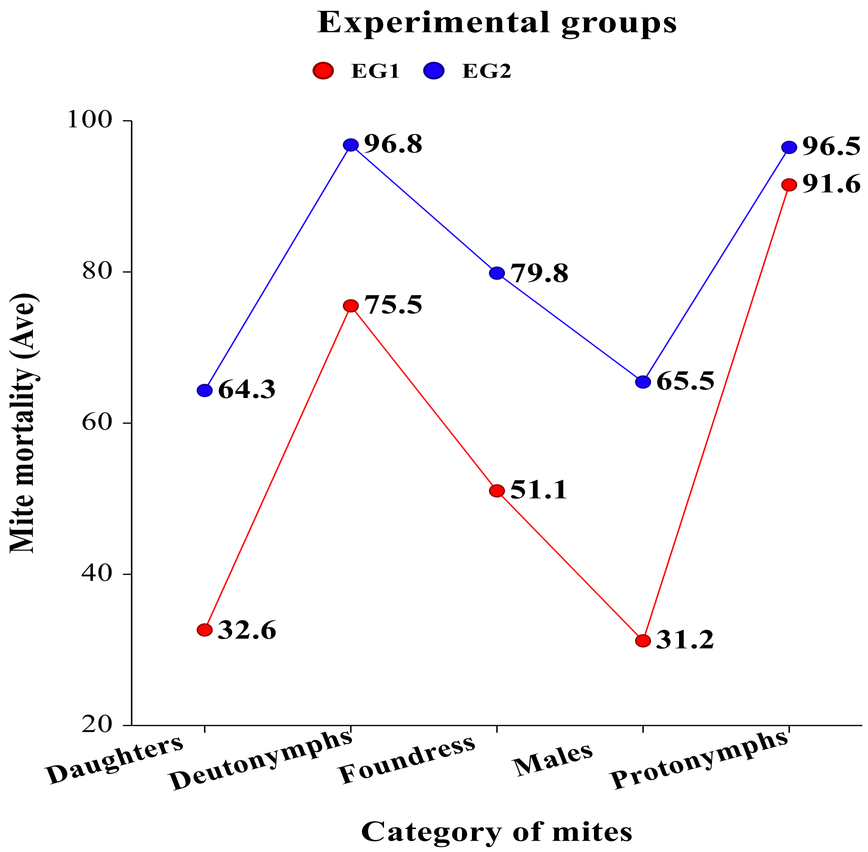


Figure 2. The effect of the treatments in the experimental groups on different categories of varroa mite.

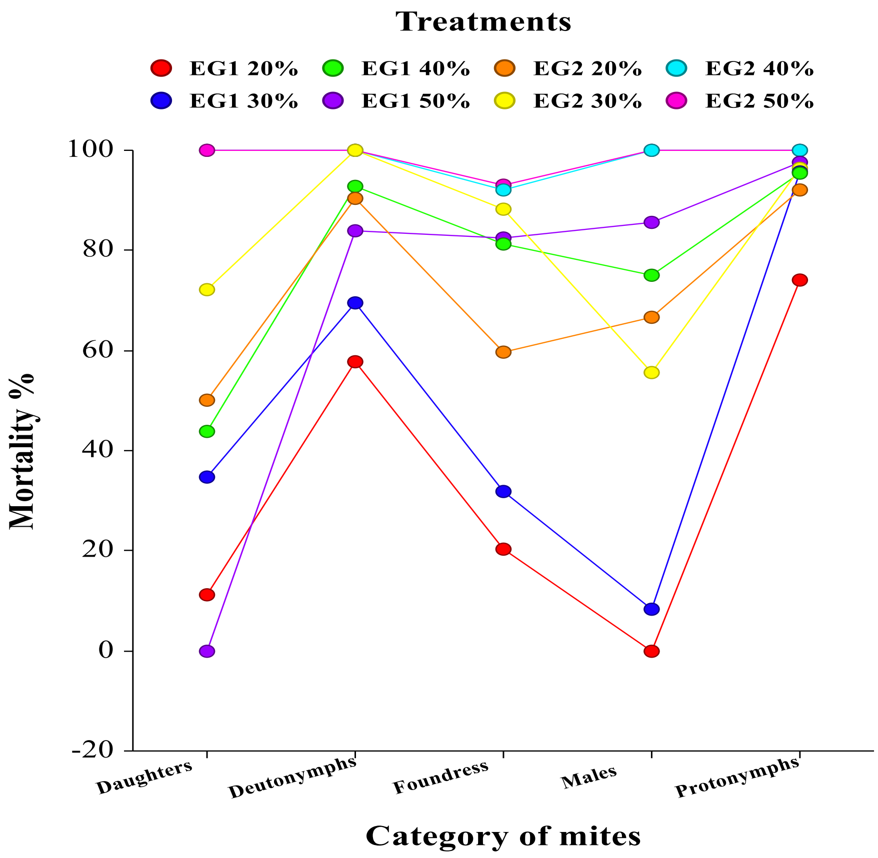


Figure 3. The effect of different treatments on each category of varroa mite found in brood.

The highest varroa mite mortality was registered in the treatments based on formic acid of 50% concentration (Ave.=88.6% in water-based formula; Ave.=95.9% in alcohol-based formula), decreasing slowly in the case of formic acid of 40% concentration (Ave.=83.1% in water-based formulas; Ave.=94.6% in alcohol-based formulas), and registering the lowest values in the treatments with formic acid of 20% concentration (Ave.=36.5% in water-based formula; Ave.=68.7% in alcohol-based formula). It is possible that the alcohol-based formulation may lead to the obtaining of ethyl formate which is an ester formed when ethanol is combined with formic acid, being an organic substance that occurs in nature. It is not known if this substance was produced in the different formulations at the treatment moment, which is the amount produced or if it would have effects on varroa mite. This topic would require special investigations.

Regarding the effect of treatments on different categories of varroa mite, as can be seen out of figures 2 and 3, the most vulnerable stages are the protonymphs and deutonymphs. This can be explained by the lack of exoskeleton sclerotization, but also by affecting their feeding which is dependent by the presence of foundress females with normal vitality. Out of the observations done on live varroa mite, including the foundress females, the most parts of these individuals were found in a low vitality state especially in the 30-50% dilutions and in many cases, the most fragile forms (males, protonymphs) were not anymore identified in the cells according with the reproductive pattern (Mondet et al, 2020) or they were found in an advanced degradation status. The low vitality means individuals with a visible lower mobility or being in a morbid status. To simplify the testing of the treatment's effectiveness performed on brood, the presence of eggs and viable protonymphs at 72 hours evaluation moment could be a better indicator of reproducing mites, which to be used in further evaluations. Taking into account the high mortality of protonymphs even in lower concentrations one can suggest that the obtained data do not reflect totally the minimal limit of optimal concentration of formic acid-based formulations, hence the preliminary nature of these researches. Out of the obtained results only a limited number of adult males were identified the most of them being included in protonymphs category, where the mortality was very high both in water-based and alcohol-based formulas. As the males' presence and vitality is very important for mating, their mortality is another important indicator for the effectiveness of treatments evaluation. In these conditions, a protocol for evaluation the vitality of varroa mite in the capped brood is very important to be established, to correctly estimate the impact of different treatments performed on brood. By brood treatment with low concentrations of formic acid one can improve the use of brushing procedure in organic and conventional beekeeping, taking into account that in the treatments applied on the whole colony the lowest concentration of formic acid used is 50% (Amrine et al. 2007).

## CONCLUSIONS

The results of this study offer useful data on the effectiveness of formic acid in lower concentrations on varroa mite mortality, when applied by brushing procedure. Being about a targeted procedure, the concentration decreasing of formic acid used is an important goal for the optimisation of new formulas and their application on capped brood whenever is necessary in the management of the apiaries in active season as part of the sustainable control of varroosis, both in organic and conventional beekeeping.


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# STUDY REGARDING THE IDENTIFICATION OF SOME ANTIBIOTIC WASTE IN TREATED COWS' MILK

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***Abstract***

*The concept of prudent usage of the antibiotics supposes that their application should have the greatest effect on human and animal health and they should determine the weakest bacteria resistance to the antibiotic used. Among the 48 milk samples assessed, 4 samples (8,33%) were positive according to the test accomplished with the Ecotest device. After 10 minutes of incubation, 91,67% of the samples had enough lactic acid. The lactic acid determined acid pH and phenolphthalein in acid environment is colourless. The test tubes containing the milk from these samples stayed white (the colour of the milk). For the rest of the milk samples (8,33%), because of the presence of antibiotic waste, the active (microbiological) substance did not develop and, since there was no lactic acid, the pH in these test tubes is slightly alkaline or neutral and the phenolphthalein becomes pink. The device used is responsive enough to find the β-lactam antibiotics in milk and it may be used at the farm level. The antibiotic concentration according to the "screening" was under the maximum admitted limit (4 µg/l) and all of the 4 samples were "screen positive".*

***Key words**: antibiotic, contamination, treatment, waste.*

## INTRODUCTION

Ideal tests offer positive results as close as possible to the "Maximum waste limit" (LMR), defined as interest levels. The tests offering positive results to values that are much higher than LMR are debatable. The tests offering positive results under LMR need an excessive number of samples necessary for confirmation.

There are several screening tests. These tests were assessed in diverse experimental conditions (Bishop et al., 1985; Macaulay and Packard, 1981; Seymour et al., 1988; Andrew et al., 2000).

Bishop et al. (1985) reported false positive results for some screening tests where milk from individual cows was used. The false positive results represent losses for the producers as the milk may be rejected for consumption (Cola & Cola, 2017). However, Macaulay & Packard (1981), reported a smaller incidence of the false positive results for three out of four screening tests assessed.

The screening tests were accepted because they reached the standards for low incidence of the false positive results but also of the false negative results (FDA, 1997). Even though the maximum limits for the waste in milk were established (MRL), some situations show that, in certain countries, the antibiotic waste contamination is still a problem (for example, Brazil: Martins-Junior et al., 2007; Bando et al., 2009; China: Bai et al., 2005; Bai & Huang, 2006; Kenya: Shitandi & Sternesjo, 2004).

Numerous studies regarding the antibiotic waste testing focus on liquid milk, so that little attention is paid to formula. The necessity to monitor the formula imports for a variety of potentially damaging substances becomes very important.

In this sens, Kneebone et al. (2010) tested the efficiency of IDEXX tests (IDEXX Laboratories Inc) for identifying antibiotic waste in 5 varieties of formula. The results suggest that the IDEXX tests (New Beta-Lactum and New Tetraidine IDEXX Snap test Kits) actually identify the waste in commercial formula samples (Nestle - 3 samples, Campina one sample and Regilait one sample) and they may be used for monitoring the antibiotic waste in formula reconstituted products.

Also, the rapid tests chosen to obtain results at the farm level proved to be very good for identifying the antibiotic waste in milk that was mixed from several animal species (Contreras et al., 1997; McEwe et al. 1996; Andrew, 2000).

The incidence of some false positive results in raw milk was correlated to several factors, including here the high levels of lactoferrin, feeding, lysozyme, milk fat, milk protein and the number of somatic cells in milk (Carlsson et al., 1989; Van Eenennaan et al., 1993; Marin et al., 2020; Andrew, 2000; Bonea, 2013, 2020).

It is interesting that the performance of the testing devices of antibiotic waste different for the breeds of milk cows. Andrew (2000) finds a growth tendency for the false positive results for the tests used to assess milk from Jersey breed compared to the tests used to assess milk from Holstein breed.

The immunologic tests are methods that detect specific interactions between the antibody and the antigen. These tests are divided in two basic categories, either direct or indirect; measuring the primary reaction antibody-antigen or the secondary reaction antibody-antibody.

The application of these immunologic tests for the analysis of antibiotic waste is made on different devices: LFD (lateral leak device), flash drives, ELISA, RIA, SPR (O´Keeffe et al., 2003; Campbell et al., 2007; Haughey & Baxter, 2006).

The rapid tests monitoring the enzymatic activity for identifying the β-lactam antibiotic class are available and represent now a well-established technology.

The enzymatic tests are generally considered as qualitative techniques that detect the presence of specific chemical waste or that are based on changing the colour reaction by assessing the final point of the test (Wang et al., 2012).

## MATERIALS AND METHODS

This study was accomplished at the Society for Milk Production S.C. Fenov Dolj, within the milk cow farm.

S.C. Fenov S.R.L. has got a genetic patrimony compose of 120 Holstein Friesian lactating cows. The Holstein Friesian breed is specialised for milk production, having an average to high body development and a spotted and black appearance. The potential for the milk production is 9510 litres per lactation. For milking the cows, the unit uses a Herringbone 6x5 room with 30 milking parlours.

During August-October 2021, 48 milk samples were taken from the quarters of 14 cows treated against mastitis with β-lactam antibiotics intramammary. The milk samples were taken after the waiting time expired and they were assessed for the presence of some antibiotic waste by means of a rapid test, using the Ekotest device and by means of a screening laboratory test where the preparation of standard milk solutions was made by diluting the solution of penicillin G stock in milk with no inhibitors up to 0.008 units/ml. concentration, and the Agar with the indicator cooled down at 60ºC and it was inoculated with a suspension of *Bacillus stearothermophilus*. 6 ml of this agar were dropped on Petri plates and they were left to solidify on a plane surface.

The antibiotic quantification was made by means of paper disks impregnated with milk with different antibiotic concentrations 0.25 x MRL; 0.50 x MRL; 1 x MRL; 1.5 x MRL; 2 x MRL on agar environment Müeller Hinton seeded with active substance (*Bacillus stearothermofilus* spores).

Each antibiotic concentration was 4 times replicated. All plates were incubated at 55ºC for 4 hours.

After incubation, the inhibition areas around the paper disks were measured by means of callipers (0.1 mm accuracy). The disk diameter is measured twice next to the inhibition and the average is calculated.

The areas having a diameter over 15 mm were considered as positive areas.

Separately, on 8 different Petri plates, paper disks with 13 mm diameters were placed, immersed in the positive milk samples identified by the EKOTEST device. The plates were incubated at 55ºC for 4 hours. After the incubation, the inhibition areas were measured.

The correlations between the antibiotic concentrations used and the diameter of the inhibition areas were analysed. The correlation coefficient was calculated by means of this calculation formula:

$$\partial = \frac{n \times \sum xy - \left(\sum x\right) \times \left(\sum y\right)}{\sqrt{n \times \sum x^2 - \left(\sum x\right)^2} \times \sqrt{n \times \sum y^2 - \left(\sum y\right)^2}},$$

where:

X = antibiotic concentration (changed into $Log_{10}$)

Y = diameter of the inhibited area

The antibiotic concentration in milk was quantified by means of the following calculation formula:

$$\overline{y} = \mathrm{a} + \mathrm{b}\,\overline{x}$$

## RESULTS AND DISCUSSIONS

Using the "screening tests" for identifying the antibiotic waste in every cow's milk is associated to reducing the incidence of this type of waste in raw material milk.

**Identifying some antibiotic waste**

A test for identifying the antibiotic waste should meet the following conditions: identifying all the components included in the definition of antibiotic waste; identifying the waste at concentrations under the maximum admitted limit (MRL).

Ekotest is a test used for determining the presence of antibiotics and inhibitors in cow milk. It is a rapid test of 10-12 minutes. Relatively cheap reagents are used and 6 samples may be simultaneously tested.

Out of the 48 assessed milk samples, 4 samples (8.33%) were positive according to the test achieved by means of the Ecotest device (Figure 1). After 10 minutes of incubation, 91.67% of the samples showed enough lactic acid.

The lactic acid determines acid pH and phenolphthalein in acid environment is colourless. The test tubes containing the milk of these samples stayed white (the colour of the milk).

For the rest of the milk samples (8.33%), because of the presence of antibiotic waste, the active (microbiological) substance did not develop and, since there was no lactic acid, the pH in these test tubes is slightly alkaline and the phenolphthalein became pink.

The device that was used is sensitive enough to find β-lactam antibiotics in milk and may be used at the farm level. The antibiotic waste amount was quantified in the laboratory.

The presence of antibiotic waste in cow milk after the waiting period expired indicates that some animals and some treatment factors may extend the antibiotic excretion into the milk.

Serious diseases influence the pharmacokinetics of the medicine and the waiting period should be adjusted.

The high-doses treatments that overcome the recommended doses extend the antibiotic excretion.

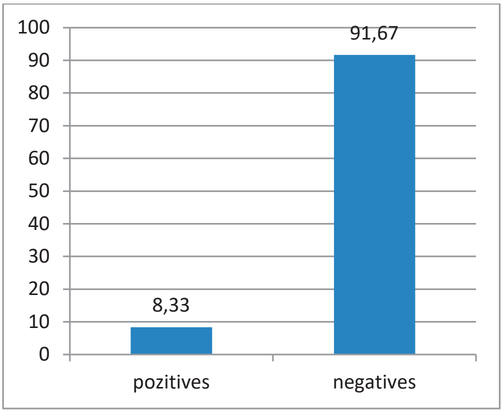


Figure 1 Assessing milk samples

**Quantifying the antibiotic waste in the positive samples**

Table 1. Determining the answer of the standard doses

| Standard doses (UI/ml): | 0.005 | 0.01 | 0.05 | 0.1 | n = 4 |
|---|---|---|---|---|---|
| Logarithm of doses (X $lg_{10}$): | -2.3 | -2 | -1.3 | -1 | $\sum(\bar{x}) =$ - 6.6 |
| Square of the logarithm of doses $X^2$: | 5.29 | 4.0 | 1.69 | 1.00 | $\sum(\bar{x})^2 =$ 11.98 $(\sum \bar{x})^2 =$ 43.56 |
| Average of the inhibition areas of the standard doses-mm $(\bar{y})$ | 15.4 | -18.8 | -23.7 | -26.8 | $\sum(\bar{y}) =$ 84.7 |
| $X \times \bar{Y}$ | -35.42 | -37.60 | -30.81 | -26.80 | $\sum \bar{x} \times \bar{y}$ = -130.63 |

$$b = \frac{n\Sigma\dot{x}\bar{y} - \Sigma\dot{x} * \Sigma\bar{y}}{n\Sigma\dot{x}^2 - (\Sigma\dot{x}^2)}$$

$$= 4\,\frac{[-130.63 - (-6.6 * 84.7)]}{4 * 11.98 - 43.56} = 8.37$$

$$a = \frac{\Sigma\bar{y} - b\Sigma\dot{x}}{n}$$

$$= \frac{[84.7 - (8.37 * -6.6)] = 34.98}{4}$$

$\bar{Y} = a + bx$

$\bar{Y} = 34.98 + 8.37X$

$$x = \text{antilog} * \frac{\bar{y} - a}{b}$$

The average diameter of the inhibition doses for the positive samples was the following:

Sample 1: $\overline{Y}$ = 15.6 mm

Sample 2: $\overline{Y}$ = 16.1 mm

Sample 3: $\overline{Y}$ = 15.8 mm

Sample 4: $\overline{Y}$ = 16.7 mm

The calculation of the antibiotic concentrations in the positive samples:

Sample 1:

$$x = \text{antilog}\frac{15.6 - 34.98}{8.37} = \text{antilog}_{10}\frac{-19.38}{8.37} = \text{antilog} - 2.31 = 0.0049\ \text{UI/ml}$$

Sample 2:

$$x = \text{antilog}\frac{16.1 - 34.98}{8.37} = \text{antilog}\frac{-18.88}{8.37} = \text{antilog} - 2.25 = 0.0056\ \text{UI/ml}$$

Sample 3:

$$x = \text{antilog}\frac{1.58 - 34.98}{8.37} = \text{antilog}\frac{-19.18}{-8.37} = \text{antilog} - 2.29 = 0.051\ \text{UI/ml}$$

Sample 4:

$$x = \text{antilog}\frac{16.7 - 34.98}{8.37} = \text{antilog}\frac{16.7 - 34.98}{8.37} = \text{antilog} - 2.18 = 0.0066\ \text{UI/ml}$$

An international penicillin unit has 0.6 μg.

Sample 1= 0.0049 UI • 0,6 = 0.00294 μg • 100 ml = 2.94 μg/l

Sample 2 = 0.0056 UI • 0.6 = 0.00336 μg • 100 ml = 3.36 μg/l

Sample 3 = 0.0051 UI • 0.6 = 0.003.6 μg • 100 ml = 3.06 μg/l

Sample 4 = 0.0066 UI • 0.6 = 0.00396 μg •100 ml = 3.96 μg/l

Maximum admitted limit (MRL) = 4 μg/l

The antibiotic concentration after "screening" is under the maximum admitted limit and all of the 4 samples, even though the samples are "screen positive" (Figure 2).

The maximum limits of waste (MRL) are the waste levels accepted for food. The MRL levels show food safety and commercial standards. The medicine waste in animal products may be dangerous for the consumers' health. For every medicine, there is a waiting period for human protection.

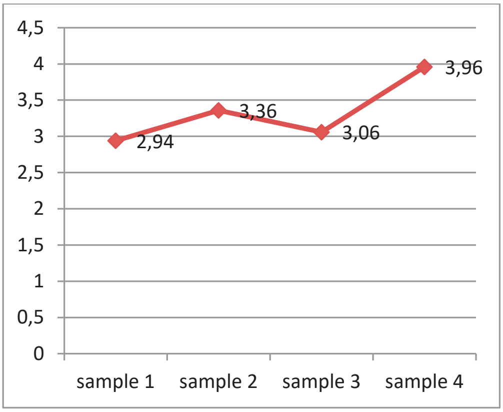


Figure 2. Antibiotic concentration (μg/l)

The waiting period is the time necessary for the antibiotic waste to reach concentrations under the tolerance levels. The maximum waste limits (MRL) stipulated by the European Union legislation guarantee the consumers' protection. Modern technologies may detect the antibiotic waste at level of part per billion (ppb).

This means that the milk dilution will never be enough to totally remove the antibiotic waste from milk. Using "screening" tests for identifying the antibiotic waste in raw material milk prevents that waste from entering the food chain. Each farm is responsible for preventing the milk from being contaminated. Using medication in a wrong way and abusing it for treating milk cows causes the contamination of the milk with waste above the established maximum limits (MRL).

Consequently, the milk becomes unusable for human consumption or for industrial processing. Moreover, the milk is also a component of other food products so that the antibiotic waste may contaminate those products.

The strategy of preventing the milk contamination with antibiotic waste should be based on correct procedures of using medication at the farm level. The presence of antibiotic waste in the cows' milk after the waiting period expired indicates the fact that some animals and some treatment factors may extend the antibiotic excretion into the milk. The test is quick (12 minutes), and the cost of a determination is small. The test identifies the inhibitory waste in milk under the "MRL" levels.

The sensitiveness of the ECOTEST method guarantees the fast identification of some antibiotic waste in cow milk. The studied milk presents no risks for the consumers' health and some of its components, after the treatment of severe mastitis, influence the test for identifying the antibiotic waste.

## CONCLUSIONS

Using the "screening" tests for identifying the antibiotic waste in raw material milk prevents it from entering the food chain. Preventing the milk contamination is the responsibility of every farm producing milk for human consumption.

The ECOTEST "screening" test that was used identified 8,33% positive samples out of the total samples. The test is rapid (12 minutes), and the cost of a determination is low. The test identifies the inhibitor waste in milk under the "MRL" levels. These levels were quantified in the laboratory.

The sensitiveness of the ECOTEST method guarantees the rapid identification of some antibiotic waste in cow milk.

The milk from S.C. Fenov S.R.L. presents no risks for the consumers' health.

After the treatment for serious mastitis, some milk components influence the test for identifying antibiotic waste. Among them, we may name the following: somatic cells, lactoferrin, lysozyme, free fat acids or sodium.

Using the antibiotic overdoses for treating sick animals should be associated to finding antibiotic waste after a waiting period.

We recommend testing milk from treated animals on the first day after the waiting period in order to identify the milk having antibiotic waste from animals with serious diseases or that had been overdosed. Research is necessary in order to find out the real prevalence of using overdoses on milk animals.

When we find the necessity to use overdoses of the same medicine, replacing it with a different antibiotic, used within the prescribed doses, may avoid the problem of antibiotic waste in milk.

Hygienically, the antibiotic waste and the contaminated substances should be as low as possible. The maximum limit of the waste in milk guarantees the consumers' protection, including for those at the end of the food chain (especially children) and offers protection from a link to the other against the accumulations that may appear in the human body.

Implementing activities of identifying and quantifying some antibiotic waste or some other harmful substance in milk within the HACCP program is feasible if the farm manager is trained and has enough information on this topic.

Controlling the risks during the primary processes of milk production determines the reduction of the contamination risk for raw material milk.

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# REVIEW OF THE FATTY ACID CONTENT OF DOMESTIC MILK AND ITS IMPORTANCE

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***Abstract***

*The fatty acids in milk from various species of domestic animals have many important benefits for the human body. The physical chemical characteristics of milk are influenced by many factors: animal nutrition, lactation period, storage conditions and treatments to which it has been subjected. Milk fat is directly determined by the characteristics and the different proportion of fatty acids in its composition. The fat contains over 70 different fatty acids, which sets it apart from all other animal fats. Fats are of vegetable and animal origin, vegetable fats are generally liquid (oils) and animal fats are solid (lard, butter). Fatty acids are of two types saturated and unsaturated. The saturated ones have only simple sigma type bonds (butyric, capronic, caprylic, capric, lauric, myristic, palmitic, stearic acid, etc.). Unsaturated acids contain a pi bond and have longer chains (palmitoleic, oleic, linoleic, etc.). It was found that cow's milk has a low fatty acid content of about 2-3%. This paper is a review of the fatty acid content of milk from different species and their importance.*

***Key words**: benefits, factors, fatty acids, milk fat, species.*

## INTRODUCTION

The content of fatty acids varies between certain limits being influenced by the breed of the animals, the way of feeding, the season in which the milk was harvested and the organoleptic and physical chemical properties of milk fat are directly determined by the different characteristics and proportion of fatty acids in its composition. Milk fat, especially short-chain, polyunsaturated, cis and trans conjugated fatty acids and other components of milk are considered to be beneficial to human health (Parodi, 2004; Ascherio, 2002; Williams, 2000). The fat content of milk rich in saturated fatty acids has been claimed to contribute to heart disease (Chisholm et al., 1996). Butyric acid is considered a potent inhibitor of cancer cell proliferation (Watkins et al., 1999). Belury (2002) demonstrated that unsaturated fatty acids, omega-3 polyunsaturated fatty acids, such as linolenic acid and conjugated linoleic acid, would help prevent breast and skin disease, with beneficial effects on the health of animals used in experiments. Butter enriched in conjugated linoleic acid reduces the incidence of breast tumors in rats (Ip et al., 1999). The concentration of fatty acids in milk is influenced by the diet of the animals, by the feed/concentrate ratio (Griinari et al., 1998).

There were seasonal differences in the diet of cows fed fresh pasture, with a higher content of fatty acids in milk (Precht & Molkentin, 2000; Lock & Garnsworthy, 2003).

Fatty acids are divided into: saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, acetylenic fatty acids, we also have fatty acids with special structure: branched, cyclic, epoxy, hydroxyl, with ketone group (Pece et al., 2007). Fatty acids are distinguished by the number of carbon atoms followed by two dots, the number of double bonds and in parentheses, the position and configuration of double bonds (Karlson, 1975).

## MATERIALS AND METHODS

The data present in this paper resulted from analyzes performed by various authors in the literature using High performance liquid chromatography (HPLC) method. Gas chromatography is currently the most commonly used pathway for fatty acid analysis. High performance liquid chromatography today covers approximately 80% of the analysis of molecular substances: organic, organo-metallic and inorganic including highly polar or thermally labile compounds as well as high molecular weight compounds (natural or synthetic). Data were analyzed from the literature on the fatty acid content of milk samples from different animal species: cattle, goats, sheep, buffalo.

## RESULTS AND DISCUSSIONS

### The fatty acid content of the milk of domestic species

The energy value of milk from different animal species is related to the concentration of certain compounds, especially the amount of fat (Barłowska et al., 2011). Milk fat makes a major contribution to the nutritional properties of milk and its technological adequacy. The fat is synthesized in the milk cells of the udder. Lipids form inclusions, which gradually increase in size and eventually migrate to the top of the cell from which they are discharged as globules into the collecting lumen (Barłowska et al., 2011). The highest concentration of conjugated linoleic acid (CLA) was found in sheep's milk followed by cow's and buffalo's milk and the lowest content was found in goat's milk (Table 1). The presence of CLA includes many properties, the most biologically active is the cis-9, trans-11 (octadecadienoic) configuration diene, it is claimed to inhibit the onset and development of skin, breast, colon, and stomach cancers (Parodi, 1999), while its trans-10, cis-12 isomer is thought to prevent obesity (Bawa, 2003; Wang & Jones, 2004). CLA would inhibit the development of osteoporosis (Watkins & Seifert, 2000), improve lipid metabolism, lower blood glucose, and stimulate the immune system (O'Shea et al., 2004). Donkey's milk is very rich in myristic acid (C 14:0), palmitic acid (C16:0), palmitoleic acid (C 16:1 trans), arachidonic acid (C 20:4, n-6), eicosapentaenoic acid (C 20:5, n-3) and C 22:6, n-6. Sheep's milk is rich in butyric acid (C 4:0), caproic acid (C 6:0); caprylic acid (C 8:0); margaric (C 17:0), stearic (C 18: 0), C 18:1 t11 trans, TFA. Cow's milk is rich in C 14: 1 c9 cis; C 16:1 c9 cis; C 18:1 c9 cis; C 18:2 c9, t11 (CLA); C 18:2 t10, c12 (CLA); C 18:2 cis. Goat's milk was found to be rich in capric acid (C 10:0); lauric acid (C 12:0); C 18:2 t9, t12 trans.

Table 1. Fatty acids of milk from domestic species (% of total fatty acids)

| Fatty acids | Cow[a] | Goat[a] | Sheep[a] | The buffalo[a] |
|---|---|---|---|---|
| C 4:0 | 3.84[b] | 2.46 | 4.06[c] | 3.90c |
| C 6:0 | 2.28[b] | 2.40 | 2.78[c] | 2.33c |
| C 8:0 | 1.69[b] | 2.53 | 3.13[c] | 2.41c |
| C 10:0 | 3.56[b] | 9.38 | 4.97[c] | 2.40c |
| C 12:0 | 3.83[b] | 4.45[a;b] | 3.35[c] | 3.09c |
| C 14:0 | 11.24 | 10.16 | 10.16[c] | 10.64 |
| C 16:0 | 26.66 | 25.64[b] | 23.11[c] | 28.02[c] |
| C 17:0 | 0.50 | 0.63 | 0.76 | 0.50 |
| C 18: 0 | 11.06[b] | 12.51 | 12.88[c] | 12.58[c] |
| Total ≤C14:0 | 23.59 | 31.37 | 28.42 | 24.76[c] |
| Total SFA | 63.94 | 68.70 | 65.17[c] | 65.86[c] |
| C 14:1 c9 cis | 0.84 | 0.22 | 0.58 | 0.67 |
| C 16:1 c9 cis | 1.68 | 0.67 | 0.39 | 1.56 |
| C 18:1 c9 cis | 24.72 | 22.03 | 23.32 | 24.10 |
| Total MUFA | 27.23 | 23.39 | 24.29[c] | 26.43[c] |
| C 16:1 trans | 0.31 | 0.38 | 0.29 | 0.37 |
| C 18:1 t11 trans | 2.01 | 1.69 | 2.69 | 2.00 |
| C 18:2 t9, t12trans | 0.45 | 0.50 | 0.44 | 0.49 |
| Total TFA | 2.76 | 2.66 | 3.15 | 2.66 |
| C 18:2 c9, t11 (CLA) | 0.59 | 0.43 | 0.60 | 0.39 |
| C 18:2 t10, c12 (CLA) | 0.036 | 0.024 | 0.032 | 0.027 |
| Σ (t11, c13, t7, c9) CLA | 0.033 | 0.030 | 0.041 | 0.027 |
| Total CLA | 0.66 | 0.48 | 0.67 | 0.49 |
| C 18:2 cis | 1.96 | 0.70 | 1.17 | 1.55 |
| C 18:3 n-3 | 0.70 | 0.82 | 0.92 | 0.68 |
| C 20:4, n-6 | 0.21 | 0.32 | 0.20 | 0.35 |
| C 20:5, n-3 | 0.15 | 0.11 | 0.09 | 0.18 |
| C 22:6, n-6 | 0.08 | 0.09 | 0.08 | 0.12 |
| Total PUFA | 3.08 | 2.04 | 2.45 | 2.67 |

[a]Talpur, 2008; [b]Ceballos et al., 2009; Barłowska et al., 2011. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; CLA, conjugated linoleic acid; TFA, trans fatty acid.

Ceballos et al. (2009) reported goat's milk fat compared to cow's milk fat that would contain 54.6 % more C 6:0 acid, 69.9% C8: 0, 80.2% C 10:0 and 56.3 % CLA and 75% less acid C 4:0. Most important of goat's milk is the high concentration of short-chain fatty acids, such as capric acid and caprylic acid, which are increasingly present in goat's milk. They have been used in therapies for patients with metabolic disorders, cholesterol problems, anemia, bone demineralization and in child malnutrition (Pop et al., 2008). The highest total content ≤ C14:0 was found in goat's milk. In the content of monounsaturated fatty acids (MUFA), cow's milk ranks first with the highest concentration found, followed by buffalo's milk. The concentration of CLA depends on the animal's diet (Michalski et al., 2005). A significant increase in the concentration of CLA in milk was observed in cows fed on pasture compared to cows fed on full mixed rations (Auldist et al., 2002; Loor et al., 2003; Schroeder et al., 2003). Feeding would influence the n-6/n-3 acid ratio in milk (Fedele et al., 2001). The 4:1 n-6/n-3 acid ratio of milk has been influenced by feeding cows fresh fodder (Haug et al., 2007), in summer the ratio may decrease, when cows are fed fresh pasture, they reach close values of 2:1. The 4:1 n-6/n-3 acid ratio of milk has been influenced by feeding cows fresh fodder (Haug et al., 2007), in summer the ratio may decrease, when cows are fed fresh pasture, they reach close values of 2:1. An argumentative report was made on the ratio of n-6 and n-3 fatty acids. People living in the Mesolithic era had an intake of n-6 and n-3 FA in a ratio of 1 to 4:1, while the diet of a modern European man reaches a ratio of 10 to 14:1 (Haug et al., 2007). Consumption of large amounts of n-3 fatty acids, consumed by many Japanese and Inuit population is defined by a lower risk of coronary heart disease and some cancers.

**The importance of acids**

Milk fat has a high content of saturated fatty acids. The majority of saturated fatty acids are palmitic acid (24-28%), myristic acid (13-14%) and stearic acid (11-12%), and the unsaturated ones are oleic acid (23-28%). Short or medium chain saturated fatty acids do not increase serum cholesterol levels, only palmitic and myristic acids have an effect in this regard (Kansal, 2002).

Experts in the fields of cancer, nutrition, and immunocompetence have come together to analyze the role of milk in the diet on the "nutritional value of milk fat" (California Dairy Research Foundation, 1996). Discussions have shown that atherosclerosis is caused by factors such as diet and genetic inheritance. Some experts believe that atherosclerosis is not a disease due to diet but a metabolic disease, but others have shown that by monitoring cholesterol and changing fat, a change in blood composition can be obtained. Consumption of trans acids is associated with an increased risk of cardiovascular disease, but this combination cannot be applied to fatty acids of animal origin such as vaccenic acid and rumenic acid (CLA) which have anti-atherogenic properties (Parodi, 2004).

Milk fat is easily digestible, this digestibility is 99% while palm oil is 91% (Kansal, 2002). The digestibility of milk fat is due to the dispersion of fat globules in the aqueous phase of milk in the form of emulsion, thus facilitating pancreatic enzymes and intestinal lipases. Milk is rich in short or medium chain fatty acids that are more easily absorbed than long chain fatty acids. The easy digestibility of milk fats makes it a valuable constituent of the diet in diseases of the stomach, intestines, liver, gallbladder, kidneys (Miron and Macovei, 2006).

Bovine milk is low in essential fatty acids (EFAs), linoleic and linolenic. The EFA requirement is 3% of the total calories, 2/3 is provided by the lipids present in cereals, vegetables, etc. There is no justification for replacing milk fats with other fats that have a higher linoleic acid content. Milk fat has a productive effect against tooth decay. Short or medium chain fatty acids with 4-12 carbon atoms that are found in high concentrations in milk fat have antifungal and antibacterial activity (Kansal, 2002). Cow's milk fat can prevent gastrointestinal infections, thus costing whole milk consumption to be associated with fewer gastrointestinal infections than skim milk consumption (Koopman et al., 1984). The results of animal studies do not support the hypothesis that milk fat has a role in the etiology of breast cancer. Daily intake of insulin-like factor-1 and biologically active estrogens in dairy products is insignificant compared to the daily exogenous secretion of these female factors, and bovine growth hormone is biologically inactive in humans (Parodi, 2004).

Milk fat contains many agents with anticarcinogenic potential, such as conjugated linoleic acid, sphingomyelin and other sphingolipids, butyric acid, 13-methyltetradecanoic acid, ether lipids, vitamins A and D. Milk fat has a protective effect against many cancers (breast, skin, stomach, prostate, colon) (Parodi, 2004).

Dairy products are rich sources of CLA, especially the cis-9, trans-11 isomer. The concentration of CLA in milk fat is 3-5 mg/g, of which the cis-9, trans-11 CLA isomer represents

80-90% (Parodi, 1997; Sehat et al., 1998). For this CLA isomer it has been proposed the name rumenic acid (Kramer et al., 1998) name resulting from foods from ruminants (beef, milk) contain large amounts of this isomer.

Numerous physiological properties have been attributed to conjugated linoleic acid. The benefits of the acid include activities such as: anticarcinogenic activity, antilipogenic activity, antiatherosclerotic activity, antidiabetogenic activity, modulator of lipid metabolism and immune function (Miron & Macovei, 2006).

Studies on animals, human culture cell lines, and human epidemiological data suggest that whole milk and dairy products rich in CLA are beneficial foods are good foods for health, because CLA in addition to its antitumor properties is a hypolipidemic and antioxidant nutrient and therefore antiatherosclerotic (Miron & Macovei, 2006).

The bactericidal activities of fatty acids depend on the length of the chain and the strain of the microorganism. Helicobacter pylori has been shown to be sensitive to monoglycerides and medium chain fatty acids (Petschow et al., 1996), and the consumption of a large amount of milk fat inhibited intestinal colonization with Listeria monocytogenes in rats. C 4:0, C 6:0, C8:0, C 16:0 and C 18:0 fatty acids at a concentration of 500 μmol /l had no bactericidal activity. C 14:0, C 18:1 and C 18:2 fatty acids killed only Compylobacter jejuni and Listeria monocytogenes while C 10:0 and C 12:0 fatty acids were found to be toxic to all pathogens tested (Sprong et al., 1999). In rats that consumed milk fat, C 10:0 and C 12:0 fatty acids accounted for 8% and 7% of free gastric fatty acids, respectively. Considering a similar human fatty acid release ratio, the calculated gastric concentration varies between 0.4-0.9 and 0.3-0.7 mol/l for C 10:0 and C 12:0 fatty acids, respectively. Both acids have been shown to be highly bactericidal at 0.5 mol/l, demonstrating that C 10:0 and C 12:0 fatty acids released during gastric digestion of milk fat can prevent gastrointestinal infections (Sprong et al., 2001).

## CONCLUSIONS

Assessing the fatty acid profile of milk from different animal species, it is found that saturated and monounsaturated fatty acids have been identified as the most concentrated in all types of milk, while polyunsaturated fatty acids have been identified in relatively low concentrations in milk. In the human diet, essential fatty acids have many benefits for the body. In the human body, fatty acids are a preferred source of energy. Dairy products and meat are often rich in saturated fatty acids.

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# STUDIES CONCERNING THE EFFECT OF THE INBREEDING ON THE VIABILITY OF LARVAE AND LIVE PUPAE PERCENTAGE (*BOMBYX MORI* L.)

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***Abstract***

*The silkworm amelioration program based on the use of the genetic resources and the inbreeding methods applicable in the case of Bombyx mori L. species targets mainly the creation of races and hybrids with superior traits. The improvement process by inbreeding is practiced in the creation of new populations and it requires the use in crossings of the inbred lines that meet the most valuable biological and productive characteristics, with a high combinative value. The possibility of rearing more silkworm generations per year, the physiological particularities and the variability of species characters constituted the base of the formation of silkworm races, lines and hybrids with high productive capacity.*

***Key words**: inbred lines, silkworm, viability of larvae, live pupae percentage.*

## INTRODUCTION

The genetic amelioration of silkworms constitutes a complex driven by systems and methods of improvement of the hereditary base, of the races productive potential and of creation of new silkworm races and hybrids (Doreswamy & Gopal, 2012). In the process of silkworm amelioration is practiced the inbreeding which constitutes the mating system of related individuals belonging to the same race (Goldsmith et al., 2005). By inbreeding, phenotypic and especially genotypic changes take place, the main effect of inbreeding being the reducing of heterozygous genotypes frequency and the increasing of the homozygous genotypes frequency (Buhroo et al., 2016). The raising of the homozygosity degree has favorable effects materialized in fixing and amplifying some characters, as well as in increasing the hereditary transmission capacity of breeders. In case of silkworms the degree of kinship is very high because the mating system of the type brother x sister is most often practiced (Braslă & Matei, 1992; Haniffa & Thatheyus, 1992; Jamuna & Subramanya, 2012). In silkworm the obtaining of differentiated lines in terms of gene frequencies is easy to achieve due to the relatively small generation interval and the large number of offspring (Kumari & Tripathi, 2017). The species also allows the selection of lines according to the general and special combinative capacity, thus retaining those lines that present the highest values of the characters, but also those that are able to provide the best combinations with other lines, being known that not every inbred line in combination with another provides the same phenotypic effect (Kuzmanov & Petkov, 2000a). The selection of lines on the basis of their combinative capacity followed by the elaboration of various schemes of their crossing, in which each line is used as maternal and paternal form, results in the maximum capitalization of the heterosis phenomenon (Kuzmanov & Petkov, 2000b).

The amelioration works meant to lead to the creation of silkworm races and hybrids have in view the achievement of some forms superior to those existents, in terms of one or more biological, technological or economic characters (Bindroo & Moorthy, 2014). The researches on the practice of the amelioration process by inbreeding associated with the works of selection in silkworms, mention the intensification of some technological characters as the silk content of the cocoons and the filament length, but also the technological characters that refers to all the stages of the evolutionary cycle, these referring to embryos viability, materialized in the hatching energy and hatching percentage, viability of larvae, percentage of their transformation in pupae, moth viability and vigor (Diniță et al., 2019; Petkov et al., 1998; Ruiz & Almanza, 2018). As a result of the application of inbreeding a greater number of generations, the negative effects of this process appear, known as inbreeding depression, which in silkworms is manifested by a decrease in the hatching percentage, viability of larvae and prolificacy (Petkov et al., 1999; Nematollahian, 2010). However, the inbreeding depression doesn't affect the qualitative features such as length, finesse, elasticity and silk filament length (Nagaraju, 2002).

The purpose of the researches was on the one hand theoretical, contributing to the study of the effects on inbreeding on phenotypic and genotypic parameters, as well as a practical one, consisting in the use of inbred lines in the hybridization process, whose end result is to obtain commercial hybrids.

## MATERIALS AND METHODS

In the experimental researches on inbreeding two line-founding races were used, both native, Alb Băneasa and Băneasa 75, respectively. The incubation process started with a number of 25 laying (lines) for each race. The silkworms rearing took place in the spring-summer season, using hibernating or non-hibernating eggs as appropriate.

In order to establish the inbred lines, families were structured within each race, practicing pairings of brother x sister type for six generations, the work stages being: lines extracting and their inbreeding a variable number of generations, until the fixation of the characters pursued in amelioration; the testing of general combinative capacity of the inbred lines by crossings with the tester race; the testing of specific combinative capacity of the inbred lines; the testing of general combinative capacity of the inbred lines by crossings with the tester race; the testing of specific combinative capacity of the inbred lines; the study of heterosis effect in hybrids obtained from inbred lines crossing.

In order to decrease the effects of the inbreeding depression was initially applied the inter-family (linear) selection based on the predefined performance criteria, continuing with the individual selection based with the performance of each family.

The inbreeding/generation coefficient was calculated according to Wright's formula:

$$F_x = (1/2)^{n_1 + n_2 + 1} \ (1 + F_A)$$

in which:

$F_x$ – the inbreeding coefficient of the individual X;

$n_1$, $n_2$ – the changes number of generations between mother or father and the common ancestor;

$F_A$ – the inbreeding coefficient of the common ancestor.

The viability of larvae has been calculated on 3 lots of 100 larvae for each line, at the beginning of the IVth stage, from the ratio between the number of cocoons and the number of larvae in the moment of the lot formation.

The percentage of live pupae was determined based on the ratio of the number of cocoons with live pupae to the total number of silk cocoons.

## RESULTS AND DISCUSSIONS

**The inbreeding effect on the larvae viability**

The 15 inbred lines from the founding race Alb Băneasa presented in $I_0$ a viability percentage within the limits 88.82 - 82.88%, the lines average in this generation being 86.07% (Table 1).

In the first inbreeding generation, 10 of the 15 lines present a lower viability compared to the control, in two of them the differences in minus being significant, while three lines present a viability superior to the control, and in other two lines there are no differences from it.

Starting with $I_2$ the analyzed character was affected by the inbreeding depression, so that 9 of the 15 studied lines presented significantly lower values than the control, aspect that was also reposted in $I_3$.

In $C_4$ the lines average is with 4.89 percent lower than the control and in $I_6$ the difference to this records 8.56 percent. Comparing the lines' average by generations of inbreeding is found that each generation presents lower values than the previous generation, differences in minus statistically significant being found only in $I_4$ compared to $I_3$ and $I_5$ compared to $I_4$.

Following the effect of inbreeding within each line, it is found that the amplitude of variation of in minus differences between $I_6$ and $I_0$ is between 4.46 percent (AB-3/2) and 13.69 percent (AB-12/9).

It is also found that in some lines (AB-20/13 and AB-22/14) the inbreeding depression it has been manifested since $I_1$, but it has been generalized starting with $I_3$.

Also, in the case of the lines from Băneasa 75 race is found a progressive decrease of the viability percentage (Table 2), but differences in minus significant to the control appeared since $I_3$, generation in which 12 of the 15 analyzed lines are under the control ($I_0$). In $I_4$, $I_5$ and $I_6$ all the inbred lines present lower values than the control, the differences in minus being statistically significant.

Table 1. The inbreeding effect on the viability of larvae in the inbred lines – Alb Băneasa (%)

| Line | Generation of inbreeding | | | | | | |
|---|---|---|---|---|---|---|---|
| | $I_0$ | $I_1$ | $I_2$ | $I_3$ | $I_4$ | $I_5$ | $I_6$ |
| | $\overline{X} \pm s_{\overline{X}}$ | $\overline{X} \pm s_{\overline{X}}$ | $\overline{X} \pm s_{\overline{X}}$ | $\overline{X} \pm s_{\overline{X}}$ | $\overline{X} \pm s_{\overline{X}}$ | $\overline{X} \pm s_{\overline{X}}$ | $\overline{X} \pm s_{\overline{X}}$ |
| AB-1/1 | 88.20±0.86 | 87.80±0.66 | 87.20±0.16 | 86.22±0,08* | 83.66±0.10** | 79.16±0.12** | 79.80±1.10** |
| AB-3/2 | 84.62±1.10 | 85.32±0.48 | 85.10±0.24 | 85.46±0.22 | 82.12±0.12** | 80.32±0.14** | 80.16±0.88** |
| AB-4/3 | 85.80±0.36 | 85.40±0.10 | 83.40±0.36** | 84.16±0.16 | 80.40±0.42** | 78.17±0.32** | 79.13±0.82** |
| AB-5/4 | 87.77±0.18 | 86.88±0.12 | 85.60±1.10** | 84.13±0.12** | 81.26±0.48** | 80.46±0.40** | 78.15±0.16** |
| AB-7/5 | 83.40±0.36 | 84.62±0.63 | 85.10±0.68 | 83.80 ± 0.16 | 80.32±0.32** | 79.90±0.44** | 75.60±0.16** |
| AB-8/6 | 82.88±0.28 | 83.30±1.10 | 82.16±0.72 | 80.22±0.22** | 79.88±0.16** | 78.10±0.30** | 75.30±0.10** |
| AB-9/7 | 87.73±0.46 | 86.82±0.88 | 86.40±0.63 | 85.40±0.18* | 83.20±0.10** | 80.42±0.18** | 78.90±0.08** |
| AB-10/8 | 86.80±0.18 | 85.70±0.66 | 83.20±0.50** | 82.60±0.60** | 82.16±0.26** | 80.14±0.12** | 79.67±0.63** |
| AB-12/9 | 88.82±0.30 | 88.20±1.10 | 85.30±0.16** | 82.20±0.54** | 80.33±1.10** | 78.16±0.16** | 75.13±0.14** |
| AB-14/10 | 83.44±0.42 | 82.30±0.60 | 83.10±0.12 | 82.10±0.30 | 81.80±0.86 | 77.33±0.30** | 76.22±0.10** |
| AB-15/11 | 85.72±0.60 | 84.10±0.48 | 80.88±0.28** | 80.60±0.12** | 80.22±0.72** | 76.35±0.48** | 77.10±0.12** |
| AB-18/12 | 87.47±0.48 | 86.12±0.36 | 82.66±0.32** | 81.44±0.16** | 79.66±0.17** | 78.20±0.36** | 76.16±0.14** |
| AB-20/13 | 86.30±0.10 | 83.13±0.76** | 82.80±0.40** | 82.20±0.42** | 80.34±0.16** | 77.16±1.10** | 77.15±0.16** |
| AB-22/14 | 85.72±0.30 | 82.14±0.68** | 83.66±0.12* | 83.20±0.16** | 81.22±0.36** | 78.32±0.46** | 77.80±032** |
| AB-25/15 | 86.40±0.42 | 85.76±0.14 | 84.40±0.40* | 83.80±0.32** | 81.10±0.18** | 79.82±0.32** | 76.40±0.40** |
| Average | 86.07±0.47 | 85.17±0.49 | 84.06±0.44* | 83.17±0.46** | 81.18±0.31** | 78.80±0.34** | 77.51±0.44** |

*P<0.05; **P<0.01; ***P<0.001.

Table 2. The inbreeding effect on the viability of larvae in the inbred lines – Băneasa 75 (%)

| Line | Generation of inbreeding | | | | | | |
|---|---|---|---|---|---|---|---|
| | $I_0$ | $I_1$ | $I_2$ | $I_3$ | $I_4$ | $I_5$ | $I_6$ |
| | $\overline{X} \pm s_{\overline{X}}$ | $\overline{X} \pm s_{\overline{X}}$ | $\overline{X} \pm s_{\overline{X}}$ | $\overline{X} \pm s_{\overline{X}}$ | $\overline{X} \pm s_{\overline{X}}$ | $\overline{X} \pm s_{\overline{X}}$ | $\overline{X} \pm s_{\overline{X}}$ |
| B75-2/1 | 89.30±0.20 | 88.76±0,46 | 87.27±0.52 | 86.40±0.36 | 76.66±0.18** | 77.08±0.16** | 72.20±0.23** |
| B75-4/2 | 86.40±0.36 | 87.32±0.82 | 86.32±0.42 | 80.80±0.72** | 77.20±0.44** | 71.88±0.22** | 70.66±0.34** |
| B75-5/3 | 87.27±1.10 | 86.88±0.16 | 87.71±0.36 | 87.59±0.36 | 63.33±0.10** | 68.20±0.46** | 65.44±0.18** |
| B75-6/4 | 85.30±0.76 | 86.30±0.14 | 85.88±0.16 | 88.39±0.38 | 64.66±0.20** | 77.50±0.38** | 73.42±0.26** |
| B75-8/5 | 83.40±0.14 | 82.86±0.32 | 82.96±0.12 | 86.40±0.42 | 76.66±0.16** | 76.50±0.14** | 64.40±0.72** |
| B75-10/6 | 88.18±0.63 | 87.75±0.48 | 87.38±0.10 | 79.20±0.14** | 74.32±0.46** | 76.94±0.12** | 76.60±1.01** |
| B75-11/7 | 87.36±0.22 | 87.20±0.56 | 86.86±0.22 | 84.40±0.16 | 77.33±0.18** | 72.97±0.16** | 73.90±2.36** |
| B75-13/8 | 88.20±0.86 | 85.30±1.06 | 87.40±0.46 | 83.20±0.10** | 74.33±0.82** | 70.93±0.28** | 70.40±1.72** |
| B75-15/9 | 82.40±0.10 | 83.66±0.84 | 81.20±0.28 | 78.59±0.12* | 72.46±0.60** | 70.84±0.10** | 69.60±1.10** |
| B75-16/10 | 88.60±0.80 | 85.40±0.36 | 84.80±0.16 | 82.00±0.62** | 70.20±0.18** | 70.11±0.26** | 70.20±0.86** |
| B75-18/11 | 87.40±1.17 | 86.80±0.24 | 85.60±0.08 | 83.60±0.46* | 74.85±0.22** | 73.40±0.40** | 72.20±0.72** |
| B75-19/12 | 88.20±0.22 | 88.10±0.28 | 87.98±0.18 | 80.32±0.32** | 78.60±0.16** | 75.00±0.26** | 71.40±0.36** |
| B75-21/13 | 84.32±0.46 | 85.12±0.42 | 85.63±0.12 | 75.52±0.16** | 76.14±0.12** | 63.62±0.18** | 66.60±0.42** |
| B75-23/14 | 82.20±1.10 | 81.20±0.72 | 80.88±0.11 | 78.44±0.46* | 77.32±0.10** | 73.79±0.60** | 70.60±0.36** |
| B75-24/15 | 84.60±2.30 | 83.30±0.16 | 83.10±0.10 | 76.77±0.48** | 75.44±0.40** | 71.25±0.30** | 69.96±0.18** |
| Average | 86.21±0.60 | 85.73±0.56 | 85.40±0.60 | 82.11±1.04* | 74.09±1.16** | 72.67±0.98** | 70.75±0.86** |

*P<0.05; **P<0.01; ***P<0.001.

Comparing the inbreeding generations between them it is observed that the lines' average presents lower values in each of them compared with the previous generation, but they only become significant between $I_4$ - $I_3$ and $I_5$ - $I_4$. Inside the lines, in most cases, it is manifested the same decreasing tendency of the value of analyzed character from a generation to another, exceptions being noted in $I_1$ at the lines B75-4/2, B75-6/4, B75-15/9, B75-21/13, in $I_2$ at the lines B75-5/3 and B75-12/8 and in $I_3$ at the lines B75-6/4 and B75-8/5 when there is an increase in viability. In other cases, such as line B75-16/10 there is a stagnation of the viability percentage starting with $I_4$. As for the values reached by the $I_6$ inbred lines, they are between 64.40 - 76.60% and the differences in minus compared to the control ($I_0$) between -11.6 percent (B75-23/14) and -21.83 percent (B75-5/3).

Also, in the case of other works that aimed to achieve inbred lines in silkworms it was noticed the decrease of the percentage of larvae viability. So, after three generations of inbreeding is noticed a decreasing of the viability of larvae with 2 - 16 percent, the maximum inbreeding depression being recorded in $I_6$, the differences to the non-inbred control reaching the maximum value of -18,9% (Craiciu et al., 1971).

**The inbreeding effect on the percentage of live pupae**

The percentage of live pupae represents an important character of evaluation of silkworm races and hybrids and their promoting in production.

According to data presented by specialized publications, the value of this character represents 92.80 - 95.90% in silkworm races widespread in Korea, 95.60 - 97.50% in those reared in Japan and 88.60 - 90.80% in those in India.

Being a character with low heritability, the live pupae percentage is affected by the related crossing, aspect highlighted also by other authors who studied the inbreeding effect in silkworms (Craiciu et al., 1971).

In the case of inbred lines from Alb Băneasa race (Table 3) it is noticed that the percentage of live pupae is not visibly influenced in $I_1$, the lines' average in this generation being very close to $I_0$. Examining the percentage of live pupae for each line in this generation there are noticed slight differences more or less than $I_0$, but not being significant. In $I_2$ the inbreeding depression is manifesting in 10 lines that are below the values of the control with statistically significant differences.

The process of inbreeding depression extends in $I_3$ when the average of the lines is with 1.82 percent lower than the control and is generalized in $I_5$, generation in which the percentage of live pupae varies between 79.16 - 84.86% with a lines' average of 81.44 %. In $I_6$ the differences in minus to the control are between 1.80 - 6.60 percent, more affected being the lines AB-15/11 (78.16%), AB-25/15 (79.86%). As a whole, the lines present in $I_6$ a percent of transforming into pupae of 78.16-4.20%, being in average with 4.25 percent lower than in $I_0$.

Similar conclusions can be drawn also from the data on the percentage of live pupae in lines from Băneasa 75 group (Table 4). In $I_1$ no negative effect of inbreeding is reported, in this generation being observed an insignificant increase tendency of the percentage of live pupae in most lines. Starting with $I_2$, 6 of the 15 lines present significant in minus differences, their number increasing at 7 in $I_3$. In $I_4$ the inbreeding depression installs in most inbred lines and its effect is accentuated in the next two generations. In $I_6$ the average percentage of live pupae is of 79.25%, with 4.35 percent lower than in $I_0$. In the same generation the inbreeding depression is manifesting by a decrease of the value of the analyzed character with 1.82 - 6.34 percent, being more obvious in the case of 3 from the 15 lines.

## CONCLUSIONS

**The viability of larvae** shows a tendency of progressive decrease with the increase of the coefficient of inbreeding depression, that become significant starting with $I_3$. The viability of larvae is in $I_6$ within the limits 75.13 - 80.16% in Alb Băneasa group of lines and 64.40 - 76.60% in Băneasa 75 group of lines. The differences in minus between $I_6$ and $I_0$ recorded by the average value of the lines represents 15.46 percent in Băneasa 75 lines and 8.56 percent in Alb Băneasa lines.

**The percent of live pupae** was influenced by the practice of re related crossings, aspect that manifested itself in the case of both groups of lines. The inbreeding depression has been installed in a small number of lines since $I_1$, expanding with the development of the inbreeding process and generalizing in $I_5$. The inbred lines in $I_6$ present a percentage of pupae between 78.16 - 84.20% in Alb Băneasa group and 76.10 - 80.86% in Băneasa 75 group, the differences in minus to $I_0$ being of 1.80 - 6.60 percent in the case of the first group of lines and of 1.82 - 6.34 percent in the case of the second group. The hatching percentage was affected by de inbreeding depression starting with $I_3$, in both groups of races. The differences in minus between $I_6$ and $I_0$ are between 6.10-15.66 percent in Alb Băneasa group of lines and 6.80-15.90 percent in Băneasa 75 group of lines.

The use of inbred lines in the process of silkworm hybridization led to the obtaining of commercial hybrids. This aspect made it possible to determine the magnitude of the heterosis effect for different characters as well as to elaborate some efficient schemes of hybridization, the work materializing by recommending some hybrid combinations for the sericultural production.

Table 3. The inbreeding effect on the live pupae percentage in the inbred lines – Alb Băneasa (%)

| Line | Generation of inbreeding | | | | | | |
|---|---|---|---|---|---|---|---|
| | $I_0$ | $I_1$ | $I_2$ | $I_3$ | $I_4$ | $I_5$ | $I_6$ |
| | $\overline{X} \pm s_{\overline{X}}$ | $\overline{X} \pm s_{\overline{X}}$ | $\overline{X} \pm s_{\overline{X}}$ | $\overline{X} \pm s_{\overline{X}}$ | $\overline{X} \pm s_{\overline{X}}$ | $\overline{X} \pm s_{\overline{X}}$ | $\overline{X} \pm s_{\overline{X}}$ |
| AB-1/1 | 87.26±0.12 | 86.22±0.40 | 85.10±0.32** | 86.00±0,52 | 85.26±0.12** | 84.86±0.18** | 84.20±0.28** |
| AB-3/2 | 86.48±0.24 | 85.28±0.36 | 84.32±0.88** | 84.12±0.44 | 85.10±0.40** | 83.28±0.26** | 82.26±0.32** |
| AB-4/3 | 87.10±0.14 | 87.27±0.14 | 86.16±0,72 | 85.26±0.66* | 83.86±0.38** | 84.10±0.40** | 84.12±0.14** |
| AB-5/4 | 84.20±0.32 | 85.46±0.12 | 86.18±0.18** | 85.30±0.18 | 83.36±0.18 | 82.66±0.52 | 82.10±0.42** |
| AB-7/5 | 84.31±0.40 | 83.32±0.26 | 84.66±0,16 | 82.26±0.24** | 80.82±0.14** | 81.82±0.38** | 80.16±0.76** |
| AB-8/6 | 85.22±0.16 | 86.26±0.34 | 85.23±0.12 | 84.48±0.36 | 82.31±0.36** | 81.45±0.18** | 80.26±0.12** |
| AB-9/7 | 83.26±0.24 | 84.46±0.42 | 81.44±0,26* | 83.25±0.40 | 80.27±0.22** | 81.23±0.10** | 81.46±0.14* |
| AB-10/8 | 84.44±0.30 | 83.88±0.11 | 82.60±0.34* | 81.88±0.44** | 79.96±0.54** | 80.44±0.16** | 80.55±0.26** |
| AB-12/9 | 86.21±0.36 | 85.22±0.17 | 84.32±0.16** | 83.36±0.16* | 81.25±0.17** | 80.18±0.27** | 81.32±0.30** |
| AB-14/10 | 87.42±0.24 | 87.36±0.24 | 85.26±0.42** | 84.48±0.40** | 84.10±0.46** | 81.28±0.35** | 81.10±0.44** |
| AB-15/11 | 83.46±0.12 | 84.42±0.30 | 85.22±0,46* | 84.10±0.52 | 81.26±0.45** | 79.86±0.14** | 78.16±0.18** |
| AB-18/12 | 84.22±0.18 | 83.18±0.44 | 83.10±0.30 | 82.86±0.64 | 82.32±0.50** | 80.32±0.44** | 80.98±0.15** |
| AB-20/13 | 85.48±0.44 | 83.44±0.28** | 82.86±0.22** | 80.62±0.24** | 81.82±0.44** | 80.42±0.12** | 80.26±0.27** |
| AB-22/14 | 85.26±0.14 | 84.16±0.36 | 83.22±0.40 | 82.44±0.38** | 80.44±0.26** | 79.16±0.34** | 80.44±0.31** |
| AB-25/15 | 86.46±0.16 | 85.20±0.40 | 84.62±0.32* | 83.18±0.22 | 81.25±0.30** | 80.30±0.52** | 79.86±0.42** |
| Average | 85.39±0.36 | 85.01±0.35 | 84.29±0.35 | 83.57±0.37* | 82.23±0.45** | 81.44±0.42** | 81.14±0.41** |

*P<0.05; **P<0.01; ***P<0.001.

Table 4. The inbreeding effect on the live pupae percentage in the inbred lines – Băneasa 75 (%)

| Line | Generation of inbreeding | | | | | | |
|---|---|---|---|---|---|---|---|
| | $I_0$ | $I_1$ | $I_2$ | $I_3$ | $I_4$ | $I_5$ | $I_6$ |
| | $\overline{X} \pm s_{\overline{X}}$ | $\overline{X} \pm s_{\overline{X}}$ | $\overline{X} \pm s_{\overline{X}}$ | $\overline{X} \pm s_{\overline{X}}$ | $\overline{X} \pm s_{\overline{X}}$ | $\overline{X} \pm s_{\overline{X}}$ | $\overline{X} \pm s_{\overline{X}}$ |
| B75-2/1 | 86.40±0.46 | 85.80±0.30 | 84.42±0.18** | 82.66±0.46** | 81.82±0.18** | 81.20±0.46** | 80.86±0.22** |
| B75-4/2 | 83.20±0.32 | 84.22±0.22 | 85.26±0.38** | 83.28±0.26 | 82.66±0,44 | 79.66±0.52** | 80.20±0,18** |
| B75-5/3 | 85.60±0.18 | 81.66±0.16** | 83.44±0.42** | 81.62±0.18** | 82.34±0.56** | 81.32±0.38** | 79.36±0.12** |
| B75-6/4 | 81.26±0.26 | 84.40±0.26** | 82.86±0.22* | 83.44±0.44** | 81.62±0.62 | 80.86±0.40 | 79.44±0.46** |
| B75-8/5 | 82.40±0.54 | 83.26±0.48 | 83.20±0.28 | 82.62±0.55 | 80.36±0.74** | 80.22±0.14** | 80.10±0.32** |
| B75-10/6 | 86.42±0.52 | 85.20±0.36 | 85.44±0.18 | 83.46±0.72** | 81.28±0.19** | 81.10±0.21** | 80.26±0.14** |
| B75-11/7 | 82.86±0.22 | 84.44±0.72* | 83.26±0.36 | 83.22±0.14 | 82.46±0.26 | 81.40±0.88 | 79.68±0.28** |
| B75-13/8 | 83.22±0.46 | 84.62±0.86 | 81.48±0,56* | 82.64±0.90 | 82.32±0.24 | 80.32±0.16** | 79.46±0.22** |
| B75-15/9 | 86.44±0.30 | 86.22±0.92 | 82.66±0.62** | 83.82±0.28** | 81.66±0.12** | 79.66±0.14** | 80.10±0.36** |
| B75-16/10 | 80.22±0.10 | 82.30±0.40** | 83.82±0.74** | 81.20±0.32 | 82.46±0.14** | 80.56±0.24 | 80.32±0.44 |
| B75-18/11 | 81.45±0.72 | 82.46±0.36 | 82.60±0.22 | 80.46±0.40 | 79.98±0.28* | 80.48±0.32 | 76.10±0.52** |
| B75-19/12 | 84.60±0.50 | 85.26±0.72 | 84.66±0.14 | 81.52±0.42 | 82.56±0.34** | 80.88±0.28** | 79.22±0.12** |
| B75-21/13 | 82.26±0.36 | 83.82±0,86 | 82.22±0.96 | 80.32±0.16** | 80.20±0.44** | 78.36±0.42** | 77.16±0.15** |
| B75-23/14 | 83.52±0.28 | 82.66±0.16 | 81.86±0.80* | 80.48±0.26** | 78.36±0.22** | 79.40±0.54** | 78.30±0.21** |
| B75-24/15 | 84.10±0.10 | 83.46±0.42 | 82.24±0.76** | 81.62±0.38** | 80.98±0.14** | 80.20±0.62** | 78.24±0.30** |
| Average | 83.60±0.51 | 83.99±0.35 | 83.29±0.31 | 82.16±0.31 | 81.40±0.32** | 80.37±0.22** | 79.25±0.34** |

*P<0.05; **P<0.01; ***P<0.001.

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# CONTRIBUTIONS TO STUDY OF MULBERRY LEAF USE BY *BOMBYX MORI* LARVAE

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***Abstract***

*In order to assess the efficiency of use of mulberry leaf by the hybrid Băneasa Super of Bombyx mori larvae, during a series of growth, some determinations were made related to the nutritional value and digestibility of the three varieties of leaf. The results showed that the advanced vegetation stage and during each period of growth of silkworm larvae, the mulberry leaf undergoes an aging process, translated by the quality decrease regarding the chemical composition. According to this aspect, in most mulberry leaf nutrients other than cellulose, a continuous decrease in digestibility was observed throughout the growing period. The results show that for the Bombyx mori larvae, from mulberry leaf, an average of 10.38 grams of dry matter is ingested and 5.92 grams of digested dry matter is required for each gram of silky coating, which indicates an efficient conversion into silk of 9.65% of the intake (ECI), and 17% of digest (ECI).*

***Key words****: capitalization, energy, larvae, leaf, mulberry.*

## INTRODUCTION

Besides the continuous improvement of the growth technologies, one of the main concerns of the specialists in sericulture is to produce biological material of high genetic value; thus, *Bombyx mori* larvae will have an increased productive potential, it will be more resilient to the environmental factors and also to diseases, and it will be capable of using nutrients offered by the mulberry leaf to the best of their advantage.
Regarding these aspects, the performances of used larvae in intensive breeding systems have greatly increased, but at the same time, in order for them to be able to reach their full potential, it is necessary to improve all the factors involved in the breeding process. From the multitude of factors that directly influence the growth process of the larvae and the economic results obtained, it is encountered also nutrition.
The quantity and especially the quality of the leaf used in feeding of larvae, directly influence the growth rate, their health and vitality, but also the quantitative and qualitative production of silk. In turn, the quality of the leaf is also influenced by many factors related to the pedo-climate conditions, season, variety of the mulberry, harvesting style and type of storage etc.
The knowledge of the nutritional value and how some factors influence the growth process, as well *Bombyx mori* larvae are harnessed to kill the nutrients in the wormwood, has attracted the attention of a significant number of researchers.
At the end of the last century, Romania was considered an important point on the map of European sericulture. Thus, in its record, Romania can boast in this field with a quite complex literature, as well as with creating new varieties and valuable hybrids of worm, as *Bombyx mori*, all considered as being the result of some decade research work of Romanian specialists.
Thus, we consider appropriate to bring a modest contribution to the study of using the mulberry leaf, derived from indigenous varieties, by larvae of breeds or hybrids created in Romania.

## MATERIALS AND METHODS

The biological material used in the experiments was represented by three groups (L1, L2, and L3) of 150 larvae of *Bombyx mori* belonging to the Romanian hybrid Băneasa Super, obtained through simple crossing between the maternal breed of Japanese type and the paternal one of Chinese type. To make it easier to track, in each batch, the 150 larvae were grouped into three subgroups (repeats) of 50 larvae each, which were grown in paper trays sized according to the age and size of the larvae, in addition, a reserve group was also formed, with 50 larvae raised separately, but under the same conditions, which served to replace the dead larvae in the experimental group.

The growth of the larvae took place in August, in an air-conditioned room, respecting all the microclimate factors.

The 1$^{st}$ group received leaves from selected mulberry hybrid, 2$^{nd}$ group of Eforie variety and 3$^{rd}$ group leaves from the Kokuso 21 variety. Each subgroup, within each batch, was given the same amount of leaf, of which samples were previously collected and subjected to chemical analysis.

Daily and at the same time from each subgroup (repartition) were collected, weighted, and registered the leftover mulberry leaves and the excreta.

The number of leftovers mulberry leaves from each subgroup were summed, the result being than divided to 3, obtaining the average quantity of leftover leaves from the 50 larvae; the value was representative for the entire batch. This value was used to calculate the digestibility coefficients of the nutrients from mulberry leaves. The same system was used to determine the excreta.

From each subgroup were collected samples of leftover, as excreta, which were homogenized in order to obtain an average sample for each batch; those samples were chemically analyzed.

Also, three subgroups of 50 larvae were raised separately, under the same environmental conditions.

During the experiment the larval mortality was observed from each batch and, if was necessary, the dead larvae were immediately replaced with ones from repartition batch.

Also, the groups were weighed at the beginning of growth (after hatching) and at the end (before budding), the difference between the two weights, divided by the number of larvae in each group, representing the body mass accumulated by larvae.

From the separated batch were extracted 10 larvae, whose content was determined in dry matter; thus, multiplying the average dry substance content of larvae, calculated from the separated batches, with the increasing body mass in the experimental batches, the average increasing of body mass of a larva was determined.

After gobbling, 15 cocoons were harvested, from which the silk wrapper was separated, weighed and its dry matter content determined, thus obtaining the average dry wool content of the silk wrapper.

Working methods aimed to determine the nutritive value of the mulberry leaves taking into account the chemical composition and the digestibility of its components.

The chemical composition was determined using the "Proximate Analysis" scheme and the digestibility (approximate digestibility) through *in vivo* method - simple digestibility with a single period control (FAO).

The chemical analyses were done on samples previously dried to 65ºC and grinded. The obtained results were processed, noted, and expressed in both fresh and dried leaves. The collected samples moisture determination was done by drying them into the hot air oven for 4-5 hours at 105ºC (Halga et al., 2005; Regulation EU, 2009).

The ashes content was determined using the incineration method of the samples (Stan & Simeanu, 2005).

To determine the protein content (RP), the Kjeldhal method was used (Stan & Simeanu, 2005).

The fat content (EE) was determined using Soxhlet method; its principle is based on the fat property of dissolving in the organic solvent (such as, petroleum ether) (Stan & Simeanu, 2005).

The raw fiber (RF) was determined by the sample acid-basic hydrolyze, then from the leaf the hydrolysable part is removed, on the filter paper remaining only the cellulose and minerals; the minerals are determined through calcination, so the raw cellulose is calculated by difference (Stan & Simeanu, 2005).

Nitrogen free extract was calculated through difference from fresh leaf or dried one. In the first case, from 100% was decreased the water, protein, fat, cellulose, and ashes percentage. In the second case, from the dry matter percentage

was obtained the raw protein, extract etherate, raw fiber, and ash percentage.
In order to determine the nutritive matter digestibility from mulberry leaves which were administered in silk larvae feeding, it was respected the digestibility principle “in vivo”, with a single control period. There were calculated the digestibility coefficients (DC% = $\frac{\text{Digest}}{\text{Intake}}$ x 100) (Al-Kirshi et al., 2013; Doliș et al., 2017; Doliș et al., 2017; Doliș et al., 2017; Halga et al., 2005; Hirano et al., 1993).
Based on the quantity of administered leaves, the leftover waste, excrete and on the data obtained from chemical analyses firstly, were found out the intake nutrients or ingest (the difference between administered quantities and the leftover) and finally the intake of the nutritive substances or digest (difference between ingest and feces). Expressing in percentage the digest from ingesta, were obtained the digestibility coefficients, which shows how much from the leaves nutrients are digested into the digestive system of the larvae.
Based on the digestible coefficients, there was calculated the digestible content for each nutrient which represents the result between the raw chemical content and the digestible coefficient which was divided to 100.
The obtained values were summed obtaining in the end the total digestible nutrients (TDN) from the mulberry leaves. The fat content was multiplied by 2.25, because it is considered that the fat has 2.25 times more energy than the others intake nutrients.
Also, because the nutritive value was expressed in TDN/kg, and the calculated values were reported to 100 g, it was multiplied with 10 (Doliș et al., 2017; Doliș et al., 2017; Doliș et al., 2017; Halga et al., 2005; Matei, 1995; Stan & Simeanu, 2005).
Regarding the energy value, the working methods were mainly the specific ones used to determine the raw energy (use of specific computation equations and regression coefficients recommended by the OKIT system), digestible (calculation equation recommended for monogastric species), and metabolic (equations recommended for monogastric animals and birds) contain (Halga et al., 2005; Pop et al., 2006; Stan & Simeanu, 2005).
The efficiency of the use of nutrients in the worm leaf by the larvae was expressed by the amount of ingested/digested dry matter required for increasing 1 gram of body mass/weight (silk wrap), respectively by the efficiency of conversion of ingested substances (ECI%)/digested (ECD%) in body mass/weight (Matei, 1995; Rahmathulla et al., 2002; Sarkar, 1993).
The main experimental data obtained were statistically processed through the arithmetic average, variance, the average standard deviation, and the variability coefficient (Cucu et al., 2004; Maciuc et al., 2015; Sandu, 1995).

## RESULTS AND DISCUSSIONS

The values regarding the mulberry chemical composition evolution throughout growth period of the silkworm larvae, were centralized and statistically processed in the Table 1a and Table 1b. For the selected Hybrid (L1), according to these data it is observed that during the experiment the values of the relative humidity of the mulberry leaf had a descending evolution, registering average values between 71.96%, in the period corresponding to the first age of the larvae and 68.24%, in the period when the larvae were in the fifth age. During the entire studied period, the mulberry leaf had a composition, on average, of 29.38 ± 0.676% dry matter and 70.62% water.
For the Eforie hybrid (L2), the average relative humidity of the mulberry leaves during the research was 70.44%, and a decreasing evolution was registered average values between 71.86% (at the first determination corresponding to the first age of the silkworm larvae) and 68.15% (to the last determination when the silkworm larvae are in the fifth age). The dry matter represented 29.56 ± 0.725%.
For the Kokuso 21 hybrid, the average relative humidity of the mulberry leaves during the research was 70.63%, and a decreasing evolution was registered average values between 72.09% (at the first determination corresponding to the first age of the silkworm larvae) and 68.86% (to the last determination when the silkworm larvae are in the $V^{th}$ age). The dry matter represented 29.37±0.575%.

Table 1a. Chemical composition of mulberry leaf in relation to the age of the silkworm larvae and the mulberry variety (%)

| Analysis | water | DM | RP | | EE | |
|---|---|---|---|---|---|---|
| | | | F* | DM** | F | DM |
| Selected hybrid | | | | | | |
| I | 71.96 | 28.04 | 6.13 | 21.86 | 0.83 | 2.96 |
| II | 71.75 | 28.25 | 6.23 | 22.05 | 0.87 | 3.08 |
| III | 71.03 | 28.97 | 6.21 | 21.44 | 1.12 | 3.87 |
| IV | 70.13 | 29.87 | 5.85 | 19.58 | 1.23 | 4.12 |
| V | 68.24 | 31.76 | 6.03 | 18.99 | 1.42 | 4.47 |
| $\overline{X}$ | 70.62 | 29.38 | 6.09 | 20.78 | 1.09 | 3.70 |
| $S_{\overline{x}}$ | - | 0.676 | - | 0.627 | - | 0.294 |
| Cv% | - | 5.147 | - | 6.744 | - | 17.775 |
| Eforie | | | | | | |
| I | 71.86 | 28.14 | 6.23 | 22.14 | 0.85 | 3.02 |
| II | 71.98 | 28.02 | 6.21 | 22.16 | 0.88 | 3.14 |
| III | 70.68 | 29.32 | 6.41 | 21.86 | 1.17 | 3.99 |
| IV | 69.53 | 30.47 | 6.00 | 19.69 | 1.22 | 4.00 |
| V | 68.15 | 31.85 | 6.06 | 19.03 | 1.38 | 4.33 |
| $\overline{X}$ | 70.44 | 29.56 | 6.18 | 20.98 | 1.10 | 3.70 |
| $S_{\overline{x}}$ | - | 0.725 | - | 0.670 | - | 0.260 |
| Cv% | - | 5.486 | - | 7.143 | - | 15.700 |
| Kokuso 21 | | | | | | |
| I | 72.09 | 27.91 | 6.31 | 22.61 | 0.79 | 2.83 |
| II | 71.66 | 28.34 | 6.28 | 22.16 | 0.88 | 3.11 |
| III | 70.31 | 29.59 | 6.23 | 21.05 | 1.14 | 3.85 |
| IV | 70.13 | 29.87 | 6.04 | 20.22 | 1.16 | 3.88 |
| V | 68.86 | 31.14 | 6.15 | 19.75 | 1.25 | 4.01 |
| $\overline{X}$ | 70.63 | 29.37 | 6.20 | 21.16 | 1.04 | 3.54 |
| $S_{\overline{x}}$ | - | 0.575 | - | 0.547 | - | 0.237 |
| Cv% | - | 4.381 | - | 5.782 | - | 14.975 |

* fresh leaves; ** dry matter

Table 1b. Chemical composition of mulberry leaf in relation to the age of the silkworm larvae and the mulberry variety (%)

| Analysis | RF | | NEF | | Ash | |
|---|---|---|---|---|---|---|
| | F | DM | F | DM | F | DM |
| Selected hybrid | | | | | | |
| I | 4.92 | 17.55 | 12.23 | 43.61 | 3.93 | 14.02 |
| II | 4.99 | 17.66 | 12.18 | 43.12 | 3.98 | 14.09 |
| III | 5.18 | 17.88 | 12.43 | 42.90 | 4.03 | 13.91 |
| IV | 5.49 | 18.38 | 13.05 | 43.69 | 4.25 | 14.23 |
| V | 6.04 | 19.02 | 13.59 | 42.79 | 4.68 | 14.73 |
| $\overline{X}$ | 5.32 | 18.10 | 12.71 | 43.22 | 4.17 | 14.20 |
| $s_{\overline{x}}$ | - | 0.271 | - | 0.183 | - | 0.143 |
| Cv% | - | 3.349 | - | 0.947 | - | 2.256 |
| Eforie | | | | | | |
| I | 4.79 | 17.02 | 12.43 | 44.17 | 3.84 | 13.65 |
| II | 4.76 | 16.99 | 12.24 | 43.68 | 3.93 | 14.03 |
| III | 5.26 | 17.94 | 12.30 | 41.95 | 4.18 | 14.26 |
| IV | 5.58 | 18.31 | 13.37 | 43.89 | 4.30 | 14.11 |
| V | 6.15 | 19.31 | 13.58 | 42.64 | 4.68 | 14.69 |
| $\overline{X}$ | 5.31 | 17.88 | 12.78 | 43.29 | 4.19 | 14.15 |
| $S_{\overline{x}}$ | - | 0.434 | - | 0.418 | - | 0.169 |
| Cv% | - | 5.412 | - | 2.163 | - | 2.667 |
| Kokuso 21 | | | | | | |
| I | 4.74 | 16.98 | 12.33 | 44.18 | 3.74 | 13.40 |
| II | 4.88 | 17.22 | 12.34 | 43.54 | 3.96 | 13.97 |
| III | 5.31 | 17.95 | 12.64 | 42.72 | 4.27 | 14.43 |
| IV | 5.44 | 18.21 | 13.09 | 43.83 | 4.14 | 13.86 |
| V | 5.93 | 19.04 | 13.41 | 43.07 | 4.40 | 14.13 |
| $\overline{X}$ | 5.26 | 17.88 | 12.77 | 43.46 | 4.10 | 13.96 |
| $S_{\overline{x}}$ | - | 0.368 | - | 0.261 | - | 0.169 |
| Cv% | - | 4.599 | - | 1.342 | - | 2.713 |

The mulberry leaves humidity influences the silkworm larvae consumption, which, especially in the early stages of life, prefers young leaves with a high percentage of water. In the data presented by different authors, the average humidity of the mulberry leaves varies between 65-75% (Ifrim, 1998; Lazăr & Vornicu, 2013).

Depending on the variety, the dry matter of the mulberry leaves varies between 23.61-27.56% (Matei, 1995).

Compared to the common mulberry (69.80-73%), the selected varieties have a higher water content (Miranda & Takahashi, 1998). The dry matter of the mulberry leaf, harvested in the same period, may register, depending on the variety / hybrid, different values, for example, between 23.61% and 27.56% (Craiciu, 1966).

Also, if in spring the humidity of the mulberry leaf is 71.85-77.81%, then it decreases to 68.42-75.64% in the summer time, and to 64.10-73.64% in autumn (Petkov, 1980).

In group L1, the raw mulberry leaf protein, related to the dry matter, had values between 21.86%, in the age I of the larvae and 18.99%, in the V$^{th}$ age; for the entire studied period the average value for this indicator was 20.78 ± 0.627%. At the end of the growth, the raw protein content of the leaf decreased by 2.86%.

In the second group L2, the raw protein had an average value of 6.18% (20.98± 0.670% from DM). It was noticed a progressive decreasing of the protein content throughout the studied period, which showed that the content was reduced by 3.11% (from 22.14% to 19.03%).

In the third group L3, the raw protein had an average value of 6.20% (21.16±0.547% from DM). It was noticed a progressive decreasing of the protein content throughout the studied period, the content decreasing by 2.86% (from 22.61% to 19.75%).

The protein content in the mulberry leaves may be considered a real indicator of the leaf's quality. The protein intake from mulberry leaves strongly influences both the silkworm larvae growth and development and, especially, the silk production of the larvae. The protein content of mulberry leaves strongly influences both the growth and development of silk larvae and, especially, their silk production.

In the literature, the raw protein from mulberry leaves has the following average values: 32.40% in the spring, 28.21% in the summer, and 24.53% in the autumn (Borcescu, 1966), during the morning 26.80% and evening 29.10% (Mărghitaş, 1995); it also varies between 22.55 and 25.73% depending on the mulberry variety (Matei, 1995).

In the literature, the raw protein from mulberry leaf is estimated to average 6.16% in fresh leaf, 20.97% in dry matter and 24.36% in its organic matter (Tzenov, 1993). The values of the raw leaf protein can vary depending on the season, the time of day, the mulberry variety / hybrid: 32.40% in spring, 28.21% in summer and 24.53% in autumn (Sarkar, 1993), 26.80% in the morning and 29.10% in the evening (Sarkar, 1993), between 22.55% and 25.73% depending on the variety (Craiciu, 1966).

The raw fat of the mulberry leaf recorded an average value of 3.70 ± 0.294%, during the larval growth period registering an increase from 2.96% (in the period corresponding to age I) to 4.47% (in the V$^{th}$ age).

For the second group L2, the fat content from the mulberry leaves had an average value of 1.10% in the fresh leaves, and 3.70% ±0.260 in DM. It is the only nutrient with a high variability, of 15.700%. The fat content increased constantly throughout the silkworm larval growth, from 0.85% to 1.38% when it was expressed in fresh leaves, or 3.2% to 4.33%, when it was reported to dry matter.

For the third group L3, the fat content from the mulberry leaves had an average value of 1.04% in fresh leaves, and 3.54% ± 0.237 in DM. It is the only nutrient with a high variability, of 14.975%.

The fat content increased uniformly throughout the silkworm larval growth, from 0.79% to 1.25% when it was expressed in fresh leaves, or 2.83% to 4.01%, when it was reported to the dry matter. The limits presented by specific literature regarding the fat content in mulberry leaves are 2.85-6.07% (Pop, 1967).

In the selected hybrid, the raw cellulose, related to the dry matter, had an average value of 18.10 ± 0.271%, registering during the larval growth an increase of 1.47% (from 17.55% to 19.02%). For the Eforie hybrid, the raw cellulose had an average value of 5.31% in fresh leaves (17.91 ± 0.434%, when in was reported to DM). Throughout the research, for a month, the raw cellulose increased by 2.29%, from 17.02% to 19.31%.

For the Kokuso 21 hybrid, the raw cellulose had an average value of 5.26% in fresh leaves, 17.88 ± 0.368%, when it was reported to DM. Throughout the research, for a month, the crude cellulose increased by 2.06%, from 16.98% to 19.04%.

The cellulose is highly responsible for aging processes of the mulberry leaves. As the cellulose content grows, the leaf becomes tougher, being more difficult to be consumed by the silkworm larvae. Regarding this aspect, in the silkworm larvae's growth are considered the most valuable mulberry varieties, the ones that have a lower cellulose content.

The values obtained for raw cellulose from mulberry leaves were comparable with the ones from literature. The raw cellulose quota varies between 12.33-14.38% to the common mulberry tree and between 10.43-13.70% to different selected varieties (Rahmathulla et al., 2004). Throughout the mulberry vegetation period, the cellulose content from leaves increased from 14.47 to 21.16% (Maciuc et al., 2015).

In group L1, the non-nitrogen extractive substances, in relation to the dry matter, had an average weight of 43.22 ± 0.183%, during the period of larval growth first registering a decrease, from the first determination to the third or from 43, 61% to 42.90%, followed by an increase until the fourth determination, reaching 43.69%, then decreasing again until the last determination, to 42.79%.

In the second batch L2 the NEF was represented by an average of 43.27 ± 0.418% from the dry matter of the mulberry leaves; the average values decreased from the first determination to the third, from 44.17% to 41.95%, then it increased to the fourth determination, being 43.89%, decreasing to the last analyses to 42.64%.

In the third batch L3, the NEF represented on average 43.46 ± 0.261% from the dry matter of the mulberry leaves; the average values decreased from the first determination to the third, from 44.18% to 42.72%, then was an increasing to the fourth determination, being 43.83%, decreasing to the last analyses to 43.07%.

In the first group L1, the raw ash accounted an average value of 14.20 ± 0.114% of the dry matter in the leaf. During the study, mulberry leaf minerals generally increased progressively (by 0.71%) from the first determination to the fifth, respectively from 14.02% to 14.73%, except for the third analysis, where a lower value was recorded even than in the first determination.

In the second group L2, the raw ash represented in average 4.19% in the fresh leaves and 14.15 ± 0.169% from dry matter. The minerals from the mulberry leaves throughout the research registered a continuous increase from one analysis to another. The average values varied from 3.84% to 4.68% to fresh leaves and from 13.65% to 14.69% from dry matter. An exception was registered to the third determination which had a higher value than the fourth one. The increasing in mineral content from mulberry leaves throughout the research was 1.04%.

In the third batch L3, the ash represented in average 4.10% in the fresh leaves and 13.96 ± 0.169% from dry matter.

The minerals from the mulberry leaves throughout the research registered a continuous increase from one analysis to another. The average values varied from 3.74% to 4.40% in fresh leaves and from 13.40% to 14.13% from dry matter. An exception was registered to the third determination which had a higher value than the fourth one. The increasing in mineral content from mulberry leaves throughout the research was 0.90%.

The data regarding the mineral content, obtained through burning the mulberry leaves are similar to those from specific literature, 9.13-17.38% (Miranda & Takahashi, 1998), 11.52-12.80% (Craiciu, 1966), 8.7-13.15% (Miranda & Takahashi, 1998).

Knowing the raw chemical composition of the mulberry leaf, using the specific calculation equations, it was possible to assess the nutritional value of the mulberry leaf based on its content of raw energy, which was, on average, over the entire studied period, of 1238 Kcal/kg, in fresh leaf, respectively 4216 Kcal/kg, in the dry matter (Table 2).

By recording the quantities of mulberry leaf administered, of unconsumed residues and excreted and determining their chemical composition (Table 3), subsequently it was possible to calculate its digestibility coefficients (Table 4), as the content of digestible substances in the leaf (Table 5).

Table 2. Raw energy of mulberry leaf

| Specification | % | | Caloric equivalent | Kcal/100 g | | Kcal/kg | |
|---|---|---|---|---|---|---|---|
| | * | ** | | * | ** | * | ** |
| L1 | | | | | | | |
| RP | 6.09 | 20.78 | 5.72 | 34.83 | 118.86 | 348.3 | 1188.6 |
| EE | 1.09 | 3.70 | 9.50 | 10.36 | 35.15 | 103.6 | 351.5 |
| CF | 5.32 | 18.10 | 4.79 | 25.48 | 86.70 | 254.8 | 867.0 |
| NEF | 12.71 | 43.22 | 4.17 | 53.00 | 180.23 | 530.0 | 1802.3 |
| | | | | | | 1237 | 4209 |
| L2 | | | | | | | |
| RP | 6.18 | 20.98 | 5.72 | 35.35 | 120.01 | 353.5 | 1200.1 |
| EE | 1.10 | 3.70 | 9.50 | 10.45 | 35.15 | 104.5 | 351.5 |
| CF | 5.31 | 17.88 | 4.79 | 25.43 | 85.65 | 254.3 | 856.5 |
| NEF | 12.78 | 43.29 | 4.17 | 53.29 | 180.52 | 532.9 | 1805.2 |
| | | | | | | 1245 | 4213 |
| L3 | | | | | | | |
| RP | 6.20 | 21.16 | 5.72 | 35.46 | 121.04 | 354.6 | 1210.4 |
| EE | 1.04 | 3.54 | 9.50 | 9.88 | 33.63 | 98.8 | 336.3 |
| CF | 5.26 | 17.88 | 4.79 | 25.20 | 85.65 | 252.0 | 856.5 |
| NEF | 12.77 | 43.46 | 4.17 | 53.25 | 181.23 | 532.5 | 1812.3 |
| | | | | | | 1238 | 4216 |

Table 3. Data necessary for digestibility coefficient calculation

| Larvae age | Specification | Quantity (g) | Chemical composition (%) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | DM | RP | EE | CF | NEF | Ash |
| L1 | | | | | | | | |
| I | Leaves | 15.50 | 28.04 | 6.13 | 0.83 | 4.92 | 12.23 | 3.93 |
| | Leftovers | 5.29 | 62.78 | 13.85 | 2.11 | 14.22 | 25.15 | 7.45 |
| | Excreta | 0.14 | 69.81 | 18.42 | 10.15 | 7.36 | 26.99 | 6.89 |
| II | Leaves | 26 | 28.25 | 6.23 | 0.87 | 4.99 | 12.18 | 3.98 |
| | Leftovers | 8.76 | 59.01 | 13.95 | 2.56 | 14.00 | 24.92 | 3.58 |
| | Excreta | 0.88 | 63.00 | 14.14 | 0.15 | 7.57 | 26.84 | 14.30 |
| III | Leaves | 77.00 | 28.97 | 6.21 | 1.12 | 5.18 | 12.43 | 4.03 |
| | Leftovers | 23.55 | 59.94 | 12.86 | 1.94 | 16.02 | 24.92 | 4.20 |
| | Excreta | 4.02 | 61.12 | 14.89 | 2.83 | 5.08 | 25.47 | 12.85 |
| IV | Leaves | 242.00 | 29.87 | 5.85 | 1.23 | 5.49 | 13.05 | 4.25 |
| | Leftovers | 66.02 | 56.21 | 12.09 | 2.66 | 17.03 | 21.05 | 3.38 |
| | Excreta | 18.98 | 60.85 | 9.77 | 2.34 | 9.22 | 25.18 | 14.34 |
| V | Leaves | 1000 | 31.76 | 6.03 | 1.42 | 6.04 | 13.59 | 4.68 |
| | Leftovers | 267.00 | 56.10 | 10.21 | 1.88 | 14.30 | 25.01 | 4.7 |
| | Excreta | 121.00 | 60.45 | 10.00 | 4.05 | 14.95 | 24.33 | 7.12 |
| L2 | | | | | | | | |
| I | Leaves | 15.5 | 28.14 | 6.23 | 0.85 | 4.79 | 12.43 | 3.84 |
| | Leftovers | 5.11 | 62.58 | 13.91 | 2.01 | 14.02 | 24.48 | 8.16 |
| | Excreta | 0.17 | 78.25 | 14.01 | 14.68 | 15.11 | 26.12 | 8.33 |
| II | Leaves | 26 | 28.02 | 6.21 | 0.88 | 4.76 | 12.24 | 3.93 |
| | Leftovers | 8.01 | 58.85 | 14.33 | 2.16 | 13.89 | 22.06 | 6.41 |
| | Excreta | 0.88 | 74.68 | 12.57 | 3.97 | 14.01 | 29.01 | 15.12 |
| III | Leaves | 77 | 29.32 | 6.41 | 1.17 | 5.26 | 12.30 | 4.18 |
| | Leftovers | 22.65 | 61.54 | 12.34 | 2.62 | 15.86 | 25.66 | 5.06 |
| | Excreta | 4.07 | 64.06 | 15.92 | 2.08 | 9.91 | 24.12 | 12.03 |
| IV | Leaves | 242 | 30.47 | 6.00 | 1.22 | 5.58 | 13.37 | 4.30 |
| | Leftovers | 65.94 | 56.49 | 12.05 | 2.02 | 15.93 | 25.67 | 0.82 |
| | Excreta | 19.99 | 64.44 | 11.98 | 2.18 | 12.01 | 26.21 | 12.06 |
| V | Leaves | 1000 | 31.85 | 6.06 | 1.38 | 6.15 | 13.58 | 4.68 |
| | Leftovers | 269.01 | 57.92 | 9.96 | 2.97 | 11.92 | 24.82 | 8.25 |
| | Excreta | 119.82 | 60.46 | 10.06 | 3.08 | 14.89 | 24.02 | 8.41 |
| L3 | | | | | | | | |
| I | Leaves | 15.5 | 27.91 | 6.31 | 0.79 | 4.74 | 12.33 | 3.74 |
| | Leftovers | 4.98 | 67.99 | 15.87 | 2.03 | 14.62 | 26.44 | 9.03 |
| | Excreta | 0.16 | 70.02 | 20.97 | 8.88 | 4.12 | 27.91 | 8.14 |
| II | Leaves | 26 | 28.34 | 6.28 | 0.88 | 4.88 | 12.34 | 3.96 |
| | Leftovers | 8.01 | 62.18 | 13.01 | 2.02 | 15.09 | 20.98 | 11.08 |
| | Excreta | 1.02 | 65.52 | 15.42 | 2.11 | 5.81 | 29.01 | 13.17 |
| III | Leaves | 77 | 29.59 | 6.23 | 1.14 | 5.31 | 12.64 | 4.27 |
| | Leftovers | 23.82 | 62.07 | 9.85 | 3.01 | 16.08 | 25.12 | 8.01 |
| | Excreta | 3.97 | 63.11 | 14.98 | 2.02 | 6.01 | 25.93 | 14.17 |
| IV | Leaves | 242 | 29.87 | 6.04 | 1.16 | 5.44 | 13.09 | 4.14 |
| | Leftovers | 66.94 | 60.01 | 10.91 | 1.91 | 16.68 | 23.55 | 6.96 |
| | Excreta | 20.31 | 62.73 | 13.18 | 2.91 | 8.19 | 28.44 | 10.01 |
| V | Leaves | 1000 | 31.14 | 6.15 | 1.25 | 5.93 | 13.41 | 4.4 |
| | Leftovers | 269.01 | 59.09 | 11.51 | 1.65 | 14.01 | 24.89 | 7.03 |
| | Excreta | 121.94 | 61.12 | 9.81 | 3.83 | 13.49 | 25.18 | 8.81 |

Table 4. Specific digestibility coefficients of Băneasa Super hybrid

| The larvae age | DM | RP | EE | CF | NEF |
|---|---|---|---|---|---|
| L1 | | | | | |
| I | 90.47 | 88.14 | 16.96 | 0.96 | 92.99 |
| II | 74.52 | 68.73 | 31.58 | 6.20 | 75.99 |
| III | 70.00 | 65.86 | 71.94 | 5.42 | 72.34 |
| IV | 71.95 | 69.97 | 63.61 | 14.32 | 72.97 |
| V | 56.41 | 63.38 | 46.62 | 18.59 | 57.41 |
| I-V | 59.04 | 64.64 | 49.57 | 18.07 | 61.42 |
| L2 | | | | | |
| I | 88.57 | 90.66 | 14.09 | 1.53 | 93.43 |
| II | 75.81 | 76.31 | 37.46 | 1.36 | 81.96 |
| III | 70.29 | 69.73 | 72.46 | 11.92 | 73.17 |
| IV | 66.15 | 63.57 | 73.11 | 19.96 | 66.04 |
| V | 56.81 | 64.34 | 36.48 | 39.39 | 58.31 |
| I-V | 58.06 | 64.78 | 45.41 | 37.07 | 60.87 |
| L3 | | | | | |
| I | 88.09 | 82.11 | 33.64 | 0.00 | 92.48 |
| II | 72.01 | 73.37 | 67.91 | 1.33 | 80.63 |
| III | 68.68 | 75.73 | 50.12 | 7.66 | 72.54 |
| IV | 60.33 | 63.40 | 61.34 | 16.80 | 63.70 |
| V | 51.11 | 60.83 | 42.07 | 23.89 | 54.27 |
| I-V | 53.77 | 62.45 | 45.35 | 23.05 | 57.44 |

By recording the quantities of the worm leaf administered, the non-consumed and excreted residues and also determining their chemical composition (Table 3), its digestibility coefficients could subsequently be calculated (Table 4) and also the content of digestible substances in the leaf (Table 5).

Following the complex phenomenon of digestion, nutrients are transformed into simple substances, which can thus be absorbed through the epithelium of the digestive tract, at different levels, thus being retained in the organism of silk larvae, representing the difference between the number of substances ingested through food and the number of appropriate substances found in droppings. Because not all the substances found in excrement are of dietary origin, some of them are of endogenous origin, which can be obtained by this difference, indicating only apparent digestibility. If you admit the fact that at *Bombyx mori* excretions are also found in their excrement, which complicates the establishment of the digestibility of nutrients in the wormwood even more accurately, the use of the approximate digestibility term seems to be more correct ((Al-Kirshi et al., 2013; Doliș et al., 2017; Doliș et al., 2017; Doliș et al., 2017; Halga et al., 2005; Hirano et al., 1993; Sabhat et al., 2011).

Throughout the study period, the dry matter digestibility of the mulberry leaf had a digestibility between 53.77%, and 59.04%, the highest values were recorded in larvae from age I (88.09 - 90.47%), after which, by the end of the larval period, there was a decrease between 31.76 and 36.98 percent.

In the literature, the main explanation for reducing the digestibility of nutrients from the worm leaf as a whole, during the growth period of the silk larvae, would be as seen from the data in Table 1, precisely the qualitative degradation of the leaf, in terms of chemical composition (Sarkar, 1993). Digestibility of the dry substance from the worm leaf decreases from 71.07% in age I, to 39.99% (for male larvae), 48.26% (for female larvae) in age V (Rath et al., 2003). The worm leaf administered to the larvae of the fifth age has an approximate digestibility between 27.99% and 32.44% (Rahmathulla et al., 2002).

The raw protein had a digestibility coefficient for the entire period studied between 60.83 and 64.64%. The digestibility of the raw protein was progressively reduced during the studied period, by 21.28 - 26.32%, from 82.11 - 90.66%, in the first larval age, to 60.83 - 64.34%, in the last one. The high digestibility of age I could be explained by the rich content in amides, simple nitrogenous substances, which are found in the young leaf and which are digested much easier than the protein nitrogenous substances, which have the weight in the old leaf.

In the literature, for raw leaf protein, the value of digestibility coefficients is between 69.21% and 78.92% (Borcescu, 1966), 60.06% and 74.69% (Petkov, 1980), 71.62% and 93.48% (Matei, 1995).

In group 1, the gross fat of the mulberry leaf had the minimum value of digestibility, of 16.96%, in the larvae of age I, and maximum, of 71.94%, in the larvae of age III.

For the second batch L2, the raw fat from the worm leaf had the minimum digestibility value of 14.09%, in the larvae of the first age and maximum of 73.11%, in the larvae of the fourth age.

For the third batch, the raw fat from the worm leaf had the minimum digestibility value of 33.64%, in the larvae of the first age and maximum of 67.91%, in the larvae of the second age.

The results of the digestibility tests regarding the raw fat in the worm leaf are generally inconclusive, as many of these can come from the intestine of the larvae and not from the leaf, which is why, we cannot speak of a determination of the digestibility of the fat itself but of the "ethereal extract", which also contains very large quantities of pigments. Thus, the big differences regarding the evolution of the digestibility of the raw fat during the studied period could be explained.

In the literature, the values of the digestibility coefficient for raw fat between 63.28% and 74.19% (Mărghitaş, 1995).

At the first batch L1, throughout the larvae period, the digestibility of raw mulberry leaf cellulose was 18.07%, the mean value being 9.10 ± 3.206%, being very low in age I, 0.96%, after which it increased progressively, with over 17%, reaching the end of the studied period up to the value of 18.07%.

For the second batch L2, during the whole larval period, the digestibility of the raw cellulose from the mulberry leaf was 37.07%, being very low in age I, 1.53%, after which it increased progressively, by over 17%, reaching the end of the period studied up to the value of 39.39%.

For the third batch L3, during the whole larval period, the digestibility of the raw cellulose from the mulberry leaf was 23.05%, being null in age I, after which it increased progressively, reaching the end of the period studied up to the value of 23.89%.

This increase in the digestibility of raw cellulose, as the larvae grow older, is in line with the development of the enzymatic equipment in their digestive tract. Thus, if at age I, in the digestive tract of the larvae, the enzymes involved in the process of cellulose digestion are as non-existent, then they gradually increase, reaching the peak at age V, at which point the weight of raw cellulose from the worm leaf it is also bigger. This aspect, however, negatively influences the digestibility of the raw leaf protein, which during the same period, is experiencing a reduction.

At the beginning of the last century, some authors found that the leaf cellulose passes undigested through the digestive tract of the larvae and later it was concluded that this substance has a digestibility of approx. 20% (Doliş, 2008; Doliş et al., 2017; Doliş et al., 2017; Doliş et al., 2017). Recently, some authors state that in the first two ages, raw cellulose would not be digested, but only from the third (8%), its digestibility reaches 21.13% in the third period (Doliş et al., 2017; Doliş et al., 2017; Doliş et al., 2017; Matei, 1995).

In the first batch L1, the non-nitrogenous extractive substances from the mulberry leaf had a digestibility throughout the studied period of 61.42%, with an average of 74.34 ± 5.670%, the digestibility coefficients registering decreasing values, from 92.99%, in the case of age larvae I, at 57.41%, in the case of those of the V$^{th}$ age.

For the second batch L2, the NEF from the leaf had a digestibility over the entire studied period of 60.87%, the digestibility coefficients registering decreasing values, from 93.43%, for the age I of larvae, at 58.31%, for the fifth age.

For the third batch L3, the NEF from the leaf had a digestibility over the entire studied period of 57.44%, the digestibility coefficients registering decreasing values, from 92.48%, for the larvae of age I, at 54.27%, in the case of those of fifth age.

According to Matei (1995), for the extracts not recorded from the leaves, the digestibility coefficients for the whole larval period record average values between 63.40% and 94.97%.

From the data in Table 3 it can be observed that the digestibility of the nutrients of the worm leaf showed a medium variability for dry matter and raw protein, high for raw fat and raw cellulose.

Knowing the value of digestibility coefficients, it was possible to calculate the digestible content for each nutrient separately, then the content of digestible substances in the leaf (Table 5).

Thus, in first group L1, when the report was made on fresh leaves, 139 g TSD/kg were obtained and when the report was made on dry matter from the mulberry leaf, its nutritional value was 474 g TSD/kg.

For the second batch L2, when the report was made for the fresh leaf, 134.81 g of Total Digestive Substance/kg were obtained, and when the report was made over the dry substance from the leaf of the mulberry, its nutritional value was 459.11 g TDS/kg.

For the third batch L3, the content of digestible substances of the leaf, so when the report was made to the fresh leaf, 148.75 g of Total Digestive Substance/kg were obtained, and when the report was made on the dried substance from the leaf of the mulberry, its nutritional value was 503.50 g TDS/kg.

The determination of the digestible energy content of the worm leaf administered in the feed of silk larvae was made based on the relative digestible content of the nutrients contained in it, using the calorific equivalents recommended for monogastric animal species (Table 6).

For the first group L1, the digestible energy from fresh leaf content was 639 Kcal / kg, and 2173 Kcal / kg for dry matter.

For the second group L2, the digestible energy content was 639 Kcal/kg in fresh leaf and 2173 Kcal/kg for dry matter.

For the third group L3, the digestible energy content was 619.94 Kcal/kg in fresh leaf and 2112.35 Kcal/kg for dry matter.

Table 5. Digestive content of mulberry leaf

| Specification | Raw chemical composition % | | Digestibility coefficients | Digestive content % | | G Total Digestive substance /kg | |
|---|---|---|---|---|---|---|---|
| | * | ** | | * | ** | * | ** |
| L1 | | | | | | | |
| RP | 6.09 | 20.78 | 64.64 | 3.94 | 13.43 | 39.40 | 134.30 |
| EE | 1.09 | 3.70 | 49.57 | 0.54 | 1.83 | 12.15 | 41.18 |
| CF | 5.32 | 18.10 | 18.07 | 0.96 | 3.27 | 9.60 | 32.70 |
| NEF | 12.71 | 43.22 | 61.42 | 7.81 | 26.55 | 78.10 | 265.50 |
| Total | | | | | | 139 | 474 |
| L2 | | | | | | | |
| RP | 6.18 | 20.98 | 64.78 | 4.00 | 13.59 | 40.03 | 135.91 |
| EE | 1.10 | 3.70 | 45.41 | 0.50 | 1.68 | 11.24 | 37.80 |
| CF | 5.31 | 17.88 | 37.07 | 1.97 | 6.63 | 19.68 | 66.28 |
| NEF | 12.78 | 43.29 | 60.87 | 7.78 | 26.35 | 77.79 | 263.51 |
| Total | | | | | | 149 | 504 |
| L3 | | | | | | | |
| RP | 6.20 | 21.16 | 62.45 | 3.87 | 13.21 | 38.72 | 132.14 |
| EE | 1.04 | 3.54 | 45.35 | 0.47 | 1.61 | 10.61 | 36.12 |
| CF | 5.26 | 17.88 | 23.05 | 1.21 | 4.12 | 12.12 | 41.21 |
| NEF | 12.77 | 43.46 | 57.44 | 7.34 | 24.96 | 73.35 | 249.63 |
| Total | | | | | | 135 | 459 |

* Reported to the fresh leaves; ** reported to DM

Table 6. Digestive energy of mulberry leaf

| Specification | Digestive content % | | Caloric equivalent (Kcal/g) | Kcal/kg | |
|---|---|---|---|---|---|
| | * | ** | | * | ** |
| L1 | | | | | |
| RP | 3.94 | 13.43 | 5.78 | 227.73 | 776.25 |
| EE | 0.54 | 1.83 | 9.42 | 50.87 | 172.39 |
| CF | 0.96 | 3.27 | 4.40 | 42.24 | 143.88 |
| NEF | 7.81 | 26.55 | 4.07 | 317.87 | 1080.59 |
| | | | | 639 | 2173 |
| L2 | | | | | |
| RP | 4.00 | 13.59 | 5.78 | 231.20 | 785.50 |
| EE | 0.50 | 1.68 | 9.42 | 47.10 | 158.26 |
| CF | 1.97 | 6.63 | 4.40 | 86.68 | 291.72 |
| NEF | 7.78 | 26.35 | 4.07 | 316.65 | 1072.45 |
| | | | | 682 | 2308 |
| L3 | | | | | |
| RP | 3.87 | 13.21 | 5.78 | 223.69 | 763.54 |
| EE | 0.47 | 1.61 | 9.42 | 44.27 | 151.66 |
| CF | 1.21 | 4.12 | 4.40 | 53.24 | 181.28 |
| NEF | 7.34 | 24.96 | 4.07 | 298.74 | 1015.87 |
| | | | | 620 | 2112 |

* Reported to the fresh leaves; ** reported to DM

The calculation of the metabolic energy of leaf administered in the feed of silk larvae was done by multiplying the digestible content of each nutrient with the energy equivalents recommended for monogastric (pigs) animal species. Considering, however, the specificity of the silkworm's digestion, and also the similarity with the digestion of the birds, for the estimation of the metabolic energy from the leaf, the energetic equivalents recommended for the birds were used (Table 7).

For the first group L1, the average metabolic energy content of fresh mulberry leaf was 597 kcal/kg, when the recommended energy coefficients for pigs were used, and 590 kcal/kg, when the recommended coefficients for birds were used. In relation to the dry matter in the leaf, the metabolic energy content was on average 2044 kcal/kg, when the recommended energy coefficients were used for pigs and 2007 kcal/kg, when the recommended coefficients for birds were used.

For the second group L2, the average content in metabolic energy from the fresh mulberry leaf was 630.24 kcal/kg, when the recommended energy ratios for pigs were used, as 630.33 kcal/kg, when the recommended coefficients for birds were used. In relation to the dry matter of the leaf, the content in metabolic energy was on average 2134.04 kcal/kg, when the recommended energy coefficients for pigs were used and 2133.59 kcal/kg, when the recommended coefficients for birds were used.

For the third group L3, the average content in metabolic energy from the fresh mulberry leaf was 576.95 kcal/kg, when the recommended energy ratios for pigs were used, respectively 571.18 kcal/kg, when the recommended coefficients for birds were used. In relation to the dry matter of the leaf, the content in metabolic energy was on average 1965.69 kcal/kg, when the recommended energy coefficients for pigs were used and 1945.78 kcal/kg, when the recommended coefficients for birds were used.

In order to determine the efficiency of use of the nutrients in worm leaf by the silk larvae, except for the intake and digestion, which were calculated during the course of the digestibility tests, it was necessary to determine the average growth rate of the larvae and the mass of the silk shell. The data necessary for calculating the efficiency of the use of the worm leaf by the larvae, as well as the results obtained in this respect, were centralized in Table 8. The data showed in this table indicate the fact that for *Bombyx mori* larvae, 10.38 grams of dry matter ingested from mulberry fruit and 5.92 grams of dry matter digested are required for each gram of silky coating, resulting an efficient conversion of the intake (ECI) in silk of 9.65% and of digestion (ECI) of 17%.

Table 7. Metabolic energy of mulberry leaf depending on the hybrid

| Specification | Digestive content % | | Caloric equivalent (Kcal/g) | | Kcal/kg | | | |
|---|---|---|---|---|---|---|---|---|
| | * | ** | | | * | | ** | |
| | | | swine | birds | swine | birds | swine | birds |
| L1 | | | | | | | | |
| RP | 3.94 | 13.43 | 5.01 | 4.26 | 197.39 | 167.84 | 684.93 | 572.12 |
| EE | 0.54 | 1.83 | 8.93 | 9.50 | 48.22 | 51.30 | 163.42 | 173.85 |
| CF | 0.96 | 3.27 | 3.44 | 4.23 | 33.02 | 40.61 | 112.49 | 138.32 |
| NEF | 7.81 | 26.55 | 4.08 | 4.23 | 318.65 | 330.36 | 1083.24 | 1123.07 |
| | | | | | 597 | 590 | 2044 | 2007 |
| L2 | | | | | | | | |
| RP | 4.00 | 13.59 | 5.01 | 4.26 | 200.40 | 170.40 | 680.86 | 578.93 |
| EE | 0.50 | 1.68 | 8.93 | 9.50 | 44.65 | 47.50 | 150.02 | 159.60 |
| CF | 1.97 | 6.63 | 3.44 | 4.23 | 67.77 | 83.33 | 228.07 | 280.45 |
| NEF | 7.78 | 26.35 | 4.08 | 4.23 | 317.42 | 329.09 | 1075.08 | 1114.61 |
| | | | | | 630 | 630 | 2134 | 2139 |
| L3 | | | | | | | | |
| RP | 3.87 | 13.21 | 5.01 | 4.26 | 193.89 | 164.86 | 661.82 | 562.75 |
| EE | 0.47 | 1.61 | 8.93 | 9.50 | 41.97 | 44.65 | 143.77 | 152.95 |
| CF | 1.21 | 4.12 | 3.44 | 4.23 | 41.62 | 51.18 | 141.73 | 174.28 |
| NEF | 7.34 | 24.96 | 4.08 | 4.23 | 299.47 | 310.48 | 1018.37 | 1055.81 |
| | | | | | 577 | 571 | 1966 | 1946 |

Table 8. Efficiency use of mulberry leaf by the Băneasa Super hybrid

| Specification | L1 | | L2 | L3 | Average |
|---|---|---|---|---|---|
| Average body mass gained during the whole larvae stage (g) | Living larvae | 5.099 | 5.039 | 5.075 | 5.071 |
| | Dry matter | 0.925 | 0.919 | 0.919 | 0.921 |
| Silky shell mass (g Dry Matter) | 0.399 | | 0.398 | 0.401 | 0.399 |
| Dry Matter of ingested leaf (g) | 4.288 | | 4.231 | 3.918 | 4.146 |
| Dry Matter of digested leaf (g) | 2.532 | | 2.457 | 2.107 | 2.365 |
| Ingested Dry Matter/Body mass Dry Matter (g) | 4.633 | | 4.607 | 4.265 | 4.502 |
| Dry matter ingested/ Body mass Dry Matter (g) | 2.736 | | 2.675 | 2.293 | 2.568 |
| Dry matter ingested/Silky shell Dry Matter (g) | 10.749 | | 10.636 | 9.767 | 10.384 |
| Dry matter digested/ Silky shell Dry Matter (g) | 6.347 | | 6.175 | 5.252 | 5.925 |
| CEI body mass % | 21.580 | | 21.708 | 23.450 | 22.246 |
| CED body mass % | 36.550 | | 37.387 | 43.610 | 39.182 |
| CEI silky shell % | 9.300 | | 9.402 | 10.240 | 9.647 |
| CED silky shell % | 15.760 | | 16.193 | 19.040 | 16.998 |

The data obtained from the experience performed, regarding the efficiency of the use of the mulberry leaf by the *Bombyx mori* larvae, are similar with those presented in the literature (Matei, 1995; Rahmathulla et al., 2002; Rath et al., 2003; Sarkar, 1993; Tzenov, 1993).

## CONCLUSIONS

From the aspects mentioned in the paper, the following conclusion may be drawn:
Regarding the dry matter from the mulberry leaves, depending on the type, the average values were: RP = 20.78 - 21.16%; EE = 3.54 - 3.70%; RF = 17.88 - 18.10%; NEF = 43.22 - 43.42% and ash = 13.96 - 14.20%.
While vegetation degree advanced during each growth period of silkworm larvae, the mulberry leaf ages and its quality regarding the chemical composition is decreasing. During the period of 30 days of research, it was noticed a moisture decreasing by 3.23 - 3.72% and of the RP with 2.86 - 3.11% and at the same time a CF increasing by 1.12% - 2.29%.
The leaves nutrients digestibility had an average of 53.77 - 58.06%. The dry matter digestibility decreased by 31.76 - 36.98%.
Digestibility coefficients of the RP (62.45 - 71.22%) and of the NEF (57.44 - 74.34%) from the mulberry leaves decreased during the study by 21.28 - 26.03% (RP) and 35.12 - 38.21% (NEF).
The CF digestibility, very low at the beginning, increased progressively till the fifth larvae stage when it was 18.59–39.39%.
Nutritional value of the mulberry leaves was 459.11 - 474 g TDN/ kg DM.
Throughout the studied period, the energy of leaf was on average 1237 - 1238 kcal/kg, in the fresh leaf, and 4209 - 4216 kcal/kg, in dry substance. For the fresh leaf, the content of digestible energy was 620 - 682 kcal/kg, and in the case of dry matter, 2112 - 2308 kcal/kg. In relation to the dry matter of the leaf, the content of metabolic energy was on average 1966 - 2134 kcal/kg, when the recommended energy coefficients for pigs were used, and 1946 - 2139 kcal/kg, when the recommended coefficients for birds were used.
For each gram of silk wrap, 9.77 - 10.75 grams of dry matter ingested from the mulberry tree are required, respectively 5.25 - 6.35 grams of digested dry substance, resulting an efficiency of conversion of ingestion (CEI) into silk of 9.30 - 10.24%, and also of digestion (CEI) of 15.76 - 19.04%.

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# RESEARCH ON THE IMPORTANCE OF UREA AT DAIRY COWS AND ITS DYNAMICS

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***Abstract***

*The present research proposed to present the dynamics of urea in Holstein cows milk (as ml/dl), in order to evidence and optimize the main factors affecting this parameter. Large amount of data, including Holstein farms along the whole country was included in the study. Statistical results proved that the feeding level of protein is a main factor influencing milk urea content. Protein intake is highly related to milk market price, therefore large differences in milk urea were revealed between hot and cold season (from 40 mg/dl in the hot season to 18 mg/dl in the cold season). Relation between milk urea level, reproduction indices and the productive life was also studied. The most affected indicator by urea excess was the calving – interval (39 mg/dl – CI of 435 days). As a result, we recommend an interval between 22 – 32 mg/dl, in order to optimize milk yield and quality, as well as the reproductive parameters and longevity.*

***Key words****: dairy cows, Holstein, milk production, reproduction, urea.*

## INTRODUCTION

Milk urea level is a high valuable indicator, providing relevant information, an indicator that can be easily obtained from milk analysis. Since the dairy sector arouses worldwide interest, large amount of information is available on this subject. Several factors are influencing milk urea level: breed (Rodriguez et al., 1997), parity (Broderick & Clayton, 1997), body weight (Kohn et al., 2001), milk yield (Godden et al., 2001), fat and protein content, DIM, and month of the year. However, feeding is the main influencing factor, more precisely the protein intake, therefore protein content from the feeding should be optimized.

If milk urea level has a low value, we can conclude that the cow is poorly fed, leading to incomplete expression of the genetic potential of animals. Otherwise, if milk urea level of is too high, we deduce that the cows will be exhausted prematurely, that the reproduction parameters will be affected, but first of all that the feed is not economical.

Also, milk urea level is used to monitor the nutritional status of dairy cows and reduce nitrogen emissions to the environment (Samore et al., 1996; Spek et al., 2013). This paper aims to identify optimal values that combine the production with the welfare of the animals, in an increased economic efficiency.

## MATERIALS AND METHODS

The present paper is based on large amount of data, obtained from Holstein Breeding Association (ACV Holstein Ro) official control production (COP). Urea content has been measured as mg/dl.

Therefore, about 10.300 Holstein dairy cows from 15 herds were been included in the survey. Lactation stage, rank, seasonal effect has been included in the research.

The season is an essential factor affecting milk urea content, since milk market is strongly correlated with seasonal milk yield in Romania. Statistical procedure used standard methods. Therefore, milk urea level (MUL) has been correlated with productive and reproductive parameters (milk yield – L305, kilograms of fat on standard lactation - FY, kilograms of protein on standard lactation - PY, calving interval - CI, service life - SL), using the correlation formula:

$$Correl(X,Y)=\frac{\sum(x-\bar{x})(y-\bar{y})}{\sqrt{\sum(x-\bar{x})^2\sum(y-\bar{y})^2}}$$

## RESULTS AND DISCUSSIONS

In order to analyze and interpret all the hypotheses the results obtained from 15 farms, located all over the country were statistically processed. In order to determine the correlation, the annual averages for the year 2021 of the milk urea level for all farms were calculated.

First of all, we will present the results obtained from the correlation of MUL with the productive parameters.

Table 1. Statistical analysis between MUL and milk yield (L305)

| Specification | Average of MUL | Milk yield - L305 |
|---|---|---|
| Farm 1 | 40 | 8369 |
| Farm 2 | 25 | 10523 |
| Farm 3 | 22 | 10643 |
| Farm 4 | 31 | 9801 |
| Farm 5 | 28 | 8771 |
| Farm 6 | 25 | 13098 |
| Farm 7 | 26 | 9838 |
| Farm 8 | 25 | 9889 |
| Farm 9 | 31 | 10469 |
| Farm 10 | 19 | 8125 |
| Farm 11 | 14 | 8358 |
| Farm 12 | 37 | 9560.1 |
| Farm 13 | 25 | 12678 |
| Farm 14 | 18 | 7599 |
| Farm 15 | 30 | 10789 |
| X + Sx | 26.4 ± 1.77 | 9900.69 ± 405.75 |
| S | 6.87 | 1571.48 |
| V% | 26.04% | 15.87% |
| r | 0.12 | |

Based on this table we can extract the following information: Farm 1, with the highest average of MUL – 40 has an average of 8,369 kg of milk and Farm 11, with the lowest average of MUL – 14 has recorded 8,538 kg of milk average on standard lactation. Almost the same quantity of milk, but with a difference of 26 mg/dl of MUL. Of course, there are many factors that can influence the milk yield, not only the MUL.

There are 4 farms that registered the same average of MUL (Farm 2, Farm 6, Farm 8 and Farm – 13, 25 mg/dl), but with significant differences regarding milk yield, 3,209 kg between Farm 6 and Farm 8. The correlation between MUL and milk yield is very weak, r = 0.12. According to others research milk yield is positively related to MUL (Godden et al., 2001), but in the same time others reported a negative relationship (Ismail et al., 1996; Trevaskis and Fulkerson, 1999).

In table 2 it is obvious that the highest FY average, 593 kg is registered at Farm 13, farm that has an average of 25 MUL. Very interesting is the fact that the lowest average of FY is found at Farm 5, farm with a higher average of MUL that Farm 13, 28 mg/dl. Relating only on this facts we will be tempted to consider that between MUL and FY is no correlation, or a negative one. Continuing study, based on the correlation result, r = 0.29, we find something quite the opposite. The correlation between these two parameters is a positive one, of course, a weak one, but however more correlation that MUL and milk yield. Positive correlation between MUL and FY were reported in others studies (Wood et al., 2003).

Table 2. Statistical analysis between MUL and FY

| Specification | Average of MUL | FY |
|---|---|---|
| Farm 1 | 40 | 448 |
| Farm 2 | 25 | 396 |
| Farm 3 | 22 | 428 |
| Farm 4 | 31 | 396 |
| Farm 5 | 28 | 324 |
| Farm 6 | 25 | 492 |
| Farm 7 | 26 | 452 |
| Farm 8 | 25 | 376 |
| Farm 9 | 31 | 502 |
| Farm 10 | 19 | 388 |
| Farm 11 | 14 | 380 |
| Farm 12 | 37 | 470 |
| Farm 13 | 25 | 593 |
| Farm 14 | 18 | 340 |
| Farm 15 | 30 | 385 |
| X + Sx | 26.4 ± 1.77 | 424.66 ± 18.05 |
| S | 6.87 | 69.92 |
| V% | 26.04% | 16.46% |
| r | 0.29 | |

Table 3. Statistical analysis between MUL and PY

| Specification | Average of MUL | PY |
|---|---|---|
| Farm 1 | 40 | 337 |
| Farm 2 | 25 | 348 |
| Farm 3 | 22 | 373 |
| Farm 4 | 31 | 336 |
| Farm 5 | 28 | 303 |
| Farm 6 | 25 | 424 |
| Farm 7 | 26 | 350 |
| Farm 8 | 25 | 318 |
| Farm 9 | 31 | 354 |
| Farm 10 | 19 | 303 |
| Farm 11 | 14 | 282 |
| Farm 12 | 37 | 326 |
| Farm 13 | 25 | 463 |
| Farm 14 | 18 | 264 |
| Farm 15 | 30 | 366 |
| X + Sx | 26.4 ± 1.77 | 343.13 ± 13.23 |
| S | 6.87 | 51.23 |
| V% | 26.04% | 14.93% |
| r | 0.22 | |

Also, in the case of the correlation between MUL and PY, as between MUL and FY we obtain a weak, but positive result, r = 0,22. Same results was obtained in other study (Stoop et al., 2007).

After the analysis of association between MUL and productive parameters the work continues by studying the relationship between MUL and reproductive parameters, and also regarding the service life (the age of reforming the cows). A negative association between reproductive parameters and high dietary protein levels have been reported by many studies (Butler et al., 1996; Chaveiro et al., 2011).

The reproductive parameter studied was calving – interval, starting from the premise that a higher MUL will determine a higher CI. A significant negative effect of MUL on the fertility at high-yielding dairy cattle was reported in a study (Siatka et al., 2020). In the same time, previous studies analyzing the correlation between MUL and conception find out that there is a negative effect of high MUL at or only after AI (Butler et al., 1996; Melendez et al., 2000).

Table 4. Statistical analysis between MUL and CI

| Specification | Average of MUL | CI |
|---|---|---|
| Farm 1 | 40 | 449 |
| Farm 2 | 25 | 424 |
| Farm 3 | 22 | 399 |
| Farm 4 | 31 | 423 |
| Farm 5 | 28 | 405 |
| Farm 6 | 25 | 390 |
| Farm 7 | 26 | 396 |
| Farm 8 | 25 | 392 |
| Farm 9 | 31 | 400 |
| Farm 10 | 19 | 412 |
| Farm 11 | 14 | 415 |
| Farm 12 | 37 | 416 |
| Farm 13 | 25 | 417 |
| Farm 14 | 18 | 407 |
| Farm 15 | 30 | 414 |
| X + Sx | 26.4 ± 1.77 | 410.60 ± 3.89 |
| S | 6.87 | 15.08 |
| V% | 26.04% | 3.67% |
| r | 0.44 | |

The present study provides the expected results. Between MUL and CI is a reasonable correlation, r = 0.44. Based on these results we can say that MUL has a bigger influence on CI than on PY for example, a double one, unfortunately, a higher CI is not desired. Also, a negative effect of MUL on conception rate at first service at dairy cows was reported (Hojman et al., 2004). Similar results were obtained in by Butler et al., 1996, they concluded that concentrations of MUL> 19 mg/dl are associated with decreased pregnancy rate. In our study, the higher CI is registered at the Farm 1, 449 days. This result is in association with a very high MUL 40 mg/dl. Farm 6, that registered the shortest CI, have a MUL of 25 mg/dl. Despite these facts there are still some uncertainly information regarding the effect of high urea on reproduction occurs mostly during the period before, surrounding, or after AI (Hammon et al., 2000; Leroy et al., 2008b). In the same time, we have to admit that are studies that found that the protein intake has no effect on fertility or conception, but is highly correlated with milk and blood urea.

Table 5. Statistical analysis between MUL and SL

| Specification | Average of MUL | SL |
|---|---|---|
| Farm 1 | 40 | 1219 |
| Farm 2 | 25 | 1325 |
| Farm 3 | 22 | 1383 |
| Farm 4 | 31 | 1518 |
| Farm 5 | 28 | 1466 |
| Farm 6 | 25 | 1205 |
| Farm 7 | 26 | 1801 |
| Farm 8 | 25 | 1459 |
| Farm 9 | 31 | 1404 |
| Farm 10 | 19 | 1922 |
| Farm 11 | 14 | 2973 |
| Farm 12 | 37 | 1612 |
| Farm 13 | 25 | 1569 |
| Farm 14 | 18 | 2391 |
| Farm 15 | 30 | 1826 |
| X + Sx | 26.4 ± 1.77 | 1671.53 ± 122.23 |
| S | 6.87 | 473.38 |
| V% | 26.04% | 28.32% |
| r | -0.65 | |

The lifetime of an animal is defined by the biological longevity and the productive longevity (Gavrila et al., 2015).

The results are very clear, MUL is undoubtedly associated with the service life. The correlation is negative and high, r = -0.65, from all parameters studied this is the most influenced one by the MUL. Of course, other factors are also contributing in determining the time when the cows are reformed, but surely MUL by early exhaustion of the body is implicated.

Another object of study was represented by urea level dynamics depending on the season. In some cases, it have been notice huge differences between the MUL registered in a summer month compared to a winter one, for example in Farm 13 in July MUL was 40 mg/dl and in December only 19. In the same time several farms have the opposite situation, higher values of MUL in the cold season and lower in the hot season. Another paper that studied this subject reported the lowest MUL in November - 11.8 mg/dl, with a maximum in June – 18.1 mg/dl (Hojman et al., 2004). The whole situation regarding the dynamics of MUL depending on the season is presented in the table 6.

Table 6. MUL dynamics depending on season

| Specification | MUL/cold season | MUL/hot season |
|---|---|---|
| Farm 1 | 38 | 43 |
| Farm 2 | 26 | 24 |
| Farm 3 | 23 | 22 |
| Farm 4 | 30 | 33 |
| Farm 5 | 29 | 27 |
| Farm 6 | 25 | 26 |
| Farm 7 | 31 | 21 |
| Farm 8 | 26 | 24 |
| Farm 9 | 26 | 24 |
| Farm 10 | 17 | 21 |
| Farm 11 | 13 | 16 |
| Farm 12 | 37 | 37 |
| Farm 13 | 17 | 32 |
| Farm 14 | 14 | 20 |
| Farm 15 | 30 | 30 |
| X + Sx | 25.36 ± 1.96 | 26.62 ± 1.85 |
| S | 7.59 | 7.17 |
| V% | 29.94% | 26.92% |

At Farm 7 can be observed a difference of 10 mg/dl between the two seasons, higher value registered in the cold one. A difference of 15 mg/dl is present at Farm 13, but the higher value is the warm season.
However, the average is almost equal 25.36 mg/dl in the cold season, respectively 26.62 in the hot season.
Therefore, the hypothesis that the feeding is done differently depending on the season, in order to the milk market is not true, or we can say that it is true in singular cases.
The main results obtained from the study are summarized in Figure 1.

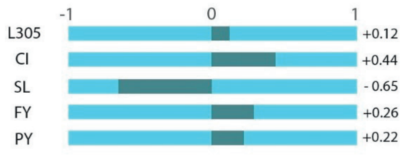


Figure 1. Correlation between milk urea level and different parameters

## CONCLUSIONS

The MUL is positive correlated with the milk yield, but in small measure, r = 0.12. So, a high protein diet is not economical in order to increase milk production.
Also, a positive association between MUL and milk quality (the amount of fat and protein) was found, greater than that with milk yield. Regarding the correlation between MUL and reproductive parameters, more exactly the CI, the result are significant. As higher is the MUL a longer CI is expected.
The biggest association was registered between MUL and SL, r = - 0.65. So, if the farmer wants that his cows to be in farm for long time, he will need to pay close attention to this indicator.
No significant differences were found between MUL in the cold season and MUL in the warm season.
Therefore, we recommend an interval of 22-32 mg/dl, in order to maintain the reproductive parameters to a satisfactory level and in the same time to optimize the milk yield and quality. A similar study, recommended for cattle management in Germany, France and Austria a range between 15-30 mg/dl (Glatz – Hoppe et al. 2020).

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# ASSESSMENT OF PLASMA BIOCHEMISTRY AND INTESTINAL MICROFLORA IN TRANSYLVANIAN NAKED NECK BREED COMPARED WITH COMMERCIAL BREEDERS'

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***Abstract***

*This study assessed to compare the plasma metabolic status and intestinal microflora of different breeders' genotypes. A total of 110 healthy female breeders (25-week-old) were divided into three groups: Transylvanians Barred Naked Neck (30 birds) and Black Naked Neck (30 birds) vs commercial Ross 308 breeders (50 birds). During a 5-weeks trial, the birds were reared on the floor system in climate-controlled conditions. They were fed a standard commercial laying breeder diet (15.26% crude protein and 11.30 MJ/kg metabolizable energy). Blood and intestinal content samples were collected at 30 weeks for analysis. Results revealed significant differences in plasma protein profile: total protein, albumin, globulin, total bilirubin increased, and uric acid decreased in the Naked Neck varieties vs Ross 308 breeds. Energy profile showed higher glucose and lower HDL-Cholesterol levels in Naked Neck varieties. Plasma mineral profile archive highest calcium and phosphorus values for Naked Neck varieties vs commercial breed, with no significant change in Ca/P ratio. There was no genotype effect on plasma enzyme activities. Cecum microflora was significantly affected by genotype, the Enterobacteriaceae (ENT) and Coliforms population count decreased, while the beneficial population Lactobacillus (LAB) spp. and LAB: ENT ratio increases in the Naked Neck breeds vs commercial breeds.*

***Key words****: laying breeders, intestinal microflora, Transylvanian Naked Neck, plasma biochemistry.*

## INTRODUCTION

It is stated that the local breeds may not compete with the specialised lines in terms of performance indicators, resources and economic efficiency. Still, they could be evaluated as dual-purpose breeds to supply niche markets and can also be used in crossbreeding with commercial breeds (Nolte et al., 2021). The local breed such as Transylvanian Naked Neck has gained attention due to its better adaptability to climatic conditions, efficient valorisation of low feed resources, disease resistance, low mortality, and conserved gene pool (Custură, 2020). Therefore, this local breed could be used for crossbreeding in meat production in alternative rearing systems (semi-intensive or free-range).

Blood biochemical responses are important markers of clinical, physiological, nutritional and health status in chickens (Gheorghe et al., 2017; Filipović et al., 2007; Silva et al., 2007). Biochemical parameter values are affected by genotype, age, sex, season, nutrition, and physiological condition (Toghyani et al., 2010). Previous studies highlighted that the host's genetics influences the microbiota composition in poultry due to the differences between individuals (Guardia et al., 2009), lines (Meng et al., 2014; Zhao et al., 2013; Gabriel et al., 2011) and genotypes (Stanley et al., 2013; Stanley et al., 2012).

The host's genotype could affect the microbial composition directly by the secretions into the gut, modifying the gut motility and epithelial cell surfaces, or indirectly *via* the feed (Gheorghe et al., 2019; Gheorghe et al., 2017; Meng et al., 2014).

The comparison of the biochemical blood profiles and intestinal microflora of Transylvanian Naked Neck breed with different breeders' genotypes has not been reported in the scientific literature. Therefore, this study assessed to compare the plasma metabolic status and intestinal microbial populations of two

Transylvanian Naked Neck varieties with a commercial breeder fed the same standard diet.

## MATERIALS AND METHODS

***Birds and experimental design***

The birds were treated according to the Directive 2010/63/EU for animals used for experimental and scientific purposes (OJEU, 2010). The Ethical Committee of the National Research-Development Institute for Animal Biology and Nutrition (INCDBNA-Balotesti, Romania) approved the trial protocol (no. 366/ 01/2021).

The trial was conducted on a total of 110 healthy female breeders (25-week-old) from two Romanian genotypes: Transylvanians Barred Naked Neck (30 birds; GGTB) and Black Naked Neck (30 birds; GGTN) compared with commercial Ross 308 breeders (50 birds; Ross 308).

The birds were housed during a 5-weeks trial at the INCDBNA-Balotesti research Biobase in climate-controlled conditions and kept on the floor system, fitted with manual feeders and nipple drinkers. A 14-h light photoperiod per day was provided. No veterinary treatment or vaccination protocol was applied during the study period.

During the trial, the birds were fed with a standard commercial laying breeders' diet. The feed (pelleted form) was administered daily in fixed amounts at 07:30, and water was available *ad libitum*. The nutrient composition of standard commercial diet fed to breeders in laying period is shown in Table 1.

Table 1. Chemical composition of laying breeders' diet

| Nutrients | % as fed basis |
|---|---|
| Dry matter | 89.29 |
| Crude protein | 15.26 |
| Methionine + cysteine | 0.64 |
| Lysine | 0.70 |
| Crude fibre | 5.30 |
| Crude fat | 4.09 |
| Ash | 9.91 |
| Calcium | 2.80 |
| Phosphorus total | 0.70 |
| Metabolisable energy (MJ/kg) | 11.30 |

***Blood and intestinal content sampling***

After a 5-week trial period, a total of 18 birds (6 female/group) were randomly selected for blood and intestinal contents collection. Blood (6 mL/bird) was taken from the wing vein in heparinised tubes in the morning. The blood were centrifuged (3000 rpm for 15 min) at 4ºC, and plasma was stored in 1.5 ml tubes at −20ºC for analysis. Birds were slaughtered by cervical dislocation and dissected. The intestinal content (small intestine and cecum) was sampled in sterile tubes for microbial determinations.

***Plasma biochemistry analysis***

The plasma profiles protein (total protein, TP; albumin, Alb; total bilirubin, TBil; plasma urea nitrogen, PUN; creatinine, Cre; uric acid, UA), energy (glucose, Glu; C, cholesterol total; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides), mineral (Mg, magnesium; Ca, calcium; IP, inorganic phosphorus), and enzyme (ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; CK, creatine kinase; GGT, gamma-glutamyl transferase; ALP, alkaline phosphatase) were assessed by dry chemistry Spotchem EZ SP-4430 analyser and specific reagent strips (Spotchem, Arkray Inc., Japan). Globulins values were calculated as a difference between plasma TP and Alb concentrations; the Alb/Glb ratio, PUN/Cre ratio, and Ca/IP ratio were also calculated. Plasma biochemistry parameters were assessed in duplicate per sample.

***Intestinal pH and microbial determinations***

The pH of the small intestine and cecum contents was assessed from fresh samples using a portable pH meter (WTW 3310, Germany).

The microbial determinations were done by conventional microbiological techniques using selective agar media. Briefly, one gram of sample was diluted 1:10 in 7 mL Brain Heart Infusion broth (Oxoid Ltd., England) plus 2 mL glycerol and homogenised. Then, 1 mL from dilutions were cultured on selective agar media or determinations of *Enterobacteriaceae* (VRBG agar; Oxoid, CM1082, England), *Enterococcus* spp. (Slanetz-Bartley agar; Oxoid CM0377, England), *Clostridium* spp. (Reinforced Clostridial agar; Oxoid CM0151, England), Coliforms (MacConkey agar; Oxoid CM0007, England), *Staphylococcus* spp. (Mannitol Salt agar, Oxoid CM0085, England), *Salmonella* spp. (Salmonella–Shigella agar; Oxoid CM0099, England), and *Lactobacillus* spp. (MRS agar; Oxoid CM0361, England). The intestinal microbial colonies were counted in duplicate per sample and expressed as $log_{10}$ cfu (colony-forming units)/g of intestinal content.

***Statistical analysis***

Data were analysed by one-way ANOVA to test the genotype effect (two Romanian Naked Neck varieties *vs* Ross 308 commercial broiler breeders) using the GLM procedure (SPSS v.20, 2011). Each bird sample was considered as the experimental unit for plasma biochemistry and intestinal determinations. The data are given as means and standard error of the mean (SEM). The statistical differences were discussed at P<0.05.

## RESULTS AND DISCUSSIONS

***Plasma biochemistry***

The effect of genotype on female breeders' plasma protein and energy profiles is shown in Table 2. Regarding the plasma protein, our study results revealed that the GGTB variety had the highest levels of TP (P=0.004) and Glb (P=0.002), followed by GGTN compared to the Ross 308 commercial breeds. A significant increase in the Alb (P=0.022) and TBil (P=0.031) values were shown on the two varieties of Naked Neck breeds (GGTB and GGTN) compared to the commercial breeds. The lowest UA concentration (P=0.001) was found on the Naked Neck vs commercial breeders. There were no significant changes in the Alb/Glb ratio, PUN, Cre and PUN/Cre ratio as effect on genotype (P>0.05).

The blood TP and Alb values are indicators of dietary protein utilization (Pavlík et al., 2007) and the haemoconcentration level (Kraus et al., 2021). A higher value of TP reflects a better health condition of chickens (Marono et al., 2017), and in layers, this may be due to an estrogenic-induced increase in globulin. The proteins are egg yolk precursors (vitellogenin and lipoproteins), synthesized in the liver, transportted to the ovary *via* plasma, and integrated into the egg cell (Ritchie et al., 1994). Creatinine (Cre), a by-product of phosphorcreatine degradation in skeletal muscles, reflects protein metabolism (Piotrowska et al., 2011). The Cre level is linked with muscle mass and changes by age and physical activities (Szabo et al., 2005).

Comparing the blood parameters of Ross 308 broilers with native Venda chickens, Mabelebele et al. (2017) reported higher TP values in indigenous breed compared to Ross 308 broilers at 42 days and no significant differences at 90 days. These authors also indicated that plasma Cre levels were lower in the indigenous breed than Ross 308 broilers. Other studies (Rehman et al., 2017; Dutta et al., 2013; Peters et al., 2011) reported variations in plasma Cre levels as genetic effects.

Kraus et al. (2021) studied the effects of genotype, housing system, and age on the blood parameters and egg quality of Czech and Slovak native hens and found no significant effect of breed on TP and Alb concentrations at 34, 42, and 50 weeks old.

The UA is the main product of nitrogen catabolism (Lumeij, 1997) and the PUN reflect the ongoing protein metabolism (Kim et al., 2012). Higher PUN concentration and lower UA levels reflect an improvement of protein catabolism (Tao et al., 2021). Similarly, several studies have been reported that the UA level varies as the effect of breeds in poultry (Eleroglu et al., 2015; Isidahomen et al., 2011; Silva et al., 2007) and UA level is higher in female birds due to ovulatory activities (Ibrahim et al., 2012).

The PUN/Cre ratio, as an important marker of renal function, obtained in our study range between normal interval (10-20 mg/dL; Washington & Van Hoosier, 2012) that indicate a good health state.

The results of the plasma energy profile (Table 2) shown that the two varieties of Naked Neck breeds (GGTB and GGTN) had higher values of Glu (P=0.027) and lower levels of HDL-C (P=0.011) than those of the Ross 308 commercial breed. The C level was slightly increased in Ross 308 hens, but no significant genotype effect (P>0.05) was found for C and TG levels.

It is known that glucose (Glu) is the primary metabolite of energy metabolism (Gallenberger et al., 2012). Cholesterol (C) can be synthesised from dietary fats and endogenously within the cells, and increased cholesterol level is a marker of a high risk of cardiovascular disease. Several factors can influence the blood cholesterol level, such as breed, sex, age, and diet composition (Toghyani et al., 2010). Our results partially agree with Abdi-Hachesoo et al. (2011), who reported lower Glu and C concentrations on Iranian indigenous hens compared to Ross 308 hens. The lower C concentration in indigenous hens may be attributed to a higher body activity and higher energy need (Abdi-Hachesoo et al., 2011; Simaraks et al., 2004). Triglycerides (TG) are synthesised in the liver from fatty acids (FAs), proteins, and glucose when the body's requirements are exceeded, and they are accumulated in fatty tissue. At the beginning of laying period, the plasma lipids such as free FAs, TG, and phospholipids increased for the yolk synthesis in oocytes (Moon, 2018). Although the TG plasma level was slightly higher in the two Naked Neck breeds varieties compared to Ross 308 the differences were not significant (P>0.05).

Table 2. Effect of genotype on plasma protein and energy profiles of female breeders

| Variables | Genotype | | | SEM | *P*-value |
|---|---|---|---|---|---|
| | Ross 308 | GGTB | GGTN | | |
| *Protein profile* | | | | | |
| TP (g/dL) | 4.10$^{bc}$ | 4.94$^{abc}$ | 4.25$^{b}$ | 0.14 | *0.004* |
| Alb (g/dL) | 2.10$^{b}$ | 2.54$^{a}$ | 2.20$^{a}$ | 0.08 | *0.022* |
| Glb (g/dL) | 2.00$^{bc}$ | 2.40$^{abc}$ | 2.05$^{b}$ | 0.05 | *0.002* |
| Alb/Glb ratio | 1.05 | 1.06 | 1.07 | 0.03 | *0.876* |
| TBil (mg/dL) | 0.20$^{b}$ | 0.32$^{a}$ | 0.30$^{a}$ | 0.02 | *0.031* |
| PUN (mg/dL) | 2.10 | 2.50 | 2.20 | 0.10 | *0.071* |
| Cre (mg/dL) | 0.17 | 0.14 | 0.13 | 0.01 | *0.481* |
| PUN/Cre ratio | 12.35 | 17.85 | 16.92 | 0.94 | *0.984* |
| UA (mg/dL) | 8.14$^{a}$ | 6.06$^{b}$ | 4.93$^{b}$ | 0.43 | *0.001* |
| *Energy profile* | | | | | |
| Glu (mg/dL) | 205$^{b}$ | 228$^{a}$ | 240$^{a}$ | 5.48 | *0.027* |
| C (mg/dL) | 112 | 96.80 | 91.60 | 4.67 | *0.190* |
| HDL-C (mg/dL) | 57.60$^{a}$ | 49.50$^{b}$ | 46.72$^{b}$ | 1.66 | *0.011* |
| TG (mg/dL) | 411 | 500 | 509 | 24.20 | *0.183* |

GGTB, Transylvanian Barred Naked Neck; GGTN, Transylvanian Black Naked Neck, SEM, standard error of the mean.
TP, total protein; Alb, albumin; Glb, globulin; TBil, total bilirubin; PUN, plasma urea nitrogen; Cre, creatinine; UA, uric acid; Glu, glucose; C, cholesterol total; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides.
$^{abc}$Means within the row with different superscripts differ significantly (P<0.05).

Regarding the plasma mineral profile (Table 3), the data show that the varieties of Naked Neck breeds (GGTB and GGTN) archive the highest values of Ca (P=0.003) and IP (P=0.028) compared to the values of the Ross 308 commercial breed. The plasma Mg concentration and Ca/IP ratio were not significantly influenced by genotype (P>0.05). Calcium and phosphorus are macro minerals needed for eggshell development in laying breeders. Feeds need to supply adequate quantities of calcium, phosphorus, and their optimum ratio due to the necessity of these minerals for eggshell synthesis and bone turnover and maintaining homeostasis in the body and circulating blood supply (Magnuson, 2015).

Abdi-Hachesoo et al. (2011) found comparable serum Ca values for Ross 308 broiler chickens and native Iranian chickens. Similar to our results, Mabelebele et al. (2017) reported a higher value of serum Ca for indigenous Venda chickens than Ross 308 at 90 days old and a similar value of serum P between these breeds. Suchý et al. (2004) reported a decrease plasma Ca level in meat-type hens compared with egg-type hens and no significant difference in P plasma levels between production types. Decreases in plasma Ca concentration had no significant influence on eggshell quality (Hester et al., 1980). Other studies noticed no link between plasma P concentration and eggshell weight (Pavlík et al., 2009; Boorman & Gunaratne, 2001).

The plasma metabolic enzyme profile of female breeders as the effect of genotype (Table 3) reveals no significant changes in enzyme activities of AST, LDH, ALT, CK, GGT, and ALP in Naked Neck breeds compared with commercial breeder.

The enzymes ALT, ALP, AST and GGT are essential indicators of liver function (Ambrosy et al., 2015). These enzymes may increase due to damaged or diseased cells, indicating the hepatic status. It was noticed that AST and ALT increased in the hyperthyroid subjects and that ALP activity is an indicator of the hen's productivity (Malik and Hodgson, 2002).

Increased plasma activity of the intracellular muscle enzyme CK is linked to an overt muscle injury as response to different pathologies and exposure to environmental stressors (Melesse et al., 2011). Additionally, plasma CK activity rises with age, and it's been suggested that genetic selection for fast- growing broilers causes changes in membrane stability and promotes intracellular enzyme export (Sandercock et al., 2009). Hocking et al. (1998) reported that feed restriction lowers the plasma CK activity in female turkeys. These authors noticed also that changes in plasma CK concentration are correlated with ovarian activity and linked with plasma TG levels.

Table 3. Effect of genotype on plasma mineral and enzyme profiles of female breeders

| Variables | Genotype | | | SEM | *P*-value |
|---|---|---|---|---|---|
| | Ross 308 | GGTB | GGTN | | |
| *Mineral profile* | | | | | |
| Mg (mg/dL) | 2.14 | 2.30 | 2.40 | 0.06 | *0.219* |
| Ca (mg/dL) | 15.28$^{b}$ | 17.52$^{a}$ | 17.63$^{a}$ | 0.43 | *0.003* |
| IP (mg/dL) | 2.88$^{b}$ | 3.32$^{b}$ | 3.80$^{a}$ | 0.26 | *0.028* |
| Ca/IP ratio | 5.31 | 5.28 | 4.64 | 0.44 | *0.170* |
| *Enzyme profile* | | | | | |
| AST (UI/L) | 256.5 | 225.8 | 227.6 | 21.71 | *0.091* |
| ALT (UI/L) | 17.2 | 15.8 | 14.4 | 0.93 | *0.497* |
| LDH (UI/L) | 302.4 | 209.2 | 301 | 32.21 | *0.104* |
| CK (UI/L) | 1004 | 825 | 989 | 89.84 | *0.363* |
| GGT (UI/L) | 45.6 | 37.4 | 41.25 | 1.55 | *0.078* |
| ALP (UI/L) | 742 | 842 | 672 | 95.83 | *0.327* |

GGTB, Transylvanian Barred Naked Neck; GGTN, Transylvanian Black Naked Neck; SEM, standard error of the mean.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; CK, creatine kinase; GGT, gamma-glutamyl transferase; ALP, alkaline phosphatase; Mg, magnesium; Ca, calcium; IP, inorganic phosphorus.
[ab]Means within the row with different superscripts differ significantly (P<0.05).

***Intestinal microflora***

As shown in Table 4, the studied genotypes had similar pH values (P>0.05) of the small intestine and cecum. The intestinal pH is one of the major's factors that influence the nutrient metabolism and health status of chickens (Recoules et al., 2017).

The previous study of Mabelebele et al. (2014) compared the gastrointestinal tract (GIT) parameters of Ross 308 broilers with native Venda chickens and reported that native breeds had lower pH value in the small intestine and a similar pH value in the cecum.

The major GIT function is the feed digestion and nutrients absorption of dietary origin in the small intestine or produced by microbial fermentation in the cecum (Apajalahti & Vienola, 2016; Scott et al., 2010). It was stated that intestinal microbiota has a major role in chickens' growth, production, reproduction, welfare, and health status (Ji et al., 2020; Yang et al., 2020). Our results showed that the small intestine microbial populations were not significantly influenced by the genotype (P>0.05), whereas significant changes in the cecum microflora were noticed as an effect of genotype (Table 4). The *Enterobacteriaceae* (P=0.044) and *Coliforms* population counts (P=0.002) decreased, while the *Lactobacillus* spp. (P=0.032) and LAB: ENT ratio (P=0.048) were increased in the naked neck breeds compared to commercial breeds.

Our findings are similar to previous studies (Stanley et al., 2012; Torok et al., 2012; Gabriel et al., 2011) reported that the differences in the microbial composition were higher in cecum content than other intestinal segments efficient and non-efficient chickens. Bikker et al. (2006) stated that the higher *Lactobacilli* counts could lower the Coliform counts, which could be attributed to the microbial colonisation resistance in the intestinal tract. Our results are in line with Bikker et al. (2006), who reported that lactobacilli suppress the activities of pathogenic bacteria (e.g., *Enterobacteriaceae* and Coliforms), improving the intestinal tract environment. It has been shown that *Lactobacillus* and *Lactococcus* spp. have biotechnological value in fermentation and bacteriocin production, with beneficial effects on the host and the reproductive performance (Yang et al., 2020). On the other hand, several studies that compared the composition of faecal microbiota in two lines selected for high or low body weight reported differences in microbiota among the two lines, and also have shown that some microbial species have shown heritability (Meng et al. 2014; Zhao et al. 2013).

Table 4. Effect of genotype on intestinal pH and microbial populations ($\log_{10}$ cfu/g) of female breeders

| Variables | Genotype | | | SEM | *P*-value |
|---|---|---|---|---|---|
| | Ross 308 | GGTB | GGTN | | |
| Small intestine | | | | | |
| pH | 6.78 | 6.65 | 6.51 | 0.06 | *0.110* |
| *Enterobacteriaceae* (ENT) | 5.26 | 4.12 | 3.63 | 1.39 | *0.288* |
| *Enterococcus* spp. | 6.05 | 5.60 | 5.36 | 0.62 | *0.444* |
| *Clostridium* spp. | 4.38 | 3.87 | 4.38 | 2.69 | *0.973* |
| Coliforms | 3.24 | 3.10 | 2.54 | 2.40 | *0.948* |
| *Staphylococcus* spp. | 3.17 | 2.77 | 2.73 | 2.45 | *0.851* |
| *Lactobacillus* spp. (LAB) | 6.50 | 6.20 | 6.15 | 1.18 | *0.431* |
| LAB: ENT ratio | 1.24 | 1.50 | 1.69 | 0.14 | *0.504* |
| *Salmonella* spp. | absent | absent | absent | - | - |
| Cecum | | | | | |
| pH | 6.40 | 6.33 | 6.20 | 0.04 | *0.207* |
| *Enterobacteriaceae* (ENT) | $6.39^{a}$ | $5.26^{b}$ | $4.70^{b}$ | 0.36 | *0.044* |
| *Enterococcus* spp. | 6.65 | 6.15 | 6.24 | 0.15 | *0.277* |
| *Clostridium* spp. | 6.32 | 5.80 | 6.65 | 0.33 | *0.152* |
| Coliforms | $6.25^{a}$ | $6.10^{a}$ | $5.80^{b}$ | 0.18 | *0.002* |
| *Staphylococcus* spp. | 5.50 | 5.52 | 6.04 | 0.22 | *0.632* |
| *Lactobacillus* spp. (LAB) | $8.80^{b}$ | $9.25^{a}$ | $9.38^{a}$ | 0.06 | *0.032* |
| LAB: ENT ratio | $1.37^{b}$ | $1.76^{a}$ | $1.99^{a}$ | 0.12 | *0.048* |
| *Salmonella* spp. | absent | absent | absent | - | - |

GGTB, Transylvanian Barred Naked Neck; GGTN, Transylvanian Black Naked Neck; SEM, standard error of the mean.
[ab]Means within the row with different superscripts differ significantly ($P<0.05$).

## CONCLUSIONS

These results revealed significant differences in certain plasma metabolites between Transylvanian Naked Neck and Ross 308 breeders: total protein and its fraction increased, and uric acid decreased; energy profile showed higher glucose and lower HDL-cholesterol levels; mineral profile archive highest calcium and phosphorus values, with no significant change in Ca/P ratio.

Genotype significantly affect cecum microbial populations, the *Enterobacteriaceae* and *Coliforms* population count decreased, while the beneficial population *Lactobacillus* spp. and LAB: ENT ratio increased in the Transylvanian naked neck breeds compared to commercial breed.

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# STUDY ON THE OFFICIAL PERFORMANCE CONTROL FOR MEAT PRODUCTION OF ABERDEEN ANGUS CATTLE BREED IN ROMANIA

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***Abstract***

*It is weel known that in order to increase progress on cattle farms it is necessary to monitor animals in order to determinate the genetic quality of animals, by assessing the quantitative and qualitative characters of the animals, estimating breeding values and setting up a database on these aspects. In our country, as a result of favorable factors, such as natural pasture potential, European subsidies, government programs for the purchasing animals, extensive-intensive growth technology systems, the price of bovine meat, population trends towards the consumption of meat obtained in ecological conditions, in Romania were imported a series of specialized breeds for meat production, including Aberdeen Angus, Galloway, Highland, Aubrac, Charolais, Limousin. Thus, in order to expand the database and monitoring the qualitative and quantitative characters of the animals, specific breeding programs to each breed were developed. Regarding the Aberdeen Angus breed, the genealogical register is held by the Aberdeen Angus Association from Sibiu county, which also leads the activity of official performances control on national level. Recordings are made in accordance with legislative framework, in herds of pure breed Aberdeen Angus cattle and also cross-breeding programs with Aberdeen Angus terminal bulls. The control purpose is to record the weights of calves between three and fourteen months old, observing the specific characters of Aberdeen Angus breed and managing all purebred bulls. Data is collected in order to provide farmers with information useful for herd management and raw data for genetic evaluations. Generally, in this paperwork it will be studied the legislative framework, the control technique, the control methods, the national evolution of performances, the stock evolution and the appreciation of the bulls.*

***Key words***: *Aberdeen Angus, breeding values, herds, official performance control, Romania.*

## INTRODUCTION

The evaluation of the main productions of the bovine species is made according to a series of quantitative and qualitative aspects. The tools for improving a population are represented from a general perspective by the official control of performance and by the herd book of the breed (Acatincăi et al., 2004; Georgescu et al., 1998; Velea et al., 2012).

The general objectives pursued by performing official performance control (OPC) are: expanding the selection base, genetic evaluation of animals, optimizing farm management. As for beef cattle, the OPC aim is to achieve the following objectives: expanding the selection base, evaluating animals performances, estimating breeding values, developing the database, optimizing farm management (Ivancia et al., 2007; Maciuc et al., 2006; www.anarz.eu).

Applying of genetic techniques and the use of advanced technologies to monitor animal performances, classical animal husbandry is becoming a precision one, that allows the staff involved in the field to record large sets of information on animal welfare, economic efficiency of the farm, social trends or resource efficiency. (Orey et al., 2008).

The Aberdeen Angus breed was imported in Romania at the end of 2008, in Sibiu county when about 120 heifers were imported from Germany (www.karpaten-meat.com).

Due to a combination of favorable factors such as the natural grazing potential of the country, national and European subsidies programs, government programs for the purchase of purebred animals, extensive-intensive breeding technology, the price of meat per kg, population trends towards consumption of meat from animals raised in ecological conditions (Gociman et al., 2019).

The manner of carrying out the official control of performances of the beef cattle on national level is carried out in accordance with a set of national, European and international laws:

- O.M. 19 from 2006, looking the rules for the assessment of breeding bovines;
- O.M. 619 from 2015, eligibility criteria, specific conditions and how the payment schemes conditions and how the payment schemes are implemented;
- Aberdeen Angus Breeding Program in Romania, chapter 8, page 20;
- E.U. regulation 1012/2016, zootechnical and genealogical conditions applicable to the breeding of pure breed animals;
- ICAR (International Committee of Animal Recording), Guidelines for Beef Cattle Production Recording.

## MATERIALS AND METHODS

In accordance with the specific legislative framework, the activity of OPC it can be performed by three specific methods: Method A, all checks are carried out by an official representative of an accredited association to carry out official performance monitoring for meat production (figure 1); Method B, all checks are carried out by the breeder or his representative; Method C, all checks shall be carried out by the breeder or his representative and by a representative of the accredited inspection association.

At the Aberdeen Angus breed, OPC is coordinated by the Romanian Aberdeen Angus Association, which at the beginning of 2022 has in control about 1253 farms, as well as by three other companies accredited by ANZ for this activity, which have in control about 44 farms. Due to the infrastructure conditions, the 100% applied method is represented by method A - all controls are performed by an official representative of an accredited association to perform the official performance control for meat production. In order to carry out the control, the accredited association has, according to the law, specialized technical personnel, respectively zootechnical engineer and equipment for weighing and restraint of animals approved metrologically annually by the National Institute of Metrology.

In order to carry out the official performance control, the department responsible for the association's database must collect, archive, update and manage the information coming from the farmers, notifying all the events on the farm, through a set of specific documents, respectively: Annex 3 (artificial breeding / seeding), Annex 4 (embryo transfer) Annex 8 (calf registration), Annex 10 (outputs / inputs). Hierarchically, this activity is followed by the actual activity of the OPC, through which the zonal responsible controller generates the control bulletins at the farms to which he is going to go (Gociman et al., 2018; www.aberdeenangus.ro).

Figure 1. Method A of OPC

At the national level OPC on Aberdeen Angus cattle it is carried out on two type of farms, the suckler herds from birth to weaning and on finishing farms. In this sense, three types of weighing are performed, systematized according to the age of the verified young bovine:

- Weight at 7 months (G200) - minimum age 90 days - maximum 250 days;
- Weight at 10 months (G300) - minimum age 251 days - maximum 319 days;
- Weight at 12 months (G365) - minimum age 320 days - maximum 410 days.

The categories of animals weight are young calves, both males and females of both with the age between 90 and 410 days, and the interval between two consecutive weighing has a minimum value of 60 days and a maximum of 210 days.

The calculation of performance involves in the first stage the determination of a weight at a reference age and then the calculation of the average daily gain according to the reference weight (www.icar.org).

**<u>Calculation method in suckler herds from birth to weaning</u>**

$$A.D.G. = (WW-BW)*1000/AW$$

*A.D.G. – average daily gain*

*WW - live weight at weaning*

*BW – birth weight*

*AW – age of weaning*

**<u>Calculation method in finishing herds after weaning to slaughter</u>**

$$A.D.G. = (W_{n-1}-W_n)*1000 / (A_{n-1} -A_n)$$

$A_{n-1}$ *- age at weight recording n-1*

*An - age at weight recording n*

$W_{n-1}$ *- live weight at weight recording n-1*

$W_n$ *- live weight at weight recording n*

With the outsourcing of the herdbooks by ANZ (National Agency for Animal Husbandry), the monitoring activity as well as the analysis of the breed and possibilities of amelioration, became the responsibility of the Romanian Aberdeen Angus Association, based in Sibiu, which was accredited on 11/18/2015, as the leader of the herdbook of the breed at the national level.

Through the assumed responsibility, they resorted to the development of a computer software for the centralization, storage and evaluation of specific data such as: information about farms, animals, performances, controls, breeding values, etc. The activity was initially carried out on the computer software provided by the state institutions called SICASA, and later, out of the desire to be able to develop the software, they transferred the activity to a new software called BIDAA (Informatic Database of Aberdeen Angus).

The OPC in beef cattle involves the weighing of cattle by an approved association, the calculation of the evolution of their performances at different youth ages, as well as the genetic evaluation of cattle according to the evolution of the average daily gain at different ages (Gociman et al., 2020).

Scales used to determine weight meet the following conditions: ensure the welfare and safety of animals, ensure safety in transport, ensure the contention of animals, checked metrologically annually, have a minimum limit (0 kg) and an upper limit (1500 kg), are disinfected daily.

In order to achieve the specific objectives of the Romanian Aberdeen Angus Association, the aim is to achieve technical coefficients at national level for a maximum period of 15 years according to the Aberdeen Angus Breeding Program in Romania (table 1).

In order to observe the influence of the official performance control on the Aberdeen Angus cattle population at national level or analyzed the following parameters: evolution of farms in the OPC, evolution of the herds in the OPC, average weight at calving, average weight at 7 months, average weight at 10 months, average weight at 12 months, average daily gain at 7 months, the average daily gain at 10 months, the average daily gain at 12 months.

The period in which the data for this study will be analyzed will be between 2018-2021.

Table 1. Technical parameters of the Aberdeen Angus breeding program in Romania

| Specification | Unit | Value |
|---|---|---|
| B.W. | Kg | 35 |
| G7 months (200 days) - heifers | Kg | 220 |
| G7 months (200 days) - males | Kg | 260 |
| G10 months (300 days) | Kg | 410 |
| G12 months (265 days) | Kg | 490 |
| A.D.G. 7 months (200 days) - heifers | g | 900 |
| A.D.G. 7 months (200 days) - males | g | 1000 |
| A.D.G. 10 months (300 days) | g | 1000 |
| A.D.G. 12 months (365 days) | g | 1200 |

## RESULTS AND DISCUSSIONS

At the national level, the official performances control activity for meat production at Aberdeen Angus cattle, at the beginning of the year 2022 is carried out in proportion of 96.6% by the Romanian Aberdeen Angus Association, the remaining 3.4% being carried out by other three associations accredited by ANZ for performing this activity.

Romanian Aberdeen Angus Association performs the OPC twice a year in each member farm, according to an annual control schedule. If in 2018 the association operated organized on three zonal routes (figure 2), at present the OPC is performed on six specific routes (figure 3).

According to statistical data provided by the Romanian Aberdeen Angus Association in 2018, they were registered with the OPC a total of 640 farms, 77% of which were under the control of the Romanian Aberdeen Angus

Association. At the beginning of 2022, the same institution records a total of 1297 farms under control, of which a share of 3.4% of farms being verified by other associations (table 2 ).


Figure 2. Territorial organization of the OPC in 2018

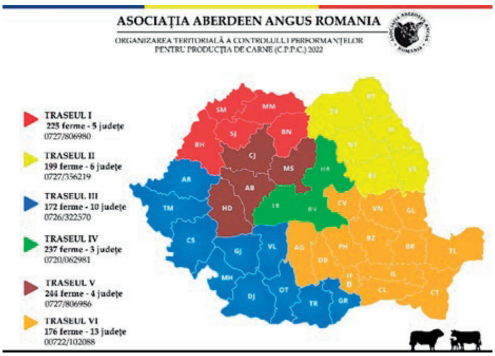


Figure 3. Territorial organization of the OPC in 2022

Table 2. Evolution of the number of farms per year and by associations

| Association | Total number of farms / per year | | | | |
|---|---|---|---|---|---|
| | 2018 | 2019 | 2020 | 2021 | 2022 |
| AAARO | 493 | 783 | 869 | 1110 | 1253 |
| ANGUS-RO | 91 | 0 | 0 | 0 | 0 |
| SOMEȘ ARIEȘ | 5 | 12 | 6 | 12 | 16 |
| NARCISA | 11 | 17 | 20 | 20 | 20 |
| ARAD | 35 | 5 | 5 | 8 | 8 |
| ACBCR | 5 | 0 | 0 | 0 | 0 |
| TOTAL | 640 | 817 | 900 | 1150 | 1297 |

Regarding the evolution of Aberdeen Angus cattle stocks at national level, according to the data registered in the genealogical register department of the Romanian Aberdeen Angus Association, at the end of 2015 our country registered a total of 10,276 purebred heads. Since then and until now, the numbers have seen continuous increases reaching the end of 2021, to have at national level only in purebred 59,343 heads (figure 4).

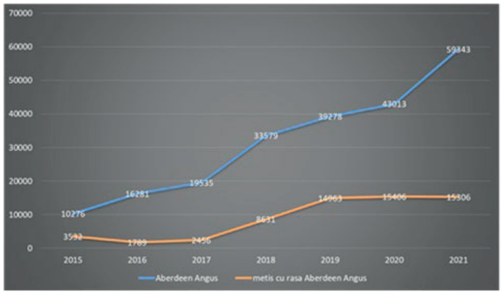


Figure 4. The evolution of Aberdeen Angus cattle in Romania in purebred and crossbreeding programs

Also, according to the data of the same institution, regarding the cross-breeding programs, at the end of 2015 in Romania were registred 3532 heads, while at this moment, respectively at the end of 2021 were registered 15,306 heads (www.bidaa.ro).

The capacity of farms in terms of total heads is also very variable, which is important in terms of increasing the level of technology and intensification. It is obvious that farms with more than 40 heads have a much greater capacity to increase the level of technology of all the factors involved in determining the level of economic profitability.

Also, that farms with a herd of 10-30 heads have a share of 40% nationally, over time at the opposite pole are the farms that have in the total structure of the herd over 100 heads with a national share of 16% (figure 5).

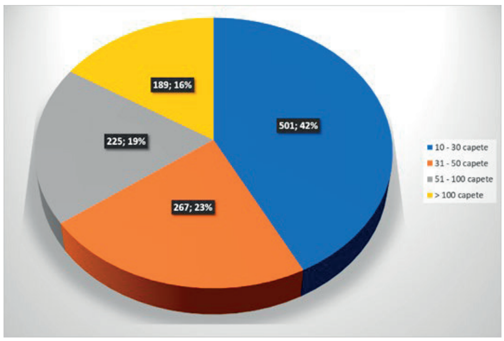


Figure 5. Share of farms at national level according to total number of heads by farm

In terms of the total number actually weighed at the end of 2018, about 9,859 heads were weighed, compared to the following years when the numbers reached 19,135 in 2019 and about 29,934 heads at the end of 2021. Regarding the parameters of achieved by analyzing the data recorded at the OPC during the years 2018, 2019, 2020 and 2021 a number of conclusions regarding the evolution of the breed at national level can be drawn (table 3).

Table 3. Evolution of average weight and daily gain per years

| Specification | Unit | 2018 | 2019 | 2020 | 2021 |
|---|---|---|---|---|---|
| B.W. | *kg* | 28 | 29 | 30 | 30 |
| G7 | *kg* | 208 | 208 | 214 | 215 |
| G10 | *kg* | 278 | 278 | 281 | 280 |
| G12 | *kg* | 327 | 315 | 311 | 313 |
| A.D.G. G7 | *g* | 892 | 887 | 917 | 929 |
| A.D.G. G10 | *g* | 816 | 828 | 880 | 834 |
| A.D.G. G12 | *g* | 791 | 780 | 770 | 775 |

Thus, from 2018 to 2021, a continuous increase in average weight and daily gain at the age of 7 months, from 208 to 215 kg, respectively from 892 to 929 grams / day. And in the case of the 10 months category, an increase in both weight and daily gain from 816 to 834 grams / day.
The 12 months category is the one that has a continuous decrease from 791 to 775 grams / day, a fact wich is influecend by certain technological factors such as: age at weaning, method of weaning, weight at weaning date, allotment, feeding before and after weaning.

## CONCLUSIONS

The results of the investigation show us the strengths of this sector respectively: the number heads at national had increase constantly which is favorable for future selection work and in terms of performances we also observe a continuous evolution which approaches to the standards set by the breeding program of Aberdeen Angus breed at national level.
In conclusion, we can say that due to the high standard of living, the constantly growing population, as well as the consumer preferences for high quality animal food, it is unanimously necessary to raise beef cattle, as the demand at European level is increasing and growth opportunities in other European countries are low, which puts Romania in a favorable position for developing this activity.
Thus, in order to achieve these goals is unanimously necessary to constantly monitor the evolution of animal performance through this activity of OPC.

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# BIOLOGICAL EFFICIENCY AND CHEMICAL COMPOSITION OF COW MILK COW FROM 'BULGARIAN RHODOPE CATTLE' WITH DIFFERENT GENOTYPE

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***Abstract***

*The milk productivity of cows from 'Bulgarian Rhodope cattle', reared on the farm of the Experimental Base of the Research Institute of Mountain Stockbreeding and Agriculture, Troyan and the farm of Deyan Filipov, in the town of Strazhitsa, was analyzed. Milk productivity, physicochemical composition, dry matter, dry fat-free residue (DFR) and energy value of milk were studied. The percentage of dry matter is a generalizing, constant feature that determines the concentration of cow's milk. The live weight of the studied animals was also determined. The biological efficiency and the coefficient of biological sufficiency of milk were calculated by formulas. The physicochemical parameters of milk of the studied animals of both genotypes showed different values. Live weight of cows bred in the area of the town of Troyan is higher than that of those reared in the area of the town of Smolyan by 27.55 kg. The coefficients for biological efficiency and biological sufficiency show that cows with a genotype typical of the region of the town of Strazhitsa gave more food production per 1 kg of live weight.*

***Key words:*** *'Bulgarian Rhodope cattle', genotype, milk yield.*

## INTRODUCTION

In the mountain and foothill regions of the Republic of Bulgaria relatively few breeds of dairy cattle are raised. A suitable and well-established breed is 'Bulgarian Rhodope Cattle'. It is obtained by complex, reproductive crossbreeding of cows from 'Rhodope Short-horned Cattle' breed with 'Brown Alpine' and 'Jersey' bulls and long-term selection (Vassilev, 1988; Gadzhev and Nikolov, 2008; Gergovska and Panayotova, 2016; Mehandzhiyski et al., 2019).

Evaluations of the biological efficiency of cows and the coefficient of biological sufficiency of milk is the research objective of Bulgarian and foreign scientists as well (Bruthen et al., 1984; Lazarenko and Gorelik, 2002; Davoodi, et al., 2013; Safina, 2018; Minabaev, 2019).

According to Karnauhov & Adrianova (2010) in some countries the nutritional value of milk is determined by the content of dry fat-free residue and protein, not excluding the assessment of productivity in protein and milk fat, because these traits are valuable in energy and biological terms.

Tagirov & Adriyanova (2008) believe that by increasing the blood percentage of the 'Holstein Friesian' dairy breed, in cases of backcrossing with local black-and-white cattle in Russia increases the level of milk productivity and preserves its environmental safety.

Khodyreva (2013) found that the cattle breed has an impact on milk productivity and its quality. When comparing two breeds and their crossings in the Urals, 'Simmental' and 'Holstein Friesian', the latter showed higher milk productivity.

Lim et al. (2020) concluded that milk obtained from 'Jersey' and cows that are crossings of 'Jersey' with other dairy cattle breeds made more efficient, processed dairy products with an appropriate energy status compared to products obtained from processed milk from cows from 'Holstein' breed. Such conclusions are reached by Cvac et al. (1982), Kozhev (2004), Iliev & Mihailova (2014).

The objective of the study is to make a comparative assessment of the biological efficiency and chemical composition of milk obtained from cows of 'Bulgarian Rhodope Cattle' with different genotypes, reared in the mountain and foot-hill regions of the Republic of Bulgaria.

## MATERIALS AND METHODS

The scientific and economic experiment was conducted in the Research Institute of Mountain Stockbreeding and Agriculture in the town of Troyan and the farm of the agricultural producer Dean Fililipov, in the town of Strazhitsa, in 2021. The objective of the study were clinically healthy, mature cows raised after the second lactation, which have completed their growth. The groups were formed on the principle of analogues of 20 cows of 'Bulgarian Rhodope Cattle' breed. In the first group were the animals from the farm of RIMSA-Troyan, and in the second from the farm of Deyan Filipov-Strazhitsa. The tested animals were studied and analyzed under identical feeding and raising conditions from a technological point of view. Milk productivity and physicochemical parameters of milk were obtained monthly after control milking: for fat content by Gerber method, for protein and casein by formal titration methods, for dry matter by calculation methods, for DFR in milk analyzer "Milco-Scan 120 B".

Live weight of the cows was determined by a measuring tape for combined measuring of height and weight.

The biological efficiency of cows (BEC) for both groups was determined by the formula of Lazarenko (1990):

**BEC=MP x DM/LW**

where **MP** is milk productivity for a 305-day-lactation, kg

**DM** is dry matter content in milk, %

**LW is live weight of cows, kg**

The coefficient for biological sufficiency (**CBS**) was calculated according to the formula of Lazarenko, Gorelik and Lykasova (2002): **CBS=MP x DFR/LW**

where MP is milk productivity for usual lactation, kg

**DFR** is dry fat-free residue, %

LW is live weight of cows, kg

The coefficient of lactation persistence (**CLP**) was determined by the formula:

**CLP= (B-A) x 100/B**

where:

A is productivity for a 100-day-lactation, kg

B is productivity for a 305-day-lactation, kg

Energy value EV (kkal) was calculate with the following formulae:

**EV (Kkal) = (PR + LACT) x 4 + (F x 4)**

Where PR is proteins

LACT is lactose

F is fats.

The results were processed biometrically by the methods of variation statistics using MS Excel and presented in tables.

## RESULTS AND RESEARCHES

The obtained data for a 100 day-lactation show higher values of milk fat in cows of the first group, from the region of Strazhitsa respectively 4.28% compared to 4.08% of the second group and higher values of milk protein in the second group in the region of the town of Troyan, respectively 3.35% compared to 3.33% of the first group for the first 100 days of lactation. The milk productivity in the first group is 1185.68 kg, and in the second 1002.76 kg.

Table 1. Milk yield, amount of milk fat and protein in milk of cows of 'Bulgarian Rhodope Cattle' for 100 and 305-day lactation

| Indicator | Breed Bulgarian Rhodope Cattle | |
|---|---|---|
| | BRC (I gr.) n=20 | BRC (II gr.) n=20 |
| First 100 days of lactation | | |
| Milk yield, kg | 1185.68 ± 112.35 | 1002.76 ± 127.21 |
| Milk fat, % | 4.28 ± 0.25** | 4.08 ± 0.14** |
| Milk protein, % | 3.33 ± 0.12*** | 3.35 ± 0.05*** |
| For a 305-day-lactation | | |
| Milk yield, kg | 3974.37 ± 23.65 | 3324.37 ± 166.22 |
| Milk fat, % | 4.49 ± 0.14** | 4.79 ± 0.36** |
| Milk butter, kg | 176.82±40.59 | 166.24±14.65 |
| Milk proteins, kg | 3.29 ± 0.09** | 3.86 ± 0.16** |
| Milk proteins, kg | 131.07±31.38 | 134.42±12.28 |

*P<0,05, **P<0,01, *** P<0,001

In the following days of lactation there was an increase in the amount of milk fat and protein in the second group in the region of Troyan, respectively by 0.61% and 0.51% and a moderate increase of 0.21% in fat and a decrease in protein by 0.04% in the second group from the region of the town of Strazhitsa.

Figure 1. Bulgarian Rhodope cattle from the farm of Research Institute of Mountain Stockbreeding and Agriculture

The milk productivity for a 305-day-lactation in the first group in the region of the town of Strazhitsa was 3974.37 kg, and in the second group in the region of the town of Troyan was 3324.37 kg, or a significant difference of 650 kg. In terms of biological efficiency coefficient, the first group is superior to the second group by 7.8%.

The achieved results justify good, average values at the level of milk productivity in both analyzed genotypes.

In addition to the quantitative indicators of milk, which are considered above in the study, we will focus on the qualitative indicators related to technology (Bruthen et al., 1984, Lazarenko, 1990).

Table 2. Biological efficiency, coefficient of biological sufficiency, coefficient for lactation persistence and energy value of milk

| **Indicators** | **Breed Bulgarian Rhodope Cattle** | |
|---|---|---|
| | **BRC (Igr.) n=20** | **BRC (IIgr.) n=20** |
| Milk yield, kg | 3974.37±23.65 | 3324.37±166.22 |
| Dry matter, % | 17.85±0.99* | 17.71±0.49* |
| DFR, % | 9.46±1.07 | 10.60±0.41* |
| Lactose | 4.83±0.23** | 5.07±0.44** |
| Live weight, kg | 431.8±22.26 | 459.35±30.54 |
| Sequence of lactation | 5±2.87 | 5±1.00* |
| BEC | 164.29 | 128.17 |
| CBS | 87.07 | 76.71 |
| CLP | 70.17 | 69.84 |
| Energy value, kkal/100 ml | 50.74 | 51.64 |

*P<0,05, **P<0,01, *** P<0,001

In terms of biological sufficiency coefficient, the superiority is of the cows from the first group with 8.81% compared to the cows from the second group. The content of DFR (Dry fat-free residue) determines its biological value and the quantitative state of the ratio between dry matter and fat (Lifanova, 2010).

According to the coefficient for lactation persistence, the difference is minimal, 0.43 points or 0.95%, again in favour of the first group of cows in the region of Strazhitsa.

There are no big differences in the energy value indicator: in the second group, in the region of Troyan, the energy value is 51.46 kcal, and in the first group, in the region of Strazhitsa is 50.74 kcal, that's a difference of 0.90 kcal., or 1.01%.

The dairy industry imposes ever higher requirements for milk quality as a raw material for the production of various delicacies and this requires increased responsibilities on the part of breeders and farmers (Lazarenko et al., 2002, Tagirov and Andriyanova, 2008).

The ratio of calcium to phosphorus in mg/kg is shown in Table 3. Calcium is an element that is actively involved in blood clotting, increases the penetration of the substrate into capillaries, synthesizes iron metabolism, increases the body's resistance to infections, participates in metabolic processes, ensures the formation of nerves and muscles. Phosphorus is also an essential element that participates in the synthesis of the protein molecule, regulates osmosis and affects tissue strength and acid-base balance in the body (Minabayev, 2019)

Figure 2. Bulgarian Rhodope cattle in the farm

Table 3. Ratio between calcium and phosphorus mg/kg

| Genotype | Milk | | |
|---|---|---|---|
| | Ca (mg) | P(mg) | Ratio |
| BRC (I gr.) | 137 | 1.0 | 1:0.73 |
| BRC (II gr.) | 142 | 0.91 | 1:0.64 |

The ratio of calcium to phosphorus in the first group of cows studied is 1:0.73, and in the second group is 1:0.64, as it is desired in the ideal product to obtain 1:0.75, and this is within expectations.

The present results correspond to and are close to the results obtained in the study on milk by Karnouhov & Adrianova (2010), Khodyreva (2013) and Safina (2018).

## CONCLUSIONS

The foot-hill and mountain climatic conditions of Bulgaria are suitable for raising cows from 'Bulgarian Rhodope Cattle' breed. Genotype affects milk productivity and milk quality. Milk productivity was higher in cows raised in the region of Strazhitsa by 650 kg compared to cows raised in the region of Troyan. The physicochemical parameters
of milk of the studied animals of both genotypes showed different values. The live weight of cows raised in the area of the town of Troyan was higher than that of those bred in the area of the town of Strazhitsa by 27.55 kg. The values of the coefficients for biological efficiency and biological sufficiency show that cows with a genotype typical for the region of the town of Strazhitsa get more food production based on 1 kg live weight.

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# MODALITIES TO REDUCE NITROGEN EMISSIONS IN SWINE FARMS: REVIEW

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***Abstract***

*The gas emissions have increased in recent years, which has exacerbated the greenhouse effect. In the livestock sector the main sources of emissions are considered to be the production, processing and transport of animal feed, manure decomposition, processing and transport of animal products (post-slaughter transport, refrigeration and packaging of animal products). A significant emission reduction, including nitrogen, are within the reach of animal producers. Adopting current best practices and technologies for feeding, raising and maintaining animal health, manure management, and greater use of biogas and energy-saving technologies would help the livestock sector to grow. reduction of greenhouse gases. This review aims to highlight the possibilities that pig farmers have in order to adopt strategies to reduce nitrogen emissions from farms.*

***Key words**: greenhouse gas, nitrous oxide emission, pig farm.*

## INTRODUCTION

This study looks over a number of nutritional and management strategies that have highlighted the dietary impact on the nitrogen (N) excreta. Environmental pollution adversely affects the ecosystem. For many years, pig farming raises a lot of policy concerns in terms of economic, environmental, and social aspects of sustainable agriculture (Habeanu et al., 2020). As a consequence, the pig farm management are directed to decrease the pollutant level by nutritional techniques and manure management. As previously stated by Petersen (2018), two nutrients such as N and phosphorus (P) contaminate water, and pig manure contribute to this large-scale pollution (Figure 1).

In pigs manure the N compounds can generate nitrous oxide ($N_2O$) as result of nitrification and denitrification processes (Zhao, 2017). Thus, from N derive nitrate and nitrite, $N_2O$ etc.

In 2019, it was anticipated globally that about 40 percent of total $N_2O$ emissions come from human activities, respectively nitrous oxide is emitted from agricultural soil management, wastewater treatment, stationary combustion, industry or chemical production, manure management, transportation and other activities, (U.S. Environmental Protection Agency).

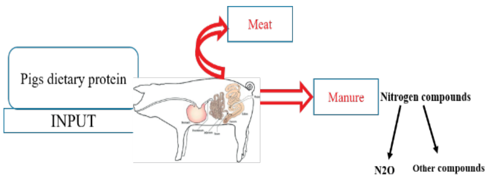


Figure 1. Nitrogen balance (adapted from Habeanu et al., 2021)

One of the most expensive nutritive substances is dietary proteins. Nitrogen is necessary for protein synthesis, as well as the synthesis of amino acids and nucleotides (Habeanu et al., 2020). Despite the fact that N is involved in a variety of metabolic processes, its detrimental environmental impact is a subject of concern. In the manure 2/3 of total nutrients is nitrogen (Millet et al., 2018). A great quantity of feed intake is excreted. The nitrogen coming from pigs' diet is converted into meat in a lower proportion than that excreted.

This paper intends to present some of the current information and concerns about climate change, as well as highlight the available nutritional options and determine which are the most cost-effective ($N_2O$ mitigation vs. diets) for reducing greenhouse gas (GHG) emissions.

## MATERIALS AND METHODS

In livestock production systems, there is a strong relationship between resource efficiency and GHG emissions. The potential for achieving emission reductions lies in providing all facilities for animal producers to use the practices already used by the most efficient operators in the field.

Taking into account these aspects, the bibliographic sources published in online scientific databases have been analyzed and presented, because many of the actions to improve efficiency and reduce greenhouse gas emissions also improve the production process, resulting in a quantitative and qualitative increase in food and higher incomes, with benefits for food security.

For livestock production systems, emissions of nitrogen oxide, methane gas and carbon dioxide are losses of nitrogen, energy and organic matter which reduce their efficiency and productivity. Possible interventions to reduce emissions are therefore largely based on technologies and practices that improve the efficiency of animal and livestock production.

## RESULTS AND DISCUSSIONS

### Climate change indicators

GHGs are gaseous compounds that, according to their molecular structure, trap heat or longwave radiation produced in the atmosphere and re-emit it back to the ground. This heat trapping phenomenon is known as the greenhouse effect that mean a natural phenomenon that allows life on Earth by maintaining the temperature of the planet at 15°C.

Climate change refers to a larger spectrum of changes that are taking place on our planet than just global warming. Global warming potential (GWP) is one aspect of climate change. In comparison to the mid-20th century baseline (of 1951-1980), the average surface temperature has risen roughly 1°C (nearly 2°F) since 1880 (Earth Observatory, 2020).

Agriculture is one of the key sectors impacted by climate change, according to recent climate assessments (National Climate Assesment, 2014; Melillo et al., 2014; Hartfield et al., 2020). Livestock products account for 17% of world calorie consumption and 33% of global protein intake, making them an important agricultural commodity for global food security (Rosegrant et al., 2009; Rojas-Downing, 2017). These assessments highlight many of the components vulnerable to climate change, requiring robust indicators to determine if the impact is increasing and our food and natural resource security is at risk.

Various models have been designed in order to assess indicators that providing information on climate change. Carbon dioxide ($CO_2$), total N outputs, $N_2O$, methane ($CH_4$ enteric or in manure) emissions are direct climate indicators for livestock sector. Carbon (C) sequestration mean carbon capture and storage which may become $CO_2$ gas.

GHGs emissions are calculated using the methodology presented in the Guide to the National Greenhouse Gas Inventory (IPCC 2006, respectively updated version in 2019) by the United Nations Environment Program, Intergovernmental Panel on Climate Change

According to the IPCC (2019), improved agricultural practices and forest-related mitigation activities can make a significant contribution to the removal of $CO_2$ from the atmosphere at relatively low cost. The idea is to stabilize the C in such a way as to avoid an atmospheric alert. These indicators are linked to the amount of feed consumed and the amount of excreta produced.

As part of attempts to mitigate climate change and enhance air quality, changes in farming methods (feed, types of livestock buildings, emission treatment systems, waste management practices, and so on) must account for these emissions (Hassouna et al., 2016; Garcia et al., 2016).

### Contribution of pigs to greenhouse gas emission

The main type of GHGs from livestock sector account 25% for $CH_4$, 32% for $CO_2$, 31% for $N_2O$ (Moran & Wall, 2011). Fluorinated gases from livestock have a lower fraction of GHG.These gases are expressed in $CO_2$ equivalent ($CO_2$ eq,) that mean their GWP.

Pigs consume feed and water in appreciable quantities, thereby their excreta is quite plentiful as well. Any protein surplus in feed that cannot be used for protein synthesis and is excreted in the urine, resulting in a high energy cost for animals and a financial loss for farmers. In addition, to being financially effective, determining the appropriate protein and energy intakes for pigs would have positive environmental repercussions by reducing pig farm nitrogenous excretions (Habeanu et al., 2019; Aquilani et al., 2019).

According to the Statistical Reports on the number of live animals on holdings transmitted by the National Sanitary Veterinary and Food Safety Authority (ANSVSA), the total number of pigs registered at the national level was 1,924,696 head on December 31, 2019 and 1,697,809 head on December 31, 2020.

The feeding strategies are designed to make the most efficient use of the fodder included in animal feed by: determining the nutritional value of pig feed with the greatest accuracy; using of the optimizing compound feed for each productive category, adapted to physiological conditions; developing recipes based on optimized nutrient structures; using recipes in such a way as to minimize waste and refusal of consumption; ensuring an adequate climate in the accommodation space.

**Nitrogen cycle**

Emissions from animal systems have been a significant concern for all livestock operations, according to Harper et al. (2004). The goal of this term influence on compromising surroundding and distant research was to determine the relationship between N emissions, ecosystems, and the environment.

Due to plant relationships, N reactivity and leachability, bacterial alteration (nitrification and denitrification) the number of N forms that can be found in the soil (urea, $NH_4$, NO, $NO_2$, $N_2O$, and various amines) and the large number of N forms that can be transported through the atmosphere (use and release; $NH_3$, $NH_4$ aerosols, $N_2$, NO, $NO_2$, $N_2O$, and various amines), obtaining a measured system analyses is difficult to found for all forms of N transport.

Pigs use feed protein nitrogen to restore deteriorated and lost digestive enzymes and other components. It has long been recognized that we should reduce the amount of pollution in our diet that is excreted as much as feasible. Many earlier investigations have shown that nitrogen output can be reduced, especially by dietary changes (Habeanu et al., 2019; Moreira et al, 2004; White et al., 2015).

Pigs retain only 32-46% of the N they consume (Dourmad et al., 2013; Millet et al., 2018). In contact with air on a manure storage platform, $NH_3$ is formed, which volatilizes in small volumes. One essential component is that which is stored in the soil and groundwater.

Diet is one approach to reduce N excretion (Monteiro et al., 2010; Dourmad et al., 2017; Wang and al., 2018).

**Nitrogen balance**

The nitrogen balance experiments provide information on net protein use, the relationship between protein intake and losses, and the relationship between protein and energy balance.

It is well recognized that N losses have an impact on the ecosystem, with the livestock sector accounting for 70% of $NH_3$ released into the environment (Habeanu et al., 2019; Kohn et al., 2005).

Protein-derived N is the most major source of N in animal metabolism. It shows us how much protein is being made and how much is being degraded. About 5 to 30% of N turned to food for human consumption, with the remainder being excreted by animals. Furthermore, too much N will sabotage efforts to achieve the Sustainable Development Goals.

Many previous studies had as main objective to evaluate N output level by using different feed formula (Dourmad et al., 2013; Mariscal-Landín et al., 2014; Untea et al., 2017; Habeanu et al., 2019, 2020 and 2021).

Thus, in 2013, Dourmad et al. presented an overview of the nutritional options for reducing pig N, P, Cu, and Zn excretion, as well as ammonia and GHG emissions, and discuss the methods that could or are now being used in practice. Their study has shown us that by improving animal nutrition efficiency the slurry excretion can be reduced. This can be an effective technique to limit the import of nutrients from outside the farm, particularly N, P, and trace elements, from a whole-farm perspective. Furthermore, due to variations in the chemical composition of the effluents, gaseous emissions from animal housing, as well as during the storage and spreading of manure, are influenced whenever livestock diet is modified.

One year later, Mariscal-Landín et al. conducted a trial in order to compare the energy metabolizability and nitrogen balance of new types of maize such as quality protein maize (QPM) to those of yellow and white maize, and to assess the apparent and standardized ileal digestibility (of protein and amino acids in QPM hybrids to those of yellow and white maize. They found out that the energy provided by QPM was utilized inefficiently compared to the energy provided by regular maize. The apparent ileal digestibility of lysine was larger in QPM than in regular maize, while the standard ileal digestibility of lysine was identical in both. The current study adds to our knowledge of QPM's nutritional content, amino acids digestibility, and N usage.

Untea et al. (2017) aimed to see how Cr as picolinate affects growth, plasma metabolites, N and fat digestibility, and pork quality (amino acids and fatty acids content of different tissues) in growing pigs. According to their findings, Cr supplements (200 ppb) increased nutrients balance and pork quality. The levels of important amino acids in tenderloin and ham samples from the Cr supplemented group were higher, showing that Cr might be used to make food functional. The mechanisms underlying these effects are unknown, and more research is needed to assess the consistency of these findings.

Habeanu et al., from 2019 to 2022, devised an experimental program with the goal of identifying and establishing the optimal nutritional solution to reduce nitrogen output and its interaction with $N_2O$. Thus, in 2019, Habeanu et al. assessed changes in performance, N metabolism, and composition of several tissues (*Longissimus dorsi* and semitendinosus muscles, heart, spleen, liver, and cecum) by combining two sources rich in n-3 fatty acids particularly alpha-linolenic fatty acids (extruded linseed and walnut meal, LE:WM, 50:50 wt/wt). Overall, their findings suggest that a diet high in n-3 fatty acids has a considerable effect on N metabolism. The addition of the LE:WM mixture decreased N excretion as well as net protein utilization. More than 40% of N was retained, resulting in increased nitrogen usage efficiency. The same team, one year later, estimated $N_2O$ production in the manure and on N metabolism of growing-finishing pigs by using three different by-products from the oil industry: mustard cakes x grapeseed cakes (MxG) and sunflower meal (SFM). Their results showed that MxG cakes are high in dietary fiber than SFM. The MxG mix determine a slight decline of performance when compared to the standard SFM. However total N output levels in pigs fed MxG mixture are lower, presumably because to the greater fiber content and fractions. The same pattern was seen in $N_2O$, which decreased by 5% in pigs fed a fiber-rich diet. The inclusion of a 15% MxG mixture in pigs' diets, although reduced growth parameters, can be considered as a valuable nutritional solution that contributes to lowering $N_2O$ and N excretion. However, in terms of performance, SFM remains an ingredient with valuable potential.

Another experiment was conducted by Habeanu et al., in 2021, in order to determine the effects of three different diets on N excretion in relation to other N metabolism indicators such as N retained (NR), N digested (ND), N digestibility, total N output (TNO), biological value of feed protein (BVFP), net protein utilization (NPU), coefficient of metabolizability (CAM), N clearance rate (CR), and blood urea N (BUN) and their correlation in males pigs. The authors look at the impact of different diets on performance and feeding efficiency, as well as enteric $CH_4$ and $CO_2$ emissions through manure. Based on the findings of their study, both peas and linseed have the ability to replace a part of soybean meal. Peas combined with soybean meal resulted in a decrease in growth performance due to a higher intake of some carbohydrates fractions. Even yet, a combination of peas, soybean meal, and linseed seemed to wipe out the discrepancies. It's worth noting that, regardless of the diet, the dietary protein level was guaranteed to be at the minimal threshold corresponding to nutritional requirements. Despite this, in pigs group fed peas diet, dry matter and organic matter intake were higher. Although peas incorporated in the diet increased significantly the total concentration of BUN, the N metabolism indicators were not significantly altered.

**Nitrous oxide emissions**

$N_2O$ is a major contributor to global warming and one of the factors which affects the earth's atmosphere When $N_2O$ emissions from the livestock sector climbed dramatically, an alarm signal was issued. $N_2O$ is a greenhouse gas with a 298-fold higher GWP than $CO_2$ ($CO_2$ eq.). Pigs are responsible for about 26% of total $N_2O$ emissions.

When there is a lack of o of oxygen and/or a nitrite accumulation during nitrification, $N_2O$ is produced as a by-product. Denitrification is the process of converting $NO_3$ to $N_2$, with various intermediate molecules ($NO_2$, NO, and $N_2O$) created along the way. Denitrification in manure is primarily carried out by heterotrophic facultative aerobic bacteria. In the presence of oxygen and/or a low quantity of degradable carbohydrates, the formation of $N_2O$ in manure is favored (Poth & Focht, 1985, Philippe & Nicks, 2015).

Therefore, it is critical to uncover the processes of dietary protein metabolism and the regulatory mechanisms that will aid in the reduction of $N_2O$ emissions. Feeding low N rations and supplementing essential amino acids such as lysine and methionine to balance the amino acids profile of pig are two nutritional approaches for reducing N excretion from animals and, as a result, $N_2O$ emissions. Other strategies include increasing fibber level into the diets.

Since 2006, Yasuyuki et al. added nitrite-oxidizing bacteria NOB to prevent $NO_2$ accumulation by boosting $NO_2$ oxidation till $NO_3$, i.e. complete nitrification, and its effect on $N_2O$ emission during pig manure composting. Such as the authors indicated in this study, $NO_2$ accumulation resulting from an unbalanced composition of nitrifying communities appeared to have a significant role in $N_2O$ emission. It was also demonstrated that improving nitrifying communities, by adding nitrite-oxidizing bacteria, could limit $NO_2$ accumulation and $N_2O$ emission.

Philippe & Nicks (2015) reviewed the mechanisms responsible for the manure production of $CO_2$, $CH_4$, and $N_2O$ by pigs. Literature emission factors are reviewed as well according to physiological stages of pig development, and an overall emission factor for the entire pig production process is provided. The impact of pig rearing conditions (including nutritional parameters) on emissions was investigated, as well as various mitigation approaches.

## CONCLUSIONS

Of the many solutions proposed to reduce nitrogen emissions, strategies based on feed solutions are the most effective in the pig farming sector.

In most cases, the benefit is twofold, namely limiting the greenhouse effect and improving animal production.

In practice, a reduction in gas emissions in the future is possible as long as farmers are aware of the need to invest in order to ensure this food adaptation to the new recommendations of researchers in the field.


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# GOAT COLOSTRUM – COMPOSITION AND IMPACT

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***Abstract***

*Colostrum is the first milk that a newborn receives immediately after birth. Its quality and timely intake are the main factors influencing the survival chances of the newborn. The composition of goat colostrum depends on the breed, age, diet and health of the animal. Immunoglobulins from the blood of the mother goat do not cross the placental barrier during pregnancy, at birth the kid does not have antibodies against the surrounding infectious agents. These immunoglobulins are concentrated in the colostrum and provide the passive immunity that the kid acquires. Goat colostrum has been shown to contain twice as much immunoglobulin G, as cattle colostrum. The specific biological properties of colostrum make it a valuable material for the development of food supplements. In recent years, these supplements have become increasingly popular on the world market as a powerful immunostimulant. The objective of the present review is to give a brief overview of the physicochemical and immunological properties of goat colostrum as well as the differences in the different breeds.*

***Key words***: *colostrum, goats, immunoglobulins, immunity, physicochemical parameters.*

## INTRODUCTION

Goats are animals distributed all over the world with an exceptional adaptive capacity. Over the years, they have become highly productive animals, transforming low-quality fodder into high-quality products intended primarily for the market.
Unlike cow's milk, goat's milk is characterized by better digestibility, alkalinity and buffering ability (Rashid et al., 2012). In this regard, the specific biological properties of colostrum make it a valuable raw material for the development of food supplements. In recent years, these supplements have become increasingly popular on the world market as a powerful immunostimulant.
Colostrum milked in the first 24 hours is the richest in fats, proteins and immunoglobulins. Moreover, there are higher values of titratable acidity in this period than in the next few days of early lactation. Colostrum is considered to have passed into milk after day 5, when all measured values meet the normal limits described in the goat's milk references. After the first to the fifth day, milk secreted by the mammary gland can be considered as transitional goat's milk, which is not quality colostrum due to low and immunological quality and is unfit for processing dairy products due to its high acidity, immunoglobulin and fat content. (Sánchez-Macías et al., 2014).
Under the legislation of many countries, milk intended for human consumption cannot contain colostrum (European Commission Regulation (EC) 1662/2006) (Romero et al., 2013), the same author notes the claims of Raynal-Ljutovac et al. (2005), which affect the negative impact of the presence of large amounts of protein in milk intended for the production and standardization of certain dairy products. It is important for the dairy industry that milk does not contain colostrum. However, the EU defines colostrum as an animal product for human consumption (Hodulová et al., 2014), in the form of food supplements.
According to Sánchez-Macías et al. (2014), the transition period (transition from colostrum to milk) is marked by nutritional, metabolic, hormonal and immunological changes that affect the incidence of infections and metabolic diseases. During this period, gradual or sometimes sudden changes in the composition and properties of colostrum can be observed (Arain et al., 2008; Sánchez-Macías et al., 2014).

Sánchez-Macías et al. (2014) cite the claims of Feagan (1979), who described the higher content of IgG in colostrum as a prerequisite for changing some physicochemical properties that make it difficult to process milk for different types of product. Less efficient pasteurization, reduced thermal stability of milk, the unpleasant tastes after pasteurization of milk, as well as reduced yield of cheese and cottage cheese associated with increased protein content are cited as such.

## RESULTS AND DISCUSSIONS

Timely suckling with colostrum is essential for the survival of newborn kids. Colostrum contains sufficient nutrients and immune-globulins that act as natural antimicrobial agents and actively stimulate the development of the newborn's immune system (Arguello et al., 2006). Colostrum suckling within a few hours after birth plays a vital role in the health and survival of the kid, as well as in building its passive immunity (Rashid et al., 2012).

In order to suckle successfully, the kid must be able to get up and move to the udder, and the mother's behavior serves only as a stimulus and guide to the udder, but in itself cannot ensure suckling, which depends on neonatal motor activity of the kid (Dwyer et al., 1999). The time it takes to stand up and find the udder is extremely important for timely suckling. The highest neonatal mortality is observed in the first 3 days, suggesting that events occurring during this period are of particular importance for survival (Nowak et al., 2000; Martinez et al., 2009).

Normal colostrum suckling and absorption is a major vital determinant of health (Khan et al., 2006) and normal growth during neonatal development and is more significant than birth weight (Chen et al., 1998).

Stoycheva et al., (2017) found in goats of Bulgarian White Dairy breed that 56% of singles and 56% of twins tried to suckle as 31% of singles and 25% of twins were suckling during the first hour after the birth.

Arguello et al., (2006) summarize the writings of Morand-Fehr, (1989) and Dos Santos et al., (1994) and confirm that the survival rate of newborn kids is related to the amount of colostrum taken in the first two days after birth and that the rectal temperature of colostrum-fed kids is higher than that of milk-fed kids. Getting enough high-quality colostrum immediately after birth is one of the most important practices in raising kids.

Colostrum synthesis

Linzell & Peaker (1974) studied changes in colostrum composition and mammary gland permeability during late pregnancy and birth in goats. Of interest are the mechanisms responsible for the ionic composition of colostrum and its conversion into milk later. The authors refer to their previous scientific publications (Linzell, 1959; Linzell & Peaker, 1971a,b), and confirmed that milk secretion begins before birth, but because milk is not milked, the pressure in the mammary gland increases to such an extent that there is a partial balance between the two phases of milk - the released milk (rich in calcium and lactose) and the extracellular fluid (rich in sodium chloride). Some evidence suggests that the increase in pressure may not be the only mechanism responsible for the ionic composition of colostrum. It is possible, in contrast to the lactation period, that there is some rupture of the epithelial tissue of the mammary gland, which at a later stage of lactation allows substances to pass directly (i.e. in both directions between extracellular fluid and milk). According to the authors, this mechanism may explain the ionic composition, but not the content of immune-globulin in colostrum. Colostrum is rich in protein and antibodies, but contains less fat and carbohydrates than mature milk.

**Colostrum composition and breed dependence**

Breed dependence in the composition of goat colostrum has been proven.

Zaharia et al. (2011) followed the change in the **fat** content of colostrum from 0 to 7 days in local breeds of Romanian goats. The authors found 4.2% fat per hour 0, followed by an increase to 8.08% at 6 hours, a decrease after 12 hours (5.14%) and an increase of 6.02% again after 24 hours, followed by a continuous decrease to average value of 2.3% on day 7. For Murciano-Granadina goat breed, Romero et al. (2013) reported 9.53% fat content in colostrum at hour 0, while for goats of Majorera-breed, the values are 7.7% (Sánchez-Macías, et al., 2014). In Beetal goats (Rashid et al., 2012), they reported a much lower fat content on the first day of 3.8%, followed by a continuous increase on the second (4.5%) and third day (5.2%).

For Bulgarian White Dairy, Zunev et al. (2004) reported 5.72% and 6.65% in crossings with Toggenburg breed for the entire colostrum period.

According to Rashid et al. (2012), low fat content of colostrum milked immediately after birth could be explained by the lower content of fatty acids, triglycerides, phospholipids and cholesterol compared to colostrum milked twenty-four hours later. This is a kind of mechanism that protects the digestive tract of the newborn.

**Lactose** is a compound that is absorbed faster than fats (Arguello et al., 2006). Rashid et al. (2012), studied the composition of colostrum in Beetal goats and found that when switched to milk, the amount of lactose increases. Lactose promotes intestinal absorption of calcium, magnesium, phosphorus and vitamin D3 (Chilliard et al., 2003; Rashid et al., 2012). This is confirmed by a number of authors who report an increase in lactose during lactation (Akinsoyinu et al., 1979, in West African dwarf goats, Hadjipanayiotou, 1995, in Damascus goat, Arguello et al., 2006, in Majorera-breed, Hodulová et al., 2014 in Czech White Shorthair goats). Romero, et al. (2013) reported 2.9% lactose per hour 0 and an increase to 4.48% per 156 hours in samples of colostrum from Murciano-Granadina goats. Sánchez-Macías et al. (2014) found that lactose increased from 2.44% per hour 0 to 5.44% over a 90-day period in Majorera-breed goats.

Zunev et al. (2004) reported 4.73% lactose both for Bulgarian White Dairy and its crossings and with Toggenburg breed for the whole colostrum period and 4.90% for mature milk on 14 days.

At birth, the kid does not have antibodies against the surrounding infectious agents because the antibodies in the mother goat's blood do not cross the placental barrier (Arguello et al., 2006; Hernández-Castellano et al. 2015). Goats receive antibodies through the first colostrum they suckle, which is why first-time suckling is essential because of the highest permeability of the intestinal mucosa in the first 24 hours after birth (Nordi et al., 2012, Moretti et al., 2013). Colostrum has a laxative effect, it helps the first defecation of the newborn kid - meconium (Rashid et al., 2012).

One of the main **proteins** in colostrum and milk is casein (Rashid et al., 2012, Soloshenko et al., 2020). The content of the main components of colostrum varies significantly during the postpartum period, at the highest concentration of proteins, including lactoferrin and immunoglobulins, is observed in the first 24 hours after birth (Soloshenko et al., 2020). Rachman et al. (2015) reported a drastic drop in lactoferrin levels in the first 48 hours after birth in three goat breeds in Indonesia.

The protein is a source of antimicrobial peptide precursors that increase the newborn's natural defenses against pathogens. The high protein content of colostrum is due to the presence of immunoglobulins, leukocytes, lactoferrin, lysozyme, polypeptides, cytokines, growth hormones, insulin-like growth factors, fibroblast growth factors, epithelial growth factors and some amino acids (Pellegrini et al., 1994; Rashid et al., 2012).

Sánchez-Macías et al. (2014) studied the colostrum of ten Majorera goats that gave birth to twins, and found that the percentage of protein in colostrum decreased by 45% by the second day after birth. From the third to the fifth day, no significant differences were observed. Which, according to them, is due to higher amounts of casein and immunoglobulins (Tsioulpas et al., 2007). In the course of the study, the percentage of protein in milk decreased, being 3.49% on the 15th day after birth and remaining at these values for up to 90 days.

Both lactose and protein are dependent on differences among breeds.

Sánchez-Macías et al. (2014) summarize their previous studies (Sánchez-Macías et al., 2010b) in the Majorera breed, as well as those of other authors, like Csapó et al. (1994) found 16.2% total protein in colostrum from White Hungarian goats, Hadjipanayiotou (1995) in turn found 16.0% protein in colostrum from Damascus goat, and Chen et al. (1998) found 16.5% in colostrum from Nubian goat. This summary confirms the presence of differences among breeds, which are more pronounced in dairy breeds, as the total protein is content lower in highly productive breeds (Pritchett et al., 1991;

Quigley et al., 1994, Sánchez-Macías et al., 2014).

According to Zazharska & Samoylenko (2016), the quality of colostrum depends mostly on the concentration of IgG. Shao et al. (2021) examined the concentration of IgG in goat colostrum and found a significant decrease from 1 to 7 days after birth. The amount of IgG decreases dramatically in the first 24 hours after birth, as it continues until the third day and remains relatively constant until day 7. The studies of Moreno-Indias et al. (2012) in Majorera goat breed found a decrease in the amount of IgG in the first 10 hours after birth by 37.4 mg/ml.

Csapó (2013) describes a very strict relationship between serum protein and colostrum IgG content.

Kessler et al. (2019) found the presence of more IgG in goat colostrum compared to sheep. They also prove differences among ten breeds of goats and sheep. Moreover, beef breeds synthesize more IgG than dairy breeds.

Castro et al. (2009) reported that birth weight and number of kids born also affected the acquisition of immunity. According to them, triplets and kids with lower birth weight (<2.8 kg) are at risk of failure of passive immunity and need special attention immediately after birth.

Keskin et al. (2007) found that sheep colostrum contained more protein, fat and lactose than goat colostrum, which was confirmed by Kessler et al. (2019) for ten breeds of sheep and goats.

Colostrum contains 4 to 10 times more **vitamins** A, D and E than milk and is a major source of these nutrients for newborns immediately after birth, as some vitamins do not cross the placental barrier (Uruakpa et al., 2002; Debier et al., 2005; Zarcula et al., 2010, Hodulová et al., 2014).

Hodulová et al. (2014) found a 14-fold higher concentration of vitamin A and a 5-fold lower concentration of vitamin E in goat colostrum in the first hour after birth than in 132 hours.

**The density** of colostrum and milk depends on indicators, such as fat, protein, total solids (TS) and dry fat-free residue. The higher the percentage of these physicochemical parameters, the higher the density.

Romero et al. (2013) found that colostrum density of Murciano-Granadina goat breed, studied immediately after birth was 1.0528 (g/ml) and at 156 hours decreased to 1.0303 (g/ml), while Sánchez-Macías et al. (2014) reported a density value of 1.0480 (g/ml) in Majorera-breed, decreasing to 1.0280 (g/ml) ninety days later.

Hernández-Castellano et al. (2015) summarizes information from some publications (Ahmad et al., 2000; Banchero et al., 2004; da Nobrega et al., 2005; Nowak & Poindron, 2006; Morales-delaNuez et al., 2011) that kids that are bottle-fed and have enough colostrum in the first days of life receive adequate, passive immune transfer, which favors their growth, development and productivity. The influence of factors such as feeding, maternal health, number of offspring on the quality of colostrum should not be overlooked.

Knight & Peaker (1982) found that single goats secreted less milk than those born to two or more kids.

According to Romero et al. (2013), pH, protein and lactose are significantly affected by the number of kids born. While chemical and physical indicators are strongly influenced by the time after birth and less by the number of born kids and the sequence of lactation (Arguello et al., 2006).

**Colostrum quality**

As already mentioned, the first-time suckling is of utmost importance. Equally significant and essential for the acquisition of passive immunity is the provision of quality colostrum (Hue et al., 2021).

Unlike expensive standard methods, the easiest, most affordable and practical device for determining the quality of colostrum in the field is the manual optical refractometer. It is used in dairy farms to measure solids. However, it can be used to assess the IgG content of colostrum, based on comparison with standard methods such as ordinary RID and ELISA.

These methods require special knowledge and consumables, and are also expensive and require technological time (Zobel et al., 2020). Therefore, the measurement of IgG using an optical refractometer is becoming more and more popular as a practical method for measuring it in the field. When using a Brix refractometer to assess the quality of goat colostrum, Brix% values less than 18.5% -

21.5% identify it as poor (Buranakarl et al., 2021).

Zobel et al. (2020), also, consider determining the quality of goat colostrum on the farm to be extremely important and a first step in improving the assessment of goat colostrum quality at the farm level. The ability to quickly determine the quality shows how well it is suitable for feeding newborn kids, and thus, facilitates the implementation of immune transfer. The authors are working on the validation of Brix refractometers and a hydrometer to determine the quality of goat colostrum. According to them, when read with a refractometer, goat colostrum shows lower values than beef. They indicate values of % Brix less than 19% for colostrum, as a reason to consider it low quality.

Rudovsky et al. (2008) measured the concentration of immunoglobulins in the colostrum of Weiße Deutsche Edelziege goats. According to them, the hydrometer allows immediate assessment of the concentration of immunoglobulin in the colostrum of goats. The method is cost-effective and easy to apply, but to be accurate, an adjustment must be made to the temperature in the barn.

### Application of goat colostrum

There are few studies in the available literature on the functional side of goat colostrum. Functional foods made from goat's milk have antioxidant, anti-inflammatory, cardioprotective, antihypertensive and antiatherogenic effects in the human body (Voloshyna et al., 2021). The authors define goat colostrum as a unique product that can be used to create cosmetics for maintaining healthy skin and treating skin diseases, as well as for the production of functional foods with antioxidant and anti-inflammatory properties and baby foods. These foods are very digestible and are an alternative source of milk oligosaccharides (van Leeuwen et al., 2020) for baby foods.

Bioactive peptides in goat's milk protein and their effects on human health, mitochondrial diseases and brain malignancies have been studied (Sharma et al., 2017).

Hyrslova et al. (2016) studied the possibility of combining goat colostrum with probiotics and recommended it as a raw material for the production of dietary supplements with bifidobacteria.

Balagayathri et al. (2021) evaluate the in vitro antimicrobial, antioxidant and anticancer activity of bioactive peptides from goat colostrum and found that they are effective against both gram-positive *(Bacillus subtilis* and *Staphylococcus aureus)* and Gram-negative *(Escheresia coli, Klebsiella pneumo)* have potent cytotoxic activity.

Romero et al. (2013), in goats of Murciano-Granadina goat breed, and Hodulová et al. (2014) in Czech White Shorthair goats, note that transitional milk after the fourth day can be used as a raw material for the food industry without affecting the quality of the product.

## CONCLUSIONS

In conclusion, despite the differences among breeds in the colostrum composition from goats, it is an indispensable source of antibodies and nutrients for the newborn kid.

There are easy and relatively accurate methods for determining its quality.

Determining and controlling the quality of goat colostrum is of a great significance for the survival and health of the newborn kid, as well as the future productivity and economic efficiency of the farm.

Goat colostrum is a valuable raw material for the pharmaceutical industry and enables the development of new food supplements and immunostimulants.

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# COMPARATIVE STUDY REGARDING THE PHENOTYPIC PERFORMANCES OF THE ASCENDANCE AND DESCENDANCE OF DAIRY CATTLE FROM HUSBANDRY HOLDINGS IN NEAMȚ COUNTY

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***Abstract***

*In this study, the productive performances of the ascendants and descendants of the cows' herd belonging to the 4 basic breeds were analyzed, as follows: Holstein, Bălțată cu negru românească (Romanian Black Spotted), Bălțată românească (Romanian Spotted), and Brună de Maramureș (Maramures Bown), from 7 farms of Neamț county, which were encoded with numbers from 1 to 7. For the analysis of the ascendants' performances - mother (M), paternal grandmother (MT), and maternal grandmother (MM) - and of the offspring' performance, 3 indicators were determined regarding the milk production: the quantity per normal lactation (kg), the percent of fat, and the percent of protein. It was found that the best milk production in normal lactation of offspring was recorded in farm 2 (6161.25 kg), where a mixed herd of Holstein and Bălțată cu negru românească breeds is raised. If we compare the phenotypic performances of the ascendants (mother of mother - MM), which realized an average milk production of 7205.44 kg, with the father's mother (MT) of 11.931 kg, and the mother's mother (MM) of 7949.83 kg we confirm that the phenotypic performance for the milk production in the offspring does not reach the level of those obtained by ancestry. This fact is due to management deficiency regarding the external factors.*

***Key words****: ancestors, cattle, descendants, milk, production.*

## INTRODUCTION

In cows, individual milk production is influenced by a number of factors which, according to nature, can be grouped as follows: genetic and physiological factors and environmental factors (Velea et.al., 2004).

The category of genetic factors includes factors directly related to the hereditary and physiological basis of cattle and influences the productive potential of their populations or individuals (Gîlcă et al., 2006).

In order to improve the genetic base of cattle in Neamț County, in late years, imports of live breeding animals and frozen semen from high biological value bulls have been made.

In the studied area there is a tendency to increase mixed breeds (milk-meat) as well as those specialized in milk production, with high productive potential.

Environmental factors that influence the phenotypic performance of cattle relate particularly to the technology of exploitation, feeding and production having a decisive role.

The operating technologies used may lead to different production and economic outcomes, generated in particular by the maintenance and operation system, the calve growth, the production and use of fodder, the use of constructions, installations and machinery, the production and work management. (Stoica et al., 2002)

Nutrition plays a key role in milk production and other environmental factors, in fact changing the degree of conversion of food to milk.

The planning of the breeding process has a decisive role in the growth and exploitation of cattle. This directly influences the herd increase, the improvement of the quality, the increase of the milk production and of the economic efficiency (Dinescu & Tontsch, 2002).

The objectives pursued in the breeding process are to achieve throughout the year an optimal structure of the herd, by physiological states and to obtain the highest fertility, the task being to obtain from each cow, each year, one calf characterized through high viability (Onaciu, 2006).

## MATERIALS AND METHODS

In order to assess the main indicators that characterize the phenotypic performances of the cattle breeds with mixed milk-meat skills and those specialized for milk raised and exploited in Neamț county, a total number of 856 cows was studied. The studied reproduction herd was of 447 cattle, consisting of: 103 heads of the Brună de Maramureș (Maramures Brown) breed, 50 heads of the Bălțată românească (Romanian Spotted) breed, 188 heads of the Bălțată cu negru românească (Romanian Black Spotted) breed, and 106 heads of the Holstein breed.

Statistical processing of milk production in the bovine herd studied has been carried out for 3 characteristics of milk production: normal milk lactation (kg), fat content (%) and protein proportion (%).

The data analysis was performed by calculating the parameters, which characterize a normal distribution, on the one hand the mean or average ($\overline{X}$), and on the other hand the dispersion indices represented by standard deviation (s), arithmetic mean error (**±s**$_{\bar{x}}$), coefficient of variability (V%).

The seven farms studied were coded from 1 to 7 and are shown in Table 1.

Table 1. Diary cattle farms encode

| Farm encode | Name of the farm | Breed |
|---|---|---|
| 1 | I.F. Anghel Oana Brândusa | Balțată cu negru românească |
| 2 | P.F.A. Muraru Constantin Felix | Holstein; Balțată cu negru românească |
| 3 | Nacu Gheorghe | Holstein; Balțată cu negru românească |
| 4 | P.F.A. Olariu Neculai Marius | Brună de Maramureș |
| 5 | SCDA Secuieni | Brună de Maramureș |
| 6 | I.I. Ciucalau Aurelia Vasilica | Bălțată românească |
| 7 | P.F.A. Pintea Ionela | Bălțată românească |

## RESULTS AND DISCUSSIONS

Table 2 shows the data on the average values and variability of the milk production indices of the ascendants for farm 1 which shows that in the case of the mother the quantity of milk averaged 6537.03 kg, while for the father's mother it was higher of 10370.07 kg, and for the mother's mother of 6511.36 kg. Thus, if we take into account the production performances written in the literature for the Bălțată cu negru românească breed, where the standard of milk production is 5000-5700 kg, the milk fat content is 3.8-3.9%, and the protein content is 3.22% (Maciuc et.al., 2015), it can be said that farm 1 has a valuable ascendance.

Table 2. Average values and variability of milk production in farm 1

| Upward | Characters | n | $\overline{X}$ | $\pm s_{\bar{x}}$ | s | V% | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| M. | Milk kg | 29 | 6537.03 | 222.847 | 1200.070 | 18.358 | 3776.00 | 9234.00 |
| | Fat % | 29 | 3.33 | 0.055 | 0.294 | 8.824 | 2.61 | 3.99 |
| | Protein % | 29 | 3.28 | 0.025 | 0.134 | 4.100 | 2.99 | 3.54 |
| MF. | Milk kg | 15 | 10370.07 | 391.752 | 1517.250 | 14.631 | 7757.00 | 11551.00 |
| | Fat % | 15 | 4.08 | 0.113 | 0.436 | 10.697 | 3.49 | 4.40 |
| | Protein % | 0 | 0.00 | 0.000 | 0.000 | 0.000 | 0.00 | 0.00 |
| M. | Milk kg | 14 | 6511.36 | 387.241 | 1448.923 | 22.252 | 2915.00 | 8595.00 |
| | Fat % | 14 | 3.16 | 0.142 | 0.533 | 16.859 | 2.40 | 4.12 |
| | Protein % | 14 | 3.23 | 0.048 | 0.179 | 5.546 | 3.03 | 3.78 |

Legend: M-mother; MF-mother of the father; MM- mother of the mother.

Table 3 shows the data on the average values and variability of the milk production indices of the ascendancy for farm 2. It was found that the average production on normal lactation of mothers is 7205.44 kg, in paternal grandmothers 8160.67 kg, and in maternal grandmothers 7949.83kg.

From the analysis of the dispersion degree, the standard deviation having the maximum value of s = 1456.494 kg milk, and the maximum coefficient of variation of V% = 20.214 in mothers, in paternal grandmothers s = 0 kg of milk, as well as the coefficient of variation V% = 0 kg, but it is necessary to specify that data were found only for two animals that achieved the same performance on the average value of milk production on normal lactation, and in maternal grandmothers s = 690.986 kg, the maximum coefficient of variation of V % = 8.692, it can be said that there is an average homogeneity regarding the phenotypic performances registered by mothers and maternal grandmothers, the farmer has the possibility to select the most valuable animals for use in multiplication.

Table 3. Average values and variability of milk production indices of farm 2

| Upward | Characters | n | $\overline{X}$ | $\pm s_{\bar{x}}$ | s | V% | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| M. | Milk kg | 16 | 7205.44 | 364.124 | 1456.494 | 20.214 | 5014.00 | 10439.00 |
| | Fat % | 16 | 3.94 | 0.068 | 0.272 | 6.890 | 3.55 | 4.44 |
| | Protein % | 16 | 3.29 | 0.054 | 0.214 | 6.520 | 2.98 | 3.75 |
| MF. | Milk kg | 2 | 11.931 | 0.000 | 0.000 | 0.000 | 11.931 | 11931.00 |
| | Fat % | 2 | 3.94 | 0.000 | 0.000 | 0.000 | 3.94 | 3.94 |
| | Protein % | 0 | 0.00 | 0.000 | 0.000 | 0.000 | 0.00 | 0.00 |
| MM. | Milk kg | 6 | 7949.83 | 282.094 | 690.986 | 8.692 | 6566.00 | 8345.00 |
| | Fat % | 6 | 4.07 | 0.157 | 0.385 | 9.458 | 3.67 | 4.65 |
| | Protein % | 6 | 3.39 | 0.109 | 0.268 | 7.907 | 3.01 | 3.65 |

Legend: M-mother; MF-mother of the father; MM- mother of the mother.

Table 4 presents the data on the average values and variability of the milk production indices of the ascendancy for farm 3 which shows that for the quantity of milk for mother had an average of 6801.41 kg, and for paternal grandparents they recorded an outstanding performance of 13352.87 kg, and to the maternal grandmothers 7015.00 kg.

From the analysis of the degree of dispersion, the standard deviation having the maximum value of s = 1716.487 kg of milk, and the maximum coefficient of variation of V% = 25.237 in mothers, in paternal grandmothers s = 3452.779 kg of milk, the coefficient of variation V% = 25.858 kg, and in maternal grandmothers s = 1397 kg, the maximum coefficient of variation of V% = 19.928, it can be said that there is a heterogeneous population regarding the phenotypic performances registered by mothers and maternal grandmothers, and in maternal grandmothers an average homogeneity is observed (this requires special attention from the farmer in the selection and mating management, because only on the basis of knowledge of performance - amount of milk, percentage of fat, percentage of protein - the parents of the next generation are chosen (Păcală, 2000).

Table 4. Average values and variability of milk production indices of farm 3

| Upward | Characters | n | $\overline{X}$ | $\pm s_{\bar{x}}$ | s | V% | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| M. | Milk kg | 27 | 6801.41 | 330.338 | 1716.487 | 25.237 | 3473.00 | 12786.00 |
| | Fat % | 27 | 3.84 | 0.079 | 0.409 | 10.645 | 2.69 | 4.65 |
| | Protein % | 27 | 3.39 | 0.042 | 0.220 | 6.502 | 2.53 | 3.65 |
| MF. | Milk kg | 15 | 13352.87 | 891.504 | 3452.779 | 25.858 | 6649.00 | 16987.00 |
| | Fat % | 15 | 4.00 | 0.111 | 0.428 | 10.706 | 3.30 | 4.37 |
| | Protein % | 2 | 3.19 | 0.085 | 0.120 | 3.774 | 3.10 | 3.27 |
| MM. | Milk kg | 8 | 7015.00 | 494.247 | 1397.941 | 19.928 | 4368.00 | 8577.00 |
| | Fat % | 8 | 3.89 | 0.114 | 0.322 | 8.285 | 3.45 | 4.36 |
| | Protein % | 8 | 3.17 | 0.125 | 0.355 | 11.194 | 2.53 | 3.65 |

Legend: M-mother; MF-mother of the father; MM- mother of the mother.

Table 5 shows the data on the average values and variability of the milk production indices of the ascendancy for the farm 4 from which it is observed that for mother, the quantity of milk averaged 5413.89 kg, for the paternal grandmothers the productive performances are not registered in the database, and for the mother's mother of 5475.00 kg.

Table 5. Average values and variability of milk production indices of farm 4

| Upward | Characters | n | $\overline{X}$ | $\pm s_{\bar{x}}$ | s | V% | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| M. | Milk kg | 9 | 5413.89 | 92.372 | 277.117 | 5.119 | 5035.00 | 5857.00 |
| | Fat % | 9 | 3.54 | 0.168 | 0.504 | 14.240 | 2.40 | 3.98 |
| | Protein % | 9 | 3.49 | 0.026 | 0.150 | 4.289 | 3.21 | 3.91 |
| MF. | Milk kg | 0 | 0.00 | 0.000 | 0.000 | 0.000 | 0.00 | 0.00 |
| | Fat % | 0 | 0.00 | 0.000 | 0.000 | 0.000 | 0.00 | 0.00 |
| | Protein % | 0 | 0.00 | 0.000 | 0.000 | 0.000 | 0.00 | 0.00 |
| MM. | Milk kg | 3 | 5475.00 | 206.730 | 358.067 | 6.540 | 5147.00 | 5857.00 |
| | Fat % | 3 | 3.33 | 0.476 | 0.825 | 24.791 | 2.40 | 3.98 |
| | Prot ein % | 3 | 3.58 | 0.057 | 0.170 | 4.765 | 3.27 | 3.92 |

Legend: M-mother; MF-mother of the father; MM- mother of the mother.

Table 6 shows the data on the average values and variability of the milk production indices of the ancestry for farm 5 which shows that in the case of the mother the quantity of milk had an average of 6258.38 kg, for the paternal grandmothers the productive performances are not recorded in the database, and to the mother's mother of 6471.00 kg.

Table 6. Average values and variability of milk production indices of farm 5

| Upward | Characters | n | $\overline{X}$ | $\pm s_{\bar{x}}$ | s | V% | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| M. | Milk kg | 34 | 6258.38 | 122.933 | 716.816 | 11.454 | 5308.00 | 8286.00 |
| | Fat % | 34 | 3.99 | 0.046 | 0.271 | 6.773 | 3.56 | 4.63 |
| | Protein % | 33 | 3.61 | 0.047 | 0.140 | 3.873 | 3.36 | 3.78 |
| MF. | Milk kg | 0 | 0.00 | 0.000 | 0.000 | 0.000 | 0.00 | 0.00 |
| | Fat % | 0 | 0.00 | 0.000 | 0.000 | 0.000 | 0.00 | 0.00 |
| | Protein % | 0 | 0.00 | 0.000 | 0.000 | 0.000 | 0.00 | 0.00 |
| MM. | Mil k kg | 9 | 6471.00 | 178.614 | 535.843 | 8.281 | 5704.00 | 6993.00 |
| | Fat % | 9 | 4.28 | 0.139 | 0.416 | 9.700 | 3.57 | 5.01 |
| | Protein % | 9 | 3.70 | 0.052 | 0.091 | 2.455 | 3.60 | 3.78 |

Legend: M-mother; MF-mother of the father; MM- mother of the mother.

Table 7 presents the data on the average values and variability of the milk production indices of the ascendancy for the farm.
From the analysis of the dispersion degree, the standard deviation having the maximum value of s = 989,237 kg of milk, and the maximum coefficient of variation of V% = 13,713 for maternal grandmothers, for paternal grandmothers s = 1303,974 kg of milk, the maximum coefficient of variation of V% = 11,734%, in the paternal grandmothers, and in the maternal grandmothers s = 487,046 kg, the maximum coefficient of variation of V% = 6,525%, a particularly favorable situation is revealed for the selection in order to improve the productive performances in the offspring.

Table 7. Average values and variability of milk production indices of farm 6

| Upward | Characters | n | $\overline{X}$ | $\pm s_{\bar{x}}$ | s | V% | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| M. | Milk kg | 11 | 7214.09 | 298.266 | 989.237 | 13.713 | 6154.00 | 9705.00 |
| | Fat % | 11 | 3.99 | 0.069 | 0.227 | 5.690 | 3.51 | 4.31 |
| | Protein % | 11 | 3.46 | 0.073 | 0.244 | 7.047 | 3.13 | 3.92 |
| MF. | Milk kg | 11 | 11113.00 | 393.163 | 1303.974 | 11.734 | 9362.00 | 13391.00 |
| | Fat % | 11 | 4.14 | 0.056 | 0.184 | 4.450 | 3.92 | 4.35 |
| | Protein % | 11 | 3.55 | 0.070 | 0.231 | 6.508 | 3.26 | 3.95 |
| MM. | Milk kg | 8 | 7464.88 | 172.197 | 487.046 | 6.525 | 6929.00 | 8139.00 |
| | Fat % | 8 | 3.87 | 0.116 | 0.327 | 8.453 | 3.48 | 4.48 |
| | Protein % | 8 | 3.32 | 0.052 | 0.147 | 4.418 | 3.13 | 3.48 |

Legend: M-mother; MF-mother of the father; MM- mother of the mother.

Table 8 shows the data on the average values and variability of the milk production indices of the ancestry for farm 7, which shows that in the case of the mother the quantity of milk averaged 5119.86 kg, while in the case of grandparents higher values were recorded of 10627.00 kg, but it is specified that we have the production performance for a single animal and cannot be representative for the entire population of cows with reference to the performance of paternal grandparents, as well as maternal grandmothers productive performance is from a single animal, respectively of 5340.00 kg.

Table 8. Average values and variability of milk production indices of farm 7

| Upward | Characters | n | $\overline{X}$ | $\pm s_{\bar{x}}$ | s | V% | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| M. | Milk kg | 7 | 5119.86 | 77.910 | 206.131 | 4.026 | 4945.00 | 5340.00 |
| | Fat % | 7 | 3.06 | 0.182 | 0.483 | 15.771 | 2.34 | 3.59 |
| | Protein % | 7 | 3.29 | 0.019 | 0.049 | 1.503 | 3.20 | 3.37 |
| MF. | Milk kg | 1 | 10627.00 | 0.000 | 0.000 | 0.000 | 10627.00 | 10627.00 |
| | Fat % | 1 | 4.34 | 0.000 | 0.000 | 0.000 | 4.34 | 4.34 |
| | Protein % | 1 | 3.36 | 0.000 | 0.000 | 0.000 | 3.36 | 3.36 |
| MM. | Milk kg | 1 | 5340.00 | 0.000 | 0.000 | 0.000 | 5340.00 | 5340.00 |
| | Fat % | 1 | 2.82 | 0.000 | 0.000 | 0.000 | 2.82 | 2.82 |
| | Protein % | 1 | 3.29 | 0.000 | 0.000 | 0.000 | 3.29 | 3.29 |

Legend: M-mother; MF-mother of the father; MM- mother of the mother.

Summarizing the data presented in all the tables, it can be stated that the limit of variation of the productive performances of the mothers was between 5119.96 kg in patient 7 and 7214.09 kg in patient 6.

One aspect that determines the difference between the productive performances is the one related to the forage conditions in all the farms. Regarding this important link of the technological flow from dairy cattle farms, it is known that the productive genetic potential of each individual can be highlighted especially by administering balanced rations regarding the quantity and also the quality (Velea et.al., 2004).

The paternal grandmothers from farm 3 are the most valuable, the average milk production per standard lactation was 13352.87 kg values obtained through testing after offspring) (Creangă et.al., 2008).

It should be noted that on farm 7, the productive performance for the parental grandparents (MT) cannot be taken into account, being recorded one single animal.

For farms 4 and 5 where the Brună de Maramureș breed is raised, the productive performances for the paternal grandparents are not registered.

Regarding the productive performances achieved by the maternal grandparents (MM) from the seven farms studied, it can be said that the maternal grandmothers (MM) from farm 2 achieved an average milk production of the best lactation, which is 7949.83 kg.

Regarding the ascending performance of the level of milk fat percentage in all the studied farms, it can be stated that the milk of the paternal grandparents (MT) from farm 6 has the highest fat percentage, of 4.14%, although farm 7 shows the highest fat percentage, at 4.34%, only animal and we cannot calculate this value as a representative for this farm.

The analysis of the percentage of milk fat in maternal grandparents (MM) shows the superiority of those on farm 5, which recorded a very good fat percentage of 4.28.

Regarding the protein content of milk from the data presented, the best performance was shown in mothers and maternal grandparents on the farm 5. In mothers, the proportion of milk protein was 3.61% and in maternal grandparents 3.70%. Thus, the milk from the Brună de Maramureș cows is suitable for the cheese industry, the yield being about 16% higher compared to other breeds (Acatincăi, 2004).

From the data presented in figure 1 regarding the performance of milk production from the seven farms studied, in the offspring, at the first two lactations, the following are revealed:

At farm 1, the average milk production per total lactation in the 1st one was 6883.45 kg and 5413.82 kg per normal lactation; it was observed that in the second one it decreases registering 6031.80 kg per total lactation and 5394.20 kg per normal lactation.

At farm 2, for the first lactation, the average value of milk per total lactation was 6733.25 kg, in the normal lactation was 6161.25 kg, and at the second lactation there was an increase observed, both in the average milk production per total lactation, which was 7737.45 kg, as well as the production obtained on normal lactation, achieving 6938.09 kg of milk (this represents an increase of 776.84 kg).

At farm 3, in the first lactation, the average value of the total quantity of milk per lactation was

6753.29 kg, at the normal lactation of 6041.68 kg, and at the second lactation there is a decrease, both in the average milk production per total lactation, which was 6448.11 kg, as well as the production obtained on normal lactation, achieving 5430.68 kg of milk.

At farm 4, at the second lactation, the average value of the total amount of milk per lactation was 6025.00 kg, at normal lactation 4673.00 kg, and at the second lactation there is a decrease, both in the average milk production per total lactation, which was 4615.00 kg, as well as the production obtained on normal lactation, achieving 4497.00 kg of milk.

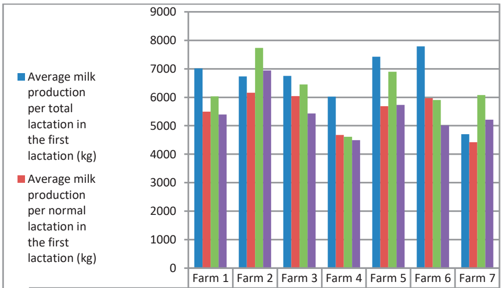
In farm 5 the average production on total lactation I is higher, respectively 7428.18 kg, compared to that obtained on total lactation II, this being 6892.11 kg. This is due to the higher number of lactation days, respectively 73.30. However, it can be seen that although the average milk production per total lactation at the second lactation decreased by 536.07 kg of milk, at normal lactation at the second lactation there is a slight increase in the average milk production of 50.82 kg, because at the second lactation an average production of 5683.26 kg of milk was registered, and at the second lactation 5734.08 kg.

At farm 6, at lactation, the average value of the total lactation was 7791.00 kg, the normal lactation was 5967.80 kg, and at lactation II there was a decrease, both in the average milk production and total lactation, which was 5901.56 kg, and of production obtained by normal lactation, reaching 5012.89 kg of milk.

At farm 7, the average value of the total lactation was 4702.00 kg at lactation, the normal lactation was 4420.67 kg, and at the second lactation there was an increase, both in the average milk production and the total lactation, which was 6074.00 kg, and of production obtained by normal lactation, reaching 5209.17 kg of milk.

In summary, it can be said that in the first two lactations, the milk production on normal lactation, in the studied farms, with the exception of farms 2, 5 and 7, at the second lactation there was a decrease in the average milk production on normal lactation, which it is mainly determined by the technological factors of operation and the poor management of these farms.

It is also found that productive performances have been achieved which can be appreciated as modest, compared to the productive performances recorded in ascendancy. (Figure 1)




| | Farm 1 | Farm 2 | Farm 3 | Farm 4 | Farm 5 | Farm 6 | Farm 7 |
|---|---|---|---|---|---|---|---|
| Average milk production per total lactation in the first lactation (kg) | 7013,05 | 6733,25 | 6753,29 | 6025 | 7428,18 | 7791 | 4702 |
| Average milk production per normal lactation in the first lactation (kg) | 5494,43 | 6161,25 | 6041,68 | 4673 | 5683,26 | 5967,8 | 4420,67 |
| Average milk production per total lactation in the second lactation (kg) | 6031,8 | 7737,45 | 6448,11 | 4615 | 6892,11 | 5901,56 | 6074 |
| Average milk production per normal lactation in the first lactation (kg)2 | 5394 | 6938,09 | 5430,68 | 4497 | 5734,08 | 5012,89 | 5209,17 |

Figure 1. Average milk production, on total and normal lactation at the first offspring

## CONCLUSIONS

The following conclusions can be drawn from the study:

The most valuable ancestry is owned by farmers: Nacu Gheorghe and I.I. Ciucălău Aurelia Vasilica, who have the highest average milk production on normal lactation in the studied animals.

In Nacu Gheorghe farm, the mothers achieved an average milk production on normal lactation of 6901.41 kg, the paternal grandmothers 13352.87 kg, and the maternal grandmothers 7015.00 kg. In this farm the Holstein breed and the Romanian Black Spotted breed are bred.

On the P.F.A. Muraru Constantin Felix, the mothers achieved an average milk production on normal lactation of 7205.44 kg, the paternal grandmothers of 8160.67 kg, and the maternal ones of 7949.83 kg. In this farm the Holstein breed and the Bălțată cu negru românească breed are raised.

On the I.I. Ciucălău Aurelia Vasilica, in mothers it was an average milk production on normal lactation of 7214.09 kg, in paternal grandmothers of 11113.00 kg and to maternal

grandmothers 7464.88 kg; the Bălțată românească breed is raised in this farm.
It should be noted that in the three farms where the ancestry is most valuable, it comes from imports, respectively from Ireland, on farms 2 and 3 for the Holstein breed and on farm 6 in Germany and for the German Spotted breed respectively (Fleckvieh).
There are significant differences between the yields obtained by farms 2, 3, and 6 compared to farms 1, 4, 5, 7.
From the analysis of the percentage of fat in milk obtained in the seven farms studied, the best results are revealed in farm 5 and farm 6. In the two farms, two breeds of cattle are raised, which are characterized morpho-productively by a mixed type, milk-meat, these being the Brună de Maramureș breed, on farm 5 and the Bălțată românească breed on farm 6, both being known for their high milk fat content.
The analysis of the quantitative indices of milk production at the first lactation on normal lactation for the seven farms studied showed that the best milk production on normal lactation was recorded on farm 2, as 6161.25 kg, followed by farm 3 with 6041 kg milk, 3rd place is occupied by farm 6 with 5967.80 kg of milk, which is justified by the valuable ancestry of the three farms.
At the same time, if we make a comparative analysis between the phenotypic performances in the offspring from farm 2, which has indeed the highest production, as 6161.25 kg of milk, with the phenotypic performances of that descendant, the mother's mother (MM), who achieved an average production of milk of 7205.44 kg, the father's mother (MT) of 8160 kg of milk, and the mother's mother (MM) of 7949.83 kg of milk.
The same happens for farm 3 where the production of milk on normal lactation is 6041.68 kg of milk, and where the ancestry is also valuable, the mothers recorded an average production of 6801.41 kg of milk, the maternal grandmothers 7015 kg, and to emphasize here the outstanding productive performance of the paternal grandparents who achieved 13352.87 kg of milk on normal lactation shows that the offspring fails to reach the phenotypic performance of the offspring.
Regarding this aspect of the difference in productive performance between offspring and offspring, it can be concluded that the performances obtained in the offspring are lower than those from the descendant, on the one hand because the cattle exploited in these two farms are not acclimatized in this area of the county. German, and on the other hand, the best maintenance conditions were not ensured, and especially a good quality feed.
A comparative study was made regarding the production performances obtained at the 1st lactation with those from the 2nd lactation registered in the 7 farms studied, which show the following:
- on farm 2, at the second lactation the average value of milk per total lactation was 6733.25 kg, at normal lactation 6161.25 kg, and at the second lactation there is an increase, both in the average milk production per the total lactation, which was 7737.45 kg, as well as the production obtained on normal lactation, achieving 6938.09 kg of milk, which represents an increase of 776.8 kg;
- in farm 5 the average production on total lactation I is higher, as 7428.18 kg, compared to that obtained on total lactation II, this being 6705.42 kg, but this is due to the higher number by 84.52 days of the total lactation duration. However, it can be seen that although the average milk production per total lactation at the second lactation decreased by 722.76 kg of milk, at normal lactation at the second lactation there is a slight increase in the average milk production of 16 kg;
- in all other farms (farms 1, 3, 4, 6, and 7), there is a downward evolution of the average milk production at the second lactation compared to the 1st lactation, which reveals the need to improve the management of the factors influencing the productive performances in dairy cattle.

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# THE INFLUENCE OF THE ADDITION OF OIL SEEDS IN THE DAIRY COW RATION ON $CO_2$ EMISSIONS

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***Abstract***

*The aim of the research undertaken was to highlight that emission reductions can be made available to producers in the steer farming sector and the adoption of current best practices and technologies for the rearing and health of animals, feed rations can be a tool that would help the dragline sector reduce greenhouse gases, and was realized on the Moara Domneasca farm on a flock of 29 dairy cows at different stages of Montbeliarde's lactation between January 2021 and September 2021. Daily milk production was established per lactation cycle, within the lactation cycle of 3 distinct stages and the establishment of two seasons, summer and winter. The influence of feed strategies applied on milk production, manure chemical composition and $CH_4$ and $CO_2$ emissions were analyzed. The milk production of cows was not influenced by the addition of vegetable oils, ranging between 22.04 l / head in the ascending phase of lactation, 19.86-20.96 l / head in the plateau phase and 19.45 l / head in the descending phase of lactation. The methane emission from enteric fermentation shows the highest values for variants 4 and 3, when 0.2 l/head/day of rapeseed oil were administered in each variant, and in version 4, 0.1 l/head/day of sunflower oil was also administered (methane emissions are 1.41 kg $CH_4$/year and 1.39 kg $CH_4$/year, respectively). The lowest emissions are recorded for nutrition variant 5 (in which equal doses, sunflower oil and rapeseed oil were administered: 0.1 l l/head/day). Also, the trend of $CO_2$ equivalent emissions closely follows the line of $CH_4$ emissions from enteric fermentation, being directly dependent.*

***Key words***: *emissions, enteric fermentation, manure, milk production.*

## INTRODUCTION

Greenhouse gas (GHG) emissions from human activities are likely to contribute to climate change. Climate change has been associated with rising sea levels, extreme weather conditions, air pollution and biodiversity loss. Such effects can harm ecosystems and human health. In order to monitor GHG emissions from human activities, initiatives to calculate and report on GHG emissions from human activities have increased. Cattle are responsible for about 30% of global GHG emissions from the livestock sector (Gerber et al., 2013). The stages along the dairy production chain include processes related to feed production (upstream), processes related to milk production on the farm (on the farm) and processes related to milk transport and processing (downstream). Significant GHG emissions from dairy production are carbon dioxide ($CO_2$), methane ($CH_4$) and nitrogen oxide ($N_2O$).

The main sources of GHG emissions from dairy production are enteric fermentation ($CH_4$), feed production (mainly $CO_2$ and $N_2O$) and manure management ($CH_4$ and $N_2O$).

Enteric fermentation and feed production each contribute about 30% to total emissions, while manure management contributes about 20%.

In this regard, various strategies have been proposed to reduce GHG emissions from activities within the traceability chain of dairy production (De Boer et al., 2011). Most strategies apply to upstream and on-farm processes (i.e. including the three main sources of GHG emissions), such as animal feed, plant or animal husbandry, or manure processing technology (Ellis et al., 2008); Wall et al., 2010; De Vries et al., 2012). From an animal husbandry research point of view, important areas of interest for reducing GHG emissions per kg of milk are feeding strategies to reduce emissions from enteric fermentation and feed production, as well as breeding strategies to improve animal productivity.

One way to reduce $CH_4$ emissions is to add oils to the ration of animals, which has generally been used to increase the energy content of rations in order to meet the energy demand of cows with high milk production. The mechanism by which oils reduce the production of $CH_4$ is to reduce the fermentation of organic matter, by directly inhibiting methanogens in the rumen by hydrogenating unsaturated fatty acids. The greatest reduction is caused by unsaturated fatty acids, which act on the hydrogen in the rumen by dehydrogenation (Boadi et al., 2004).

Grainger and Beauchemin (2011) also reported that supplementation with fat rations often reduces carbohydrate fermentation due to the effects of fats on cellulolytic bacteria and protozoa, while starch fermentation remains unaffected.

Also, cows that received flaxseed oil in a percentage of 6% reduced their methane emissions by 27 to 37% (Md Najmul, 2018).

The aim of the research was to evaluate in a dairy farm the effectiveness of the application of nutritional strategies based on the addition of vegetable oils (sunflower oil and rapeseed oil) to obtain accurate data to calculate greenhouse gas emissions. greenhouse effect ($CH_4$, $CO_2$).

## MATERIALS AND METHODS

The research was carried out at the Moara Domnească didactic farm of the University of Agronomic Sciences and Veterinary Medicine in Bucharest, on a herd of 29 dairy cows in different stages of lactation of the Montbeliarde breed between January and September 2021.

The rations included fibrous fodder (alfalfa hay), pickled fodder (corn), fodder beet, concentrated fodder and minerals, vegetable by-products (beer brewery) for a judicious use of local fodder resources (Table 1).

Table 1. Rations administered to dairy cows during the experimental period

| Lactation phase | Upward phase | | Plateau phase | | | | | | | | Down phase | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Experimental variant | V1 | | V2 | | V3 | | V4 | | V5 | | V6 | |
| Fodder | Fodder quantity (kg) | Fodder quantity (%) | Fodder quantity (kg) | Fodder quantity (%) | Fodder quantity (kg) | Fodder quantity (%) | Fodder quantity (kg) | Fodder quantity (%) | Fodder quantity (kg) | Fodder quantity (%) | Fodder quantity (kg) | Fodder quantity (%) |
| Lucerne hay | 3.00 | 7.63 | 3.00 | 7.88 | 3.00 | 7.97 | 3.00 | 7.88 | 3.00 | 7.82 | 3.00 | 7.31 |
| Corn soiled | 25.00 | 63.65 | 25.00 | 65.67 | 25.00 | 66.40 | 25.00 | 65.64 | 25.00 | 65.17 | 25.00 | 60.91 |
| Fodder beet | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.50 | 1.30 | 4.00 | 9.74 |
| Beer brewery | 3.00 | 7.64 | 3.00 | 7.88 | 3.00 | 7.97 | 3.00 | 7.88 | 3.00 | 7.83 | 3.00 | 7.31 |
| Corn grains | 2.30 | 5.86 | 1.50 | 3.94 | 1.20 | 3.19 | 1.30 | 3.41 | 1.40 | 3.65 | 1.50 | 3.65 |
| Barley grains | 2.10 | 5.35 | 2.00 | 5.25 | 2.00 | 5.31 | 2.00 | 5.25 | 2.00 | 5.21 | 2.00 | 4.87 |
| Wheat bran | 1.50 | 3.82 | 1.50 | 3.94 | 1.50 | 3.98 | 1.40 | 3.67 | 1.20 | 3.13 | 1.30 | 3.17 |
| Sunflower meal | 2.00 | 5.09 | 1.80 | 4.73 | 1.70 | 4.51 | 2.00 | 5.25 | 2.00 | 5.21 | 1.20 | 2.92 |
| Sunflower oil | 0.20 | 0.51 | 0.20 | 0.52 | 0 | 0 | 0.10 | 0.26 | 0.10 | 0.26 | 0 | 0 |
| Rapeseed oil | 0.10 | 0.25 | 0 | 0 | 0.20 | 0.53 | 0.20 | 0.52 | 0.10 | 0.26 | 0 | 0 |
| Calcium carbonate | 0.07 | 0.18 | 0.07 | 0.19 | 0.04 | 0.11 | 0.06 | 0.16 | 0.03 | 0.08 | 0.04 | 0.10 |
| Dicalcium phosphate | 0.01 | 0.02 | 0 | 0 | 0.01 | 0.03 | 0.03 | 0.08 | 0.03 | 0.08 | 0.01 | 0.02 |
| Total | 39.27 | 100.00 | 38.07 | 100.00 | 37.65 | 100.00 | 38.09 | 100.00 | 38.36 | 100.00 | 41.05 | 100.00 |
| Ration contribution | | | | | | | | | | | | |
| DM (kg) | 17.07 | | 15.99 | | 15.62 | | 16.02 | | 15.88 | | 15.61 | |
| NE (Mj) | 101 | | 91 | | 91 | | 92 | | 90 | | 88 | |
| PDIN (g) | 1601 | | 1491 | | 1447 | | 1512 | | 1501 | | 1348 | |
| PDIE (g) | 1494 | | 1374 | | 1330 | | 1368 | | 1367 | | 1325 | |
| Ca (g) | 106.34 | | 103 | | 93.34 | | 106.82 | | 95.51 | | 93.92 | |
| P (g) | 70.07 | | 64.05 | | 64.16 | | 69.99 | | 68.37 | | 59.05 | |

where: DM – dry matter; NE – net energy for milk production; PDIN - true protein absorbable in the small intestine when N is limiting in the rumen; PDIE - protein digested in the small intestine when rumen-fermentable energy is limiting; Ca – calcium; P – phosphorus.

From the point of view of milk production, the existing cows on the farms have average productions of about 5920 l of milk / lactation / year, with a duration of lactation of 295.17 days.

In this research, the influence of the addition of vegetable oils, respectively sunflower oil and rapeseed oil, in 5 variants of rations intended for dairy cows was tested. In the 6th experimental variant of the ration, which corresponded to the descending phase of lactation, no oil was administered, but 4 kg of fodder beet was used, because there are researches that indicate the action of this fodder with effect to reduce the greenhouse gas emissions.

## RESULTS AND DISCUSSIONS

**The influence of feed strategies applied on milk production.**

The monitoring of milk quantity and quality (Table 2) was carried out with the DairyPlan C21 system, which made it possible to identify the animals in the milking parlor, to monitor reproduction and ruminating.

The milk production of cows was not influenced by the addition of vegetable oils, ranging between 22.04 l/head in the ascending phase of lactation, 19.86-20.96 l/head in the plateau phase and 19.45 l/head in the descending phase of lactation. The protein content of milk varied between 3.17-3.23% in the ascending phase of lactation, 3.27-3.39% (3.30% being registered in the summer ration variant) in the plateau phase and 3.08% in the descending phase.

The acidity of the milk was between 6.48 and 6.52, and all the milk samples complied with the recommendations regarding the maximum limit of the total number of germs (10 x 104 NTG/ml), the average being 8.6 x 104 NTG/ml.

Although milk production was expected to increase in response to dietary lipid supplementation after peak lactation (Wu and Huber, 1994), no such increase was found in the current study. This is consistent with other studies (Bell et al., 2006; Roy et al., 2006; Chilliard et al., 2009; Benchaar et al., 2015).

The lack of effect of vegetable oils on milk fat concentration is not consistent with some studies (Chelikani et al., 2004; Bell et al., 2006), which reported a lower milk fat content without changes in milk production and other constituents due to high levels of ration oils.

The lack of effect of vegetable oils on milk fat can be attributed to the lack of changes in rumen fermentation in diets based on pickled fodder.

**The influence of feed strategies applied on manure chemical composition**

Regarding the chemical composition of the manure (Table 3), 3 samples were analyzed for each variant of ration and it was noted that in the case of using rations with fodder beet the proportion of water in manure determined by drying in the oven at $105^0$C was 78.44%, while in the case of other rations the water content was 73.09-75.98%.

The ash content determined by calcination at a temperature of $550^0$ C varied between 8.12% for the fodder beet variant in the ration and 10.11% for the ration variant administered in the ascending phase of the lactation curve.

The nitrogen content determined by the Kjeldahl method was lower in the ratio of ratio 5 (0.37%), and in the other variants the nitrogen values were between 0.40-0.67%.

**The influence of feed strategies applied on shelter's methan emissions**

Methane emission from enteric fermentation were estimated using IPCC method 2. The calculation of the methane emission shall be carried out on the basis of equations 10.19, 10.20, 10.21 from *IPCC Good Practice Guidance and Uncertainty Management in National Greenhouse Gas Inventories, 2006:*

$$Emisions = EF_{(T)} * \frac{N_{(T)}}{10^6}$$

where:

*Emisions* = methane emission from enteric fermentation (kg $CH_4$/year);

$EF_{(T)}$ = emissions factor for dairy cow (kg $CH_4$/head/year);

$N_{(T)}$ = the herd of animals of the species / category T;

T = category of animal

$$\text{Total } CH_{4\ \text{ENTERIC}} = \sum_i E_i$$

where:

Total $CH_{4\text{Enteric}}$= total methane emissions from enteric fermentation (kg $CH_4$/year)

$E_i$ = emissions from animal categories

$$EF = \left[\frac{EB * \left(\frac{Ym}{100}\right) * 365}{55.65}\right]$$

where:

EF= emission factor (kg $CH_4$/head/year)

EB = gross energy (MJ /head/year)

Ym= methane conversion factor, which is the percentage of raw energy in the administered feed converted to methane

55.65 (MJ/kg $CH_4$) = energy content of $CH_4$

*Gross energy (GE)*

Using the calority of the gross energy of each ration: 1 g crude protein (PB) = 5.72 kcal;1 g raw fat (GB) = 9.5 kcal,1 g crude fiber (CelB) = 4.79 kcal, 1 g SEN (non-nitrogenous extractive substances) = 4.17 kcal, the formula for calculating GE was:

**GE (kcal/kg) = 5.72·PB + 9.5·GB + 4.79·CelB + 4.17·SEN**

where:
GE = gross energy intake (kcal/kg);
PB = crude protein (%);
GB = raw fat (%);
CelB = crude fiber (%);
SEN = non-nitrogenous extractive substances (%).

The values of gross energy and digestible energy for feed constituents of the rations and the total energy value of the rations delivered to the dairy cows expressed in GE and DE are given in Table 4. Digestible energy (DE) was calculated by applying the three simple rule according to the relationship: ED % = (ED/EB)·x 100.

The following equation shall be used to calculate the values of Ym: Ym = -0.0038 x (ED%)2 + 0.3501 x ED% – 0.811 (Cambra-Lopez equation, 2008) The equation for calculating the enthic $CO_2$ emission shall be (Users' guide for estimating carbon dioxide, methane, and nitrous oxide emissions from agriculture using the State inventory tool, 2019):

**$CO_2$ enteric (kg/year) = (Emission $CH_4$ x 25 GWP) / 1,000,000,000**

The values obtained for the methane emission from enteric fermentation and the $CO_2$ equivalent are given in Table 5, figures 1 and 2, respectively.

Table 2. The quantitative, qualitative and microbiological parameters of milk production

| Exp. variant | Lactation phase | Milk production (l) | Milk protein (%) | Milk fat (%) | pH | Total number of germs (NTG/ml x $10^4$) |
|---|---|---|---|---|---|---|
| V1 | The upward phase | 22.04±0.46 | 3.25±0.06 | 3.48±0.43 | 6.51±0.03 | 8.7±0.09 |
| V2 | The upward phase | 20.34±0.98 | 3.17±0.11 | 3.51±0.35 | 6.48±0.02 | 8.5±0.08 |
| V3 | Plateau phase | 19.86±0.77 | 3.09±0.08 | 3.53±0.55 | 6.48±0.04 | 8.6±0.08 |
| V4 | Plateau phase | 20.88±0.85 | 3.18±0.10 | 3.57±0.73 | 6.50±0.03 | 8.5±0.10 |
| V5 | Plateau phase | 20.96±1.01 | 3.21±0.09 | 3.56±0.52 | 6.52±0.04 | 8.7±0.09 |
| V6 | Down phase | 19.45±0.67 | 3.02±0.08 | 3.49±0.41 | 6.49±0.03 | 8.6±0.08 |

Table 3. Chemical composition of manure obtained in experimental period

| Feed variant | Water (%) | Ash (%) | N (%) |
|---|---|---|---|
| V1 | 73.09 | 10.11 | 0.67 |
| V2 | 75.35 | 8.74 | 0.40 |
| V3 | 75.98 | 9.06 | 0.55 |
| V4 | 73.26 | 9.97 | 0.61 |
| V5 | 75.22 | 8.46 | 0.37 |
| V6 | 78.44 | 8.12 | 0.45 |

Table 4. Value of gross energy (GE) and digestible energy (DE) of the component feeding stuffs and rations delivered to cows during the experimental period

| **Feed** | **GE (Mj/kg)** | **DE (Mj/kg)** | **Total ration GE (Mj)** | **Total ration DE (Mj)** | **Ration variant** |
|---|---|---|---|---|---|
| Lucerne hay | 16.25 | 8.26 | 322.95 | 212.88 | V1 |
| Fodder beet | 2.37 | 1.87 | 301.16 | 194.45 | V2 |
| Beer Brewery | 3.78 | 2.35 | 293.70 | 188.37 | V3 |
| Corn soiled | 4.78 | 2.78 | 302.76 | 196.03 | V4 |
| Maize | 16.57 | 14.20 | 298.94 | 192.99 | V5 |
| Barley | 16.14 | 13.05 | 289.20 | 185.18 | V6 |
| Sunflower rot | 17.88 | 12.56 | | | |
| Soybean rot | 17.87 | 16.05 | | | |
| Wheat bran | 16.57 | 10.69 | | | |
| Sunflower oil | 36.98 | 35.39 | | | |
| Soybean oil | 33.47 | 32.58 | | | |

Table 5. $CH_4$ emission from enteric fermentation

| Experimental variant | GE (Mj/zi) | DE (Mj/zi) | DE(%) | Ym | EF | Head number | $CH_4$ emissions (kg/year) | $CO_2$ x $10^{-9}$ Emissions (t/year) |
|---|---|---|---|---|---|---|---|---|
| 1 | 322.95 | 212.88 | 65.917 | 5.755 | 121.91 | 10 | 1.219 | 30,477 |
| 2 | 301.16 | 194.45 | 64.567 | 5.952 | 117.57 | 10 | 1.176 | 29,392 |
| 3 | 293.7 | 188.33 | 64.123 | 6.014 | 115.84 | 12 | 1.390 | 34,753 |
| 4 | 302.76 | 196.03 | 64.748 | 5.927 | 117.69 | 12 | 1.412 | 35,306 |
| 5 | 298.94 | 192.99 | 64.558 | 5.953 | 116.73 | 10 | 1.167 | 29,182 |
| 6 | 289.20 | 185.18 | 64.032 | 6.026 | 114.31 | 12 | 1.372 | 34,292 |
| | | | | | | | **7.736** | **193.403** |

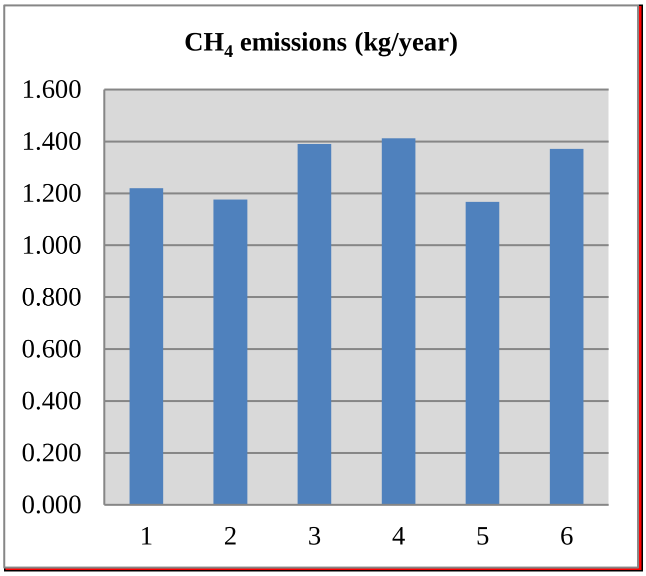


Figure 1. Methane emissions from enteric fermentation in the 6 variants

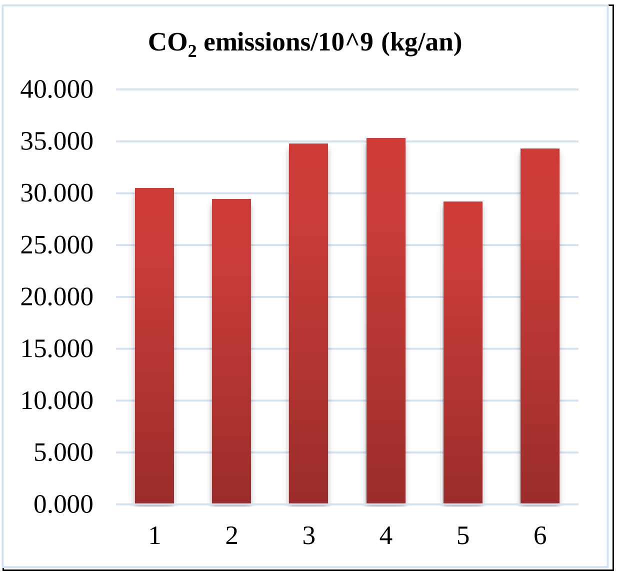


Figure 2. $CO_2$ equivalent emissions in the 6 variants

Table 6. Dynamics of methane emissions from enteric fermentation in the 2 experimental variants

| Experimental variant | $CH_4$ emissions (kg/year) Experimental Faze 2 (2020) | $CH_4$ emissions (kg/year) Experimental Faze 3 (2021) | $CH_4$ emission reduce (%) |
|---|---|---|---|
| 1 | 1.112 | 1.219 | 9.62 |
| 2 | 1.294 | 1.176 | -9.12 |
| 3 | 1.453 | 1.39 | -4.34 |
| 4 | 1.538 | 1.412 | -8.19 |
| 5 | 1.229 | 1.167 | -5.04 |
| 6 | 1.536 | 1.372 | -10.68 |
| **Total** | **8.163** | **7.736** | **-5.23** |

From the analysis of the data presented in table 5 it can be seen that the methane emission from enteric fermentation shows the highest values for variants 4 and 3, when 0.2 l/head/day of rapeseed oil were administered in each variant, and in version 4, 0.1 l/head/day of sunflower oil was also administered (methane emissions are 1.41 kg $CH_4$/year and 1.39 kg $CH_4$/year, respectively). The lowest emissions are recorded for nutrition variant 5 (in which equal doses, sunflower oil and rapeseed oil were administered: 0.1 l l/head/day). Also, the trend of $CO_2$ equivalent emissions closely follows the line of $CH_4$ emissions from enteric fermentation, being directly dependent.

Compared to the methane emissions from enteric fermentation in Experimental Stage 2 (2020), when oils were not used in rations, the methane emissions from enteric fermentation decreased (Table 6). Rations supplementing to dairy cows with vegetable fats reduced daily $CH_4$ production, which is consistent with research by Odongo et al. (2007), Martin et al. (2010), Grainger and Beauchemin (2011).

## CONCLUSIONS

The strategies based on feed solutions are the most effective in the dairy farming sector, the benefit being twofold, respectively limiting the greenhouse effect and improving animal production. Milk production has evolved upwards, respecting the lactation curve, the addition of oils not quantitatively or qualitatively influencing milk production.

The lowest emissions are recorded for nutrition variant in which equal doses, sunflower oil and rapeseed oil were administered (0.1 l l/head/day). Also, the trend of $CO_2$ equivalent emissions closely follows the line of $CH_4$ emissions from enteric fermentation, being directly dependent.

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# ANIMAL WELFARE AND ITS ASSOSSIATIONS WITH FARM SIZE AND STOCKMANSHIP CHARACTERISTICS ON EUROPEAN BREEDING-TO-FINISHING PIG FARMS

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***Abstract***

*Animal health and welfare (AHW) has become an important aspect for sustainable development in livestock farming. Therefore, this paper aims to analyse the AHW performance of 27 breeding-to-finishing pig farms across six European countries and to investigate associations with number of sows in production, number of sows per worker and percentage of family labour. AHW indicators were aggregated to themes, namely "Hunger and thirst", "Comfort", "Injuries and disease", "Pain by management", "Appropriate behaviour" and "Human-animal relationship". On a scale from 0 to 100 (worst to best AHW), lowest median theme score was found for "Comfort" (39) and highest for "Human-animal relationship" (78). AHW performance varied considerably between farms, indicating a potential for improvement, especially regarding "Comfort". Number of sows per farm in production correlated negatively with "Hunger and thirst" ($r_s$ = -0.81), "Comfort" (-0.44) and "Appropriate behaviour" (-0.61). Number of sows per worker also correlated negatively with "Hunger and thirst" (-0.56). Family labour did not correlate with any of the themes. We conclude that some aspects of animal welfare especially regarding comfort (e.g., space allowance and enrichment material), require improvement measures across these six European countries. These problems may increase with farm size and number of sows per worker. Therefore, incentives to implement animal welfare improvement measures are needed for all farms to encourage farmers to construct housing systems above the minimum legal requirements.*

***Key words**: animal-based indicators, comfort, family labour, farm size, multi-criteria analysis.*

## INTRODUCTION

Due to the competitive forces of the market farmers will also need to produce in an efficient way. This have led to the development of husbandry systems which might compromise the animals' health and welfare, as in the case of the pig sector (e.g., fully slatted floor systems). In such systems, pigs are not able to fulfil their natural behavioural needs which may result in abnormal behaviour such as tail biting and stereotypies (Terlouw et al., 1991, Valros and Heinonen, 2015). Therefore, animal health and welfare (AHW) has increasingly become a concern of society (European Commission, 2016, Bozzo et al., 2019) and is seen by some scholars as an important component of the concept of sustainability (Tucker et al., 2013).

Furthermore, an intensification of pig production took place with an increase in farm size (Robbins et al., 2016) and number of sows per worker (Martel et al., 2008). Even though farm size has increased, family farms remain predominant in the EU (Eurostat - Statistics Explained, European Commission). However, with increasing farm size, family farms depend also on external workers. There is also evidence, that with increasing farm size the number of animals per worker may increase and therefore less time might be spent per animal so AHW may be compromised (Robbins et al., 2016). On the other hand, others found no impact (Moinard et al., 2003; Meyer-Hamme et al., 2018) or even positive effects in dairy farms due to a professional management (Robbins et al., 2016).

As part of the Era-Net project SusPigSys (Sustainable Pig Production Systems) we aimed at developing a multi criteria assessment tool to analyse sustainability of pig farms with AHW as a separate dimension beside the three commonly used dimensions, namely economy, environment and social well-being (Hörtenhuber et al., 2021).

The aims of this paper are to describe the integrated AHW performance of breeding-to-finishing pig farms across six European countries and to analyse the correlations between size, number of sows per worker and percentage of family labour.

We hypothesised that farm size might not have a strong effect on AHW, whereas number of sows per worker might correlate negatively and family labour might correlate positively with the AHW performance.

## MATERIALS AND METHODS

*Development of the AHW component within the SusPigSys Multi-Criteria Assessment Tool*

The SusPigSys assessment tool was developed in an iterative process, resulting in a multi-criteria assessment tool, which summarises indicator scores into subtheme and theme scores in several steps (Munsterhjelm et al., 2021).

First, AHW indicators were selected from previous projects, including ProPig (Leeb et al., 2015) and Welfare Quality® (Welfare Quality®, 2009). A detailed protocol was refined in iterative discussions within the consortium and stakeholder workshops, as well as by including pilot farm visit experiences (Munsterhjelm et al., 2021). As a result, the protocol consisted of indicators that can be assessed through an interview including records and management-based indicators (e.g. productivity data, castration method) as well as direct observations to assess resource- (e.g. amount of bedding) and animal-based indicators (e.g. tail lesions).

Second, indicators were scaled from 0 (= worst) to 100 (= best) to obtain a uniform scale across all indicators. This was done based on literature, own project data and expert opinion (Hörtenhuber et al., 2021).

Third, indicators were attributed to subthemes and subthemes further to themes. The AHW dimension consisted of six themes:

1. Hunger and thirst (AHW1)
2. Comfort (AHW2)
3. Injuries and disease (AHW3),
4. Pain by management (AHW4)
5. Appropriate behaviour (AHW5)
6. Human-animal relationship (AHW6).

Fourth, weights were allocated to indicators within themes and subthemes within themes through a Delphi-like approach (Mukherjee et al., 2015), similar to the methodology of Averos et al. (2013).

*Data collection*

The tool was then applied on 27 breeding-to-finishing farms in six countries: Austria (7 farms), Finland (2), Germany (4), Netherlands (4), United Kingdom (6), and Poland (4). One national contact person conducted the interview in the local language and one out of two trained observers performed direct observations on the pigs. Before the visit started, an inter-observer reliability test with sufficient agreement was carried out (Ruckli et al., submitted).

It has to be kept in mind that our sample is not representative for all pig farms in the EU, since the main aim was to include a large range of different farm types so to develop and test the assessment protocol.

*Statistical analysis*

Statistical analyses were undertaken in SAS 9.4 (SAS Institute Inc., 2016). Spearman correlation coefficients ($r_s$) were calculated to investigate associations between farm characteristics (e.g., size, number of sows per worker, percentage of family labour) and AHW theme scores.

Furthermore, correlations were calculated between the indicators which contributed most to the theme (Ruckli et al., submitted) with the farm characteristics. Absolute $r_s$ values greater than 0.4 and p-value smaller than 0.05 were regarded as indicating a relevant association and highlighted in bold in Table 2.

## RESULTS AND DISCUSSIONS

*Farm characteristics*

Table 1 summarizes farm management characteristics of the 27 breeding-to-finishing pig farms. The sample included a variety of production systems including 8 conventional, 5 organic and 14 farms certified according to other labels. Productivity, as well as feed and bedding characteristics show the large variation across farms and can be used to understand the different AHW theme scores.

Number of sows in production (farm size) correlated positively with number of sows per worker ($r_s$ = 0.55) and negatively with family labour ($r_s$ = -0.49). Number of sows per worker and percentage of family labour did not correlate.

Table 1. Farm management-characteristics of 27 breeding-to-finishing pig farms in Austria (7 farms), Finland (2), Germany (4), Netherlands (4), United Kingdom (6), and Poland (4)

| | Min | M | Max |
|---|---|---|---|
| Sows in production (n) | 17 | 150 | 1022 |
| Number of sows per worker | 31 | 80 | 263 |
| Percentage of family labour | 0 | 50 | 100 |
| **Productivity** | | | |
| Litters sow$^{-1}$ y$^{-1}$ (n) | 1.5 | 2.3 | 2.4 |
| Piglets born alive sow$^{-1}$ y$^{-1}$ (n) | 14 | 29 | 39 |
| Piglets weaned sow$^{-1}$ y$^{-1}$ (n) | 14 | 25 | 33 |
| Lactation length (d) | 25 | 28 | 49 |
| Mortality suckling piglets (%) | 0 | 13.4 | 26 |
| Mortality weaners (%) | 0 | 2.9 | 18 |
| Mortality finishers (%) | 0 | 2.0 | 6 |
| **Feed** | | | |
| FCR BU (kg feed kg$^{-1}$ BM$^{-1}$) | 1.7 | 3.6 | 6.6 |
| FCR FU (kg feed kg$^{-1}$ BM$^{-1}$) | 1.8 | 2.9 | 5.2 |
| **Bedding, manure management system and electricity** | | | |
| Bedding (kg sow$^{-1}$ year$^{-1}$) | 0 | 76 | 248 |
| Bedding (kg weaner$^{-1}$ year$^{-1}$) | 0 | 0 | 55 |
| Bedding (kg finisher$^{-1}$ year$^{-1}$) | 0 | 0 | 37 |

n = number. Min = minimum, M = median, Max = maximum. FCR = feed conversion ratio. BU = breeding unit, FU = finishing unit. BM = body mass, FW = fresh weight.

*Theme scores*

AHW theme scores of the 27 breeding-to-finishing farms are presented in Figure 1. Overall, only a few farms scored between 0 and 20 and between 80 and 100, except for one theme, i.e. "Human-animal relationship" (AHW6). This is in line with Meyer-Hamme et al. (2018) and indicates that most farms still have a potential for improvement, especially regarding the "Comfort" (AHW2), which was found to have the lowest median score (39). This theme covers aspects such as *Space allowance, Proper sick pen* and *Proper creep area*. Detailed analysis can be found in Ruckli et al. (2016).

"Human-animal relationship" (AHW6) had the highest median score (78). This theme focused on the perception of the farmer regarding his/her relationship with the pigs. It included questions such as how important it is for animal welfare to avoid force when moving pigs, or whether good overall farm performance is directly linked to good pig welfare. We assume that answers to this questions can partly be explained by "social desirability bias" (King & Bruner, 2000) and should be carefully interpreted. This means that assessors might have inadvertently influenced the farmers, who may have felt obligated to agree with e.g., not using force when moving pigs, since that would be considered to be expected of them by society as the 'right answer'. However, Scott et al. (2001) (cited in Hubbard et al., 2007) points out that there is an inexorable link between the economic wellbeing of the farmers and the welfare of animals.

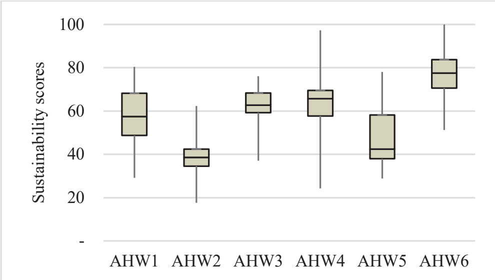


Figure 1. Boxplot of AHW theme level sustainability scores of 27 breeding-to-finishing farms.

Scores of 0 represent worst and scores of 100 represent best sustainability scores. The horizontal bold line denotes the median. The box denotes the first and third quartile. The vertical line denotes the maximum and minimum value.

AHW1 (Hunger and thirst), AHW2 (Comfort), AHW3 (Injuries and disease), AHW4 (Pain by management) AHW5 (Appropriate behaviour), AHW6 (Human-animal relationship).

*Hunger and thirst (AHW1)*

Both, the size of the farm expressed as the number of sows in production ($r_s$ = -0.81) and the number of sows per worker (-0.56) correlated negatively with "Hunger and thirst" (AHW1; Table 2). In contrast, no correlation was found with the percentage of family labour. We explain the correlations of this theme with size and number of sows per worker by the high contribution of the indicator *Quality of roughage* (Ruckli et al., submitted). We found that farm size correlated negatively with the indicator *Quality of roughage* ($r_s$ = -0.59, $p <$ 0.00). Roughage is very beneficial for pigs (Olsen et al., 2000) since they are used to explore, root and graze several hours a day in a semi-natural environment (Stolba and Wood-Gush, 1989). However, one of the reasons for not providing roughage is related to the extra labour needed which in turn leads to an increase in the production costs. This is in line with Czekaj et al. (2013) who found that there is a slight tendency that relatively large farms are less likely to provide any rooting substrate for pigs since it leads to additional labour and therefore higher production costs. Furthermore, slatted floors can get clogged and slurry-based systems do not allow a lot of structure (e.g., leftover from the roughage or bedding material) in the slurry. Providing roughage is not yet mandatory on most EU farms except on organic ones (Council Directives 2007/834/EC and 2008/889/EC).

*Comfort (AHW2)*

Farm size correlated negatively with the theme "Comfort" (AHW2; $r_s$=-0.44), but neither number of sows per worker or percentage of family labour correlated with this theme. We found that space allowance, which contributes to a high degree to this theme score, was decreasing with increasing farm size ($r_s$ = -0.51, $p < 0.00$). Space allowance, however, is crucial for pigs to separate their functional areas such as resting, feeding and defecating (Stolba and Wood-Gush, 1989) and therefore presents an important aspect for "Comfort". Larger farms might have often just followed the legal minimum requirements of the EU (Council Directive 2008/120/EC) when constructing a farm. Interestingly, no correlation was found with number of sows per worker even though farms with higher animal welfare level e.g. organic farms (Council Directives 2007/834/EC and 2008/889/EC) are usually more labour intensive (Tuyttens, 2005) and therefore the number of sows per annual working unit is usually lower compared to conventional farms. One explanation could be that other indicators such as the *Presence of a proper sick pen* might have overruled this effect. A proper sick pen with a soft floor (e.g., bedding) as well as easy access to feed and water is highly relevant for the comfort of sick pigs since they are more vulnerable, climate sensitive and need a soft lying area. Many farms did not have a proper sick pen independent of the farm size, number of sows per worker or percentage of family labour: We assume that sick pigs are often seen as not profitable and therefore sick pens are often used as additional pens. We suggest that farmers need to be more aware of this highly relevant topic.

*Injuries and disease (AHW3)*

No correlation was found between the theme "Injuries and disease" (AHW3) with farm size, number of sows per worker or percentage of family labour. This was at first surprising, since we were expecting better performance on farms with less sows per worker, since they might be able to spend more time per pig. It could be that farms with more sows per worker might have been able to afford more technology (e.g. automatic feeding) (Robbins et al., 2016) in order to replace manual labour and were therefore able to spend a similar amount of time with observation of pigs. Furthermore, highest influence on this indicator had mortality (Ruckli et al., submitted), which did not increase with increasing farm size or number of sows per worker.

Table 2. Spearman correlations ($r_s$) of farm management characteristics (rows) with AHW theme scores (columns). Correlations with an absolute $r_s \geq 0.4$ and p-value $\leq 0.05$ are shown in bold font

| Farm characteristic | AHW1 | AHW2 | AHW3 | AHW4 | AHW5 | AHW6 |
|---|---|---|---|---|---|---|
| Number of sows in production | **-0.81** | **-0.44** | 0.21 | -0.38 | **-0.61** | 0.26 |
| Number sows per worker | **-0.56** | 0.01 | 0.01 | 0.06 | -0.23 | 0.01 |
| Percentage of family labour | 0.22 | 0.04 | 0.09 | 0.20 | 0.13 | -0.35 |

AHW1 (Hunger and thirst), AHW2 (Comfort), AHW3 (Injuries and disease), AHW4 (Pain by management) AHW5 (Appropriate behaviour), AHW6 (Human-animal relationship).

*Pain by management (AHW4)*

No correlation of farm size, number of sows per worker and percentage of family labour was found with "Pain by management" (AHW4). Highest contribution to this theme had the indicator *Hospitalisation* (= pigs present in pens that should have been separated), *Castration, Tail docking* and *Nose rings* (Ruckli et al., submitted). Overall, pigs needing hospitalisation were only observed on few farms and this was irrespective of farm size, number of sows per worker or proportion of family labour. Also, none of the farms used nose rings for sows

*Appropriate behaviour (AHW5)*

Farm size correlated negatively with the theme “Appropriate behaviour” (AHW5; -0.41). However, no correlations were found with number of sows per worker and percentage of family labour.

The negative correlation between size and “Appropriate behaviour” (AHW5) can be partly explained by the highest contributing indicator *Space allowance,* as it negatively correlated with the size of the farm, as described for “Comfort”. Furthermore, we found that larger farms were less likely to provide other enrichment measures (e.g. roughage, objects). However, an enriched environment is crucial for pigs to fulfil their internal needs (e.g. rooting) and thus to reduce the risk for tail biting (Schroder-Petersen & Simonsen, 2001) and other abnormal behaviour (Terlouw et al., 1991).

*Human-animal relationship (AHW6)*

No correlations of farm size, number of sows per worker and percentage of family labour were found with the theme “Human-animal relationship” (AHW6).

At first, it was surprising that we did not find any correlation with size and number of sows per worker. Other studies found that with increased farm size, farmers might be more profitability-orientated and therefore other values might replace animal welfare (Robbins et al., 2016). This seems not to be the case in our study. Furthermore, with increasing number of sows per worker, less time is spent per animal (Robbins et al., 2016). This can result in less positive interactions with humans but also in less negative interactions (Robbins et al., 2016). However, one explanation could be the “social desirability bias” as discussed above.

We were also expecting that the percentage of family labour would positively correlate since with an increasing intensification and therefore more animals per worker, family farms might be replaced by companies, which may threaten AHW (Fraser, 2005). However, even though some farms did not have any family internal labour, we only included farms that are a family owned business and no farms owned by companies. Moreover, we interviewed the farm manager who was not necessarily the person working with the pigs. It would be interesting to interview not only the farm manager but all employees working with pigs about their perception on the human-animal relationship in order to receive a more comprehensive picture.

Nevertheless, when working with family and hired labour, one important aspect is to train people in order to improve the welfare on the farm (Boivin et al., 2003). Therefore, training of workers, hence high quality husbandry and skilled labour might be more important than the percentage of family internal labour.

## CONCLUSIONS

Our findings indicate that there is still room for AHW improvement on most of the breeding-to-finishing pig farms in our sample, especially aspects such as comfort (space allowance) and appropriate behaviour (enrichment). On one hand, relatively large farms in our sample provided less roughage and space allowance, which might be partly due to lower number of sows per worker. On the other hand, the proportion of family labour did not to have an impact on the AHW performance. Therefore, we conclude that the size of the farm and the number of sows per worker could be considered as risk factors for impaired animal welfare, whereby the percentage of family labour seems not to play an important role.

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# POSSIBILITIES TO REDUCE $CO_2$ EMISSIONS BY USING ELECTRIC MOTORS WITH HIGH ENERGY EFFICIENCY

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***Abstract***

*The latest Intergovernmental Panel on Climate Change (IPCC) report said that without immediate and deep emissions reductions across all sectors, limiting global warming to 1.5°C is beyond reach. According to a new IEA (International Energy Agency) analysis, $CO_2$ emissions rose by 6% in 2021 to 36.3 billion tonnes, their highest ever level, as the world economy rebounded strongly from the Covid-19-pandemic crisis. The direct greenhouse gas emissions ($CO_x$, $CH_4$, $NO_x$) come mostly from agriculture (crops cultivation) and the livestock sector. Indirect reduction of $CO_2$ emissions in livestock farms and the food and beverage industry can involve using electric motors with high energetic efficiency. Electric motors represent worldwide, around 50% of electricity consumption. A recent study highlights that if the world's 300 million industrial motor-driven systems were replaced with optimized, high-efficiency equipment, global electricity consumption could be reduced by 10%. This paper analyses the International and European Commission Regulations for efficiency and the new Ecodesign measures for electric motors.*

**Key words:** *carbon dioxide emissions, energy efficiency class, energy policies and regulation, induction motor.*

## INTRODUCTION

The IEA (*International Energy Agency)* works with countries worldwide to shape energy policies for a secure and sustainable future (IEA, 2021). The burning of coal, natural gas, and oil for electricity and heat is the largest single source of global greenhouse gas emissions. In the last ten years, average annual global greenhouse gas emissions were at their highest in human history (IEA, 2021).

Approximately 45 % of the world's electricity is used for electric power motors in buildings and industrial applications. Many research suggests that if the 300 million electric motor-driven systems in active service worldwide were replaced with optimized, high-efficiency equipment, we could reduce global electricity consumption by up to 10%. (Floeck, 2021). In industrial applications, motors are used for pumping, fans, air, liquid compression, conveyance, and other mechanical handling and processing (Waide et al., 2011). The use of high-efficiency electric motors is a current goal in the global industry due to the need to reduce energy costs and not pollute the environment.

By the Kyoto Protocol, developed countries have committed to meet a target of a 20% reduction in carbon emissions by 2020, with an extra stipulation of minimum motor efficiency restrictions (Shyi-Min, 2016). A transition to energy-efficient motor systems would reduce the global electricity demand by motor systems by 20 to 30% in 2030, depending on the actual development and implementation of energy efficiency and environmental policies globally. Using more efficient motors, countries can save 300 TWh per annum of electricity in 2030, saving 200 Mt of $CO_2$ emissions (equivalent to the annual electricity generated by approximately 60 coal-fired power plants with a capacity of 1,000 MW) (U4E, 2017).

For the carbon emissions of electricity use, there is no such thing as a default value. The $CO_2$ intensity of electricity production (kg $CO_2$/MWh electricity) depends on the fuel mix used for electricity generation and on the calculation method used. Moreover, different approaches – often related to the type of study carried out – exist to determine the $CO_2$ emission reduction from (future) electricity savings (Harmsen & Graus, 2013).

In livestock farms, most of the equipment is driven by electric motors: for the preparation and distribution of feed, manure evacuation systems, ventilation, milking installations, water pumps, etc. Also, the processing units of animal products use electricity as the main source of operation of mechanized or automated systems. Such motors are commonly used in pumps, fans, compressors, extruders, mixers, agitators, centrifuges, conveyors, etc. Thus, electric energy reductions can be made through process optimization and technological and manufacturing behavioral changes. Electric motor-driven systems (EMDS) are the most important type of electric load in the industry. In the food industry or livestock farms, EMDS plays an important role, the electric drives forming the link between the source of electricity and most mechanical processes that require a large amount of energy (Waide & Brunner, 2011). For example, in the European Union, they are estimated to account for about 70% of all industrial electricity consumption (Waide & Brunner, 2011). Thanks to the highly efficient motors, users also save energy, reduce their operating costs, and reduce their $CO_2$ emissions.

Motor efficiency is the ratio of mechanical power output to the electrical power input, usually expressed as a percentage, and describes how an electric motor converts electrical energy into mechanical energy. Not all electrical energy that goes into a motor is converted to usable mechanical energy. It is known that the efficiency of electric motors is over 95%, the percentage of 5% representing the losses (dissipated as heat) due to the thermal effect produced during operation. This loss is evidenced by a rise in motor temperature.

The electric motor market has witnessed a major change in the last decade in several aspects: in structure, with company mergers contributing to a more global market, in content with energy-efficiency policies, and in its economy due to increasing electricity prices, all aspects contributing to pushing the market towards more energy-efficient electric motors (De Almeida et Al., 2019)

The most used electric motors in the animal husbandry and food industry are the three-phase or single-phase asynchronous AC motors (Figure 1). The electric motor system includes equipment for supplying power, starting the motor and varying its speed, mechanically transmitting its motion to drive equipment (e.g., pumps, fans, compressors, production machines), and additional controls for subsequent parts of the process.

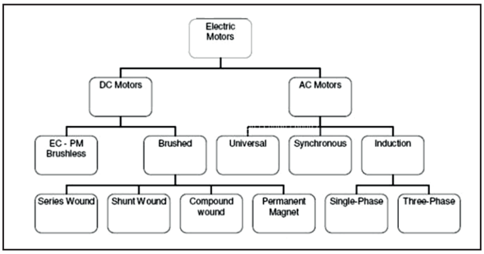


Figure 1. The application categories for the electric motors (Waide & Brunner, 2011)

Most developed countries have implemented energy efficiency regulations similar to that of the European Union. Several regions have adopted a minimum energy efficiency to restrict the sale and use of industrial motors.

## MATERIALS AND METHODS

Energy efficiency label is one of the most important ways to implement the energy efficiency standard for electric motors. Label design should be based on the grade setting specified in the standard. However, various countries have different practices in numbering high and low-efficiency products (APEC, 2008). Ecodesign regulations require manufacturers to decrease the energy consumption of their products by establishing minimum energy-efficiency standards. Requirements for individual product groups are created under the EU's Ecodesign Directive (2009/125/EC). As an alternative, industry sectors may also sign voluntary agreements to reduce the energy consumption of their products (Monforti-Ferrari et al., 2015).

Since motors have long lifetimes, sometimes 20 years or more, the electricity waste is locked-in for decades. An Integrated Policy Approach to fully transforming a market includes Standards and Regulations, Supporting Policies, Finance and Financial Delivery Mechanisms, Monitoring, Verification, enforcement (MVE), Environmental Sustainability, and Health (U4E, 2017).

In 1998, in Europe, three-phase low-voltage electric motors were classified and marketed by the European Committee of Manufacturers of

Electrical Machines and Power Electronics (CEMEP) in three efficiency classes called EFF3, EFF2, and EFF1, based on a voluntary agreement between the motor manufacturers and the European Commission (IEC, 2008). This classification system has proven useful and was initially adopted by many countries around the world. Later, other countries developed their own national systems, different from the European system. Following international debates between electric motor manufacturers, a common international standard has been established to replace all (different) national systems. In order to harmonize the standards describing motor energy efficiency, the International Electrotechnical Commission has developed the International Efficiency (IE) classes through collaboration with the National Electrical Manufacturers Association (NEMA), CEMEP (Table 1), the Japan Electrical Manufacturers' Association (JEMA), the Institute of Electrical and Electronics Engineers (IEEE) and other international organizations (CSA in Canada, COPANT in South America, AS/NZS in Australia and New Zealand, JIS in Japan, GB in China etc.) (Fong et.al., 2020).

In October 2008, the old classification system of the three-phase AC motor was replaced with IEC 60034-30 into three commercially available energy-efficiency classes: *IE3 Premium Efficiency; IE2 High Efficiency* (similar to 50 Hz operation with Eff1), and *IE1 Standard Efficiency* (the lower class and similar in 50 Hz operation with Eff2).

The *IE4 Super Premium Efficiency* class is described in the actual IEC 60034-30-1 standard, published in 2014, for direct online operated AC motors and in the IEC TS 60034-30-2 for variable speed AC motors (Ferreira F et al., 2016). In IEC TS 60034-30-2, minimum efficiencies for the highest efficiency class IE5 or *Ultra Premium Efficiency* are also specified for the first time. An IE5 motor will have a 20% loss reduction with respect to an equivalent IE4 motor. (European Commission 2009, 2019, 2021).

Under the current regulation, motors must reach the IE2, IE3, or IE4 efficiency level depending on their rated power and other characteristics (Gavrilă et al., 2016).

For instance, three-phase motors with a rated output between 0.75kW and equal to or below 1000kW had to achieve the IE3 level by July 2021. Motors between 75kW and 200 kW must meet the IE4 level as of July 2023. The EU is the first place worldwide making the IE4 level mandatory for some categories of motors (Table 2). Thanks to highly efficient motors, users also save energy, reduce operating costs and reduce $CO_2$ emissions.

Table 3 specifies the start dates for implementing the new standards in other non-European countries. Around 50% of industrial energy demand in the European Union comes from electric motors. This represented 2020 some 578 million tons of $CO_2$ emissions. The extension of the efficient motors under the regulation will avoid an additional 40 million tonnes of $CO_2$ each year. With that, the annual energy bill of EU households and industry will be reduced by approximately €20 billion by 2030 (European Commission, 2019, 2021; Bauer Gear Motor, 2022).

The advantage of energy-efficient motors is that they are constructed with improved manufacturing techniques and superior materials and usually have higher service factors, longer insulation and bearing lives, lower waste heat output, and less vibration, increasing reliability. The previous Commission Regulation 640/2009/EG regarding electric motors' Ecodesign requirements were replaced by the new Commission Regulation (EU) 1781/2019 (European Commission, 2021). Several Ecodesign regulations focus on industrial components largely employed in the food sector, such as ventilation units (Reg. 1253/2014), power transformers (Reg. 548/2014), heaters and water heaters (Reg. 813/2013), hot water storage tanks (Reg. 814/2013), water pumps (Reg. 547/2012), industrial fans (Reg. 327/2011) and electric motors (Reg. 4/2014) (Monforti-Ferrario et al., 2015). In 2021 Regulation (EU) 2019/1781 was amended by the Commission Regulation (EU) 2021/341, which aims to clarify and improve some aspects of some of the ecodesign regulations adopted in 2019 (European Commission, 2021).

Table 1. Energy efficiency standard of CEMEP, IEC and NEMA

| Efficiency Class | IEC *(International Electrotechnical Commission)* | CEMEP *(European Committee of Manufacturers of Electrical Machines and Power Electronics)* | NEMA *(National Electrical Manufacturers Association- USA)* |
|---|---|---|---|
| Ultra Premium | IE5 | - | |
| Super Premium | IE4 | - | Super Premium |
| Premium | IE3 | - | Premium |
| High | IE2 | EFF1 | High |
| Standard | IE1 | EFF2 | Standard |
| Below Standard | - | EFF3 | |

(Source: IEC)

Table 2. Overview of efficiency requirements from EU regulations from 2017

| Three-phase motors for continuous operation up to 1000V 50/60Hz | | Year of implementation of minimum efficiency requirements | | |
|---|---|---|---|---|
| | | from 01/01 2017 | from 01/07 2021 | 2023 |
| Power | Mode | 2-; 4-; 6-pole | 2-; 4-; 6-; 8-pole | |
| ≥ 0.12 < 0.75 kW | Starter | - | IE2 | |
| | FC | - | | |
| ≥ 0.75 < 75 kW | Starter | IE3 | IE3 | |
| | FC | IE2 | | |
| ≥ 75 ≤ 200 kW | Starter | IE3 | IE3 | IE4 for 2-;4-;6- pole motors |
| | FC | IE2 | | |
| > 200kW < 375 kW | Starter | IE3 | IE3 | |
| | FC | IE2 | | |
| ≥ 375 ≤ 1000kW | Starter | - | IE3 | |
| | FC | - | | |

(Source: European Commission)

This will require minimum requirements for a wider scope of motors with a power range from 0.12 kW – 1000 kW relative to the previous 0.75 – 375 kW, and will also include 8-pole motors. Also, this new regulation will abolish the former requirement of an IE2 motor plus a converter instead of IE3 (De Almeida, 2017). Also, maximum losses for converters between 0.12 kW and 1000 kW at IE2 are requested. From 2023, IE4 will be required for motors between 75 kW and 200 kW (IEC 60034-1). Figure 2 illustrates IE motor efficiency for 50/ 60 Hz current frequency. For any mechanical system driven by an electric motor, very important it is also the operating mode. IEC uses ten duty cycle (S1 to S10) designations to describe electrical motor operating conditions. These cycles refer to the sequence and duration in time of all aspects of a typical operation, including starting, running with no load, running with a full load, electric braking, and rest. On the motor's nameplate, it is important to be specify the efficiency class and the operating mode (duty cycle) (Figure 3).

## RESULTS AND DISCUSSIONS

According to ANRE *(Romanian Energy Regulatory Authority)* data, at the level of the year 2020 of the total electricity produced in Romania 16.51% came from coal 15.92% from natural gas (ANRE, 2020). Many consumers on livestock farms still use electricity from these conventional sources. This means an indirect increase in $CO_2$ emissions through electric motors with low energy efficiency.

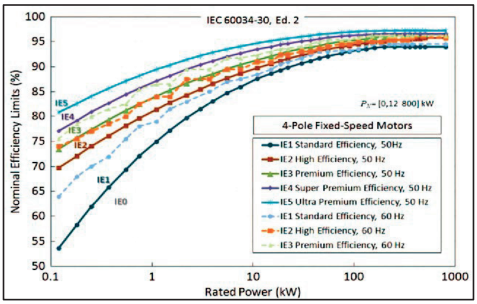


Figure 2. IEC 60034-30 efficiency classification standard, representing classes from IE1 to IE5 for 4-pole electric motors

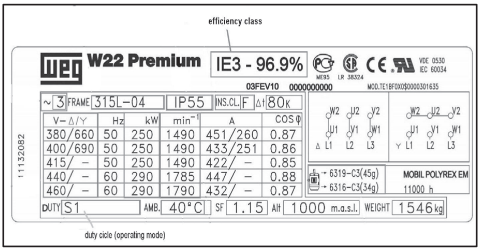


Figure 3. Example of an electrical motor nameplate

Promoting market uptake of efficient motors and drives is an important contribution to the fight against climate change. The EU supports the Super-Efficient Equipment and Appliances Deployment (SEAD) Initiative bringing together countries worldwide to cooperate in promoting efficient appliances (European Commission, 2021).

AC induction motors are a cheap and cost-effective means of converting electrical energy *($P_{input}$)* into rotational mechanical power *($P_{output}$)*. They are an effective way to continuously operate pumps, fans, compressors, conveyors, etc., at a fixed speed. These motors are mass-produced by many manufacturers around the globe and sold in standard catalog types and sizes (Waide et al., 2011).

The efficiency *($\eta$)* of a 3-phase induction motor can be calculated with the formula:

$$\eta = \frac{P_{output}}{P_{input}} \cdot 100\%$$

this means:

$$\eta = \frac{P_{ouput}}{P_{output} + P_{losses}} = \frac{P_{output}}{P_{output} + P_{fixed} + P_{var}} \cdot 100\%$$

The relative importance of the different kinds of IM losses *($P_{losses}$)* depends on motor size.

The *motor losses* (fixed or variable) can be split into five major areas (Table 4) (IEC 60034-31):

· *copper losses* (determined from input power, voltage, current, rotational speed, and torque);

· *iron losses* (determined from input power, voltage, current, rotational speed and torque);

· *rotor losses* (determined from input power, voltage, current, rotational speed and torque);

· *friction and windage losses* (determined from input power, voltage, current, rotational speed and torque);

· *additional load losses* (PLL) (much more difficult to determine, IEC/EN 60034-2-1 specifies different methods of determining PLL which involve low, medium or high uncertainty) (Saidur, 2010).

In spite of the wide sort of electric motors available in the market, three-phase squirrel-cage induction motors (SCIMs) represent by far the vast majority of the market of electric motors (Ferreira F et al., 2016). However, there are some relatively recent entrances in the marketplace, such as the line-start permanent magnet motors (LSPMs) and the synchronous reluctance motors (SynRMs), the latter requiring a variable-speed drive (VSD) or electronic controller. Consequently, the evaluation of direct-on-line (DOL) performance only makes sense for SCIMs and LSPMs (Ferreira et al., 2016). The Synchronous reluctance motor (SynRM) has become commercially viable as a high-efficiency alternative to the induction motor (Ozcelik et.al., 2019).

The core idea of a SynRM motor is that the rotor has no windings or magnets, just electric steel plates stacked together to form a rotor package. However, synchronous reluctance motors require electronic control to produce the rotating magnetic field compared to induction motors. Rotor position feedback is used to control phase energization in an optimal way (De Almeida et al., 2004, 2017).

Unlike in an induction motor, a SynRM rotor has no induced current and thus no losses. This makes SynRM the perfect combination of simplicity and efficiency (Figure 4) (Fereira et al., 2016). A Motor-Driven Unit (MDU) consists of the core components of a motor system: electric motor, variable speed drive (VSD), mechanical transmission, and end-use application, like a pump or fan (Figure 5) (De Almeida et al., 2019; Kulterer, 2021).

Table 3. Legal starting dates for energy efficiency standards

| Country | Efficiency Class | Power Range | Start Date |
|---|---|---|---|
| Canada | NEMA Premium | 0,75 kW ... 375 kW | 28. June 2017 |
| USA | NEMA Premium | 0,75 kW ... 375 kW | 01. June 2016 |
| Mexico | NEMA Premium | 0,75 kW ... 375 kW | 13. January 2017 |
| Columbia | IE2 | 0,12 kW ... 370 kW | 31. August 2018 |
| Brazil | IR3 (IE3) | 0,12 kW ... 370 kW | 01. August 2019 |
| Switzerland | IE3 + IE2 for inverter duty<br>IE2 for VSD<br>IE2 (3 phase)<br>IE3 (3 phase)<br>IE2 (1 phase)<br>IE4 (3 phase) | 0,75 kW ... 375 kW<br>0,12 kW ... 1.000 kW<br>0,12 kW ... <0,75 kW<br>0,75 kW ... 1.000 kW<br>>= 0,12 kW<br>75 kW ... 200 kW | 01. January 2017<br>01. July 2021<br>01. July 2021<br>01. July 2021<br>01. July 2023<br>01. July 2023 |
| Europe | IE3<br>IE2 inverter-driven<br>IE2 for VSD<br>IE2 (3 phase)<br>IE3 (3 phase)<br>IE2 (1 phase)<br>IE4 (3 phase) | 0,75 kW ... 375 kW<br>0,75 kW ... 375 kW<br>0,12 kW ... 1.000 kW<br>0,12 kW ... <0,75 kW<br>0,75 kW ... 1.000 kW<br>>= 0,12 kW<br>75 kW ... 200 kW | 01. January 2017<br>01. January 2017<br>01. July 2021<br>01. July 2021<br>01. July 2021<br>01. July 2023<br>01. July 2023 |
| Turkey | IE3 + IE2 inverter-driven<br>IE2 for VSD<br>IE2 (3 phase)<br>IE3 (3 phase)<br>IE2 (1 phase)<br>IE4 (3 phase) | 0,75 kW ... 375 kW<br>0,12 kW ... 1.000 kW<br>0,12 kW ... <0,75 kW<br>0,75 kW ... 1.000 kW<br>>= 0,12 kW<br>75 kW ... 200 kW | 01. January 2017<br>01. July 2021<br>01. July 2021<br>01. July 2021<br>01. July 2023<br>01. July 2023 |
| Saudi Arabia | IE3 | 0,75 kW ... 375 kW | 16. August 2018 |
| India | IE2 | 0,12 kW ... 1.000 kW | 04. August 2018 |
| China | IE3 | 3-phase: 0,12 kW ... 1000 kW<br>1-phase: 0,12kW … 3,7kW with starting capacitor<br>0,12kW … 2,2kW with running capacitor<br>0,25kW … 3,7kW with starting + running capacitor | 01. June 2021 |
| South Korea | IE3 | 0,75 kW ... 200 kW<br>0,75 kW ... 375 kW (4 and 6 pole) | 01. October 2018 |
| Japan | IE3 | 0,75 kW ... 375 kW | 01. April 2015 |
| Taiwan | IE3 | 0,75 kW ... 200 kW | 01. July 2016 |
| Singapore | IE3 | 0,75 kW ... 375 kW | 01. October 2018 |
| Australia<br>New Zealand | IE2 | 0,73 kW ... 185 kW | 15. May 2019 |

Source: https://www.bauergears.com/energy-efficiency/energy-regulations-standards

Table 4. Typical losses in an AC induction motor

| Typical losses in 4-pole motors | | Factors affecting these losses |
|---|---|---|
| **Stator losses** | 30-50% | Stator conductor size and material |
| **Rotor losses** | 20-25 % | Rotor conductor size and material |
| **Core losses** | 20-25% | Type and quantity of magnetic material |
| **Additional load losses** | 5-15% | Primarily manufacturing and design methods |
| **Friction and windage** | 5-10% | Selection/design of fan and bearings |

Source: IEC 60034-31

Regulating the entire Motor-Driven Unit (MDU) would translate into 1400 TWh of cost-effective electricity savings (7% of the World motor systems electricity consumption), with a corresponding reduction in emissions of 469 Mton of $CO_2$ eq by 2040 (De Almeida et al., 2019).
The productivity premium resulting from intelligent speed control can be utilized in two ways: to increase productivity and keep production stable while reducing energy consumption. For example, pump and fan applications can cut energy consumption by two-thirds (ABB, 2004). The relationship between centrifugal pump or fan speed and its energy demand is known as the cube law because the demand for power increases with the cube of the speed.
This means that a small increase in speed requires a lot more power, but also that a modest speed reduction can give significant energy savings. A pump or a fan running at half speed consumes only one-eighth of the power compared to one running at full speed (ABB, 2016).

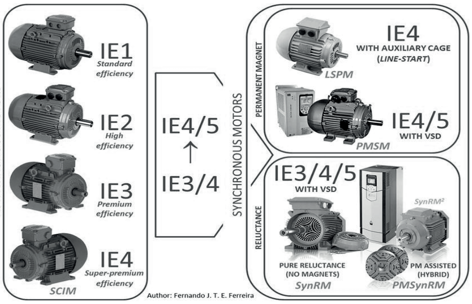


Figure 4. Examples of commercial three-phase motor technologies with high-efficiency class. (Ferreira et al., 2016)

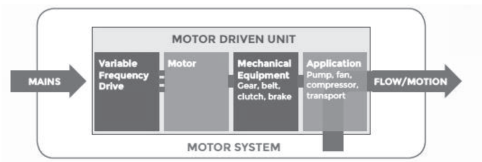


Figure 5 Motor-driven unit (Kulterer, 2021)

A centrifugal pump delivers maximum output, and the excess is reduced at the valve, where the surplus energy is wasted through friction (Falkner, 2008).

Reducing motor speed to meet the actual demand of the process often means substantial energy savings and reduced operating costs. An electric drive enables a process to achieve the right speed and torque while maintaining its accuracy the ability to bring a process up to speed slowly prevents the sudden shock loading that can damage the motor and the driven machine over time (Ahonen et al. 2016).

An example of saving energy is given by De Almeida (2004), who shows that in the case of using pumps with variable speed drive, and efficiency of almost 72% can be obtained compared to a classic system where the efficiency is only 31%. Figure 6 exemplifies the possibility of increasing the energy efficiency of the pumps provided with variable speed drive (U4E, 2017).

Many studies have discussed energy efficiency standards for electric motors in the industry and compared different countries in terms of technology, regulatory, and trend aspects regarding motor systems. For example, to reduce energy consumption and carbon emissions at a food processing units, if is it replaced all the old inefficient motors in compressors and cooling systems with modern IE5 motor and drive packages, it is possible to reduce the energy consumption of the unit by 14% and the carbon dioxide emissions by 131 tonnes per year. (IEA,2021).

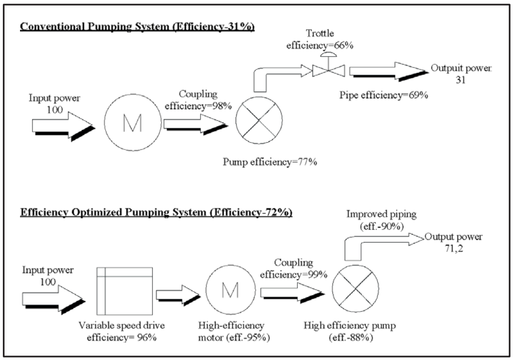


Figure 6. The efficiency of an electric motor pumping system, showing the energy-saving potential (U4E, 2017)

## CONCLUSIONS

Many countries' regulatory agencies have introduced and implemented regulations to encourage the development and use of higher-efficiency electric motors.

According to the European Commission Regulations from 1 January 2017, all line-start motors with a rated output of 0.75–375 kW should not be less efficient than the IE3 efficiency level. VSD motors should not be less efficient than the IE2 level. There are about 8 billion electric motors in use in the EU, consuming nearly 50% of the electricity the EU produces. Upgrading to IE5 SynRM motor, we can reduce energy losses by 40% compared to IE3 and save electricity 18 TWh in a year. All measures aim to reduce carbon dioxide ($CO_2$) emissions by 6 million tonnes over the motor lifetime (ABB, 2016). Besides energy saving on the consumer side, using sources with high energy conversion efficiency and renewable energy sources is also important. It results in a significant reduction in greenhouse gas emissions.

The most common barriers to applying energy-efficient technologies identified in the field include split budgets (different capital and operations budgets), risk of failure, lack of internal incentives, and market structure.

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# PERFORMANCE OF NATURE CHICKEN WAS GIVEN RATION CONTAINING MEAL OF BREAD WASTE

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***Abstract***

*Nature chicken is Indonesian local chickens that widely maintained and are very popular in community, because of their distinctive taste and flavour. Expired bread is a food factory waste that has the potential to be used as a feed ingredient for corn substitute rations, because it contains high gross energy and crude protein. The objective of this research was knowing the optimal dosage level of expired bread meal on nature chicken performance. The completely randomized design-based research used 100 one week old nature chicks, with five treatments and four replications. The ration treatments used were of R1( ration with 10% Expired Bread Meal), R2 (ration with 15% Expired Bread Meal), R3 (ration with 20% Expired Bread Meal), R4 (ration with 25% Expired Bread Meal) and R5 (ration with 30% Expired Bread Meal. Parameters measured were feed consumption, final body weight, feed conversion, carcass, giblet and inedible organs. The result can be concluded that ration with 25% expired bread meal gave the optimum performance, therefore it can be used as alternative on nature chicken.*

***Key words**: expired bread meal, internal organ, nature chicken, performance.*

## INTRODUCTION

The native chicken is raised as dual-purpose chicken and can be productive in a harsh environment as well as in the low quality of diets. Native chickens have good adaptations to the environment. Consumer demand for local chicken meat is increasing every year. To support the high productivity of native chickens, one of them is by providing good quality feed so that it can meet the needs of producing eggs and optimal body weight. The ration costs incurred reach 60-70% of the total production costs. Efforts that can be made to reduce the cost of rations are to make ration formulations by utilizing alternative feeds that are cheap but can meet the nutritional needs of livestock. Expired Bread meal is an alternative source of energy and low in price. Therefore, expired bread is expected to replace some of the energy source feed ingredients such as yellow corn.

Expired bread meal from bakery products that have contains 12.25% protein and 2980 kcal/kg metabolic energy so that it can be classified as an energy source (Leeson & Summers, 2005). The results of the analysis show that the expired bread meal contains crude protein 10.25%, crude fiber 12.04% crude fat 13.42%, calcium 0.07%, phosphorus 0.019% and gross energy 4217. The results of the above analysis indicate that expired bread meal is a food factory waste that has the potential to be used as a feed ingredient for native chicken rations as a substitute for corn, because it contains high gross energy and protein. The protein content in the expired bread meal greatly affects the achievement of body weight in native chickens. Protein in the ration is needed for tissue growth, tissue repair, and production as well as part of the structure of enzymes so that protein is known as one of the main constituents of cells and body tissues. Therefore, protein plays an important role in achieving the desired carcass weight (Reddy & Quddratullah, 1996). The resulting body weight gain is an illustration of the quality of the ration given. The increase in body weight resulted from good quality rations. The quality of the protein ration will affect the intake of protein into the meat so that the amino acids are fulfilled in the chicken's body. Weight gain is caused directly by the availability of tissue-forming amino acids, so the consumption of protein rations is directly related to the growth process (Sawosz et al., 2018). Expire Bread meal also contains various nutrients including beta carotene, thiamin (vit. B1) and minerals such as iodine and calcium (Astawan, 2007). However, the expired bread has bad flavour/taste, damage nutrients, containing fungi and toxins (poisons). Poultry is very sensitive to mycotoxin. There are several species of fungi that produce toxin, such as *Aspergillus* sp, mycotoxins, *Penicilium* sp. and *Fusarium* sp. (Lesson and Summers, 2005). Swammy et al. (2004) reported that mycotoxins affected significantly the decreasing of broiler weight gain from 21 to 42 weeks of age. According to Afzaland Zahid (2004), the levels tolerance of mycotoxin in poultry feed is about 28 ppb that is not affect health and performance of boilers. The use of mycotoxin detoxification enhance antibody which improves body weight and decreases feed conversion significantly. The toxicity level of aflatoxin in feed was 1,2 ppm (Leeson & Summers, 2005). This is considering that the expired bread contains mycotoxin that was sensitive to poultry.

From the results of the study, using expired bread mixed with Garlic Straw in the ration can replace 30% of the use of yellow corn, it has no effect on egg production and efficiency of ration. If 60% yellow corn is used in the ration, the addition of 18% expired bread meal still provides the same quality as using 60% yellow corn (Suasta, 2001). The use of baby food waste in broiler rations, the best performance was obtained at the level of 20% (Djaenudin et al., 2004). According to the calculation of the addition of expired bread meal up to 30% in the metabolic energy ration and the content of food substances for native chickens is still fulfilled. By looking at the composition of the expired bread meal, it is suspected that this material will be easily digested like yellow corn meal but must be proven biologically. Therefore, the purpose of this study is to see how the addition of various levels of expired bread meal in the ration has on the performance of native chickens.

## MATERIALS AND METHODS

The study used 100 local day-old chickens (DOC) without sex separation (straight run).

DOC body weight has an average coefficient of variation of 8.13%. The cage used is cage-shaped, as many as 20 units with a length of 0.7 m, width 0.5 m, and height of 0.7 m. Each cage unit consists of 5 chicks and is equipped with a round feeder-shaped feeder and a round-water drinking container made of plastic, and a 25-watt incandescent lamp. Chicken rearing is carried out from the age of 1 day to 12 weeks, the provision of rations and drinking water is carried out *ad libitum*.

The study was conducted by experimental method and using a completely randomized design (CRD) with five kinds of ration treatments and repeated four times. The ration treatments used were of R1 (ration with 10 % expired bread meal), R2 (ration with 15 % expired bread meal), R3 (ration with 20 % expired bread meal), R4 (ration with 25 % expired bread meal) and R5 (ration with 30 % expired bread meal), given to native chickens up to 12 weeks of age ad-libitum. The ration is based on the crude protein content of 17 % and metabolizable energy of 2,850 kcal/kg (Widjastuti, 1996). The composition of the experimental ration used in the study is shown in Table 1 and the nutrient content and metabolizable energy is shown in Table 2.

Parameters measured were feed consumption, body weight gain, feed conversion, carcass weight, giblet weight and inedible organ of native chicken.

Tabel 1. The composition of the experimental ration

| Feed ingredients | R1 | R2 | R3 | R4 | R5 |
|---|---|---|---|---|---|
| | % | | | | |
| Expired bread meal | 10.00 | 15.00 | 20.00 | 25.00 | 30.00 |
| Rice bran | 12 .50 | 12.50 | 12.50 | 12.50 | 12.50 |
| Yellow corn | 48.00 | 44.00 | 40.50 | 36.50 | 32.00 |
| Coconut meal | 5.50 | 5.50 | 5.50 | 5.50 | 5.50 |
| Soybean meal | 15.00 | 14.00 | 12.25 | 11.50 | 11.00 |
| Fish meal | 8.00 | 8.00 | 8.00 | 8.00 | 8.00 |
| Bone meal | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| $CaCO_3$ | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Amount | 100 | 100 | 100 | 100 | 100 |

Table 2. Nutrient Content and Metabolizable Energy of Experimental Ration

| Nurient content | R1 | R2 | R3 | R4 | R5 |
|---|---|---|---|---|---|
| Metabolizable energy (kkal/kg) | 2,865 | 2,870 | 2,882 | 2,838 | 2.899 |
| Crude protein (%) | 17.28 | 17.13 | 15.05 | 17.03 | 17.18 |
| Crude Fat (%) | 6.47 | 6.54 | 6.69 | 6.99 | 7.00 |
| Crude Fibre (%) | 4.89 | 4.97 | 5.15 | 4.92 | 5.35 |
| Calcium (%) | 1.05 | 1.27 | 1.54 | 1.87 | 1.98 |
| Phosphor (%) | 0.61 | 0.63 | 0.63 | 0.62 | 0,62 |
| Lysin (%) | 1.42 | 1.41 | 1.40 | 1.36 | 1.38 |
| Methionin (%) | 0.35 | 0.38 | 0.43 | 0.45 | 0.42 |

Note: R1 = Ration contain 10% Expired bread meal; R2 = Ration contain 15% Expired bread meal; R3= Ration contains 20% Expired bread meal; R4=Ration contains 25% Expired bread meal; R5=Ration contains 30% Expired bread meal

## RESULTS AND DISCUSSIONS

The results of addition of expired bread meal in the ration on the feed consumption, body weight gain, feed conversion, carcass weight, gizzard weight and inedible organ for each treatment can be seen in Table 3.

Table 3. Average feed consumption, body weight gain, feed conversion, carcass weight, gizzard weight and inedible organ

| Observed variables | Treatment | | | | |
|---|---|---|---|---|---|
| | R1 | R2 | R3 | R4 | R5 |
| Feed Intake (g) | 3545.50 a | 3454.30 a | 3305.45 a | 3140.37 a | 2954.30 b |
| Body weight gain (g) | 820.20 a | 810.40 a | 780.90 a | 720.40 a | 680.20 b |
| Feed Conversion | 4.32 a | 4.26 a | 4.23 a | 4.36 a | 4.54 a |
| Carcass weight | 535,70 a | 526.76 a | 507.59 a | 498.87 a | 450.13 a |
| Gizzard weight | 52.40 a | 53.80 a | 54.10 a | 54.45 a | 55.10 a |
| Inedible organ weight | 202.35 a | 232.23 a | 252.33 a | 285.10 a | 282.35 a |

Note : Different superscript shows significant differences.

### Feed Intake

From Table 3, it can be seen that the average feed consumption in various treatments ranges from 3140.37 – 3545.50 grams. The results of statistical analysis showed that the addition of waste bread meal to the ration have significant effect (P< 0.05) on feed consumption. Feed consumption of R5 was significantly lower than those of R1, R2, R3 and R4. Feed consumption of R1, R2, R3 and R4 were not significantly different, these were caused by the low proportion of expired bread meal. The consumption of rations can be influenced by the condition of the ration itself, including taste, color and taste. The low feed consumption of R5 was probably because of the excess proportion of expired bread meal that caused different flavor. Expired bread meal might be grown by fungi. The content of mycotoxin was estimated still bellow the toxic level. The tolerant levels of mycotoxin in the poultry feed was 28 ppb (Afzal & Zahid, 2004). The substitution of yellow corn with more expired bread meal, with a lighter ration color makes chickens more interested in consuming more rations. The addition of up to 25 percent of expired bread meal showed a very good ration composition due to the right balance between yellow corn and expired bread meal. According to opinion Sarastani et al. (2002) reported that long of storage may cause several decreasing of nutrition quality, safety, flavor (taste) and texture.

### Body weight gain

The results of the variance showed that the treatment had a significant effect (P<0.05) on body weight gain. Body weight gain in treatment R1, R2, R3, and R4 was higher as a result of increased consumption. The more rations consumed, the faster the growth rate achieved. The substitution of corn with 30% expired bread meal (R5) significantly decreased weight gain. The decrease of weight gain was caused by a taste or flavour and mycotoxin from expired bread meal. According Watt et al. (2003) reported that mycotoxin in the feed decreased the weight gain of broiler (from 1 to 21 days).

The body weight gain of R1, R2, R3 and R4 was not significantly different, it may be caused by mycotoxin content in R1 -R4 was still in the normal range that did not interfere the growth process. The composition of the ingredients used in making bread is one of them is milk and it is a source of protein and calcium for the body, with the addition of expired bread meal can increase the calcium content in the ration which can then be utilized by the body, especially bones and muscles so that it will affect the increase in body weight.

Expired bread meal is a food factory waste that has the potential to be used as an ingredient in chicken rations as a substitute for corn, because it contains high gross energy and protein. The bread was made of several materials such as wheat flour, eggs, sugar, margarine, cooking oil and some materials. All of these components are source of protein and amino acids. The quality of the protein ration will affect the intake of protein into the meat so that amino acids are fulfilled in the chicken's body. Bodyweight gain is caused directly by the availability of tissue-forming amino acids, so that the consumption of protein rations is directly related to the growth process.

**Feed Conversion**

In Table 3, it can be seen that the addition of expired bread meal to the conversion value gives a positive increase. The use was expired bread meal 0-30 % in the ration had not affected on feed conversion ratio, although the R5 had the lowest feed consumption and body weight gain. The addition of expired bread meal which is an energy source can increase palatability.

This is because the composition of the ingredients used in making bread is milk. Milk contains lactose which can be used as a medium for the growth of beneficial bacteria in the digestive tract of chickens such as lactobacillus. These bacteria anaerobically produce lactic acid which can increase bile secretion into the small intestine so that it can improve and increase cell metabolism, resulting in increased absorption of nutrients in the jejunum and ileum.

The increased absorption of substances will in turn improve the efficiency value of the use of rations, so that it will affect the conversion value of the ration.

**Carcass weight**

Carcass weight is closely related to the live weight of chickens at harvest time. In addition, part of the ration that is very influential for carcass formation is the protein content of the ration (Widjastuti et al., 2021). Carcass weights were not significantly different (P>0.05) in all using of expired bread meal 10% - 30% (R1-R5), though the R5 had the lowest feed consumption and body weight gain. This is because to the bread was made of several materials such as wheat flour, eggs, sugar, margarine, cooking oil and some materials. All of these components are source of protein and amino acids. In the R5 treatment, expired bread meal was used more as a substitute for corn so as to provide better feed quality. Therefore, even though the consumption of R5 feed was the lowest, it could still produce the same carcass weight. Expired bread meal mixture in the form of milk has complete amino acids, especially essential amino acids. The function of protein is mainly to build muscle/meat. Carcass is the part of the chicken that contains the muscle/meat. The proportion of expired bread meal on R5 was higher than on R1, R2, R3 and R4, so it may contain more complete amino acids, so that although feed consumption and body weight gain decreased, carcass weight did not differ significantly. Research Quentin et al. (2005) concluded that amino acids affect carcass production.

**Giblet Weight**

Giblet is an edible product consisting of a combination of heart, liver, and gizzard organs. The average weight of native chicken obtained from this study can be seen in Table 3. The results of the analysis of diversity showed that the addition of expired bread meal gave no significant difference (P>0.05) on the giblet weight of native chicken. This can be interpreted that the giblet weight in each treatment is still in the normal range, although there is an increase in the average weight and percentage of giblet in treatments R4 to R5, but this is not significantly different.

The giblet weights that were not significantly different (P>0.05) were thought to be caused by the final live weights that were not significantly different. Live weight is the result of the process of growth and development of livestock which is supported by various internal and external factors, such as the nation and the ration used. Genetics as well as the intake of ration nutrients that can be absorbed are factors that can affect the size, shape, and body composition of an animal. Ideally, the external and internal organs will also increase along with the increase in live weight. The sizes of body parts will proportionally adjust to live weight, this is so that each organ is able to work optimally on the individual livestock. In line with the statement Setiadi et al. (2012) that live weight affects giblet weight. The higher the live weight, the higher the giblet weight produced.

In addition, there was no increase in giblet weight, because basically the enlargement of the heart, liver, and gizzard organs was more influenced by their activity. One of the activities that can improve the performance of these organs is in digesting the crude fiber content in the ration. Poultry will increase their metabolic ability to digest crude fiber thereby increasing the size of the heart, liver, and gizzard (Hetland et al., 2005). However, the provision of too high crude fiber will reduce the consumption of rations, because the chickens will feel full longer, causing a lower final live weight to be produced. According to Haryadi et al. (2015), when the crude fiber in the ration exceeds the requirement, the livestock will need more energy to digest. Thus, the energy that can be used to digest protein and other substances will be reduced. The crude fiber content of the ration used in this study was < 6%, the crude fiber content was not higher than the standard requirement for native chickens, namely a maximum of 7%-8% (SNI, 2013). In addition, the addition of expired bread meal in the ration proved that there was no significant increase in the performance of the heart, liver, and gizzard. The crude fiber content in the treatment rations in this study could still be tolerated by livestock so that it did not have a significant effect on increasing the size/weight of the heart, liver, and gizzard produced.

### The in-edible part

In-edible parts consist of bone and slaughtered waste which includes blood, feathers, head, feet, gastrointestinal system including digestive and intestinal fat, abdominal fat, as well as other wastes such as windpipe, lungs, reproductive organs, pancreas, spleen and kidney (Murawska et al., 2011). The results of the analysis showed that giving expired bread meal to the ration had no significant effect ($P>0.05$) on the in-edible weight of native chickens. Nature chickens at the age of 12 weeks, the weight of the internal organs has exceeded the maximum growth limit, so that the increased body weight is not followed by an increase in the weight of the internal organs (Dennis, 2016). The internal organs except for the reproductive organs in the livestock body are parts of the body of livestock that are ripe early because they are important in providing metabolic products for growth, as well as the head and legs, because the head is a very important organ in regulating all livestock life, namely the brain, while the legs are an important tool in finding food from the time of hatching. The weight of the in-edible part shows results in the range of 139.8-162.1 grams. Forest et al. (1975) stated that the percentage of in-edible parts decreased with increasing live weight. If the in-edible weight of this study is calculated as a percentage, the results obtained are 24.67-35.9 percent. The percentage of in-edible varies between 20-38 percent of body weight (Card & Nesheim, 1976). By looking at the composition of expired bread meal, it is suspected that this material will be easily digested, such as yellow corn meal, it can maintain digestive function in the body and the work of the digestive tract does not experience disturbances so that the weight that cannot be eaten remains in normal condition.

## CONCLUSIONS

1. The use of expired bread meal up 30% in diet produced optimal performance compared to 30%. The use expired bread meal 30% decreased feed consumption and body weight gain.
2. The use expired bread meal up to 25% in the ration can be used as alternative source of energy on nature chicken.

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# THE EFFECT OF DIET ON GROWTH PERFORMANCES, CARCASS AND MEAT QUALITY CHARACTERISTICS OF LAMBS FROM TSIGAI BREED

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***Abstract***

*The aim of this study was to determine the effects of diet on growth performances, carcass and meat quality of Tsigai lambs – rusty variety fed with different diets, in order to improve meat quality and meat sensory characteristics of lambs; forty male lambs (L1 and L2, n = 20 heads/ group) were used in experiment from birth up to 5 months. No significant differences (p > 0.05) were found between the two lots with regard at final weight and average daily gain. The diet had a significant effect between L1 and L2 groups in terms of hot carcass weight and hot slaughter yield (p < 0.001), cold carcass weight and cold slaughter yield (p < 0.01), as well for commercial yield (p < 0.05). The juiciness and overall difference were strongly influenced by the diet administered in the present study, significant differences (p <0.001) being recorded between meat from the two groups. The diet administered to L1 influenced positively the eating qualities of lamb meat, resulting in a more juicy and tender meat, in which the specific lamb taste was attenuated.*

***Key words**: diet, lamb, meat, sensory characteristics, Tsigai.*

## INTRODUCTION

The latest official numbers available in Romania show that the country's sheep population reached about 10.4 million heads (FAOSTAT, 2021), the breed structure being dominated by indigenous, unimproved breeds (Turcana and Tsigai) (Gavojdian et al., 2016).
At Turcana sheep, Gavojdian et al., (2013) reported that the weight of lambs at different age are influenced from genotype, and not from rearing system, altough housing and feeding condition of lamb are superior under semi-intensive rearing practices. The Romanian Rusty Tsigai is a native sheep bred in central Transylvania.
The total number of Tsigai has a large population size (2 500 000) (Kusza et al., 2011), but some of varieties are endangered. The Tsigai sheep had an important role (Kukovics & Kume, 2005) in the development of Turkish Kivircik populations (Marmara and Trakya) which are critically endangered bred in North-West Turkey for meat, wool and milk (Elmaci et al., 2006). Managed semi-intensively under European temperate conditions, Gavojdian et al. (2015) reported high value for litter size in Tsigai ewes, value that might be attributed to the good feeding and management condition.
The unimproved breed Tsigai is kept extensively in mountainous and sub-mountainous regions with large pasture areas. Like Turcana, Tsigai sheep is a multi-purpose breed with focus on cheese production. With regard to the potential of Tsigai breed for meat production, in recent years, the breeders believe it would be more efficient to keep the pure breed for meat than for milk production (Ilişiu et al., 2012). The breeders have considered that using crossbreeding of the foreign breeds specialized by meat production for half-breed lamb production would make the growth of the Tsigai breed more efficient (Ilișiu et al., 2013). To improve meat production at Tsigai sheep, were imported specialized breeds for meat production to improve the skills of fattening and carcass quality, namely: Suffolk, Ile de France, Merinofleisch, German Blackface. The obtained results were in all cases higher than those obtained from the Tsigai breed (Ilişiu et al., 2010), but under the potential of improved breed (for lamb). The risk of losing the pure breed Tsigai is relevant and becomes evident because many sheep herders would like to increase the

genetic quality of these high performance breeds in their herds.

Taking into account the above mentioned aspects, the improvement of Tsigai sheep for meat production needs to be done in purebred through selection and adaptation of feed technologies according to the objectives pursued in the selection process.

The feeding is one of the major costs in animal production and they can affect the composition of the carcass (Lewis et al., 2002) and the degree of fattening. Despite the importance that breed and feed type have individually in determining the quality of the carcass, it is possible that the interaction between two or more factors also affects quality and characteristics of lamb carcasses (Díaz et al., 2002; Mustafa et al., 2008; Ríos-Rincón et al., 2014).

The aim of this study was to determine the growth performances, carcass and meat quality of Tsigai lambs – rusty variety fed with different diets, in order to improve meat quality and meat sensory characteristics of lambs.

## MATERIALS AND METHODS

The present research was conducted in Experimental Base Reghin of Research and Development Institute for Sheep and Goat Palas - Constanta, Mures County, 46°46' N/ 22°42'E; 395 m altitude; annual rain fall varies between 650-700 mm; average temperatures 19/–3°C during summer/winter).

The growth performances of the lambs from Tsigai breed were followed from birth up to the age of 5 months. The experiment included two lots of lambs (20 heads/lot) born in January-March interval. At birth or shortly thereafter, lambs were identified with ear tags and weighed (±0.1 kg). Sex, date of birth, type of birth, dam and ram group were recorded. The lambs were also weighed monthly (±0.1 kg) up to 5 month age. Ewes and their lambs were kept together under the same management condition. Until weaning, the lambs were creep fed (*ad libitum*, 135 g DP and 10.89 MJ NE) and weaned at 66 days and 58 days, respectively. The structure of concentrated fodder was: 30% corn flour, 30% barley flour, 25% corn grain, 11.25%, sunflower groats, 2.25% calcium and 1.5% salt. After weaning, the two lots (L1, L2) of lambs were fed with different diet up to 5 months, the lambs are raised on shelter, and the diet was offer *ad libitum*. For the lot 1, the concentrate fodder was the same like in the suckling period (from birth up to end of fattening), while for the lot 2, the fattening period was divided into two phases (6 April-6 June and 7 June - 15 July) (Table 1). Additionally, for both lots, in the ration was added hill hay.

Table 1. Structure of concentrate fodder used in fattening experiment with lambs from Tsigai sheep

| Characteristics | After weaning | | | |
|---|---|---|---|---|
| | 6 April - 6 June | | 7 June - 15 July | |
| | L1 | L2 | L1 | L2 |
| Corn flour (%) | 30.0 | 4.00 | 30.0 | 20.0 |
| Barley flour (%) | 30.0 | 4.00 | 30.0 | 20.0 |
| Corn grain (%) | 25.0 | 90.00 | 25.0 | 50.0 |
| Sunflower groats (%) | 11.25 | 1.50 | 11.25 | 7.5 |
| Calcium (%) | 2.25 | 0.30 | 2.25 | 1.5 |
| Salt (%) | 1.5 | 0.2 | 1.5 | 1.0 |
| Dry matter/kg concentrated fodder | 820 | 830 | 820 | 830 |
| Digestible protein g/kg dry matter | 135 | 100 | 135 | 118 |
| NE MJ/kg dry matter | 10.89 | 11.29 | 10.89 | 10.89 |

Calculated composition was derived from tabular values based on ingredient composition of the experimental diet (NRC, 2007).

At the end of the fattening, 6 Tsigai male lambs (3 from each lot) were brought to the abattoir for small and large animals from Reghin city. The lambs were subjected to a fast of 24-hour, were weighed and then slaughtered after electrical stunning. After the removal of non-carcass components, lamb carcasses were weighed and chilled at 4 ºC for 24 h and then carcass weights were recorded.

Commercial dressing percentage was calculated based on pre-slaughter live weight, while warm and cold slaughter yield was calculated based on empty body weight. Carcasses were split into two halves and the: gigot, shoulder + arm, cutlet and rest carcasses were determined. For determining of the *Longissimus dorsi* (*LD*) area, the semi-carcass was sectioned between the D12 and D13 vertebra and between 3 and 4 lumbar vertebrae, perpendicularly on the axis of the backbone, the shape of the *Longissimus dorsi* muscle being copied on transparent paper. The leg muscle section area from the half of the femur was sectioned, perpendicularly on its longitudinal axe, the shape of this section being copied on transparent paper. The size of the areas were determined on the computer.

In order to be used in meat quality analyses, *LD* muscle was removed from right side of the carcasses at 24 h post-slaughter and were packed. For sensory evaluation, meat samples were frozen and kept at –18°C until the day before the panel evaluation. Meat samples, which were served to untrained panellists, were prepared according to the methodology descrybed by AMSA (2015). Sensory characteristics of cooked samples were assessed by 24 panellists using the degree of difference test. The panellists assessed the lambs breed difference in juiciness, tenderness, flavour, appearance, the difference of specific lamb taste and overall difference. The scale used has a seven point category (scale 1= no difference, 2 = very small difference, 3 = small difference, 4 = moderate difference, 5 = big difference, 6 = very big difference, 7 = extremely big difference).

The traits investigated were classified as lamb, carcass, and meat traits. Early growth traits consisted of birth weight (BW); weaning weight (WW), post-weaning weights at 5 months (W5M). Carcass traits included warm carcass weight (WCW), cold carcass weight (CCW), hot slaughter yield (HSY), cold slaughter yield (CSY) and commercial yield (CY). Meat sensory characteristic refers to juiciness, tenderness, flavor, appearance, the difference of specific lamb taste and overall difference. In order to determine the effect of diet on growth performance, carcass and meat quality characteristics, the mean comparisons between the two groups of the variables were carried out using independent samples Student t-test of the JASP procedure.

## RESULTS AND DISCUSSIONS

The effects of fed ration on lamb growth performance are presented in Table 2. Lambs' ages in the two groups in the research were smaller with cca. 10 days at birth, weaning and at end of fattening (5 months), weaning weight and weight at the end of fattening were similar in the two lots.

The experimental plan was to test the diet starting from the same body weight. Although the two lots were fed with the same fed up to weaning, and they had the same body weight at birth and weaning, the average daily gain from birth to weaning were significantly higher in lot 2 (who is about 10 days younger than in lot 1). Post-weaning, the average daily gain was not significant between the 2 lots.

The values obtained regarding daily dry matter intake, net energy and digestible protein are given in Table 3. The data table shows that during fattening, the highest dry matter intake was recorded in L1. The evolution of specific consumption (SC) of feed throughout the fattening period has major importance in the sense that the economic efficiency of fattening is dependent on this indicator. The evolution of specific consumption (SC) of feed during fattening period are shown in Table 4.

Table 2. Means ± SE (standard errors) for birth weight, final live weight and average daily gain in lambs fed with different rations

| Characteristics | L1 | L2 | P value |
|---|---|---|---|
| Birth weight, kg | 4.44 ± 0.16 | 4.44 ± 0.15 | 1.000 |
| Weaning weight, kg | 20.16 ± 0.48 | 20.17 ± 0.51 | 0.983 |
| Weight at final of fattening, kg | 42.23 ± 1.07 | 40.18 ± 1.41 | 0.367 |
| Total weight birth – weaning, kg | 15.72 ± 0.48 | 15.73 ± 0.48 | 0.982 |
| Total weight weaning – final of fattening, kg | 22.07 ± 0.84 | 20.01 ± 1.31 | 0.982 |
| ADG[d] birth – weaning, g | 238.10 ± 7.67[a] | 277.10 ± 11.06[b] | < 0.001 |
| ADG[d] weaning – 5 months, g | 225.83 ± 5.93 | 225.33 ± 8.80 | 0.963 |
| ADG[d] birth – 5 months, g | 227.19 ± 5.96 | 226.76 ± 8.86 | 0.968 |
| Weaning age, days | 66.35 ± 1.45[a] | 57.90 ± 2.11[b] | < 0.001 |
| Age at end of fattening, days | 166.35 ± 1.45[a] | 157.90 ± 2.11[b] | ***, < 0.001 |
| Slaughter age | 166.35 ± 1.45[a] | 157.90 ± 2.11[b] | < 0.001 |

[a,b] Means in the same line with different superscripts are significantly different. [d] ADG, average daily gain

Table 3. Daily dry matter intake (DMI), NE (net energy) and digestible protein (DP) during fattening period

| Characteristics | L1 | L2 |
|---|---|---|
| DMI kg/animal | 0.94 | 0.85 |
| NE MJ/animal | 13.06 | 11.83 |
| DP g/kg animal | 127.00 | 100.00 |

Table 4. Specific consumption (SC) during fattening period

| Characteristics | L1 | L2 |
|---|---|---|
| NE MJ/kg gain | 46.67 | 46.91 |
| DP g/kg gain | 583.58 | 506.91 |

Compared to the two lots, it is noticed that the highest protein consumption was recorded to the L1, while, the energy consumption was the same.

The effects of the ration on carcass quality characteristics are shown in Table 5. The diet had a significant effect on hot and cold carcass weight, and on hot, cold and commercial yield as well as on leg of muscle section area

The lambs from L1 presented higher live weight, hot and warm carcass weight, hot, warm and commercial yield and leg of mutton muscle area than L2. There were significant differences ($p < 0.001$) between L1 and L2 groups in terms of hot carcass weight and hot slaughter yield. In terms of cold carcass weight and cold slaughter yield were found significant differences ($p < 0.01$). With regard al commercial yield and leg of mutton muscle section area, were significant differences between the two lots ($p < 0.05$). The lambs from the group 1 were slaughtered at higher slaughter weight than their group 2 counterparts although all lambs were at similar starting weight in the research. These differences in final live weight might be explained by the protein and energy levels during fattening period.

Due to the very high variation of body weight at the end of fattening (36-51 kg in L1 and 26.5-55 kg in L2), for slaughter were chosen lambs with close body weights both within and between the lots. The evaluation of the carcass quality was based on the appreciation of the share of main cut sections of carcass (leg of mouton, shoulder + arm, cutlet and rest carcasses) and the share of the basic tissues that physically compose the carcass, namely the amount of muscle mass, bones and adipose tissue, the determinations being made both for the half-carcass and for the main cut portions (Table 6 and Table 7). Statistical data processing confirms the existence of significant differences ($p<0.01$) for the characters represented by the weight of the half-carcass and the amount of muscle mass in the half-carcass.

Table 5. Means ± SE for certain carcass quality characteristics of lambs fed with different diet

| Characteristics | Lot 1 (n = 3) | Lot 2 (n = 3) | **T-Test** | The significance of differences |
|---|---|---|---|---|
| Live weight, kg | $37.00 \pm 0.58^a$ | $35.33 \pm 0.33^b$ | 2,5 | *, p < 0.05 |
| HCW, kg | $19.37 \pm 0.42^a$ | $17.22 \pm 0.36^b$ | 3.879 | ***, p < 0.001 |
| CCW, kg | $18.43 \pm 0.36^a$ | $16.73 \pm 0.39^b$ | 3.191 | **, p < 0.01 |
| HSY, % | $52.34 \pm 0.79^a$ | $48.72 \pm 0.66^b$ | 3.536 | ***, p < 0.001 |
| CSY, % | $49.77 \pm 0.25^a$ | $47.35 \pm 0.76^b$ | 3.024 | **, p < 0.01 |
| CY, % | $60.41 \pm 0.52^a$ | $57.68 \pm 0.99^b$ | 2.442 | *, p < 0.05 |
| Leg of mutton muscle section area, $cm^2$ | $112.48 \pm 8.56^a$ | $92.08 \pm 1.85^b$ | 2.326 | *, p < 0.05 |
| LD muscle section area at 12 ribs, $cm^2$ | $14.60 \pm 0.26$ | $14.79 \pm 2.00$ | -0.096 | ns, p > 0.05 |
| LD muscle section area at 3-4 lumbar vertebrae, $cm^2$ | $13.57 \pm 0.55$ | $10.77 \pm 2.34$ | 1.916 | ns, p > 0.05 |

[a,b] Means in the same line with different superscripts are significantly different

In the study performed, it was found that between the two lots, there are significant differences ($p<0.01$) for the gigot weight and for the total amount of meat in the gigot. Shoulder + arm analysed as a region of butchery cut from the carcasses, highlights the fact that in terms of percentage participation in the total weight of carcass muscle mass, in group 1 the values are higher, therefore the meat/bone ratio is higher to this lot. At the cutlet level, the determinations made on half-carcasses show that the meat/bone ratio is higher in group 2, while for the rest of the carcass, the meat/bone and meat/fat ratio is higher in group 1. Meat/bone and meat/fat ratio of 3.06 and 4.01, respectively from semi-carcass of lot 1, are superior to the semi-carcass of lot 2. The differences between the two groups (L1 and L2) in terms of lean, fat and bone percentages were probably caused by different protein and energy levels in the diet. Furthermore, increased slaughter weight causes a change in carcass composition, which ap- pears as a decrease in lean ratio and an increase in the fat ratio, especially subcutaneous fat, in the carcass (Díaz et al., 2002; Santos-Silva et al., 2002; Majdoub-Mathlouthi et al., 2013).

Table 6. Means ± SE for the main cut sections of carcass from lambs submitted to fattening

| Characteristics | Lot 1 (n = 3) | Lot 2 (n = 3) | **T-Test** | The significance of differences |
|---|---|---|---|---|
| Live weight, kg | 37.00 ± 0.58$^{a}$ | 35.33 ± 0.33$^{b}$ | 2.500 | *, p < 0.05 |
| CCW, kg | 18.43 ± 0.36$^{a}$ | 16.73 ± 0.39$^{b}$ | 3.191 | **, p < 0.01 |
| Leg of mutton, kg | 6.57 ± 0.20$^{a}$ | 5.73 ± 0.15$^{b}$ | 3.341 | **, p < 0.01 |
| Cutlet, kg | 2.23 ± 0.03$^{a}$ | 1.63 ± 0.07$^{b}$ | 8.050 | ***, p < 0.001 |
| Shoulder + arm, kg | 3.13 ± 0.17 | 2.93 ± 0.18 | 0.824 | ns, p > 0.05 |
| Carcass rest, kg | 6.50 ± 0.35 | 6.43 ± 0.28 | 0.173 | ns, p > 0.05 |

$^{a,b}$ Means in the same line with different superscripts are significantly different

Table 7. Means ± SE for tissue structure of lambs fed with different diet

| Characteristics | Lot 1 (n = 3) | Lot 2 (n = 3) | **T-Test** | The significance of differences |
|---|---|---|---|---|
| Half-carcass, kg, from wich: | 9.22 ± 0.25 $^{a}$ | 8.38 ± 0.19 $^{b}$ | 3.344 | ***, p < 0.001 |
| Leg of mutton, kg: | 3.28 ± 0.10 $^{a}$ | 2.87 ± 0.07 $^{b}$ | 3.341 | ***, p < 0.001 |
| - meat, kg | 2.08 ±0.04 $^{a}$ | 1.70 ± 0.10 $^{b}$ | 3.507 | ***, p < 0.001 |
| - bone, kg | 0.67 ± 0.06 | 0.65 ± 0.03 | 0.250 | ns, p > 0.05 |
| - fat, kg | 0.53 ± 0.04 | 0.52 ± 0.07 | 0.196 | ns, p > 0.05 |
| Meat/bone ratio | 3.10 | 2.62 | - | - |
| Meat/fat ratio | 3.92 | 3.27 | - | - |
| Cutlet, kg | 1.54 ± 0.07 | 1.37 ± 0.06 | 1.858 | ns, p > 0.05 |
| - meat, kg | 0.95 ± 0.06 | 0.86 ± 0.01 | 1.467 | ns, p > 0.05 |
| - bone, kg | 0.38 ± 0.02 | 0.31 ± 0.01 | 1.703 | ns, p > 0.05 |
| - fat, kg | 0.21 ± 0.02 | 0.20 ± 0.03 | 0.340 | ns, p > 0.05 |
| Meat/bone ratio | 2.50 | 2.77 | - | - |
| Meat/fat ratio | 4.52 | 4.30 | - | - |
| Shoulder + arm, kg: | 1.57 ± 0.08 | 1.47 ± 0.09 | 0.824 | ns, p > 0.05 |
| - meat, kg | 0.97 ± 0.03 | 0.90 ± 0.10 | 0.632 | ns, p > 0.05 |
| - bone, kg | 0.35 ± 0.29 | 0.35 ± 0.08 | 0.000 | ns, p > 0.05 |
| - fat, kg | 0.25 ± 0.06 | 0.22 ± 0.02 | 0.555 | ns, p > 0.05 |
| Meat/bone ratio | 2.77 | 2.57 | - | - |
| Meat/fat ratio | 3.88 | 4.09 | - | - |
| Carcass rest, kg: | 2.83 ± 0.20 | 2.67 ± 0.08 | 0.758 | ns, p > 0.05 |
| - meat, kg | 1.85 ± 0.13 $^{a}$ | 1.46 ± 0.02 $^{b}$ | 3.055 | **, p < 0.01 |
| - bone, kg | 0.51 ± 0.08 | 0.54 ± 0.04 | -0.324 | ns, p > 0.05 |
| - fat, kg | 0.47 ± 0.06 | 0.66 ± 0.08 | -1.978 | ns, p > 0.05 |
| Meat/bone ratio | 3.63 | 2.70 | - | - |
| Meat/fat ratio | 3.94 | 2.21 | - | - |
| Total meat, kg | 5.85 ± 0.16 $^{a}$ | 4.74 ± 0.17 $^{b}$ | 3.734 | ***, p < 0.001 |
| Total bone, kg | 1.91 ± 0.08 | 1.86 ± 0.12 | 0.405 | ns, p > 0.05 |
| Total fat, kg | 1.46 ± 0.11 | 1.60 ± 0.12 | - 0.824 | ns, p > 0.05 |
| Total meat, % | 64.84 ± 2.09 | 61.72 ± 1.86 | 0.653 | ns, p > 0.05 |
| Total bone, % | 21.19 ± 0.27 | 23.27 ± 1.04 | 0.721 | ns, p > 0.05 |
| Total fat, % | 16.17 ± 1.18 | 20.08 ± 1.71 | -1.879 | ns, p > 0.05 |
| Meat/bone ratio | 3.06 | 2.55 | - | - |
| Meat/fat ratio | 4.01 | 2.96 | - | - |

$^{a,b}$ Means in the same line with different superscripts are significantly different.

The effects of diets on meat sensory characteristics are presented in Table 8. The juiciness and overall difference perception of the difference between the members of the evaluation committee were strongly influenced

by the diet administered in the present study registering very significant differences (p <0.001) between the meat from the two groups. On a scale of 1 to 7, it is found that the juiciness has an average value of 5.21 in group 1 and 3.71 in group 2. The perception of the panelists indicated differences represented statistically (p <0.05) between the 2 groups regarding the tenderness and the specific lamb taste. Therefore, it can be seen that the diet administered to group 1 influenced positively the eating qualities of lamb, resulting in a more juicy and tender meat, in which the specific lamb taste was attenuated. Hopkins et al. (2006) reported that tenderness and intramuscular fat level were significant predictors of the consumer sensory traits.

Table 8. Means ± SE for meat sensory characteristics of lambs reared with in different diets

| Characteristics | L1 | L2 | **T-Test** | The significance of differences |
|---|---|---|---|---|
| Juiciness | 5.21 ± 0.24[a] | 3.71 ± 0.27[b] | 4.185 | ***, p < 0.001 |
| Tenderness | 4.38 ± 0.26[a] | 3.58 ± 0.27 | 2.113 | *, p < 0.05 |
| Flavor | 3.96 ± 0.34 | 3.58 ± 0.28 | 0.861 | ns, p > 0.05 |
| Appereance | 4.21 ± 0.20 | 4.00 ± 0.26 | 0.632 | ns, p > 0.05 |
| The difference of specific lamb taste | 4.67 ± 0.31[a] | 3.88 ± 0.26[b] | 1.961 | *, p < 0.05 |
| Overall difference | 5.50 ± 0.28[a] | 3.92 ± 0.34[b] | 3.616 | ***, p < 0.001 |

[a,b] Means in the same line with different superscripts are significantly different.

Meat tenderness can be affected by the structure of the connective tissue, carcass fatness and collagen levels of meat (Sañudo et al., 2000; Díaz et al., 2002). The diet did affect the meat juiciness, tenderness, the difference of specific lamb taste and overall difference in the current study. Moreover, there were positive and very significant correlations (p <0.001) between tenderness and juiciness and between tenderness and flavor. Positive and very significant correlations (p <0.001) were also recorded between juiciness, tenderness, aroma and specific lamb taste according to the Pearson correlation results (Table 9).

Table 9. Coefficient of correlation among for meat sensory characteristic

| Characteristics | Juiciness | Tenderness | Flavor | Appereance | The difference of specific lamb taste | Overall difference |
|---|---|---|---|---|---|---|
| Juiciness | 1.00 | | | | | |
| Tenderness | 0.77*** | 1.00 | | | | |
| Flavor | 0.49*** | 0.70*** | 1.00 | | | |
| Appereance | 0.27 | 0.42 | 0.46*** | 1.00 | | |
| The difference of specific lamb taste | 0.57*** | 0.61*** | 0.58*** | 0.43 | 1.00 | |
| Overall difference | 0.62*** | 0.63*** | 0.53*** | 0.38 | 0.55*** | 1.00 |

*** $P < 0.001$.

## CONCLUSIONS

We found that growth rate, carcass weight, dressing percentages are influenced by the diet. On the other hand, the lambs fed higher level of protein during entire fattening period had higher values in terms of lean/bone and lean/fat ratio than lambs fed smaller level of protein. Furthermore, results of sensory analyses indicate that the meat was juicier and more tender in the lambs fed with a higher protein level compared to the lambs fed with a smaller protein level.


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# PROTEIN METABOLISM IN EPITHELIOCYTES OF THE LARGE INTESTINE IN FETUSES OF BLACK-SPOTTED CALVES

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***Abstract***

*The article shows the dynamics of total proteins and the features of protein metabolism at the fetal stage of calf development. This is important for diagnosing intestinal diseases in newborn calves due to their rather high mortality from such diseases. It was revealed that at the early fetal stage of development, a villous-cryptal gradient of the distribution of total proteins in the cytoplasm of epithelial cells is formed. With the growth of the fetus, at the midfetal stage of development, an increase in the content of total proteins is observed in the epithelial cells of the villi. In a differentiated study, it was found that the decrease in the color of total proteins in 4-5-month-old fetuses is due to the action of acidic proteins. The intensity of the reaction to the main proteins does not decrease, but even increases. In fetuses of 6 months, acidic proteins are again accumulated in epithelial cells, by birth, the ratio of acidic and basic proteins levels off. The epithelium of the large intestine at the neonatal stage undergoes adaptive histochemical restructuring.*

***Key words:*** *epitheliocytes, fetal stage of development, large intestine, protein metabolism, total proteins.*

## INTRODUCTION

The study of the patterns of development of the digestive system, including the large intestine, is an important prerequisite for the development of a nutrition system, prevention and diagnosis of various diseases (Reid, 1993).
A significant number of studies on the digestive system of animals touch upon general issues of the structure and patterns of growth of the gastrointestinal tract in ontogeny. The morphology of the small and large intestines of cattle has been studied quite well. At the same time, the histochemistry of the large intestine has not been studied deeply enough, in particular, we are talking about protein metabolism in enterocytes (Furness, 2000; Teltsov, 2002).
An analysis of the dynamics of total proteins and the characteristics of protein metabolism at the fetal stage of calf development is important for diagnosing intestinal diseases in newborn calves due to their rather high mortality from such diseases (Zdorovinin et al., 2021).

## MATERIALS AND METHODS

The material of the study was the fruits of the black-motley breed of cattle, their large intestine (its components: blind, colon, rectum). Pieces of tissue were taken from different sections of the colon: cranial, middle, and caudal. The material was fixed by a standard method for histological and histochemical studies. For the statistical analysis of all results, such indicators as the arithmetic mean, the error of the arithmetic mean, the correlation coefficient, and the probability of error were used.

## RESULTS AND DISCUSSIONS

At the early fetal stage of fetal development (2-5 months), simultaneously with the formation of true intestinal villi, a villous-cryptal gradient in the distribution of total proteins in the cytoplasm of epitheliocytes is formed. The nuclei of epithelial cells at the top of the villi contain granules of total proteins, which are distributed throughout the karyoplasm. In the center of the karyoplasm, small granules are visible, and larger, intensely colored ones are located near the nuclear envelope. The cytoplasm is filled with small granules, which are located closer to the periphery of the cell and therefore the perinuclear region remains free. A dark band is visible in the area of the brush border. In the epithelial cells of the lateral surface of the villus,

the color intensity in the area of the brush border is preserved. In the karyoplasm, an increase in the number of large, intensely stained granules of total proteins is observed. In the cytoplasm of epitheliocytes, the granules are distributed evenly throughout the cell, in contrast to the epithetliocytes of the villus apex. Goblet cells have large granules of total proteins in their contents, which are distributed throughout the cell and form a light blue staining in the form of a mesh.
With the growth of the fetus, at the midfetal stage of development (5-7 months), an increase in the content of total proteins is observed in the epithelial cells of the villi. This is evidenced by an increase in the number of large intensely colored granules. The granules are distributed throughout the cell. Closer to the cell periphery, an increase in the intensity of the reaction to proteins is noted. In the region of the brush border, a dark-colored band is visible, which loses its intensity of reaction in epitheliocytes lying in the region of the base of the villus. In a 7-month-old fetus, epithelial cells are characterized by an increased content of total proteins. In the nucleus, large granules are visible, distributed throughout the karyoplasm. A dark band with thickenings is visible along the nuclear membrane.
Goblet cells also contain large amounts of total protein granules. Some of the cells have a light content, which, apparently, is due to the fact that the cells have had time to secrete the contents of their glands into the intestinal cavity and have not yet had time to accumulate a sufficient amount of protein to give an intense staining. In the epithelial cells of the villi, there is a decrease in the color intensity from the base of the villus to its apex (Figure 1). In the area of the brush border, there is also an increase in the intensity of color in the cryptal-villous direction.

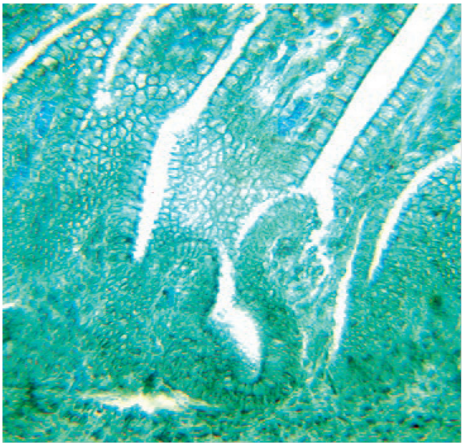

Figure. 1. Section of the wall of the caecum of a 5-month-old fetus.
Positive reaction for proteins according to Bonheg. About 20 × Ok.10

In the colon at the early fetal stage of development (2-5 months), the nuclei of villi enterocytes contain intensely stained clumps of total proteins. In the cytoplasm of epithelial cells, total proteins are presented in the form of granularity. The grains are distributed throughout the cytoplasm. The highest intensity of the content of total proteins is noted in the area of the brush border. A similar picture is observed in the epithelial cells of the lateral surfaces of the villi. In the region of the base of the villi, the nucleus and cytoplasm of epitheliocytes contain a large amount of total proteins. They are presented in the form of large grains, colored in dark blue, in the region of the brush border, the color intensity disappears.
By the age of 5 months, there is an increase in the content of total proteins in the epithelial cells of the villus apex. The most intense staining is noted in the nucleus and cytoplasm of epithelial cells, where total proteins are presented in the form of large granules. A large number of total proteins are observed in the region of the nuclear envelope and brush border. The nuclei of the epitheliocytes of the lateral surface of the villus also contain a large amount of total proteins, but

the intensity of their coloration is weaker than in the nuclei of the epithelial cells of the apex of the villi. The cytoplasm of the epithelial cells of the lateral surface of the villus, as well as the cytoplasm of the epithelial cells of the apex of the villus, contain a large amount of total proteins. In the area of the brush border, the color intensity is preserved.

In the epithelial cells of the base of the villi, the intensity of the reaction to proteins is preserved. In the nucleus and cytoplasm, total proteins are isolated in the form of large colored granules. Crypt epithelial cells are characterized by a high content of total proteins. In the nucleus, there is intense staining of granules of total proteins distributed throughout the karyoplasm.

A large amount of total proteins is observed in the region of the nuclear membrane, where they are presented in the form of a uniform band. In the cytoplasm of epithelial and goblet cells, total proteins in the form of large granules are distributed throughout the cell. In the area of the basement membrane, there is an intense reaction to proteins. At the midfetal stage of development (5-7 months), in the nuclei of the epitheliocytes of the top of the villi of the colon, an intense reaction to granule proteins is noted. This indicates an increased content of total proteins. In the cytoplasm of epitheliocytes, total proteins are presented in the form of small grains distributed over the entire area. A particularly intense reaction to total proteins is observed in the area of the brush border. A similar picture of the content of total proteins is observed in the epithelial cells of the lateral surface and the base of the villus. The nuclei of crypt epithelial cells have granules of total proteins located in the region of the nuclear envelope. In the karyoplasm, granules occur in separate groups. In the cytoplasm, total proteins are detected in the form of fine grains, which occupy the area of the entire cell.

Goblet cells are also characterized by an increased content of total proteins distributed throughout the cell. In the area of the basement membrane, an intense reaction is noted in the form of a dark blue stripe.

At the late fetal stage of development (7-9 months) and before birth, an increased content of total proteins persists. They are detected in the nucleus and cytoplasm of epithelial and goblet cells in the form of large dark blue granules. The granules fill the area of the entire cell, which gives the cells a dark shade. In the region of the nuclear envelope, total proteins are presented as an intensely colored band. In the region of the brush border, a reaction is observed, the intensity of which is maintained from the mouth to the bottom of the crypts.

In the epithelial cells of the crypts, with the growth of the fetus, an increase in the content of total proteins in the nucleus and cytoplasm is noted. A similar picture is observed in the intestinal endocrinocytes of the crypts. The persistence of the intensity of the reaction of the granules is also noted in the neonatal period in the goblet cells of the crypts, which, apparently, is associated with the specific functions of these cells.

In the rectum of 3-month-old fetuses, in the nuclei of epithelial cells of the tops of the villi, total proteins are detected in the form of small grains distributed throughout the karyoplasm. The most intense reaction of total proteins is noted in the region of the nuclear envelope. Throughout the cytoplasm of epitheliocytes, total proteins appear as a delicate light blue mesh. A narrow dark stripe is noted in the region of the brush border.

In the epithelial nuclei of the lateral surfaces of the villi, an intense reaction of protein granules is visible. An increase in the intensity of the reaction of protein granules is also noted in the cytoplasm. To the base of the villi in the karyoplasm and cytoplasm of epithelial cells, an increase in the intensity of color is noted. Dark blue granules are visible.

Fruits 4-5 months. in epithelial cells, in contrast to the epitheliocytes of a 3-month-old fetus, there is a slight decrease in the intensity of the reaction to proteins. Obviously, this is due to a decrease in the content of total proteins in the nucleus and cytoplasm of epitheliocytes. Goblet cells, like epithelial cells, contain granules of total proteins, some goblet cells have a light content. Apparently, by the time the material was taken, the cells had managed to release the secret of their glands into the intestinal cavity. A decrease in the intensity of the reaction is also noted in the area of the brush border, which indicates a decrease in the amount of total proteins.

At the midfetal stage of development (5-7 months), the nuclei of the epithelial cells of the apex of the rectal villi show an increase in the intensity of the reaction to proteins. This is confirmed by the appearance of intensely stained granules of total proteins in the karyoplasm. They are distributed throughout the karyoplasm, and near the nuclear membrane, the granules seem to merge and form a darkly colored strip. In the cytoplasm of epitheliocytes, granules of total proteins are distributed throughout the cell. A dark colored strip is visible in the area of the brush border.

Epithelial cells of the lateral surfaces of the villi are characterized by an increased content of total proteins. This can be judged by the intense staining of epithelial cells. Large, intensely stained granules are visible in the nucleus and cytoplasm. An increase in color intensity is also observed in the area of the brush border. Goblet cells have a similar intensity of reaction. An increased content of total proteins is also observed in the epithelial cells of the base of the villi.

The epithelial cells of the crypts are dark blue in color. In the nuclei of epitheliocytes and goblet cells, a high protein content is noted, which gives the cells a dark color. A dark stripe is visible near the nuclear membrane, but it is not the same along the entire length, some thickenings are noted in places. In the cytoplasm of epitheliocytes, total proteins are presented in the form of medium-sized granules distributed throughout the cell. The highest staining intensity is observed at the apical pole of the cells. In some goblet cells, the cytoplasm is lighter and granules of total proteins are visible in it, located in separate granules, forming a mesh. Consequently, at the midfetal stage of fetal development, a cryptal-villous distribution gradient of total proteins is formed in the epithelial cells of the colon mucosa.

At the late fetal stage of development (7-9 months) and before birth, destruction of epithelial cells is noted (Figure 2). Large clumps of total proteins of dark blue color are visible in the nuclei. A dark band with separate thickenings is visible near the nuclear envelope. A large number of granules is also observed in the cytoplasm of epitheliocytes. A similar picture is observed in goblet cells. On the lateral surface of the crypts, goblet cells have a darker staining. At the apical pole, a dark, almost black coloration is seen. Obviously, the cells are preparing to release a secret with a high protein content into the intestinal cavity. The epithelial cells of the base of the crypts are characterized by a slight decrease in the color intensity of the granules of total proteins distributed throughout the cell. The crypts contain a large number of dark-colored granules of total proteins. In the nuclei of epitheliocytes and goblet cells, dark blue granules of total proteins are observed, distributed throughout the karyoplasm. In the cytoplasm, the total protein is secreted in the form of small granules distributed throughout the cell and forming a dense network. At the apical pole of the cells, more intense staining is observed.

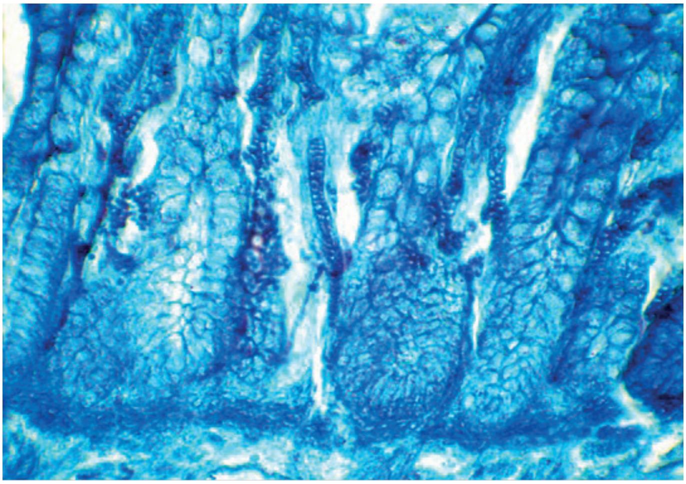

Figure. 2. A section of the wall of the rectum of a 9-month-old fetus. Positive reaction for proteins according to Miquel-Calvo. About. 40 × Approx. 15

In epithelial cells, the crypts of the nucleus contain separate large dark blue granules. The entire karyoplasm is filled with small granules. Closer to the nuclear envelope, they merge and form a dark band. This indicates an increased content of total proteins in this region of the nucleus. Small light-colored granules of total proteins are observed in the cytoplasm. Their number is greater at the periphery of the cell, as evidenced by intense staining in this area. The perinuclear region remains free of granules of total proteins. Goblet cells have dark-colored granules of total proteins that are evenly distributed throughout the cell. A dark band is observed in the region of the basement membrane.

At the late fetal stage of development (7-9 months) and before birth, the epithelial cells of the caecum are characterized by an increased content of total proteins. The nuclei of epitheliocytes contain large granules of dark blue color, they are especially numerous in the region of the nuclear membrane. Darkly stained granules of total proteins are also found in the cytoplasm of epitheliocytes. A dark band is visible in the area of the brush border. A similar picture of the content of total proteins is observed in goblet cells. In the cell secretion, intensely stained granules are visible, distributed throughout the cell. In the epithelial cells of the bottom of the crypts, a slight increase in the intensity of staining is observed. In the karyoplasm, individual granules of total proteins are visible, the color intensity of which is somewhat higher than in the nucleus of the epithelial cells of the mouth of the crypts. In the cytoplasm, small intensely stained granules of total proteins are visible, distributed throughout the cell. In the region of the brush border, their greatest accumulation is observed.

In the nuclei of the epithelial cells of the crypts, separate large granules of a dark blue color are visible, which form an intense staining in the form of a strip near the nuclear membrane. The karyoplasm is occupied, small dark blue granules. In the cytoplasm, granules of total proteins form a dark blue mesh. The most intense staining of the granules is noted at the apical pole of the cell.

In goblet cells, the color intensity of the granules is similar to the color intensity of the granules of epithelial cells. Only along the cell periphery is a darker band visible.

In epithelial cells, basic proteins create a blue, soft background. Acidic proteins are intensely stained in the karyoplasm, the nucleolus of the nucleus, the cytoplasm of crypt enterocytes, and in the brush border of epitheliocytes. The main proteins are presented in the form of a homogeneous network in the nucleus and cytoplasm. As the epitheliocytes move from the bottom of the crypts to its mouth, they accumulate.

In a differentiated study, it was found that the decrease in the color of total proteins in 4-5-month-old fetuses is due to the action of acidic proteins. The intensity of the reaction to the main proteins does not decrease, but even increases. In fetuses of 6 months, the accumulation of acidic proteins in epithelial cells is again taking place, by birth the ratio of acidic and basic proteins is leveling off. However, the villous-cryptal gradient in the distribution of total proteins in epitheliocytes in fetuses disappears after 4.5-5 months. Intestinal endocrinocytes have the same reaction to total proteins as epithelial cells. The cytoplasm of goblet cells contains much less total proteins, while the nuclei of goblet cells practically do not differ from the nuclei of epitheliocytes.

Thus, in the dynamics of the content of total proteins in enterocytes, three successive stages are revealed: up to 3-4 months - accumulation of total proteins; from 3-4 months up to 6 months – decrease in acidic proteins and gradual accumulation of basic proteins; from 6 months before birth - accumulation of total proteins in epitheliocytes.

According to the measurement of the average histochemical coefficient, the intensity of the reaction to SH-groups of proteins in fetuses before birth is 4.1 points, while in newborn calves it is 3.7. Goblet cells of the rectum contain -SH-groups, while in the colon and cecum they are not detected in goblet cells. The epithelium of the large intestine at the neonatal stage undergoes adaptive histochemical restructuring. It is characterized by the restructuring of the nucleoprotein, carbohydrate and protein metabolism of epitheliocytes.

## CONCLUSIONS

In the dynamics of protein substances of cellular different of enterocytes of the large intestine at the fetal stage of development, successive stages can be traced. The first - in fetuses up to 3-4 months. there is an increase in the concentration of acidic and total (basic) proteins.

The second - in fetuses from 3-4 to 6 months. the concentration of acidic acids decreases and the content of total proteins increases.

The third - in fetuses from 6 months. before birth, accumulation of total proteins in enterocytes is observed.

The formation of a cryptal-villous gradient in the distribution of total proteins occurs at 2-5 months. development of the fetus, and the caudal-cranial gradient - in fetuses for 5-7 months. The epithelium of the large intestine at the neonatal stage undergoes adaptive histochemical restructuring. It is characterized by the restructuring of the nucleoprotein, carbohydrate and protein metabolism of epitheliocytes.

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# UNDERSTANDING THE PERCEPTION AND BEHAVIOUR OF ROMANIAN CONSUMERS REGARDING THE USE OF NANOTECHNOLOGY IN FOOD AND FOOD PACKAGING

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***Abstract***

*The lack of a consistent number of studies to indicate the benefits and safety of using food nanotechnology more accurately, as well as the novelty of the field, lead to reluctance from the consumer's side. At the same time, researchers may feel discouraged by the intransigence of the consumer perception, coupled with a restrictive legislative framework. This results in a circular argument in which all actors in the food field are involved: consumer resistance to change leads to the demobilization of the scientific and academic community. This quantitative research was based on a questionnaire used to explore the acceptance of Romanian consumers (n = 359) of food products obtained or packaged using nanotechnology. In this sense, the extent to which consumers are informed about the use of nanotechnology in the agri-food sector has been determined. The research has been designed in such a way to identify the factors that influence consumers' perceptions in accepting nano-food or food packaged using nanotechnology. The aim was also to identify the demographic characteristics of consumers that would accept foodstuffs obtained or packaged using nanotechnology. The results of this research showed that consumers would more easily accept nanotechnology if it were applied to packaging rather than when it is directly applied to food. Familiarization with the term "nanotechnology" may lead to stronger opinions, either positive or negative. Food industry players could turn their attention to the presentation of concepts and benefits, as well as the risks associated with nanotechnology, to encourage consumers to form their own educated opinions. Such results may reveal an early openness from the consumers' side towards nanoengineering in general and a first step in overcoming food neophobia.*

***Key words***: *consumer studies, food innovation, nanotechnology, food packaging, nanomaterials.*

## INTRODUCTION

Nearly 50 years after conceptualizing the notion, nanotechnology is the science capable to revolutionize the major fields of human civilization: industry, culture, and society. Norio Taniguchi is the scientist reclaiming the paternity of the "nanotechnology" term (Taniguchi, 1974). The development of research in nanotechnology has led to the elaboration of a new class of materials called "nanomaterials". The special properties of these materials allow for improvements in lifestyle, offering alternative solutions in communication systems, medicine, and new developments in food safety and quality (Jafari & McClements, 2017).

Food contains both natural nanomaterials, such as milk casein mycelium or certain organisms found in plant or animal cells (DNA, ribosomes, enzymes, antibodies), and artificially created nanomaterials, which are deliberately added to improve food quality and safety. Some nanoparticles are not an intrinsic part of food but may come into contact with it due to their inclusion in food packaging or in nanosensors.

Nanomaterials for the food industry are used in food processing as food additives, in the manufacture of packaging, in the development of nutraceuticals and increasing nutrient bioavailability, and in the production of sensors especially designed to detect toxins, pathogens or pesticides (Dasgupta et al., 2015; Marin et al., 2017; He et al. 2019).

Despite de advantages proposed using nanotechnology in the food industry, a noteworthy aspect that can make it difficult to market foods that present novelties and innovations is related to consumer behaviour and perception. In this sense, consumer study and education become essential for the survival of newly introduced food products. Notable research has shown the importance of taking the "consumer" factor into account when developing innovative products (Chen et al., 2020; Lakomaa et al., 2021).

Given the challenge to place genetically modified organisms (GMOs) on the market, consumer acceptance of modern technologies cannot be taken for granted. While consumers consider cost, safety, and quality in purchasing decisions, they are also emotional beings and have their own considerations regarding production practices and ethics, factors that play an increasingly significant role in such decisions (Dagevos, 2013).

As with any emerging technology, there are still considerable knowledge gaps (Erdem, 2018). Existing uncertainties have led to a significant increase in consumer concerns, especially regarding the effectiveness of nanotechnology, long-term side effects and the real ability to ensure safety (Gupta et al., 2017).

Over time, consumer perception regarding nanotechnology, in general, has been extensively studied (Siegrist et al., 2007; Capon et al., 2015; Giles et al., 2015). The available research shows that attitudes towards nanotechnology are particularly positive in many application areas. Consumers expect the benefits of nanotechnology to show in the pharmaceutical and medical fields and for technological development, in contrast to the agri-food sector. Food-related applications tend to be a public concern compared to other applications, with consumers being reluctant to buy food resulting from nanotechnology. Priest & Greenhalgh (2011) had similar conclusions - the participants in their study consider that nanotechnology brings benefits for medicine, but for the agri-food field the benefits were not as obvious.

Our study aims to explore the current perception of Romanian consumers regarding the use of nanotechnology. To our knowledge, no other similar research has been conducted in this sense.

## MATERIALS AND METHODS

There are three distinct paradigms in research that are often used to guide research methodology and analysis: positivism, interpretivism, and realism. The *research philosophy* adopted in this study is "Positivism". Positivism is a philosophy suitable for natural sciences, in which the researcher is objective, independent of the study, and usually presents the results of the analysis in a quantifiable manner. Researchers who choose positivism use existing theory to develop and assess hypotheses, to confirm or invalidate them, or to suggest directions for further research (Candy, 1989; Kivunja & Kuyini, 2017).

As the scientific literature on consumer behavior and perception on the use of nanotechnology for the food industry is limited and outdated (the most relevant papers were written in 2005-2006), we wanted to examine the consumer perception in 2021. The uniqueness of the study also consists of addressing the Romanian consumers.

For this study, the "deductive" *approach* has been used. The deductive research approach is closely aligned with quantitative analysis, and it is based on testing one or more theories to examine "cause and effect", to anticipate results, to separate facts from opinions, and to control research in such a way to establish relationships between data sets (Robson & McCartan, 2015). In this approach, the theory comes from the analysed scientific literature.

For operational purposes, to make the collection more efficient, the use of a single research method was chosen, namely the survey method. It is interesting to note that although Ahmed and Sil (Ahmed & Sil, 2012) recognize the benefits of multi-method research, they suggest that validating data from often different methods is difficult, prone to errors and variations in interpretations.

The *instrument* used to collect the data is a *questionnaire*. The online software "SurveyMonkey" was used to manage the surveys. The survey consists of 14 questions. The first and second questions concern the identification of the respondents - they are designed to obtain general information about their sex and age. The following two questions were asked to investigate whether respondents had heard of the term "nanotechnology" and what they associate it with. Next, it has been introduced a section that summarizes what nanotechnology is and some of its possible applications. After reading this part, respondents are asked a set of questions about their knowledge of using nanotechnology for food. They are also asked to indicate what are the sources they consider to be reliable if they want to inform themselves about nanotechnology.

Respondents address statements related to their need for information on the use of nanotechnology for food, in the form of appropriate product labelling. Questions 8 to 11 were designed to provide information on consumer perceptions. These refer to the benefits and risks associated by consumers with certain applications of food nanotechnology. Questions 12-14 are demographic, with respondents providing information about the last form of education completed, who is the person in charge of shopping, and monthly income (expressed in Romanian currency – LEI) for the entire household.
In addition to "SurveyMonkey", "Microsoft Excel" was used for its PivotTable function.

## RESULTS AND DISCUSSIONS

The survey was designed to assess the level of consumer acceptance of the use of nanotechnology. 359 respondents participated to the study – 120 males (33.43%) and 239 females (66.57%). This kind of information is needed to make future correlations about the type of consumers who might be open to nanotechnology in food production and packaging, as well as to describe those who would be reluctant to the use of this technology. The gender fragmentation was correlated as closely as possible with the statistical data resulting from the report of the National Institute of Statistics and Economic Studies (INSSE), stating that Romania's population consists of 51.1% women and 48.9% men. However, it was also taken into account that women are more willing to participate in market research than men. Smith (2008) observed a trend in which participants' gender influences their desire to take part in questionnaires, especially when administered in the traditional (paper) way; the influence is slightly lower when the survey is electronic.
Regarding the age distribution of respondents (Figure 1), most are between 30-39 years old (28.69% or 103 respondents). Respondents aged between 21 and 29 is the second most represented category (24.79% or 89 respondents). The lowest percentage belongs to the participants who fall into the group of 60 years old and more, 8.64% (31 people). The lowest age to participate in the survey was 18 years old. Those who did not meet this requirement were automatically excluded from the study.

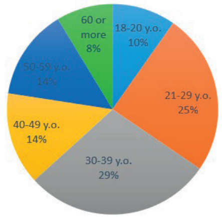


Figure 1. Distribution of sample population by age

Figure 2 depicts the results obtained after asking the participants whether they are familiar with the term "nanotechnology". It can be observed that more than two-thirds of the respondents state that they are familiar with the term "nanotechnology". Questions in future sections will check this statement and try to highlight whether the participants in the study have relevant knowledge about this technology.

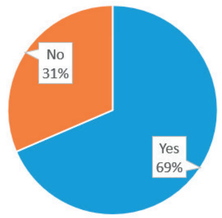


Figure 2. Answers regarding the knowledge of the term "nanotechnology"

Brown & Kuzma conducted a similar study in 2013. They concluded that the general population has little or limited knowledge about nanotechnology, which influences the decision to buy the by-products. The lack of knowledge as an impediment in accepting a new technology was highlighted even more recently, in 2020, by van Giesen et al. - they explained that it is difficult for consumers to form an opinion when they are not sufficiently informed about a topic. When trying to explore respondents' knowledge regarding nanotechnology they were asked in an open-ended question to note what they associated the term with. Most study participants referred to the technology in question using terms in the lexical field of the words "small," "future," "robotics," "scale," "novelty," "evolution," "movies," or "microchips".

For further understanding of the respondents' knowledge about nanotechnology, participants were asked to indicate whether they "agreed", "disagreed" or "did not know" on two statements. One refers to a possible similar behaviour of nanomaterials when used in larger scales and the other one states that nanotechnology involves materials that are not visible to the human eye.

Table 1 shows 38.72% of respondents believe that nanomaterials behave similarly to larger scales, but 28.69% disagreed with this statement. One-third of the participants did not know how to answer this question. Regarding the visible spectrum of nanomaterials, most of the respondents agreed that they are not visible to the naked eye.

The same set of statements also sought to deliver information on consumers' perceptions regarding the need to regulate the use of nanotechnology in food or food packaging. The general will is that regulators should have strict control over food nanotechnology. However, a difference of one per cent (5 respondents) can be observed between the nanotechnology used for food packaging and nanotechnology used for food production. Thus, 77.37% of the participants agree that the use of nanotechnology for food packaging should be strictly regulated, and 78.77% of respondents believe that when nanotechnology is applied to food, it is necessary to have strict legislative control. These results are in line with the scientific literature that mentions consumers may be more open to nanotechnology when it manifests itself on the outside of food (Giles et al., 2015; Zhou & Hu, 2018).

Consumers, however, although reluctant to consume food obtained or packaged using nanotechnology, agree (81.34%) with the statement that nanotechnology could be a possible solution to reduce the consumption of the planet's resources. This is an important finding that may indicate a future openness and acceptance of nanotechnology if it manifests its concrete benefits. The fact that so many respondents agree with this statement also underscores that human nature is not only based on cognition but also on affectivity as well as the care for future generations.

Many factors determine how consumers might respond to the use of new nanotechnologies. These include, but are not limited to, media coverage, past individual experiences with other innovative technologies, general attitudes, beliefs, knowledge, and preferences. Among these factors, the level of trust a person has in the food system (producers, processors, traders) and in the regulatory process that oversees it is also a critical issue. In the case of the introduction of new technologies, trust is a fundamental pillar (Roosen et al., 2015; Gupta et al., 2017). This perspective is especially true when consumer knowledge and experience about new technologies are limited, and consumers have the support of expert advice. The experts represent the mechanism that reduces the complexity when consumers judge the risks and benefits of new technologies (Gupta et al., 2017; Siegrist & Cvetkovich, 2020). On the other hand, a lack of trust in institutions could hinder the adoption of modern technologies and generate resistance to new policies (Hobbs & Goddard, 2015).

Table 1. Set of questions addressed to determine the knowledge of consumers regarding nanotechnology

| Statement | Answers | | | Total |
|---|---|---|---|---|
| | Agree | Disagree | I do not know | |
| Nanomaterials have similar behaviour to the materials on larger scales | 139 (38,72%) | 103 (28,69%) | 117 (32,59%) | 359 (100%) |
| Nanotechnology implies materials not visible to the human eye | 323 (89,97%) | 20 (5,57%) | 16 (4,46%) | 359 (100%) |
| The use of nanotechnology for food production should be strictly regulated | 282 (78,7%) | 14 (3,91%) | 62 (17,32%) | 358 (100%) |
| The use of nanotechnology for food packaging should be strictly regulated | 277 (77,37%) | 22 (6,15%) | 59 (16,48%) | 358 (100%) |
| Nanotechnology may help in reducing the consumption of the Planet's resources | 292 (81,34%) | 14 (3,90%) | 53 (14,76%) | 359 (100%) |

As education is one of the main contributors that could lead to changes in consumer perception and behaviour, it is relevant to identify the main actors in the food field that are trustworthy, as an information source for interested consumers.

Because nanotechnology is a recent technology and the public has very little related knowledge, it is therefore important to gain confidence and

be able to trust those institutions responsible for the development and regulation of the food in question.
Table 2 shows that participants have the greatest confidence when it comes to information about nanotechnology coming from scientists (from universities, research institutes). The National Veterinary Sanitary and Food Safety Authority (ANSVSA) also enjoys a high degree of trust. The next most trusted information source for consumers is the Consumer Associations. These results are like those obtained by Erdem in 2018 (Erdem, 2018). His study on British consumers showed that they have the greatest confidence in nanotechnology in government institutions, researchers, and consumer associations - in that order.
Respondents participating in our study are reluctant to receive information from food industry actors (producers, distributors, traders). British consumers have a similar perspective (Erdem, 2018). However, other studies show that industry representatives are willing to work with public institutions and consumers to assure a smooth functioning of the food chain (Baicu, 2016). The Romanian consumers show little trust in the information they encounter on the Internet, social media, and mass media. Less than 15% of the respondents trust or rely heavily on these possible sources of information.
Considering the importance of food for human beings, education about the technical and rational aspects of new food technologies may not be sufficient to determine consumer acceptance. Price, good taste and comfort are some of the proposed key considerations in today's market. For a third of the European consumers, moral and ethical issues are important in decision-making about the food they eat. Rollin et al. (2011) point out that the Europeans are likely to get over the risks associated with nanotechnology, if they perceive the benefits of it but also if they conclude it is a morally acceptable technology.
The same authors indicate the need for clear labelling of food, as it increases the consumers' perception of self-control (Rollin et al., 2011).
Table 3 supports the above statements and shows that Romanian consumers in 2021 want to be informed about the use of nanotechnology in the food they could consume. Thus, in the case of the statement that Consumers are sufficiently informed about the regulation of nanomaterials, over 70% of them expressed their disagreement or total disagreement.

Table 2. Set of questions addressed to determine the level of trust that consumers would grant to different food field stakeholders regarding the information on nanotechnology they would issue

| Stakeholder | Answers | | | | | Total |
|---|---|---|---|---|---|---|
| | Very reliable | Reliable | Neutral | Somewh at reliable | Not reliable | |
| The National Veterinary Sanitary and Food Safety Authority | 55 (15,36%) | 133 (37,15%) | 95 (26,54%) | 54 (15,08%) | 21 (5,98%) | 358 (100%) |
| Consumer Associations | 39 (10,86%) | 131 (36,49%) | 103 (28,69%) | 62 (17,27%) | 24 (6,69%) | 359 (100%) |
| Food industry (producers, distributors, traders) | 13 (3,63%) | 68 (18,99%) | 114 (31,84%) | 82 (22,91%) | 81 (22,63%) | 358 (100%) |
| Scientists (research institutions, universities) | 132 (36,87%) | 150 (41,90%) | 42 (11,73%) | 28 (7,82%) | 6 (1,68%) | 358 (100%) |
| Mass-media | 5 (1,39%) | 36 (10,03%) | 78 (21,73%) | 101 (28,13%) | 139 (38,72%) | 358 (100%) |
| Internet, social media | 6 (1,68%) | 37 (10,34%) | 96 (26,82%) | 102 (28,49%) | 117 (32,68%) | 358 (100%) |

Concerning labelling, the respondents show their need to be informed if the food was produced using nanotechnology. Slightly lower percentages are recorded when study participants were asked to express their views on the need for a label stating that the food packaging contains nanomaterials.
This difference may suggest a greater openness of the consumers when nanotechnology is used in packaging rather than when it is used in food. This result is also in line with previous studies showing that there may be a greater receptivity towards nanotechnology when applied externally.
The need to inform consumers is also emphasized by the responses regarding a food label to indicate the use of nanotechnology despite having stricter legislation for manufacturers. As in the previous set of statements, consumers expressed their agreement (49.16%) or total agreement (37.43%) to keep a nanotechnology symbol on the label if the food contains nanomaterials, but the percentages are lower (agreement - 43.18%; total agreement - 34.82%) for the use of the symbol when the food packaging contains nanomaterials.

Table 3. Set of statements with regards to the need of the consumers to be informed about nanotechnology

| Statement | Answers | | | | | Total |
|---|---|---|---|---|---|---|
| | Totally agree | Agree | Neutral | Disagree | Totally disagree | |
| The consumers are sufficiently about addressed about regulating food nanotechnology | 12 (3,35%) | 13 (3,63%) | 73 (20,39%) | 167 (45,81%) | 96 (26,82%) | 358 (100%) |
| When nanotechnology is applied to food, no label is needed to indicate this | 8 (2,23%) | 19 (5,31%) | 42 (11,73%) | 148 (41,34%) | 141 (39,39%) | 358 (100%) |
| When nanotechnology is applied to food packaging, no label is needed to indicate this | 13 (3,63%) | 37 (10,31%) | 48 (13,37%) | 149 (41,50%) | 112 (31,20%) | 259 (100%) |
| If the relevant legislation is stricter, no label is needed to indicate the use of nanotechnology in food production | 134 (37,43%) | 176 (49,16%) | 32 (8,94%) | 10 (2,79%) | 6 (1,68%) | 358 (100%) |
| If the relevant legislation is stricter, no label is needed to indicate the use of nanotechnology in food packaging | 125 (34,82%) | 155 (43,18%) | 34 (9,47%) | 34 (9,47%) | 11 (3,06%) | 359 (100%) |

Another step taken to understand consumers' perceptions of the risks and benefits associated with nanotechnology applications in food production, consisted of presenting the respondents a series of possible applications of nanotechnology in food production. The research participants were asked to specify whether any of these applications would lead them to purchase the product. Table 4 shows that if nanomaterials reduced the chances of a food product to make them sick, the participants to the study would be interested in such a food benefit. Also, favourable answers were given to the options in which nanotechnology would lead to more nutritious products, or if the products would keep their freshness longer. The use of nanomaterials in food packaging could also be a factor that would convince consumers if the application would determine a longer shelf life. The "I would not buy" option is the least popular answer.

Table 4. Determining factors to buy food containing nanomaterials

| Nanotechnology applications that would determine the Romanian Consumers to buy food products containing nanomaterials -statements- | Answers | |
|---|---|---|
| | % | Count |
| Nanomaterials would lower the chances that a food product would make me sick | 71,59 | 257 |
| Food becomes more nutritious | 40,39 | 145 |
| Food is fresh for longer | 36,49 | 131 |
| Nanotechnology would indicate if the food contained allergens | 16,71 | 60 |
| The food packaging has unique features | 20,06 | 72 |
| If the nanomaterials are used in the food packaging and this would lead to a longer shelf-life of the food product | 28,69 | 103 |
| I would not buy | 8,08 | 29 |

The results of our study are consistent with those presented in the current scientific literature, pointing out that when consumers perceive potential benefits, there may be more interested and confident about modern technologies.

Figure 3 shows that more than half (54%) of the research participants would trust a large, well-known company, if they were to buy nanofoods. On the other side, a boutique shop specialized in commercializing food with unique features would only attract 29% of the respondents willing to buy nano-based food.

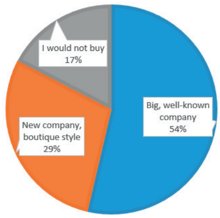


Figure 3. Consumer preferences regarding the type of company where they would buy food obtained or packaged with the use of nanotechnology

This question was addressed in order to understand what kind of companies would be successful, in an initial phase, in the production and marketing of nano food products, so what kind of company could open such a market. The results are similar to those obtained in previous research, which aimed to study the possible benefits of changes in European legislation governing novel foods, namely the simplification of procedures to be followed by companies to place such a product on the market (Baicu & Popa, 2016). Current research has also shown that, in theory, small and medium-sized food companies would have more opportunities to enter the market under the new regulation, as the time and money invested in the authorization procedure would be reduced. However, after the empirical analysis, the effect of the new procedures is different in the opinion of the consumer. Consumers would be suspicious of nano foods and would prefer to buy them from large, well-known companies that they consider reliable. It is appropriate to emphasize the consumer's willingness to buy traditional food from smaller companies, boutique or local companies, given that food manufactured on a larger scale will not provide the same specific quality. However, as far as nano-foods are concerned, consumers would still be cautious and believe that larger companies would be better suited to this situation. Therefore, small businesses will face barriers when trying to enter the market with foodstuffs obtained or packaged using nanotechnology. Small and medium-sized enterprises will have to wait for another stage, when consumers will gather more information and confidence about nanotechnology food after buying such products from large companies.

Figure 4 depicts the possible consumer concerns that would prevent them from buying food that uses nanotechnology. The most notable issue that would prevent consumers from buying nano-foods is that scientific studies have not gathered enough data to draw conclusions about the long-term effects of nanomaterials on human health. This factor is particularly important, as it shows the willingness of respondents to buy such food if the research in the field were to advance and the potential risks would be presented by the scientific community. Study participants point to the lack of solid data on risks and no other reasons, such as ethics. Another popular response came from respondents' awareness that they were not well enough informed about nanotechnology. Most study participants realize the need for education. Therefore, a risk communication system about nanotechnology could support this part of the population. At the same time, communication channels should be established and implemented to ensure continuing education on nanotechnologies and newly developed products. According to this research, consumers are willing to receive information from researchers, food safety authorities, but also from consumer associations. Therefore, the communication channels could initially include these three actors, and later are the industry representatives that could take part in the communication process. Another proposal would be to prepare educational programs as a first step, and then to include consumers in consultations and decision-making discussions.

From the answers provided by the survey participants, we also point out that part of the consumers still prefers traditional foods instead of innovative ones, but this group of respondents is not the majority. The possible high price of food using nanotechnology would also not be the first barrier for consumers.

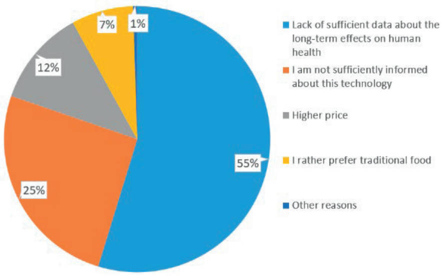


Figure 4. Factors that would determine a reserved behaviour in buying nanotechnology-based food

Table 5 shows the answers of the participants regarding a set of statements addressed to assess the perceived naturalness of the use of nanotechnology. The respondents were expected to either agree or disagree with the proposed statements.

The trend shows that consumers are relatively open to the use of food nanotechnology. Like the previous results of this study, a lower level of reticence is shown regarding the use of nanotechnology for food packaging rather than for food production. A generally positive attitude is expressed for encouraging research for the development of nanotechnology both for food and for food packaging. A higher number of respondents encourages the use of nanotechnology for food packaging (agree– 43.45%; totally agree – 22.84%) than for food production (agree – 14.76%; totally agree – 38.16%).

Table 5. Perceived natural nature of the use of nanotechnologies in the food industry

| Statement | Answers | | | | | | |
|---|---|---|---|---|---|---|---|
| | Totally agree | Agree | Neutral | Disagree | Totally disagree | I do not know | Total |
| I would buy food carrying a label that it was produced using nanotechnology | 53 (14,76%) | 149 (41,50%) | 77 (21,45%) | 22 (6,13%) | 30 (8,36%) | 28 (7,80%) | 359 (100%) |
| I would buy food carrying a label that the packaging was obtained using nanotechnology | 80 (22,28%) | 171 (47,63%) | 59 (16,43%) | 11 (3,06%) | 16 (4,46%) | 22 (6,15) | 359 (100%) |
| I would eat food carrying a label that it was produced using nanotechnology | 55 (15,32%) | 133 (37,06%) | 87 (24,23%) | 21 (5,85%) | 30 (8,36%) | 33 (9,19%) | 359 (100%) |
| I would eat food carrying a label that the packaging was obtained using nanotechnology | 79 (22,01%) | 163 (45,40%) | 69 (19,22%) | 12 (3,34%) | 15 (4,18%) | 21 (5,85%) | 359 (100%) |
| I would offer food obtained using nanotechnology to my family | 51 (14,21%) | 121 (33,70%) | 93 (25,91%) | 27 (7,52%) | 33 (9,19%) | 34 (9,47%) | 359 (100%) |
| I would offer food packaged with nanomaterials to my family | 72 (20,06%) | 146 (40,67%) | 79 (22,01%) | 14 (3,90%) | 18 (5,01%) | 30 (8,36%) | 359 (100%) |
| Nanotechnology for food production is acceptable | 53 (14,76%) | 137 (38,16%) | 93 (25,91%) | 15 (4,18%) | 21 (5,85%) | 40 (11,14%) | 359 (100%) |
| Nanotechnology un food packaging is acceptable | 82 (22,84%) | 156 (43,45%) | 66 (18,38%) | 9 (2,51%) | 12 (3,34%) | 34 (9,47%) | 359 (100%) |
| The use of nanotechnology for food production should be encouraged | 71 (19,78%) | 132 (36,77%) | 82 (22,84%) | 12 (3,34%) | 23 (6,41%) | 39 (10,89%) | 359 (100%) |
| The use of nanotechnology for food packaging should be encouraged | 93 (25,91%) | 149 (41,50%) | 60 (16,71%) | 10 (3,79%) | 14 (3,90%) | 33 (9,19%) | 359 (100%) |
| The use of nanotechnology in the food sector may contribute to reducing world hunger | 81 (22,56%) | 109 (30,36%) | 78 (21,73%) | 16 (4,46%) | 19 (5,29%) | 56 (15,60%) | 359 (100%) |

A control question was included. It refers to the consumers perception regarding the use of nanotechnology in the food sector as a possible factor to reduce world hunger. The answers are comparable to the ones received and presented in an earlier set of questions (Table 1).

The last three questions of the survey are demographic and useful to correlate and determine which type of consumer might be more open to food nanotechnology.

From Figure 5 it is observed that most (36%) of the respondents hold a bachelor's degree. The second most representative group (29%) is that of master's degree graduates. 23% of the participants only finished high school. 7% of the respondents hold a doctorate degree.

The graph in Figure 6 shows that 40% of respondents are the only ones responsible for shopping in their households, while more than half (54%) of them share this task with someone else.

Regarding respondents' income, most (24%) are part of the households where they earn a total of over 8500 LEI per month. The second most represented category (16%) is that of those living in households where the total income is between 6501-8500 LEI per month, almost equal to those with incomes between 2500-3500 LEI (15%) per month. The complete results are shown in Figure 7.

One of the goals of this research was to describe the consumer open to the applications of nanotechnology in the food field. As a results, several correlations of the survey findings were made.

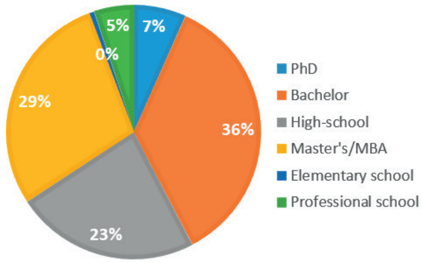


Figure 5. Completed level of education by the research participants

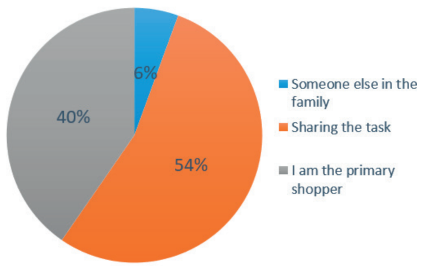


Figure 6. Who is doing the groceries in the participants' household

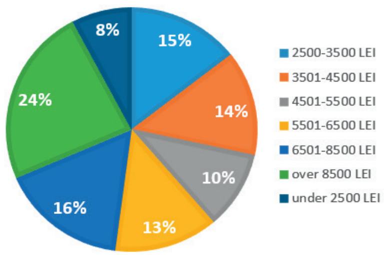


Figure 7. Total average income in the research participants' households

Table 6. Influence of the age factor on the familiarity with the nanotechnology term

| Are you familiar with the term of „nanotechnology"? | Age | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Answers | 18-20 y.o. | % of total per age group | 21-29 y.o. | % of total per age group | 30-39 y.o. | % of total per age group | 40-49 y.o. | % of total per age group | 50-59 y.o. | % of total per age group | 60 y.o. or more | % of total per age group | Total answers |
| Yes | 22 | 63% | 53 | 59% | 78 | 76% | 33 | 65% | 37 | 74% | 23 | 74% | 246 |
| No | 13 | 37% | 36 | 41% | 25 | 24% | 18 | 35% | 13 | 26% | 8 | 26% | 113 |
| Total respondents | 35 | 100% | 89 | 100% | 103 | 100% | 51 | 100% | 50 | 100% | 31 | 100% | 359 |

**Correlation between age and the familiarity with the *"nanotechnology"* term.** This correlation helps in performing an analysis of how age plays a role regarding the familiarity with the term "nanotechnology". The results of this correlation are presented in Table 6. It can be observed that, compared to the number of participants in each age category, the highest percentages of respondents who declared they were familiar with the term are recorded for the groups 30-39 years old, 50-59 years old and over 60 years old. The least familiar with the "nanotechnology" term are young adults aged 21-29 - 41% of them admit they do not know the term.

**Gender influence on the intention to consume food obtained or packaged using nanotechnology.** Although the general intention of the research participants is positive in terms of the consumption of food products to which nanotechnology has been applied, differences in perception can be observed depending on how the technology is applied (on the food or on the packaging), but also depending on the sex of the respondents. Table 7 shows that shy over half of the women surveyed agree to eat foods whose label states that they were produced using nanotechnology (40.58% - agreement and 12.55% - total agreement). Similar answers were given by men (30% - agreement and 20.83% - total agreement). However, the fragmentation of opinions is more pronounced among men, who also generate many negative responses (35% total agreement or disagreement), compared to women who chose to be neutral.

On the other hand, when the use of nanotechnology is applied to the food packaging, men are much more open than women (Table 8). If among women the percentages remain similar to those for food products obtained using nanotechnology, among men there are more positive answers and fewer negative opinions: 41.66% - agreement, 33.33% total agreement and only slightly more than 10% disagreement (5.83% disagreement; 5% total disagreement). These results indicate a need for possible nanotechnology education or popularization programs depending on the audience.

Table 7. Intention to eat food obtained using nanotechnology depending on the gender of the respondents

| I would eat food carrying a label that it was produced using nanotechnology | Number of answers relative to the gender of the participants | | | | |
|---|---|---|---|---|---|
| Answer | Male | % of total (m) | Female | % of total (f) | Total |
| Agree | 36 | 30% | 97 | 40,58% | 133 |
| Totally agree | 25 | 20,83% | 30 | 12,55% | 55 |
| Disagree | 12 | 10% | 9 | 3,76% | 21 |
| Totally disagree | 21 | 17,5% | 9 | 3,76% | 30 |
| Neutral | 21 | 17,5% | 66 | 27,61% | 87 |
| I do not know | 5 | 4,16% | 28 | 11,71% | 33 |
| Total | 120 | 100% | 239 | 100% | 359 |

Table 8. Intention to eat food packaged using nanotechnology depending on the gender of the respondents

| I would eat food carrying a label that the packaging was obtained using nanotechnology | Number of answers relative to the gender of the participants | | | | |
|---|---|---|---|---|---|
| Answer | Male | % of total (m) | Female | % of total (f) | Total |
| Agree | 50 | 41,66% | 113 | 47,28% | 163 |
| Totally agree | 40 | 33,33% | 39 | 16,31% | 79 |
| Disagree | 7 | 5,83% | 5 | 2,09% | 12 |
| Totally disagree | 6 | 5% | 9 | 3,76% | 15 |
| Neutral | 16 | 13,33% | 53 | 22,17% | 69 |
| I do not know | 1 | 0,83% | 20 | 8,36% | 21 |
| Total | 120 | 100% | 239 | 100% | 359 |

According to the responses received from the respondents, the women who did not express their agreement had neutral answers regardless the application of nanotechnology. A deeper familiarity with the concept could lead them to form other opinions. As men are more receptive to the applications of nanotechnology in food packaging, it is likely that a rigorous presentation of the risks and benefits of using nanotechnology in food production could lead them to consider such an application as well.

**Correlation between education level and the willingness to eat food obtained using nanotechnology.** The higher the education level of the respondents, the greater their openness to eat of food products obtained or packaged using nanotechnology.

Tables 9 and 10 display the results obtained after correlating the level of studies declared by the survey participants in relation to the way they would behave regarding the consumption of food products obtained using nanotechnology or packaged using nanotechnology.

Table 9. Influence of education level on the willingness to eat food obtained using nanotechnology

| I would eat food carrying a label that it was produced using nanotechnology | Number of answers relative to the education level of the respondents | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Answers | PhD | % of total | Bachelor | % of total | High school | % of total | master/MBA | % of total | Elementary school | % of total | Professional school | % of total | Total answers |
| Agree | 14 | 58,33% | 45 | 35,15% | 28 | 33,33% | 40 | 38,83% | - | - | 6 | 33,33% | 133 |
| Totally agree | 2 | 8,33% | 21 | 16,4% | 11 | 13,09% | 17 | 16,5% | - | - | 4 | 22,22% | 55 |
| Disagree | - | - | 9 | 7,03% | 6 | 7,14% | 3 | 2,91% | 1 | 50% | 2 | 11,11% | 21 |
| Totally disagree | 2 | 8,33% | 8 | 6,25% | 13 | 15,47% | 4 | 3,88% | - | - | 3 | 16,66% | 30 |
| Neutral | 4 | 16,66% | 33 | 25,78% | 17 | 20,23% | 31 | 30,09% | - | - | 2 | 11,11% | 87 |
| I do not know | 2 | 8,33% | 12 | 9,37% | 9 | 10,71% | 8 | 7,76% | 1 | 50% | 1 | 5,55% | 33 |
| Total | 24 | 100% | 128 | 100% | 84 | 100% | 103 | 100% | 2 | 100% | 18 | 100% | 359 |

Table 10. Influence of education level on the willingness to eat food packaged using nanotechnology

| I would eat food carrying a label that the packaging was obtained using nanotechnology | Number of answers relative to the education level of the respondents | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Answers | PhD | % of total | Bachelor | % of total | High school | % of total | master/MBA | % of total | Elementary school | % of total | Professional school | % of total | Total answers |
| Agree | 16 | 66,66% | 56 | 43,75% | 38 | 45,23% | 47 | 45,63% | - | - | 6 | 33,33% | 163 |
| Totally agree | 2 | 8,33% | 28 | 21,87% | 17 | 20,23% | 26 | 25,24% | - | - | 6 | 33,33% | 79 |
| Disagree | - | - | 4 | 3,12% | 4 | 4,76% | 1 | 0,97% | 1 | 50% | 2 | 11,11% | 12 |
| Totally disagree | 1 | 4,16% | 4 | 3,13% | 5 | 5,95% | 4 | 3,88% | - | - | 1 | 5,55% | 15 |
| Neutral | 3 | 12,5% | 29 | 22,65% | 15 | 17,87% | 20 | 19,41% | - | - | 2 | 11,11% | 69 |
| I do not know | 2 | 8,33% | 7 | 5,46% | 5 | 5,95% | 5 | 4,85% | 1 | 50% | 1 | 5,55% | 21 |
| Total | 24 | 100% | 128 | 100% | 84 | 100% | 103 | 100% | 2 | 100% | 18 | 100% | 359 |

58.33% of the participants holding a PhD degree expressed their agreement on the possibility to consume food products obtained using nanotechnology, while 8.33% expressed a total agreement in this regard. The doctorate diploma holders form the smallest group of participants that stand neutral on this issue. Among the respondents with a master's degree or MBA diploma, 38.83% agree and 16.5% fully agree to consume nanofoods. 35.15% of the respondents who have completed a bachelor's degree agree to consume food obtained using nanotechnology, and 16,4% express their total agreement. Generally positive answers are also provided by high school and post-high school graduates. Thus, 33.33% of those who obtained a post-secondary education diploma would consume products obtained with the help of nanotechnology, and 22.22% expressed their total agreement in this regard.

The trend is similar in terms of consumer behaviour towards using nanomaterials for food packaging: the more advanced the level of education of the survey participants, the stronger their intention to consume the mentioned products. However, it is interesting to note that for all categories of respondents (regardless of the form of education completed), the total number of positive responses (agreement or total agreement) is about 11.2% higher in this case, which shows that among the respondents, the use of nanotechnology for food packaging is easier to accept rather than when nanotechnology is used in food production.

The results are similar to those reported in the scientific literature (McCarron, 2016; Kapteina, 2016; Smith et al., 2008) also show that respondents with higher levels of education were “significantly more likely to have heard about nanotechnology” and those with higher education are “more likely to perceive that the benefits outweigh the risks, while those with a lower level of education perceive that the risks outweigh the benefits”.

**Correlation between the income category of the household to which the respondent belongs on the intention to consume food obtained or packaged nanotechnology.**

The intention of the respondents to eat food products obtained or packaged using nanotechnology was also correlated with the monthly average income of their household. These correlations are depicted in Table 11 (for products obtained using nanotechnology) and in Table 12 (for products packaged using nanotechnology).

After analysing the obtained date, it can be observed that the monthly income of the household does not play a significant role, the scores obtained being similar to each other, regardless of the application of nanotechnology.

Table 11. The influence of the income category of the household to which the respondent belongs on the intention to consume food obtained with the use of nanotechnology

| I would eat food carrying a label that it was produced using nanotechnology | Number of answers relative to the income group of the respondents | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Answers | 2500-3500 LEI | % of total | 3501-4500 LEI | % of total | 4501-5500 LEI | % of total | 5501-6500 LEI | % of total | 6501-8500 LEI | % of total | Over 8500 LEI | % of total | Under 2500 LEI | % of total | Total answers |
| Agree | 21 | 40,38% | 18 | 36% | 12 | 32,43% | 23 | 47,91% | 18 | 30,5% | 30 | 35,29% | 11 | 39,28% | 133 |
| Totally agree | 7 | 13,46% | 6 | 12% | 6 | 16,21% | 5 | 13,51% | 11 | 18,6% | 18 | 21,17% | 2 | 7,14% | 55 |
| Disagree | 5 | 9,61% | 2 | 4% | 1 | 2,7% | 2 | 4,16% | 5 | 8,47% | 5 | 5,88% | 1 | 3,57% | 21 |
| Totally agree | 4 | 7,69% | 4 | 8% | 2 | 5,4% | 1 | 2,08% | 10 | 16,9% | 7 | 8,23% | 2 | 7,14% | 30 |
| Neutral | 10 | 19,23% | 17 | 34% | 12 | 32,43% | 14 | 29,16% | 12 | 20,3% | 14 | 16,47% | 8 | 28,57% | 87 |
| I do not know | 5 | 9,61% | 3 | 6% | 4 | 10,81% | 3 | 6,25% | 3 | 5,08% | 11 | 12,94% | 4 | 14,28% | 33 |
| Total | 52 | 100% | 50 | 100% | 37 | 100% | 48 | 100% | 59 | 100% | 85 | 100% | 28 | 100% | 359 |

Table 12. The influence of the income category of the household to which the respondent belongs on the intention to consume food packaged with the use of nanotechnology

| I would eat food carrying a label that the packaging was obtained using nanotechnology | Number of answers relative to the income group of the respondents | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Answers | 2500-3500 LEI | % of total | 3501-4500 LEI | % of total | 4501-5500 LEI | % of total | 5501-6500 LEI | % of total | 6501-8500 LEI | % of total | Over 8500 LEI | % of total | Under 2500 LEI | % of total | Total answers |
| Agree | 23 | 44,23% | 22 | 44% | 17 | 45,94% | 25 | 52,08% | 27 | 45,76% | 37 | 43,59% | 12 | 42,85% | 163 |
| Totally agree | 10 | 19,23% | 9 | 18% | 6 | 16,21% | 11 | 22,91% | 20 | 33,89% | 20 | 23,52% | 3 | 10,71% | 79 |
| Disagree | 5 | 9,61% | 2 | 4% | 1 | 2,7% | - | - | 1 | 1,69% | 1 | 1,17% | 2 | 7,14% | 12 |
| Totally disagree | 3 | 5,76% | 1 | 2% | 2 | 5,4% | 1 | 2,08% | 2 | 3,38% | 5 | 5,88% | 1 | 3,57% | 15 |
| Neutral | 7 | 13,46% | 13 | 26% | 8 | 21,62% | 11 | 22,91% | 8 | 13,55% | 15 | 17,64% | 7 | 25% | 69 |
| I do not know | 4 | 7,69% | 3 | 6% | 3 | 8,1% | - | - | 1 | 1,69% | 7 | 8,23% | 3 | 10,71% | 21 |
| Total | 52 | 100% | 50 | 100% | 37 | 100% | 48 | 100% | 59 | 100% | 85 | 100% | 28 | 100% | 359 |

However, respondents with higher income tend to be more open to consuming food obtained or packaged using nanotechnology. Thus, in the case of using nanotechnology at the food product level, the most receptive consumers are those who come from households where the monthly income is over 8500 RON. The results obtained regarding the consumption of packaged products using nanotechnology, did not generate a uniform model that would lead to conclusions about a possible correlation between receptivity or reluctance towards nano-packaging and monthly revenues.

In their research, Yue et al. (2015) also observed that individuals with higher incomes tend to have positive attitudes towards nano-foods as well as towards genetically modified organisms. The same finding was made by Kapteina in 2016, that analysed how the demographic factors influence consumer behaviour regarding the use of nanotechnology in the food sector. Moreover, Tran et al. (2019) reported that the higher-income participants in their study are willing to pay more for food packaging (involving nanotechnology) if they lead to a safer food product, but also for food (beef, in that case) which would become safer as a result of the use of nanotechnology (Tran et al., 2019). Wang et al. (2019) correlated the higher incomes of the participants in their study with the desire to buy premium products and products containing ingredients that come from organic farming. There is a trend in the scientific literature that consumers with high levels of education and high incomes are more willing to try to eat special and new foods. This type of consumer demonstrates a greater ability to weigh the risks and benefits the new technologies bring to the food industry.

**Correlation between the familiarity with the term "nanotechnology" and intention to eat food obtained or packaged using nanotechnology.** Table 13 and Table 14 display the results of the correlation between the participants' answers to the question "Are you familiar with the term *nanotechnology*?" and their intention to consume food obtained or packaged using nanotechnology. We wanted to validate or invalidate the hypothesis that consumers who are familiar with the term nanotechnology would be more receptive and open to consuming foods that have been of this technology.

More than half of the consumers participating in the study expressed their agreement or total agreement on the consumption of food products obtained using nanotechnology (Table 13). The fact that the respondents were familiar with the term nanotechnology led to several answers of total agreement: 18.69% when the term was known and 7.96% when the term was unknown to the participants. The answers suggesting resistance to nanofood consumption were also influenced by the familiarity with the term "nanotechnology" - of those familiar, 18.28% expressed disagreement or total disagreement with the consumption of nano foods, while respondents unfamiliar with the term offered a percentage slightly higher than 5% in terms of disagreement or total disagreement with the consumption of such products.

Similar results have been obtained regarding the intention to consume foods whose label states that the packaging was made using nanotechnology. However, the percentages of individuals open to such an application are higher than in the case of nano-foods, whether or not the term nanotechnology is known to them. 72.76% of respondents who are familiar with the term *nanotechnology* are more receptive to nano-packaging, compared to 55.74% who would consume nano-packaged food, although they do not know the term. It is also noteworthy that 30% of people who declare they were unfamiliar with the term nanotechnology preferred to remain neutral when asked if they would consume such packaged foods.

Table 13. Familiarity with the *nanotechnology* term and the willingness to eat nanotechnology-based food

| I would eat food carrying a label that it was produced using nanotechnology | Results relative to the respondents' answers to the question "Are you familiar with the term *nanotechnology*?" | | | | |
|---|---|---|---|---|---|
| Answers | Yes | % of total | No | % of total | Total |
| Agree | 91 | 36,99% | 42 | 37,16% | 133 |
| Totally agree | 46 | 18,69% | 9 | 7,96% | 55 |
| Disagree | 19 | 7,72% | 2 | 1,76% | 21 |
| Totally disagree | 26 | 10,56% | 4 | 3,53% | 30 |
| Neutral | 45 | 18,29% | 42 | 37,16% | 87 |
| I do not know | 19 | 7,72% | 14 | 12,38% | 33 |
| Total | 246 | 100% | 113 | 100% | 359 |

Table 14. Familiarity with the nanotechnology term and the willingness to eat food packed with the use of nanotechnology

| I would eat food carrying a label that the packaging was obtained using nanotechnology | Results relative to the respondents' answers to the question "Are you familiar with the term *nanotechnology*?" | | | | |
|---|---|---|---|---|---|
| Answers | Yes | % of total | No | % of total | Total |
| Agree | 111 | 45,12% | 52 | 46,01% | 163 |
| Totally agree | 68 | 27,64% | 11 | 9,73% | 79 |
| Disagree | 10 | 4,06% | 2 | 1,76% | 12 |
| Totally disagree | 12 | 4,87% | 3 | 2,65% | 15 |
| Neutral | 35 | 14,22% | 34 | 30,08% | 69 |
| I do not know | 10 | 4,06% | 11 | 9,76% | 21 |
| Total | 246 | 100% | 113 | 100% | 359 |

The data obtained from this correlation indicates that familiarization with the term "nanotechnology" may lead to stronger opinions, either positive or negative. Food industry players could turn their attention to explain concepts and benefits, as well as the risks associated with nanotechnology, to encourage consumers to form their own educated opinions.

## CONCLUSIONS

The results of this research showed that nanotechnology would be more easily accepted by consumers if it is applied to packaging rather than if it is directly applied to food. Moreover, when consumers perceive potential benefits, there may be more openness to new technologies.

If consumers were to buy food obtained or packaged using nanotechnology, more than half (54%) of the participants in the study would trust a large, well-known company detrimental to new companies that specialize in food with special features.

Familiarization with the term "nanotechnology" can lead to stronger opinions, either positive or negative. Food industry players could turn their attention to raising awareness about concepts and benefits, as well as about the risks associated with nanotechnology, to encourage consumers to form their own educated opinions.

Such results may indicate an early openness from the consumers side towards nanoengineering in general and a first step in overcoming food neophobia.

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# EFFICIENT VALORIZATION OF DEFATTED WHEAT GERMS IN BREAD MAKING BASED ON DOUGH PROPERTIES AND BREAD QUALITY

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***Abstract***

*Wheat germs rich sources of biologically active compounds and are therefore among the most valuable by-products of the milling industry. Effect of defatted wheat germ powder addition on dough rheological properties and bread quality prepared with white wheat flours with different gluten index was investigated in this study. Different percentages of defatted wheat germ powder (5 and 10 g/100 g flour) were added to the white wheat flour samples, and dough rheological properties were tested using various Mixolab protocols. Defatted wheat germ powder addition increased the water absorption, dough development time, stability and weakening. Significant positive correlation between dough weakening or stability and Wixo parameters were registered. The Chopin+ torque values associated to starch gelatinization and retrogradation decreased from 2.03 to 1.90 Nm and from 3.21 to 2.68 Nm, respectively. Finally, the baking test indicated that bread samples with 10% defatted wheat germ powder had significantly lower specific volume and higher crumb firmness compared to the controls. Overall the results indicated that the addition of 5% defatted wheat germ powder to the wheat flour allows preparing bread with acceptable quality.*

***Key words***: *bread quality, defatted wheat germ, rheological properties, white wheat flour.*

## INTRODUCTION

There is an increasing worldwide concern for the sustainable development of healthy and nutritionally balanced foods. In this respect, the use of by-products rich in nutrients and biologically active compounds for developing value-added food products is of high interest. In particular the fortification of the bakery products while valorizing these kinds of by-products might be an efficient way to overcome some nutrition security issues. A common food habit consists on the consumption of white wheat flour bread, which is poor in vitamins, minerals and fibers and has low nutritional value. Bran is the main by-product obtained by cereals milling, but germs can be considered the most valuable by-product, even if are obtained in much smaller quantities than bran. Considering that the wheat germs represent 2-3% of the kernel, it appears that, at least theoretically, about 25 million tons of wheat germs should be separated annually worldwide (Boukid et al., 2018; Sun et al., 2015). Beyond these important quantitative resources, wheat germs are the most valuable anatomical part of the grain from a nutritional point of view. Thus, wheat germs contain appreciable amounts of proteins with a balanced essential amino acids ratio (Sun et al., 2015), unsaturated fatty acids (Boukid et al., 2018), dietary fiber (Marti et al., 2014), minerals, vitamins (E, thiamine, riboflavin, niacin), and compounds with antioxidant properties, mainly phenolic (Sun et al., 2015), flavonoids, phytosterols and polycosanols (Boukid et al., 2018).

The valorization of the nutritional potential of this anatomical component of the wheat grain depends on their isolation, storage and stabilization in order to prevent lipid oxidation (Boukid et al., 2018). The main strategies tested for getting wheat germs stabilization consist on: physical – thermal, radiation, thermal/mechanical treatments (Gomez et al., 2012; Marti et al., 2014; Sun et al., 2015), chemical treatment – alkalis, acids, supercritical carbon dioxide (Boukid et al., 1918; Ma et al., 2014) and biological treatment – fermentation (Marti et al., 2014). Each type of strategy approached has advantages and disadvantages, which finally influence the quality of the end product in which the wheat germs are added (Boukid et al., 2018).

The aim of this study was to investigate of the effect of wheat germ powder addition on the technological behavior of dough prepared with wheat white flours by using different protocols specific to the Mixolab device, namely Chopin+, Simulator and Wixo, and on bread quality. The three Mixolab protocols were selected to highlight the effect of wheat germs addition on the behavior of the dough during the dual mixing and temperature constraints (Chopin+ protocol), during mixing at constant speed (80 rpm) and temperature (30°C) (Simulator protocol), and during mixing at increasing speed from 80 to 240 rpm and constant temperature (30°C) (Wixo protocol).

## MATERIALS AND METHODS

***Flour samples selection and preparation of the mixtures***

Four samples of superior white wheat flours (WF) produced by three major Romanian producers - Boromir, Pambac and Dobrogea, were purchased from the Galati market. Preliminary analyzes performed on these flours indicated that all samples had quality indices that respect the admissibility conditions of the SR 877:1996 – ash content max 0.48%, protein content min 11%, gluten content min 28%, gluten deformation 5-12 mm, granularity rest on 180 mesh min 8% and sifted through 125 mesh max 70%. We selected four flour samples with various gluten quality, which were considered representative for this study. The gluten quality was assessed on the basis of the gluten deformation index and the gluten index.

Each of the four white wheat flour samples was supplemented with defatted wheat germ powder (G), purchased from Pronat (Romania). Two different supplementation levels were considered in the study, namely 5% (samples coded WF1 … WF4+5G) and 10% (samples coded WF1 … WF4+10G).

***Proximate composition of wheat white flours and wheat germ powder***

The proximate composition of the white wheat flours and wheat germ powder was determined as follows: moisture content by SR ISO 712:2005 (ASRO, 2008), protein content by using a nitrogen-to-protein conversion factor of 6.25 (semimicro-Kjeldahl method, Raypa Trade, R Espinar, SL, Barcelona, Spain), fat content through Soxhlet extraction method with ether (SER-148; VELP Scientifica, Usmate Velate (MB), Italy), crude fiber content using the Fibretherm Analyser (C. Gerhardt GmbH & Co. KG, Germany) and ash content by SR ISO 2171:2002 (ASRO, 2008).

***Gluten quantity and quality of wheat white flours***

Gluten quantity and quality of white wheat flours were estimated based on the wet gluten content and gluten index (SR ISO 21415-2:2007, ASRO, 2008) (Glutomatic 2200, Perten Instruments AB), and on gluten deformation (Bordei et al., 2007).

***Rheological properties of the dough samples***

Rheological properties of the dough samples prepared with white wheat flours alone or in admixture with 5 and 10% wheat germ powder were evaluated using the Simulator, Wixo and Chopin+ protocols of the Mixolab device (Chopin Technology, Villeneuve La Garenne, France).

The Simulator protocol was used for determining the mechanical properties of dough subjected for 30 minute at 80 rpm mixing at constant temperature of 30°C (Dubat and Boinot, 2012). The main parameters registered from the Simulator curves are water absorption (WA), development time (DT), stability (S) and weakening (W).

The Wixo protocol was used for determining the mechanical properties of the dough samples upon subjecting them to mixing at constant temperature (30°C) at speed of 80 rpm for 4 min, followed by 240 rpm for 4 min, (Dubat and Lebrun, 2019).

The Chopin + protocol was used for determining the thermo-mechanical properties of the dough during the dual mixing (80 rpm) and temperature constraints. The torque values were measured while running a thermal diagram consisting of: a 8 min plateau at 30°C, followed by temperature increase by 4°C/min up to 90°C; the second plateau of 7 min at 90°C was followed temperature decrease by 4°C/min to 50°C; the third plateau of 5 min at 50°C (Dubat and Boinot, 2012).

The main parameters selected from the Mixolab curves registered while running the Chopin+

protocol are: consistency after 8 min of mixing at constant temperature (30°C), C2 torque reflecting the protein weakening while increasing the temperature, C3 torque reflecting starch gelatinization, C4 associate to the hot gel stability and C5 torque estimating the extent of starch retrogradation upon cooling down to 50ºC (Dubat & Boinot, 2012). Other Mixolab curve parameters measured were: α – associated to the speed of heat related protein weakening was determined as the slope of curve delimited by the end of the 30°C plateau and C2; β - associated to starch gelatinization speed under the effect of heat and measured as the slope of curve delimited by C2 and C3 torque values; and (C5-C4) that defines starch retrogradation degree.

***The bread-making procedure***

The following recipe was used to prepare the bread: 100 g of white wheat flour alone or in admixture with wheat germ powder was supplemented with 1.5% salt, 3% compressed baker's yeast (Pakmaya, Rompak SRL, Pascani, Romania) and water. The amount of water used for preparing the dough was decided based on the water absorption capacity determined for each flour mixture by means of the Mixolab tests. The one stage method was selected to prepare the dough samples. Further on, the bread-making procedure proposed by Banu et al. (2010) was followed.

***Bread analysis***

After cooling to room temperature for two hours, the bread samples were characterized in terms of specific volume, crumb firmness and color.

The specific volume of the breads was determined using the rapeseed displacement method (SR 91:2007; ASRO, 2008).

The firmness of the crumb was measure with the MLFTA apparatus (Guss, Strand, South Africa) by using a probe with diameter of 7.9 mm. Measurements were performed on two different bread slices taken from the center of each bread sample. The parameters proposed by Banu et al. (2017) were selected for performing the test: penetration speed of 5 mm/s, trigger threshold force of 1.96 N, and penetration depth of 25 mm.

The lightness (L*), a* (green - red) and b* (blue - yellow) chromatic components of the crumb were measured using the Chroma Meter CR-410 (Konica Minolta Sensing Americas Inc., Ramsey, NJ, USA). Three measurements were taken on different areas for each sample and the overall color change (DE) induced by wheat germs powder addition in respected to the corresponding white wheat flour sample was determined as follows:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

***Statistical analysis***

The experimental measurements were done in triplicate, and the results are reported as average together with standard deviation values. The differences between samples with different levels of white wheat flour substitution with wheat germ powder were determined through one-way ANOVA with a 95% confidence interval.

## RESULTS AND DISCUSSIONS

***Proximate composition of wheat white flours and wheat germ powder***

The proximate composition of wheat germ powder and of the four white wheat flours selected after preliminary tests are shown in Table 1. The wheat germ powder used in this study contains higher contents of proteins (30.42%) and minerals (ash of 3.17%) compared to the wheat flour samples. No important differences were found among the white wheat flour samples considered in the study; the coefficient of variations was low for all quantified chemical components (Table 1). In particular, the protein and ash contents of the white wheat flours varied between 11.20-11.55% and 0.46-0.48%, respectively.

All white wheat flour samples had high gluten content, which varied between 30.3 and 31.8%, but gluten deformation and gluten index presented high coefficients of variation, of 33.10% and 11.78%, respectively. The values of gluten deformation and gluten index varied from 5.00 to 11.80 mm, and from 67 to 88%, respectively (Table 2). These variations, indicating the existence of important differences on the gluten qualities, were in fact the main criteria for selecting the flour sample used in the experiment.

Table 1. Proximate composition of wheat germ powder and white wheat flours (WF)

| Sample | | Moisture, % | Protein,% | Fat, % | Fiber, % | Ash, % | Starch, % |
|---|---|---|---|---|---|---|---|
| Wheat germ powder | | 13.87 | 30.43 | 0.89 | 1.59 | 3.17 | 53.29 |
| Wheat flour | WF1 | 11.71 | 11.25 | 0.88 | 0.36 | 0.48 | 87.03 |
| | WF2 | 11.64 | 11.20 | 0.90 | 0.34 | 0.47 | 87.09 |
| | WF3 | 11.29 | 11.35 | 0.89 | 0.36 | 0.48 | 86.92 |
| | WF4 | 11.35 | 11.55 | 0.94 | 0.35 | 0.46 | 86.70 |
| | Average±SD | 11.50 ±0.21 | 11.34 ±0.15 | 0.90 ±0.03 | 0.35 ±0.01 | 0.47 ±0.01 | 86.49 ±0.17 |
| | CV, % | 1.81 | 1.37 | 2.91 | 2.72 | 2.03 | 0.20 |

SD - standard deviation; CV – coefficient of variation

Gluten deformation provides information on the gluten quality, being useful mainly for assessing the elasticity and viscosity of the gluten. The high deformation ability of the gluten when is left to rest is an indication of the low quality (Mironeasa et al., 2016). Gluten index is a parameter related to the protein network. The gluten samples with large amounts of high molecular weight proteins have high value of the gluten index. It is therefore expected to identify relationships between this parameter and those related to greater protein quality (Collar et al., 2007).

Table 2. Gluten quantity and quality of white wheat flours (WF)

| Wheat flour | Wet gluten, % | Gluten deformation, mm | Gluten index, % |
|---|---|---|---|
| WF1 | 31.80 | 11.80 | 88.00 |
| WF2 | 30.30 | 5.00 | 67.00 |
| WF3 | 30.40 | 10.50 | 83.00 |
| WF4 | 31.10 | 8.50 | 75.00 |
| Average ±SD | 30.90±0.70 | 8.95±2.96 | 78.25±9.22 |
| CV, % | 2.26 | 33.10 | 11.78 |

SD - standard deviation; CV – coefficient of variation

***Dough rheology***

*Simulator protocol*

The effect of wheat germ powder addition on the rheological properties of white wheat flour was first measured using the Simulator protocol. The main parameters registered in the Simulator curves for all flour samples considered in the study are presented in Table 3. Simulator curves presenting the average torque values determined for each supplementation level of the white wheat flour with wheat germ powder are displayed in Figure 1.

As can be seen in Figure 1 and Table 3, the incorporation of wheat germ powder into white wheat flour caused significant changes in the rheological behaviour of the dough during kneading at temperature of 30°C.

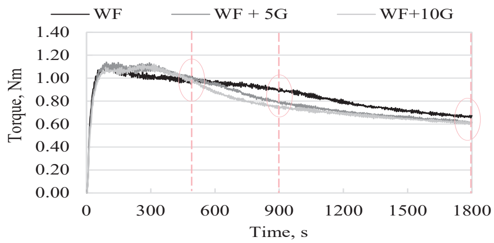


Figure 1. Simulator curves of white wheat flour with 0% (WF) 5% (WF+5G) and 10% (WF+10G) wheat germ powder. The average torque values are presented for each supplementation level. The red ellipsoids indicate the zones of the Simulator curves with major differences among samples with different amounts of wheat germ powder.

The wheat germ powder addition affected the rheological properties of the white wheat flour, through diluting the gluten and altering the water behavior because of the charges occurring in the ratio between the main components of the mixture, particularly between proteins and fiber (Gomez et al. 2012). Thus, WA and DT increased, while S and W decreased with increasing the addition level of wheat germ powder to the white wheat flour.

The increase of WA from 60.4 to 61.3% (Table 3) with increasing the level of wheat germ powder addition could be explained by the cumulative effect of the higher fiber and protein contents (Marti et al., 2014; Sun et al., 2015). Replacement of the white wheat flour with wheat germ powder resulted in the increase of the fiber and protein contents, mainly globulins (Zhu et al., 2006). Our observations regarding the increase of WA through incorporation of wheat germ powder into wheat white flour are in agreement with the literature. Marti et al. (2014) reported an increase of WA from 63.5 to 65.8%, Sun et al. (2015) noted an increase from 64.5 to 65.3%, while Gomez et al. (2012) reported an increase of WA from 58.2 to 59.6%, upon supplementing the control sample with 10% wheat germs.

On the other hand, our results regarding the DT increase from 2.5 min, for wheat white flour, to 3.70 and 4.80 min when adding 5 and 10% wheat germ powder, respectively (Table 3), contradicts other studies. For example, Sun et al. (2015) did not registered significant changes of DT, while Marti et al. (2014) reported significant decreases of DT, from 18 min, for the control sample, to 5.9, 6.3 and 7.2 min through the addition of 3, 10 and 20% wheat germ. The explanation for this behaviour could rely on the higher fiber content in the wheat germ powder used in this study, compared to those reported in other studies. Basically, the dough needs a longer time to develop due to the increase of the fiber content, which requires a longer time for water absorption (Torbica et al, 2010), compared to the wheat dough that have a lower dough development time because of the fast hydration and direct interaction between available thiol groups while kneading, leading to inter- and intramolecular sulphur bridges (Patrascu et al., 2017).

Table 3. The effect of wheat germ powder (G) addition on the rheological properties of white wheat flour (WF) measured using Simulator protocol

| Sample | | WA, % | DT, min | S, min | W, Nm |
|---|---|---|---|---|---|
| WF | WF1 | 61.0 | 2.40 | 10.80 | 0.15 |
| | WF2 | 60.4 | 2.60 | 8.58 | 0.17 |
| | WF3 | 61.0 | 3.20 | 9.30 | 0.15 |
| | WF4 | 59.2 | 1.80 | 10.30 | 0.14 |
| | Average ±SD | 60.4±0.85 | 2.50±0.58 | 9.75±1.00 | 0.15±0.01 |
| | CV, % | 1.40 | 23.09 | 10.22 | 8.25 |
| WF+5G | WF1+5G | 61.5 | 4.80 | 8.01 | 0.29 |
| | WF2+5G | 61.2 | 4.50 | 7.40 | 0.32 |
| | WF3+5G | 61.2 | 3.30 | 8.50 | 0.19 |
| | WF4+5G | 59.6 | 2.70 | 8.70 | 0.21 |
| | Average ±SD | 60.9±0.86 | 3.70±1.10 | 8.15±0.58 | 0.25±0.06 |
| | CV, % | 1.42 | 29.85 | 7.11 | 24.71 |
| WF+10G | WF1+10G | 61.9 | 4.80 | 7.00 | 0.31 |
| | WF2+10G | 61.7 | 5.40 | 6.90 | 0.33 |
| | WF3+10G | 61.5 | 5.40 | 8.10 | 0.29 |
| | WF4+10G | 60.1 | 3.40 | 7.80 | 0.32 |
| | Average ±SD | 61.3±0.82 | 4.80±0.94 | 7.45±0.59 | 0.31±0.02 |
| | CV, % | 1.33 | 19.86 | 7.94 | 5.47 |

WA – water absorption; DT – development time; S – stability; W – weakening; SD - standard deviation; CV – coefficient of variation

However, the addition of wheat germ powder decreased the dough stability from 9.75 to 7.45 min, and increases the weakening from 0.15 to 0.31 Nm (Table 3). Interesting are the trends of the simulator curves registered for the samples with different levels of wheat germ powder (Figure 1). After 7 minutes (420 s) of kneading, the dough consistencies of all samples have extremely close values, but in the next 8 minutes (480 s) the consistency values decrease with different speeds: by 10% in the case of wheat flour, up to near 0.91 Nm, and by 25% and 27% in the case of the samples with 5% and 10% wheat germ powder added, up to near 0.76 and 0.73 Nm, respectively. In the next 15 minutes, practically until the end of the test, the decrease of the dough consistency attenuation was notices in the case of all samples; the wheat flour finally registering a torque of 0.67 N, while the flour supplemented with wheat germ powder had torque values of 0.61 and 0.62 Nm, respectively. The effect of wheat germ powder on the dough stability can be attributed to the deterioration of the protein matrix quality, in respect to the control sample consisting on white wheat flour. Even if the wheat germs have large quantities of proteins, they mainly consist of globulins. Therefore, their incorporation into the wheat flour produce the so-called gluten dilution effect. In addition the globulins from the wheat germs compete with gluten proteins for dough water (Sun et al., 2015). Based on microscopy studies, Zhan et al. (2019) noted that the addition of more than 4% wheat germ accelerates the loss of gluten network properties and separates the starch granules from the protein matrix. The incorporation of wheat germ causes a redistribution of water in the dough and the breaking of -S-S- bonds in the dough, the final effect consisting on the reduction of the dough stability (Sun et al., 2015; Marti et al., 2014). The main responsible for breaking the -S-S- bonds from the gluten network is glutathione, a chemical component found in the wheat germ, and the most affected protein fraction is the high molecular weight glutenin, which undergoes a depolymerization process (Zhan et al., 2019).

Analyzing the behaviour of the dough for each of the four flour samples, can be seen how the addition of the wheat germ powder differently influences the dough stability (Table 3, Figure 1), depending on the gluten network quality, assessed based on gluten deformation and gluten index (Table 2). For example, even if the doughs prepared with WF1 and WF2 have different stabilities, upon the addition of 10% wheat germ powder, they rich rather close stability values (Table 3). Regarding the weakening, it can be seen that samples prepared with WF1 and WF2 flours and 5% of wheat germ powder have significantly higher values compared to WF3 and WF4, but the weakening values are very close for all flours supplemented with 10% wheat germ powder (Table 3).

*Wixo protocol*

The most important parameters of Wixo curves registered for the samples prepared with white wheat four flour samples with various gluten amounts and quality are presented in Table 4. Additionally, the Wixo curves represented using the average torque values calculated for each supplementation level of the white wheat flour with wheat germ powder are presented in Figure 2.

The dough is formed while mixing at 80 rpm in the first 4 minutes of the test; the dough consistency measured at the end of this stage is named $C_{end}$ FI.

The dough is formed while mixing at 80 rpm in the first 4 minutes of the test; the dough consistency measured at the end of this stage is named $C_{end}$ FI. When the speed increases to 240 rpm, the dough consistency suddenly increases at a value of $C_{start}$ FII.

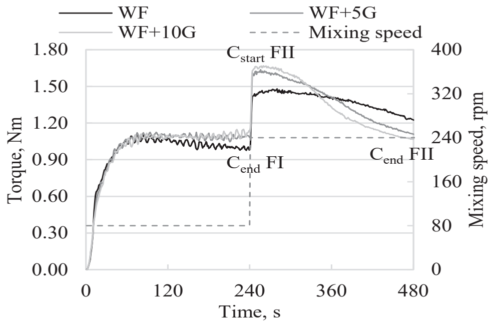


Figure 2. Wixo curves of the white wheat flour with 0% (WF) 5% (WF+5G) and 10% (WF+10G) wheat germ powder. The average torque values are presented for each supplementation level.

The higher difference between $C_{start}$ FII and $C_{end}$ F I define a dough resistant (Dubat and Lebrun, 2019). According to the results from Table 4, the wheat germ powder addition to the white wheat flour results in the increase of the difference between $C_{start}$ FII and $C_{end}$ F I consistency values, most probably because of the contribution of the fibers residing from wheat germ powder. A significant positive correlation of 0.82 ($p<0.01$) between DT (Table 3) and ($C_{start}$ FII - $C_{end}$ FI) (Table 4) was observed.

The final dough consistency measured at the end of the Wixo protocol is named $C_{end}$ FII. During the 4 minutes of kneading at 240 rpm, the consistency of the dough decreases with a much higher speed in the case of samples with the wheat germ powder addition compared to the corresponding wheat white flours. Thus, if in the case of white wheat flour the decrease was about 8%, from 1.30 to 1.25 Nm, in the case of samples with wheat germ powder addition the extents of the decrease were about 25 and 31%, from 1.51 to 1.13 Nm, and from 1.56 to 1.07 Nm, respectively. These results suggest the weakening of the gluten network by the addition of wheat germ powder. A positive correlation of 0.93 ($p<0.01$) was obtained between weakening (W) measured with Simulator protocol and ($C_{start}$ FII - $C_{end}$ FII). At the same time, the low values of $C_{end}$ F II indicate a low dough stability. In fact S measured with the Simulator protocol was positively correlated (0.88, $p<0.01$) with $C_{end}$ FII.

Table 4. The effect of wheat germ powder (G) addition on the rheological properties of white wheat flour (WF) measured using the Wixo protocol

| Sample | | $C_{start}$ FII – $C_{end}$ FI, Nm | $C_{end}$ FII, Nm | $C_{start}$ FII - $C_{end}$ FII, Nm |
|---|---|---|---|---|
| WF | WF1 | 0.41 | 1.23 | 0.21 |
| | WF2 | 0.35 | 1.18 | 0.17 |
| | WF3 | 0.38 | 1.31 | 0.07 |
| | WF4 | 0.30 | 1.26 | 0.00 |
| | Average ±SD | 0.36±0.05 | 1.25±0.05 | 0.11±0.10 |
| | CV, % | 13.03 | 4.37 | 84.76 |
| WF+5G | WF1+5G | 0.44 | 1.14 | 0.44 |
| | WF2+5G | 0.42 | 1.07 | 0.44 |
| | WF3+5G | 0.46 | 1.17 | 0.36 |
| | WF4+5G | 0.35 | 1.13 | 0.28 |
| | Average ±SD | 0.42±0.05 | 1.13±0.04 | 0.38±0.08 |
| | CV, % | 11.47 | 3.72 | 20.16 |
| WF+10G | WF1+10G | 0.47 | 1.08 | 0.52 |
| | WF2+10G | 0.47 | 1.01 | 0.54 |
| | WF3+10G | 0.47 | 1.08 | 0.49 |
| | WF4+10G | 0.38 | 1.09 | 0.41 |
| | Average ±SD | 0.45±0.05 | 1.07±0.04 | 0.49±0.06 |
| | CV, % | 10.06 | 3.47 | 11.66 |

SD - standard deviation; CV – coefficient of variation

*Chopin+ protocol*

The thermo-mechanical properties of the wheat white flour supplemented with the wheat germ powder measured using Chopin+ protocol are shown in Table 5 and, as average, in Figure 3.

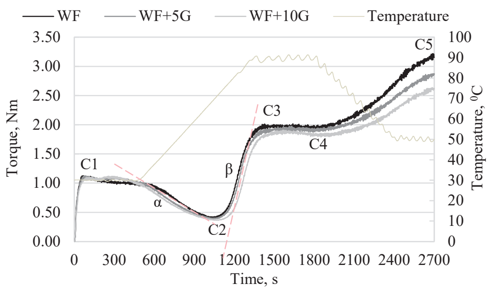


Figure 3. Mixolab curves of white wheat flour with 0% (WF) 5% (WF+5G) and 10% (WF+10G) wheat germ powder. The average torque values are presented for each supplementation level.

When the dough was subjected to mixing and temperature constraints, by increasing the temperature from 30 to 56±2°C, the consistency decreased to C2 value due to protein weakening. The C2 values decreased with the wheat germ powder addition to the wheat white flour (Table 5). Moreover, the addition of wheat germ powder to the white wheat flour samples determined the increase of the protein weakening speed during heating (α) (Table 5). These results suggest a higher weakening of the samples supplemented with wheat germs powder during heating, most likely due to the poor proteins quality residing from the wheat germ compared to the gluten protein from wheat flour. A significant positive correlations of 0.98 ($p<0.01$) between gluten index and C2 (0.98, $p<0.01$) was observed, while between gluten index and α, a significant negative correlation of 0.92 ($p<0.05$) was registered.

However, the decrease of C2 through wheat germ powder addition is not so pronounced, mainly in case of the sample with 5% addition. A possible explanation of this behaviour could be the high thermo-stability of the globulin fractions (Zhu et al., 2006), prevailing in the wheat germ. In fact, C2 is registered in the case of samples with the 10% wheat germ powder addition after a longer mixing time, about 18 minutes, and at a temperature of about 58°C, compared to 17 minutes and at 55-56°C in case of dough prepared from white wheat flour. All these appear over the effect of glutation from wheat germ powder on protein network during kneading.

Table 5. The effect of wheat germ powder (G) addition on the rheological properties of the white wheat flour (WF) measured using Chopin+ protocol

| | Sample | C2, Nm | α, Nm/min | C3, Nm | TC3, °C | β, Nm/min | C4, Nm | C5, Nm | C5-C4, Nm |
|---|---|---|---|---|---|---|---|---|---|
| WF | WF1 | 0.46 | 0.07 | 1.98 | 77.3 | 0.65 | 1.98 | 3.22 | 1.24 |
| | WF2 | 0.35 | 0.11 | 2.00 | 77.1 | 0.58 | 1.82 | 2.83 | 1.01 |
| | WF3 | 0.46 | 0.09 | 2.09 | 78.5 | 0.52 | 2.00 | 3.48 | 1.48 |
| | WF4 | 0.38 | 0.10 | 2.06 | 78.2 | 0.59 | 1.99 | 3.30 | 1.31 |
| | Average± | 0.41± | 0.09± | 2.03± | 77.78± | 0.59± | 1.95± | 3.21± | 1.26± |
| | SD | 0.06 | 0.02 | 0.05 | 0.68 | 0.06 | 0.08 | 0.27 | 0.20 |
| | CV, % | 13.70 | 18.46 | 2.45 | 0.87 | 9.66 | 4.32 | 8.51 | 15.49 |
| WF +5G | WF1+5G | 0.43 | 0.06 | 1.84 | 78.6 | 0.52 | 1.96 | 2.96 | 1.00 |
| | WF2+5G | 0.32 | 0.10 | 1.82 | 79.2 | 0.57 | 1.78 | 2.62 | 0.84 |
| | WF3+5G | 0.43 | 0.08 | 1.98 | 82.1 | 0.49 | 1.93 | 3.00 | 1.07 |
| | WF4+5G | 0.38 | 0.08 | 2.03 | 82.0 | 0.57 | 1.94 | 2.89 | 0.96 |
| | Average± | 0.39± | 0.08± | 1.92± | 80.48± | 0.54± | 1.90± | 2.87± | 0.97± |
| | SD | 0.05 | 0.02 | 0.10 | 1.84 | 0.04 | 0.08 | 0.18[b] | 0.10 |
| | CV, % | 13.36 | 20.41 | 5.32 | 2.28 | 7.57 | 4.47 | 6.16 | 10.20 |
| WF +10 G | WF1+10G | 0.39 | 0.07 | 1.88 | 84.3 | 0.20 | 1.84 | 2.72 | 0.88 |
| | WF2+10G | 0.31 | 0.07 | 1.76 | 84.6 | 0.24 | 1.63 | 2.42 | 0.79 |
| | WF3+10G | 0.40 | 0.08 | 1.94 | 84.8 | 0.30 | 1.84 | 2.79 | 0.95 |
| | WF4+10G | 0.37 | 0.09 | 2.00 | 83.3 | 0.35 | 1.93 | 2.78 | 0.85 |
| | Average± | 0.37± | 0.08± | 1.90± | 84.25± | 0.27± | 1.81± | 2.68± | 0.87± |
| | SD | 0.04 | 0.01 | 0.10 | 0.67 | 0.07 | 0.13 | 0.17 | 0.07 |
| | CV, % | 10.97 | 12.35 | 5.44 | 0.79 | 24.23 | 6.95 | 6.48 | 7.67 |

SD - standard deviation; CV – coefficient of variation

Our results indicated a significant positive correlation of 0.86 ($p < 0.01$) between C2 and $C_{start}$ FII-$C_{end}$ FII. Smaller but significant positive correlations of 0.46 ($p < 0.01$) were observed between C2 and $C_{end}$ FII, as well as between $C_{start}$ FII-$C_{end}$ FI.

The addition of wheat germ powder decreased the maximum consistency of the dough due to the starch gelatinization (C3), decreased the starch gelatinisation speed (β), and increased the starch gelatinization temperature (TC3) and time when the dough reached the maximum C3 value. Thus, in case of the samples including wheat germs powder the C3 values were reached after 25-27 minute and at temperatures of 81-83°C, compared to the about 23 minute and 77-78°C in case of wheat white flour. These differences are mainly due to the fact that the wheat germ powder has high fiber content.

The wheat germ powder addition decreased the dough consistency during heating to 90°C (C4) from 1.95 to 1.81 Nm, indicating a reduced starch gel stability during heating. This reduction of the consistency can be attributed to the intake of amylase brought by the wheat germ powder (Sun et al., 2015). Starch retrogradation during cooling from 90 to 50°C and the starch retrogradation degree, (C5-C4), decrease through wheat germ powder addition to the white wheat flour (Table 5).

***Bread properties***

The effect of the wheat germ powder addition on the properties of the bread samples prepared using the four different types of white wheat flour was estimated by determining the bread specific volume, crumb firmness and colour (Table 6). Overall, the specific volume of the bread samples decrease, from 452.1 to 394.33 and 310.50 $cm^3$/100 g, with increasing the level of wheat germ powder to the white wheat flour from 0 to 10%. A significant positive correlation of 0.57 ($p < 0.01$), was registered between specific volume and C2. A small decrease by 12.8% of the specific volume was registered in case of samples with 5% wheat germ powder addition, compared to the control sample, while further increase of the wheat germ powder level in the samples up to 10% caused an additional decrease of 21.3% of the specific volume. The addition of wheat germ powder influenced the crumb firmness. When compared to the control samples, a 17% increase of the crumb firmness was observed in case of the bread samples supplemented with 10% wheat germ powder, whereas in case of the bread samples with 5% wheat germ powder, the firmness increase was of 3.8%. Significant correlations of 0.77 ($p<0.01$) between crumb firmness and specific volume, and of 0.84 ($p<0.01$) between firmness and C2, were registered.

The specific volume decrease and the crumb firmness increase with the level of wheat germ powder addition to the white wheat flour might be the result of the following factors: the gluten network was affected, the competition of the different constituents of the flours for moisture varied with the mixture formula, the presence of glutathione, and the interaction between wheat flour and germ constituents. Any of those factors could affect the gas holding capacity of the dough during. Moreover, the reduction of the dough viscoelasticity due to changes occurring in the starch hydration capacity could negatively affect the bread quality (Gomez et al., 2012; Ma et al., 2014; Sun et al., 2015).

The wheat germ powder addition increase the total color change (ΔE) of the bread crumb (Table 6). The ΔE had lower values (ΔE of 1.76±0.10) in case of the bread samples with 5% wheat germ powder addition, compared to the samples with 10% wheat germ powder addition (ΔE of 7.89±0.34).

Table 6. The effect of wheat germ powder (G) addition on the proprieties of the bread prepared with white wheat flour (WF)

| Sample | | Specific volume, $cm^3/100$ g | Firmness, g force | ΔE |
|---|---|---|---|---|
| WF | WF1 | 483.10 | 561.84 | - |
| | WF2 | 403.40 | 755.51 | - |
| | WF3 | 470.90 | 617.41 | - |
| | WF4 | 452.10 | 685.32 | - |
| | Average±SD | 452.38±35.05 | 655.02±83.89 | - |
| | CV, % | 7.75 | 12.81 | - |
| WF+5G | WF1+5G | 443.94 | 584.56 | 1.62 |
| | WF2+5G | 333.48 | 793.66 | 1.84 |
| | WF3+5G | 422.84 | 638.73 | 1.79 |
| | WF4+5G | 377.06 | 704.32 | 1.79 |
| | Average±SD | 394.33±49.24 | 680.32±90.04 | 1.76±0.10 |
| | CV, % | 12.49 | 13.24 | 5.58 |
| WF+10G | WF1+10G | 340.30 | 676.01 | 8.12 |
| | WF2+10G | 280.50 | 857.08 | 8.09 |
| | WF3+10G | 320.50 | 755.23 | 7.96 |
| | WF4+10G | 300.70 | 775.06 | 7.40 |
| | Average±SD | 310.50±25.72 | 795.79±74.37 | 7.89±0.34 |
| | CV, % | 8.28 | 9.35 | 4.29 |

SD - standard deviation; CV – coefficient of variation

## CONCLUSIONS

The addition of defatted wheat germ powder to the wheat white flour influenced dough behavior measured using the Mixolab device under different conditions: during dual mixing and temperature constraints (Chopin+ protocol), during mixing at constant speed of 80 rpm and temperature of 30°C (Simulator protocol), and during mixing at constant temperature of 30°C and subsequent mixing speeds of 80 and 240 rpm (Wixo protocol). Significant correlations were obtained between different parameters defined by Simulator, Wixo and Chopin+ curves. The baking test indicated that the use of 10% defatted wheat germ powder in admixture with the white flour significantly altered the bread specific volume and crumb firmness.

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# INFLUENCE OF INJECTION LEVEL AND QUANTITIES OF BRINE INGREDIENTS ON THE SENSORY QUALITY OF BEEF PASTRAMI

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***Abstract***

*The study aimed to sensory evaluate and compare the colour, aroma, texture and taste attributes of six beef pastrami samples. The experimental batches were obtained in the Meat Processing Microsection of the University of Life Sciences Iasi and were formed to differ by two variation factors: the level of brine injected into the product (F1) and the amounts of ingredients introduced (salt/rapeseed oil/alfalfa powder) into the brine solution (F2). The most evident and significant differences (P < 0.01) were due to the increase in the percentage of brine introduced into the product. Thus, the sensory attributes for colour, aroma, texture (especially tenderness and juiciness) and saltiness were significantly influenced by the amount of brine injected. The batch that stood out with superior sensory characteristics was batch A because it showed favourable average scores for the attributes of tenderness, juiciness, elasticity, and the specific flavours of the brine additives did not negatively influence the characteristic flavour of the product.*

***Key words:*** *alfalfa powder, beef pastrami, injection, rapeseed oil, sensory evaluation.*

## INTRODUCTION

Although beef consumption has been declining steadily since 2007 and is expected to fall by a further 5% by 2030, it remains the third most consumed meat worldwide after pork and poultry (OECD-FAO, 2021).

In 2021, global beef consumption was 6.27 kg per capita, while pork and chicken consumption was significantly higher at 11.76 kg per capita and 15.10 kg per capita respectively (OECD Meat Consumption, 2021).

Meat is a major source of a balanced diet that contributes to the development and maintenance of optimal physical and intellectual performance.

Beef is a nutritious source, rich in proteins with essential amino acids (with high bioavailability), minerals (especially iron), vitamins and other bioactive compounds (coenzyme Q10, carnitine, anserine, creatine) (Martini et al., 2019; Ribas-Agusti et al., 2019; Cardoso et al., 2020). Mwangi et al. (2019) state that 100 g of beef provides more than 25% of the recommended daily intake of protein, niacin, vitamin $B_6$, vitamin $B_{12}$, zinc and selenium and more than 10% of the recommended daily intake of phosphorus, iron and riboflavin.

Pastrami is a traditional and well-known meat product, which can be obtained from different species (lamb, mutton, goat, pork or beef), with different muscle structures and anatomical regions of the carcass. Before the development of the method of preservation by refrigeration/ freezing, the manufacture of pastrami aimed to preserve the meat by salting, partial drying, the addition of aromatic herbs and spices, smoking and boiling (using steam) (Ibrahim, 2001; Roșca & Roșca, 2014). The shelf life and microbiological safety of pastrami are mainly due to low water activity, drying and modification of microbial flora after maturation (Karabiyikli et al., 2015).

Although attempts are made to reduce the salt content of processed meat products to decrease its effect on hypertension (Yalçın & Şeker, 2016), sodium chloride is a multifunctional ingredient added to meat products to improve sensory properties, microbiological quality and some technical characteristics such as water holding capacity (Ruedt et al., 2022). In addition, Er Demirhan & Demirhan (2021) showed that besides antimicrobial, preservative and shelf-life extension roles, salt added to meat products also acts on textural attributes (hardness, cohesiveness, elasticity, chewiness).

Rapeseed oil is valued due to its optimal fatty acid composition with a favourable ω-6/ω-3 ratio (Wirkowska-Wojdyła et al., 2021). Shtonda & Semeniuk (2021) used vegetable oils (rapeseed, olive and sunflower) for marinating some beef and pork varieties, obtaining positive results for sensory characteristics: more tender texture, higher juiciness, pleasant aroma, taste and appearance.

Alfalfa (*Medicago sativa* L.) has become a product of interest in recent years, with the potential for introduction into human consumption, due to its content in slow-release carbohydrates, proteins, minerals and vitamins (Mielmann, 2013). Moreover, Aziz et al. (1968) demonstrated the antioxidant effects of alfalfa extract, effects attributed to its property to inhibit linoleic acid oxidation.

Consumer acceptability of meat and meat products is closely related to intramuscular fat content, fatty acid composition (polyunsaturated and monounsaturated) and sensory characteristics (colour, tenderness, juiciness, odour and flavour) (Mwangi et al., 2019). The importance of sensory analysis of food products is mainly due to the increasing influx of new products in the market, and the need for companies to evaluate and determine the attributes of new and competing food products already on the market (Schilling & Pham, 2012).

The most important sensory attributes of beef for consumers are flavour, tenderness and juiciness, mainly due to the amount of connective tissue it contains (Schilling & Pham, 2012). Thus, this work aims to evaluate the sensory attributes of six experimental batches of beef pastrami obtained by injecting two salting solutions (containing salt, rapeseed oil and alfalfa sprout powder in different amounts) at three different injection levels (10%, 20% and 30%).

## MATERIALS AND METHODS

The research was based on the production of six experimental batches of beef pastrami in the Meat Processing Microsection of the University of Life Sciences Iasi and their sensory evaluation. The differentiation of the six batches was done in two steps, first by the content of the ingredients added to the injection solution and second by the level of solution injected in each batch. Thus, experimental batch A had as injection solution a liquid containing 4% salt, 5% rapeseed oil and 0.3% alfalfa sprout powder, and experimental batch B was injected with a solution containing 5% salt, 8% rapeseed oil and 0.6% alfalfa sprout powder. Three different sub lots were formed from each batch by the percentages of brine injected, hence sub lots $A_1$/ $B_1$ were injected with 10% brine solution, sub lots $A_2$/ $B_2$ were injected with 20% brine solution and sub lots $A_3$/ $B_3$ were injected with 30% brine solution.

*Sample preparation*

The raw material used for the pastrami was beef from adult animals purchased from a local slaughterhouse. The pieces of meat were sorted and trimmed to remove fat and connective tissue. The meat pieces were weighed before and after injection. The injection was performed in three stages for each solution formed (with 10%, 20% and 30% brine) with the INWESTPOL injection machine (10 needle version). The machine was set according to the category of meat used, at 12 strokes per minute and a pressure of 1.8 bar. After injection, the meat pieces were tied and placed on the racks for heat treatment. The heat treatment was carried out in the INDU imax500 heat treatment cell according to the following smoking programme: smoking (for 60 minutes at 82°C); smoke ignition (for 5 minutes at 82°C); smoking (for 40 minutes at 82°C); boiling (for 90 minutes at 83°C); baking (for 20 minutes at 89°C). After heat treatment, the products were cooled to reach refrigeration temperature, vacuum-packed and stored at 2-4°C until the sensory evaluation session.

*Sensory evaluation*

The batches obtained were sensory evaluated in terms of colour, aroma, texture and taste characteristics using a 9-point hedonic rating scale. From the descriptive sensory terminology specific for beef meat, 15 attributes were selected: colour uniformity, colour intensity, overall aroma, metallic aroma, pungent (woody) aroma, grassy aroma, rancidity, tenderness, hardness, elasticity, juiciness, acid, salty, bitter and umami taste.

The panellists were instructed how to use the hedonic scale and trained to understand the attributes assessed. The sensory evaluation consisted of three tasting sessions (three repetitions) organised to evaluate the six experimental batches. The evaluation team consisted of 8 members, four women and four men (between 22 and 27 years of age). The sensory evaluation sessions were conducted in the Sensory Analysis Laboratory of the University of Life Sciences Iasi, a space equipped with boards to separate the evaluators so that individual perception could not be influenced. Each assessor was provided with plain water and unsalted biscuits to rinse the palate after each assessed sample.

The samples were prepared in a separate room, coded, placed on plates and presented to the assessors in blind so that only the organiser of the assessment knew the identity of each sample. The results obtained from the sensory evaluation questionnaire were collected and statistically processed with the Data Analysis function of Excel software using the two-way ANOVA test.

Analysis of variance (ANOVA) was used to determine the presence of a statistically significant difference between the mean scores of the six samples, according to the two differentiating factors (quantities of ingredients introduced into the brine and levels of brine injected).

## RESULTS AND DISCUSSIONS

The colour of the six beef pastrami samples was assessed by the panellists in terms of intensity and uniformity. On the 9-point scale, the samples with the highest mean scores for colour intensity were $A_1$ and $B_1$ (Table 1), the percentage of brine introduced showing distinctly significant differences ($P < 0.01$) between experimental batches. The ingredients added to the brine solution significantly ($P < 0.05$) influenced the perception of colour intensity of the evaluated samples.

Table 1. Sensory evaluation of color of beef pastrami as influenced by the salting solution and the injection proportions

| | Attributes | n | Batch no. | | | | | | Effect | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Batch A | | | Batch B | | | F1 | F2 | F1 x F2 |
| | | | $A_1$ | $A_2$ | $A_3$ | $B_1$ | $B_2$ | $B_3$ | | | |
| COLOUR | Colour intensity | 24 | 8.5 ±0.34 | 8.04 ±0.65 | 7.50 ±0.43 | 8.75 ±0.19 | 8.25 ±0.45 | 7.70 ±0.38 | 1.18E-11** | 0.039* | 0.983 $^{ns}$ |
| | Colour uniformity | | 7.33 ±0.89 | 7.41 ±0.68 | 6.66 ±0.92 | 7.58 ±0.31 | 7.45 ±0.34 | 6.33 ±0.21 | 9.5E-06** | 0.936$^{ns}$ | 0.296 $^{ns}$ |

n = number of evaluations per sample; ±SD = standard deviation; F1 = percentage of brine injected; F2 = proportions of ingredients added to the brine; F1 x F2 = the effect of the interaction of the two factors; ns = $P > 0.05$; * = $P < 0.05$; ** = $P < 0.01$.

As for the colour uniformity of the six batches, significant differences ($P < 0.01$) were observed due to the change in the volume of solution injected, but the amounts of ingredients added to the brine and the interaction between the two factors did not generate significant changes between the batches ($P > 0.05$). However, a higher uniformity was observed for batches that were injected with 10% brine, meaning that at this injection level the brine was evenly distributed throughout the mass of the product.

The results of the sensory aroma evaluation of the experimental batches are shown in Table 2. The overall aroma intensity of the six samples was scored with mean scores ranging from a minimum of 5.87±0.63 (for batch $A_3$) to a maximum of 6.62±0.41 (for batch $B_2$). Overall aroma intensity was mainly influenced by the level of brine introduced in the experimental batches ($P < 0.01$).

The metallic flavour was identified as more intense in the samples with the lowest percentage of brine introduced, thus this factor had a significant influence ($P < 0.01$) on the perception of the evaluators. The pungent, rapeseed oil-specific and grassy aroma had mean scores directly and significantly ($P < 0.01$, $P > 0.05$) influenced by the percentage of brine injected and the proportions of ingredients introduced in the brine solution. An inversely proportional relationship was observed between meat-specific metallic flavour and pungent flavour and alfalfa flavour specific to the ingredients added to the solution. Thus, sample

3 from batch B showed the lowest mean score for metallic flavour (1.33±0.23) and the highest scores for pungent flavour (1.83±0.75) and alfalfa flavour (2.04±0.65).

For rancid flavour, all samples of the experimental batches received subunit mean scores, with distinctly significant differences (P < 0.01) due to different percentages of brine, and significant differences (P > 0.05) due to different amounts of ingredients introduced into the brine solution.

Table 2. Sensory aroma of beef pastrami as influenced by the salting solution and the injection proportions

| | Attributes | n | Batch no. Batch A $A_1$ | $A_2$ | $A_3$ | Batch B $B_1$ | $B_2$ | $B_3$ | Effect F1 | F2 | F1 x F2 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| AROMA | Overall intensity | 24 | 6.04 ±0.65 | 6.37 ±0.41 | 5.87 ±0.63 | 6.25 ±0.45 | 6.62 ±0.41 | 6.08 ±0.60 | 0.002** | 0.069 ns | 0.986 ns |
| | Metallic aroma | | 2.62 ±0.24 | 1.75 ±0.28 | 1.37 ±0.24 | 2.50 ±0.43 | 1.66 ±0.31 | 1.33 ±0.23 | 3.21E-20** | 0.357 ns | 0.931 ns |
| | Pungent aroma | | 0.79 ±0.43 | 1.29 ±0.30 | 1.58 ±0.25 | 0.83 ±0.40 | 1.62 ±0.24 | 1.83 ±0.75 | 3.22E-10** | 0.049* | 0.508 ns |
| | Grassy aroma | | 1.00 ±0.52 | 1.33 ±0.40 | 1.83 ±0.31 | 1.12 ±0.46 | 1.75 ±0.36 | 2.04 ±0.65 | 1.92E-08** | 0.027* | 0.552 ns |
| | Rancid aroma | | 0.20 ±0.17 | 0.62 ±0.41 | 0.54 ±0.78 | 0.37 ±0.24 | 0.83 ±0.66 | 0.95 ±0.73 | 0.002** | 0.027* | 0.652 ns |

n = number of evaluations per sample; ±SD = standard deviation; F1 = percentage of brine injected; F2 = proportions of ingredients added to the brine; F1 x F2 = the effect of the interaction of the two factors; ns = P > 0.05; * = P < 0.05; ** = P < 0.01.

In terms of texture, the attributes assessed were hardness, tenderness, elasticity and juiciness, the average scores obtained are shown in Table 3. The level of injection influenced the perception of all texture attributes evaluated to the greatest extent (P < 0.01), whereas the ingredients, added to the brine in different proportions influenced significantly only the juiciness of the samples (P < 0.01).

Table 3. Sensory evaluation of texture of beef pastrami as influenced by the salting solution and the injection proportions

| | Attributes | n | Batch no. Batch A $A_1$ | $A_2$ | $A_3$ | Batch B $B_1$ | $B_2$ | $B_3$ | Effect F1 | F2 | F1 x F2 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| TEXTURE | Hardness | 24 | 2.54 ±0.43 | 1.66 ±0.40 | 1.29 ±0.56 | 2.83 ±0.57 | 2.20 ±0.43 | 1.16 ±0.75 | 1.22E-16** | 0.053 ns | 0.079 ns |
| | Tenderness | | 5.04 ±0.56 | 6.04 ±0.30 | 6.79 ±0.43 | 4.91 ±0.60 | 5.75 ±0.63 | 6.54 ±0.86 | 1E-19** | 0.078 ns | 0.852 ns |
| | Elasticity | | 2.58 ±0.34 | 2.70 ±0.84 | 3.62 ±1.28 | 2.66 ±0.31 | 3.66 ±0.84 | 3.00 ±0.52 | 3E-08** | 0.322 ns | 0.736 ns |
| | Juiciness | | 4.95 ±0.56 | 5.62 ±0.41 | 6.41 ±0.42 | 4.64 ±0.41 | 5.20 ±0.60 | 6.16 ±0.66 | 1E-17** | 0.006** | 0.851 ns |

n = number of evaluations per sample; ±SD = standard deviation; F1 = percentage of brine injected; F2 = proportions of ingredients added to the brine; F1 x F2 = the effect of the interaction of the two factors; ns = P > 0.05; * = P < 0.05; ** = P < 0.01.

The freshness of the batches after heat treatment increased significantly (P < 0.01) with increasing levels of introduced brine. These results are similar to those obtained by Boles and Shand

(2001) who studied how anatomical region and injection percentage influence the tenderness and cooking yield of beef. Moreover, as the amount of brine introduced increased, the amount of water in the product also increased. This may explain the higher average scores given by the evaluators to the juiciness attribute for samples with a higher percentage of salting solution introduced.

The elasticity of the experimental samples was significantly influenced by the percentage of brine injected (P < 0.01). The samples with the highest elasticity were $A_3$ (with 30% brine injected) for the first experimental batch and $B_2$ (with 20% brine injected) for the second experimental batch, with mean scores of 3.62±1.28 and 3.66±0.84.

The taste of the experimental batches was evaluated in terms of salty, sour, bitter and umami tastes. The percentage of brine introduced significantly (P < 0.01) influenced the perception of salty taste. Although the two brine solutions had different amounts of salt, no significant differences (P > 0.05) were found between batches due to this variable. In contrast to the results obtained by McDonald et al. (2001), where increasing the injection level led to a decrease in the intensity of the salty taste, in the present study the salty taste was perceived as more intense the higher the injection percentage. Therefore, the salty taste was identified by the evaluators with higher mean scores in samples $A_3$ and $B_3$, those where the injection percentage was 30%.

Sour taste can be associated with the pungent flavour identified as the two attributes obtained similar mean scores, with the factor of different ingredient ratios showing distinctly significant differences between experimental batches (P < 0.01).

Bitter taste and umami were very poorly identified by the evaluators in the samples under analysis, obtaining the lowest mean scores ranging from 0.29±0.21 ($A_1$) to 1.20±0.69 ($B_3$) for bitter taste and from 0.87±0.54 ($A_1$) to 1.41±0.60 ($B_2$) for umami taste (Table 4).

Table 4. Sensory evaluation of taste for beef pastrami as influenced by the salting solution and the injection proportions

| Attributes | | n | Batch no. Batch A $A_1$ | $A_2$ | $A_3$ | Batch B $B_1$ | $B_2$ | $B_3$ | Effect F1 | F2 | F1 x F2 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| TASTE | Salty | 24 | 2.37 ±0.24 | 3.12 ±0.63 | 3.45 ±1.12 | 2.62 ±0.50 | 3.37 ±1.28 | 3.58 ±1.07 | 1.84E-06** | 0.19 ns | 0.934 ns |
| | Sour | | 1.04 ±0.30 | 1.16 ±0.23 | 1.33 ±0.57 | 1.87 ±0.37 | 1.83 ±0.14 | 1.91 ±0.34 | 0.336 ns | 2.52E-11** | 0.555 ns |
| | Bitter | | 0.29 ±0.21 | 0.91 ±0.42 | 0.75 ±0.71 | 0.58 ±0.34 | 1.00 ±0.60 | 1.20 ±0.69 | 0.0002** | 0.019* | 0.431 ns |
| | Umami | | 0.87 ±0.54 | 1.08 ±0.68 | 1.00 ±0.60 | 1.29 ±0.56 | 1.41 ±0.60 | 1.04 ±0.56 | 0.175 ns | 0.042* | 0.859 ns |

n = number of evaluations per sample; ±SD = standard deviation; F1 = percentage of brine injected; F2 = proportions of ingredients added to the brine; F1 x F2 = the effect of the interaction of the two factors; ns = P > 0.05; * = P < 0.05; ** = P < 0.01.

## CONCLUSIONS

Based on the results obtained from the sensory evaluation of the six experimental batches of beef pastrami, it was found that the colour characteristics were significantly influenced by the percentage of brine injected into the meat (P < 0.01). The highest mean values for colour intensity and uniformity were recorded for the samples injected with the lowest percentage of brine (10%). Aroma attributes were also significantly affected by increasing the amount of brine (P < 0.01). By the additions used in the brine solution (rapeseed oil and lucerne powder), specific flavours (pungent aroma, grassy aroma) were identified in the products obtained, whose intensity was significantly influenced by the two variation factors (P < 0.01). The texture of the experimental batches was significantly influenced (P < 0.01) by the amount of brine injected into the meat, samples injected with 30% brine solution showed higher tenderness and juiciness compared to those injected with lower percentages of brine. In addition, juiciness was higher in batch A, possibly due to the lower oil and alfalfa powder content of the brine, which facilitated diffusion of the solution into the product mass. The most intense taste identified by the evaluators was salty, significantly influenced ($P < 0.01$) by the percentage of brine in the product; while sour, bitter and umami tastes were poorly identified in the experimental batches.

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# PHYSICOCHEMICAL COMPOSITION AND FATTY ACIDS IN KEFIR FROM MILK OF "BULGARIAN WHITE DAIRY" GOAT BREED AND ITS CROSSINGS

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***Abstract***

*The physicochemical parameters of kefir on the 3rd and 14th day of the storage process produced from the milk of 'Bulgarian White Dairy' (BWD) goat breed and its crossings with 'Toggenburg' (TG) and 'Anglo-Nubian' (AN) were studied. The main groups of fatty acids in kefir on the 14th day of storage were identified and a qualitative assessment of milk fat was made based on lipid indices. The highest content of protein, fat and dry matter in kefir on the 3rd and 14th day was found in BWDxAN breed (5.42%, 5.34%; 4.77%, 4.66% and 15.84%, 15.74%), and the lowest in kefir from BWD (5.07%, 4.99%; 4.14%, 4.05%; 14.92%, 14.15%). Kefir from the milk of BWDxAN has the highest content of saturated fatty acids (SFAs), and monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) predominate in kefir from BWD. The content of SFAs in the studied kefir was from 3.0g/100g to 3.59 g/100 g. Therefore, kefir products are determined as high in content of saturated fatty acids (over 1.5 g/100 g product) and low content of trans fatty acids (0.12-0.17 g/100 g product).*

**Key words:** *fatty acids, goat milk, kefir, lipid indices, physicochemical composition.*

## INTRODUCTION

Milk and dairy products are some of the best accepted and widely consumed foods. To obtain them, fermentation technologies are used with the participation of various lactic acid bacteria, thus increasing their dietary potential (Nacheva et al., 2018). Consumption of goat's milk products is associated with health benefits beyond their nutritional value and compared to other types of milk, goat has unique bioactive characteristics such as high digestibility, alkalinity, high buffering capacity and certain therapeutic values associated with healthy feeding (Park & Haenlein, 2007).

Fermented milk, in particular kefir, is an extremely suitable form for the absorption of nutrients in milk on the one hand and for the impact of lactic acid bacteria on various body functions on the other (Slacanac et al., 2010). One of the possible alternatives to avoid and reduce the negative impact of milk proteins in people allergic to cow's milk is to include goat's milk and fermented products in their diet. One of the most useful products for human health in the group of fermented lactic acid foods is kefir. It is a traditional drink originating from the Caucasus region, but is consumed worldwide, the result of two fermentations with kefir grains - lactic acid and alcohol (Guzel-Seydim et al., 2011). It has all the beneficial characteristics of lactic acid drinks, providing the body's needs for calcium, and at the same time is a dietary lactic acid product with high absorption, rich in many beneficial bacteria and suitable for all ages (Roshtunkina, 2010). The starter culture of kefir, which is the kefir grains are small with an irregular shape, yellowish-white, firm granules that resemble miniature cauliflower and are a formation containing a matrix of proteins, fats, sugars and symbiotic association of lactic acid bacteria, such as *Lactobacillus kefiri*, *Leuconost*oc, *Lactococcus* and *Acetobacter* and fermenting and non-fermenting lactose yeasts (*Kluyveromyces marxianus*, *Saccharomyces*

*unisporus*, *Saccharomyces cerevisiae* and *Saccharomyces exiguus*) (Oliveira et al., 2013).
Milk fat also plays an important role in the production of fermented beverages, as interest in various omega-3 and CLA fatty acids, which have a significant effect on metabolism, has recently increased.
The possibilities for including goat's milk as a component in functional products are limited and have not been sufficiently studied. Therefore, the aim of the present study is to produce kefir from goat's milk of 'Bulgarian White Dairy' breed (BWD) and its crossings with 'Toggenburg' (BWDxTG) and Anglo-Nubian (BWDxAN), to establish its physicochemical composition on the 3rd and 14th day of storage as well as to study its fatty acid profile in view of its healthy effect on the human body.

## MATERIALS AND METHODS

Milk from experimental animals at the Research Institute of Mountain Stockbreeding and Agriculture - Troyan are used, that have been raised in one herd under the same production conditions consisting of three groups - 'Bulgarian White Dairy' and its crossings with 'Toggenburg' and 'Anglo-Nubian'. The rearing system is pasture-barn based, as in the period of April-November the animals were on a natural pasture of transitional type and in the barn, during the rest of the year. Several batches of kefir were prepared at the beginning (April), the middle (June) and the end (September) of the lactation period. For this purpose, the milk is pasteurized at a temperature of 85-90˚C, with a delay of 10-15 s., cooled to 29˚C and fermented with dry kefir leaven. It is poured into suitable containers and allow to ferment at 29°C for 16-18 hours, then cool and transfer to 0-4°C for refrigerated storage. Samples of the obtained kefir batches were examined to determine their physicochemical composition on the 3rd and 14th day of storage and are presented on arithmetic mean for the lactation period. The main groups of fatty acids in kefir on the 14th day of the storage process were determined and lipid indices were calculated.
The samples were tested in the technological laboratory for milk and dairy products at RIMSA - Troyan.
The following indicators were found on the Food Scan device (Lab, 78800): protein, fat, dry matter, water content, salts.
The titratable acidity was determined with 0.1 NaOH (BDS 1111-80), the active acidity with pH meter, the density by weight method, calcium by complexometry (according to Kodratenko et al., 1981), and the fatty acid composition with gas chromatograph "Shimadzu 2010" with flame ionization detector and column CP 7420 (100m x 0.25mm id, 0.2 μm film). The analysis was performed in the laboratory "Technology of milk, dairy products and fats" at the Institute of Cryobiology and Food Technology - Sofia.
Based on the obtained fatty acid composition, the following indices were calculated:
1) Atherogenic index (AI) - calculated on the basis of the content of medium-long fatty acids - C12: 0, C14: 0 and C16: 0 and the groups of monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) (Chilliard et al., 2003):

$$AI = \frac{C12:0 + 4 \times C14:0 + C16:0}{MUFAs + PUFAs}$$

2) Thrombogenic index (TI) - according to Ulbricht and Southgate (1991): TI=(C14:0+C16:0+C18:0)/0.5×C18:1+0.5×ΣMUFAs+0.5×ΣPUFAn6+3×PUFAn−3+(PUFAn3/PUFAn6)
3) Lipid preventive score (LPS) according to the equation of Richard and Charbonnier (1994):
LPS=FAT+2xSFA-MUFA-0.5PUFA
4)Ratio between hyper and hypocholesterolemic fatty acids (h/H);
h/H=(C18:1n-9+C18:1n-7+C18:2n-6+C18:3n-3+C18:3n-6+C20:3n-6+C20:4n-6+C20:5n-3+C22:4n-6+C22:5n-3+C22:6n-3)/(C14:0+C16:0).
The variational-statistical data processing is done through Statistica software package. The mean values of the groups were compared according to the tables of Student-Fisher t-test.

## RESULTS AND DISCUSSIONS

The chemical composition of kefir depends on many factors such as the type of milk and technological conditions (Sady et al., 2007).
In the studied kefir samples on the 3rd day of the storage process (Table 1.) the content of protein, fat and dry matter is the highest in kefir from the

milk of BWDxAN (5.42%, 4.77%, 15.84%), and the lowest in kefir of BWD breed (5.07%, 4.14%, 14.92%), as the results are statistically significant.

Much lower results indicated Nacheva et al. (2017) for kefir from goat's milk on the 1st day of storage, respectively (3.41%; 3.47%; 12.20%), as well as Akgul et al., (2018) for dry matter and protein (10.01%; 3.23%) for kefir of 5th day of cow's milk, which was probably due to differences in technological process as well as differences in the composition of milk from different breeds of animals.

Table 1. Composition of kefir on the 3rd day of storage, % (n = 9)

| INDICATORS | Breed groups | | |
|---|---|---|---|
| | **BWD** | **BWDxTG** | **BWDxAN** |
| | **x±Sx** | **x±Sx** | **x±Sx** |
| **Protein,%** | 5.07±0.144 | 5.34±0.139 | 5.42±0.078 |
| **Fat,%** | 4.14±0.050 | 4.73±0.081b*** | 4.77±0.209a* |
| **Water content,%** | 85.25±0.385 | 84.39±0.269 | 84.14±0.081a* |
| **Salts,%** | 0.86±0.055 | 0.82±0.044 | 0.81±0.067 |
| **Dry matter,%** | 14.92±0.302 | 15.75±0.229b* | 15.84±0.105a* |
| **Titrable acidity,T°** | 80.33±0.837 | 82.78±1.160 | 83.11±1.976 |
| **Active acidity, pH** | 4.46±0.018 | 4.50±0.029 | 4.52±0.035 |
| **Density,%** | 1.051±0.003 | 1.065±0.010 | 1.071±0.012 |
| **Ca, mg %** | 0.160±0.003 | 0.161±0.003 | 0.155±0.005 |

Note: a: BWD/BWDxAN; b: BWD/BWDxTG *P≤0.05;***P≤0.001.

The water content varied from 84.14% for BWDxAN to 85.25% for kefir from BWD, and the salts were in the range of 0.81% for BWDxAN to 0.86% for kefir from BWD.

The titratable and active acidity were the highest in BWDxAN - 83.11 T° and 4.52, which was close to the results indicated by Nacheva (2019) pH-4.50 and titratable acidity - 98°T in kefir from goat's milk fermented with the addition of lactulose.

Chen et al. (2009) also found, as in the present study, a decrease in active acidity and an increase in titratable acidity in kefir produced with starter cultures and kefir grains with different fermentation cycles.

The density and calcium content varied within relatively narrow limits between the different batches of kefir (1.051 – 1.071%; 0.154 - 0.160%). The lowest density was found in kefir from BWD with 1.051, which was because of the lower content of dry matter and milk fat.

The composition of kefir on the 14th day of storage is presented in Table 2.

Table 2. Composition of kefir on the 14th day of storage, % (n = 9)

| INDICATORS | Breed groups | | |
|---|---|---|---|
| | **BWD** | **BWDxTG** | BWDxAN |
| | **x±Sx** | **x±Sx** | **x±Sx** |
| **Proteins,%** | 4.99±0.145 | 5.27±0.136 | 5.34±0.044 |
| **Fat,%** | 4.05±0.066 | 4.55±0.038b*** | 4.66±0.192a* |
| **Water content,%** | 85.78±0.325 | 84.86±0.153b* | 83.68±0.140a**c*** |
| **Salts,%** | 0.85±0.037 | 0.78±0.050 | 0.80±0.025 |
| **Dry matter,%** | 14.15±0.290 | 15.14±0.160a* | 15.74±0.069a*c* |
| **Titrable acidity,T°** | 84.33±1.020 | 87±1.345 | 88±2.696 |
| **Active acidity, pH** | 4.40±0.003 | 4.43±0.020 | 4.44±0.012 |
| **Density,%** | 1.062±0.009 | 1.060±0.004 | 1.064±0.005 |
| **Ca, mg %** | 0.155±0.004 | 0.161±0.003 | 0.157±0.003 |

Note: a: BWD/BWDxAN; b: BWD/BWDxTG *P≤0.05;***P≤0.001.

The values for protein, fat and dry matter were the highest as well as on the 3rd day of kefir storage from BWDxAN milk (5.34%, 4.66%, 15.74%), and the lowest for kefir from BWDxAN (4.99%, 4.05%, 14.15), as there was statistical reliability for fat and dry matter, respectively ($p<0.05$), ($p<0.001$) for BWDxAN and BWDxTG.

The reduction in dry matter, fat, and protein found in the present study from the 3rd day to the 14th day of storage coincided with data by Irigouen et al. (2005) and Nacheva et al. (2017). Irigouen et al. (2005) indicated a value for fat and dry matter - 3.59-3.54% and 11.5-11.4% on the 14th day of kefir storage with 1% and 5% kefir grains.

Changes in the amount of fat are mainly due to the action of lipases obtained from kefir grains during fermentation (Vujicic et al., 1992). However, the most significant changes in fat values occurred after the 14th day of storage and were associated with the development of molds, which are the main lipolytic agents in fermented milk (Tamime & Deeth, 1980).

The active acidity ranged from 4.40 in BWD to 4.44 in BWDxAN, and the titratable with 84.33-88 Tº, as in all three batches of kefir there was a decrease on the 14th day of active acidity, and there was an increase in titratable acidity.

Sung-Ho Yoo et al. (2013), as well as in the present study, found in the production of kefir by two-stage fermentation a decrease of pH from zero to 24 days of storage, respectively, from 3.99 - 3.94 and 4.52 - 4.45 and increase in the titratable acidity from 0.60 to 0.71 and 0.83% - 0.90%.

According to Kang et al. (2013), the pH decreases due to the increase in acidity in the early stage of storage caused by the continuing metabolic activity of lactic acid bacteria.

Paseephol et al. (2008) did not find significant differences in pH values (4.2-5.5) and titratable acidity (0.81.0%) among samples of yogurt on the 1st day and after the 28th day of storage at 4℃. The acidity of all samples increased slightly (1.0-1.2%) and the pH decreased slightly (4.1-4.3). This is a proof for the continuous metabolic and enzymatic activity of the mixed cultures during low temperature storage.

The results for the main groups of fatty acids in kefir on the 14th day of storage are presented in Table 3.

Modern aspects concerning the composition of milk fat in ruminants relate mainly to the content of conjugated linoleic acid (CLA), which can reduce the risk of many diseases such as obesity, atherosclerosis, cancer etc. (Lawson et al., 2001). CLA-containing products contribute to the reduction of body fat by inhibiting lipogenesis and stimulating lipolysis (Raff et al., 2009).

The amount of conjugated linoleic acid in ruminants changes during the season due to changes in diet (Chilliard et al., 2005).

The differences between the three batches of kefir were insignificant and ranged from 0.59 g/100 g fat in BWD to 0.61 g/100 g fat in BWDxTG.

The amount of trans-isomers during the analyzed period was high in kefir from BWDxAN with 3.89 g/100 g fat, and of cis-isomers in kefir from BWD with 26.45 g/100 g fat, as the differences were statistically insignificant.

Table 3. Main groups of fatty acids in kefir on the 14th day of storage, g/100 g fat

| Groups of fatty acids | Breed groups | | |
|---|---|---|---|
| | BWD | BWDxTG | BWDxAN |
| | x±Sx | x±Sx | x±Sx |
| **ΣCLA** | 0.59±0.123 | 0.61±0.091 | 0.60±0.0116 |
| **Σ C-18:1 trans forms** | 3.28±0.554 | 3.40±0.628 | 3.89±0.834 |
| **Σ C-18:1 cis forms** | 26.45±2.030 | 25.48±1.799 | 25.08±1.655 |
| **ΣSFAs** | 71.89±2.354 | 73.16±2.449 | 73.88±2.232 |
| **ΣMUFAs** | 25.38±1.817a* | 24.37±1.701a* | 24.71±1.521 |
| **ΣPUFAs** | 5.75±0.908 | 5.32±1.015 | 5.12±0.855 |
| **Σ n-3** | 0.64±0.092 | 0.65±0.092 | 0.59±0.087 |
| **Σ n-6** | 5.04±0.778 | 4.83±0.920 | 4.33±0.759 |
| **Σn-6/Σn-3** | 7.90±0.423 | 7.43±0.354 | 7.33±0.523 |
| **ΣBranched chain fatty acids** | 1.30±0.138 | 1.29±0.205 | 1.24±0.231 |
| **CLA** | 0.51±0.091 | 0.55±0.063 | 0.53±0.091 |

Note: a: BWD/BWDxTG; *$P \leq 0.05$

The total amount of SFAs, which are the main components of milk fat and are considered harmful to human health because they increase cholesterol (USDA, 2002), was relatively high in the studied samples, respectively 71÷73 g/ 00 g fat ($p<0.05$ in BWD/BWDxTG).

The amounts of MUFAs and PUFAs predominated in kefir from the milk of BWD goats with 25.38 g/100 g fat and 5.75 g/100 g fat, while the lowest values were found in BWDxTG with 24.37 g/100 g fat and 5.32 g/100 g fat.

As it is known, C-18:2 and C-18:3 perform a number of important functions as C-18:2 is characterized by very high biological activity, as in the body in the presence of vitamin $B_6$ it passes into the essential arachidonic acid (C-20:4) (Gladky & Fedyakina, 2006).

Vieira et al. (2015) found in kefir from cow's milk on the 14th day of storage values close to the present study for SFAs, MUFAs and PUFAs, respectively 67.2 g/100 g fat, 28.2 g/100 g fat, 4.54 g/100 g fat.

Vital for metabolism fatty acids from the group omega-3, omega-6 are in the range of 0.59-0.64 g/100 g fat and 4.33-5.04 g/100 g fat, which is close to the results obtained by Nacheva et al., (2018) omega-3 - 0.52 g/100 g fat, omega-6 - 4.13 g/100 g fat in kefir from goat's milk.

The qualitative assessment of milk fat was made on the basis of the following indicators: lipid preventive score, atherogenic and thrombogenic index and the ratio between hyper- and hypocholesterolemic fatty acids (Table 4).

Lipid preventive score (LPS) calculated by equation, is used to assess the preventive effect of a fat on the risk of cardiovascular disease. In the studied samples with the highest LPS is kefir from BWDxTG with 9.89 g/100 g fat, and the lowest in BWD breed with 8.12 g/100 g fat, and the results are statistically significant, which means that the product has a well-balanced fatty acid composition.

The atherogenic index giving the relationship between the sum of basic saturated and unsaturated fatty acids and the thrombogenic index expressing the ratio between saturated fatty acids and monounsaturated and polyunsaturated omega-3 and omega-6 fatty acids (Ghaeni et al., 2013) are the highest in kefir from the milk of BWDxAN - 4.52; 3.27, and the hypocholesterolemic index in kefir from BWD - 0.56.

Vieira et al. (2015) indicate for kefir from cow's milk on the 14th day of storage values for AI, TI and h/H of 1.94; 2.98; 0.82.

Table 4. Kefir indices on the 14th day of storage, g/100 g product

| Indices | BWD | BWDxTG | BWDxAN |
|---|---|---|---|
| | x±Sx | x±Sx | x±Sx |
| **LPS** | 8.12±1.588 | 9.89±0.643a* | 9.20±0.632 |
| **AI** | 3.11±0.244 | 4.30±0.840 | 4.52±0.891b* |
| **TI** | 2.16±0.363 | 2.14±0.550 | 3.27±1.163 |
| **h/H** | 0.56±1.476 | 0.42±1.886 | 0.43±1.746 |
| **Trans fatty acids** | 0.12±0.593 | 0.14±0.810 | 0.17±0.847 |
| **SFAs+TFAs** | 3.0±0.502 | 3.30±.555 | 3.59±0.482 |

Note: a: BWD/BWDxTG; b:BWD/BWDxAN *P≤0.05

Trans fatty acids (TFAs), naturally obtained and important in human nutrition, varied in individual kefir from 0.12 g/100 g fat in BWD to 0.17 g/100 g fat in BWDxAN, which gives us reason to refer to products with a low TFAs content according to Regulation (EC) No 1924/2006.

## CONCLUSIONS

The highest content of protein, fat and dry matter in kefir on the 3rd and 14th day of storage was found in milk from BWDxAN breed (5.42%, 5.34%; 4.77%, 4.66% and 15.84%, 15.74%), and the lowest in kefir from the milk of BWD breed (5.07%, 4.99%; 4.14%, 4.05%; 14.92%, 14.15%).

Water content and active acidity decreased, and titratable acidity increased from the 3rd to the 14th day of storage.

The results for protein, fat and dry matter decreased in all three batches of kefir on the 14th day of storage compared to the 3rd day.

Kefir from the milk of BWDxAN had the highest content of saturated fatty acids, while MUFAs and PUFAs predominated in kefir from purebred goats.

The content of saturated fatty acids in the studied kefir from goat's milk was from 3.0 g/100 g fat to 3.59 g/100 g fat. Therefore, it is defined as products with high content of saturated fatty acids (over 1.5 g/100 g product) and low content of trans fatty acids (0.12-0.17

g/100 g product) according to Regulation (EC) No 1924/2006.

LPS values show a well-balanced fatty acid composition of kefir from the milk of the three breed groups, which defines it as a product with pronounced health effects.

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# STUDY ON CONSUMER CONFIDENCE IN THE FOOD PRODUCTS LABELLING SYSTEM

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***Abstract***

*The research revealed that when consumers read this information, they are most interested in the list of ingredients, substances causing allergies or intolerance, the date of minimum durability or use-by date and the net quantity on the food. In addition to the descriptive analysis that revealed how these factors influence consumer confidence in the information read on labels, the results of the study showed gender differences in the importance of reading label information. The frequency of reading label information is influenced by people who engage in daily physical activity. Regarding the frequency of reading label information, the χ2 test revealed significant differences between food poisoners and healthy people. The results of this study may be useful to companies and institutions dealing with food safety. They, through specially designed programmes (as exist in other countries for the uninformed, in our case discussing men, the elderly and those with no formal education), can improve the frequency of reading and confidence in label information.*

***Key words****: labels, food products, consumer information, frequency of reading information.*

## INTRODUCTION

Recently, both producers and consumers have been increasingly interested in the features on labels. Consumers are equally interested in: food safety (Caswell & Joseph, 2008); technological processes underlying the production of food (Harper et al., 2007); nutrition declaration on labels; nutritional components provided by the food consumed (Gracia & Magistris, 2016); a healthy lifestyle (World Health Organizations, 2003); rational consumption of food to avoid food waste (Stenmarck et al. 2016; Marin et al., 2019).
Producers are interested in presenting, in a competitive way, the quality characteristics of food products (Gracia & Magistris, 2016) to convince consumers to frequently purchase products from a particular brand (Liljander et al., 2009).
Due to food safety issues reported in recent decades, it has been necessary to develop legislation at European level to be implemented in all EU countries.
Thus, the European Parliament and Council approved Regulation 1169/2011 where clarifications are made with reference to the information to consumers about the characteristics of food products (Euro Commerce and Food Drink Europe, 2013). In Romania, ANPC has developed a legislative guide for consumer protection, which details legislative aspects of food labelling and marketing (ANPC, 2015).

## MATERIALS AND METHODS

### Materials

For this study we interviewed 151 people, 104 female and 47 male, from both urban (98 people) and rural (53 people) areas. In order to identify whether the objectives pursued in the study varied by age, one of the items targeted this characteristic.
The people participating in this study fall into the following categories: 20-30 years (129 people), 31-40 years (11 people), 41-50 (3 people), 51-60 (7 people) over 60 (2 people). Educational level is also a factor influencing the objectives of this study.
For this item we had 4 response variants, namely: high school level (40 persons), professional level (6 persons), university studies (97 persons), post-graduate studies (8 persons).

**Methods**

To carry out this study, we designed a questionnaire with 18 items. The questionnaire was structured in 2 sections: a. socio-demographic data (4 items); b. trust in the information on the labels.

**Data processing methodology**

Interpretation of the questionnaire was done through numerical (graphs) and summative presentation of the data. The test of differences significance was carried out using the Pearson chi test (test of association).

**RESULTS AND DISCUSSIONS**

The frequency of reading the information (Table 1) on the label is influenced by young, urban people and the number of years spent in university education.

It seems that when consumers read this information, they are most interested in the list of ingredients, substances causing allergies or intolerance, the date of minimum durability or use-by date and the net quantity of the food.

Another aspect of the study was to identify the extent to which there is confidence in the information provided by producers on food labels (Table 2).

The majority of respondents (43.05%) have a neutral attitude towards the truthfulness of the information on labels. Only 11.26% were in total agreement with this item. Out of the total number of people who do physical activity on a daily basis (42), about a quarter agreed that the information on the labels is consistent with the characteristics of the product.

People who have been diagnosed with food poisoning and who read label information more frequently are more confident about the truthfulness of the information on the label (52.55%).

Lately, people have become more health-conscious, which is why they are more careful about the food they buy. The survey found that only 6.62% of respondents never read the ingredient list and 23.81% always read the ingredient list on the label. In terms of age category, of those who read the ingredient list very often, 88.74% are in the 20-30 age group.

Table 1. Frequency of reading label information by individual characteristics

| Specification | | Never | | Rarely | | Sometimes | | Very often | | Ever | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | N=9 | 5.96% | N=24 | 15.89% | N=61 | 40.40% | N=30 | 19.87% | N=27 | 17.88% |
| Gender | Female | 5 | 55.56 | 14 | 58.33 | 42 | 68.86 | 23 | 76.67 | 20 | 74.07 |
| | Male | 4 | 44.44 | 10 | 41.67 | 19 | 31.14 | 7 | 23.33 | 7 | 25.93 |
| Domicile | Urban | 7 | 77.78 | 14 | 58.33 | 44 | 72.13 | 18 | 60 | 15 | 55.56 |
| | Rural | 2 | 22.22 | 10 | 41.67 | 17 | 27.87 | 12 | 40 | 12 | 44.44 |
| Level of education | High school studies | 2 | 22.22 | 6 | 25 | 15 | 24.60 | 10 | 33.33 | 7 | 25.93 |
| | Professional studies | 1 | 11.11 | 1 | 4.17 | 1 | 1.63 | 0 | 0 | 3 | 11.11 |
| | University studies | 6 | 66.67 | 17 | 70.83 | 43 | 70.49 | 19 | 63.34 | 12 | 44.44 |
| | Postgraduate studies | 0 | 0 | 0 | 0 | 2 | 3.28 | 1 | 3.33 | 5 | 18.52 |
| Age | 20-30 | 9 | 100 | 22 | 91.67 | 55 | 90.16 | 26 | 86.66 | 17 | 62.97 |
| | 31-40 | 0 | 0 | 2 | 8.33 | 4 | 6.56 | 2 | 6.67 | 3 | 11.11 |
| | 41-50 | 0 | 0 | 0 | 0 | 1 | 1.64 | 0 | 0 | 2 | 7.41 |
| | 51-60 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 6.67 | 4 | 14.81 |
| | over 60 | 0 | 0 | 0 | 0 | 1 | 1.64 | 0 | 0 | 1 | 3.70 |
| Smoking frequency | Yes | 5 | 55.56 | 11 | 45.83 | 17 | 27.87 | 9 | 30 | 9 | 33.33 |
| | Occasionally | 0 | 0 | 1 | 4.17 | 9 | 14.75 | 3 | 10 | 5 | 18.52 |
| | No | 4 | 44.44 | 12 | 50 | 35 | 57.38 | 18 | 60 | 13 | 48.15 |
| Body weight | Underweight | 0 | 0 | 2 | 8.30 | 3 | 4.92 | 1 | 3.33 | 1 | 3.70 |
| | Normal | 8 | 88.89 | 18 | 75.00 | 53 | 86.89 | 26 | 86.67 | 21 | 77.78 |
| | Overweight | 1 | 11.11 | 4 | 16.70 | 5 | 8.19 | 3 | 10 | 5 | 18.52 |
| Frequency of physical activity | Daily | 2 | 22.22 | 9 | 37.60 | 16 | 26.23 | 6 | 20 | 9 | 33.33 |
| | Weekly | 2 | 22.22 | 5 | 20.83 | 13 | 21.31 | 9 | 30 | 5 | 18.52 |
| | Occasionally | 2 | 22.22 | 7 | 29.17 | 25 | 40.98 | 13 | 43.33 | 10 | 37.04 |
| | Monthly | 0 | 0 | 3 | 12.50 | 3 | 4.92 | 2 | 6.67 | 2 | 7.41 |
| | Never | 3 | 33.34 | 0 | 0 | 4 | 6.56 | 0 | 0 | 1 | 3.70 |
| Food poisoning | Yes | 5 | 55.56 | 7 | 29.17 | 17 | 27.87 | 10 | 33.33 | 16 | 59.26 |
| | No | 4 | 44.44 | 17 | 70.83 | 44 | 72.13 | 20 | 66.67 | 11 | 40.74 |

Table 2. Dynamics of truthfulness of information on labels in relation to respondent characteristics

| Specification | | Total disagreement | | Disagreement | | Neutral | | Agreement | | Total agreement | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | N=1 | 0.66% | N=9 | 5.96% | N=65 | 43.05% | N=59 | 39.07% | N=17 | 11.26% |
| Gender | Female | 1 | 100 | 7 | 77.78 | 43 | 66.16 | 41 | 69.50 | 12 | 70.59 |
| | Male | 0 | 0 | 2 | 22.22 | 22 | 33.84 | 18 | 30.50 | 5 | 29.41 |
| Domicile | Urban | 1 | 100 | 8 | 88.89 | 40 | 61.53 | 36 | 61.01 | 13 | 76.47 |
| | Rural | 0 | 0 | 1 | 11.11 | 25 | 38.47 | 23 | 38.99 | 4 | 23.53 |
| Level of education | Secondary school | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | High school studies | 0 | 0 | 1 | 11.11 | 23 | 35.38 | 14 | 23.73 | 3 | 17.65 |
| | Professional studies | 0 | 0 | 0 | 0 | 3 | 4.62 | 2 | 3.39 | 1 | 5.88 |
| | University studies | 1 | 100 | 7 | 77.78 | 37 | 56.92 | 38 | 64.40 | 13 | 76.47 |
| | Postgraduate studies | 0 | 0 | 1 | 11.11 | 2 | 3.08 | 5 | 8.47 | 0 | 0 |
| Individual monthly income (RON) | Less than 1500 | 1 | 100 | 3 | 33.33 | 32 | 49.23 | 26 | 44.07 | 7 | 41.18 |
| | 1501-3000 | 0 | 0 | 2 | 22.22 | 15 | 23.08 | 15 | 25.43 | 4 | 23.53 |
| | 3001-5000 | 0 | 0 | 4 | 44.45 | 7 | 10.76 | 8 | 13.56 | 0 | 0 |
| | 50001-8000 | 0 | 0 | 0 | 0 | 2 | 3.08 | 4 | 6.78 | 4 | 23.53 |
| | 8000-10000 | 0 | 0 | 0 | 0 | 1 | 1.54 | 1 | 1.69 | 0 | 0 |
| | More than 10000 | 0 | 0 | 0 | 0 | 2 | 3.08 | 1 | 1.69 | 1 | 5.88 |
| | other | 0 | 0 | 0 | 0 | 6 | 9.23 | 4 | 6.78 | 1 | 5.88 |
| Age | 20-30 | 1 | 100 | 7 | 77.78 | 57 | 87.69 | 49 | 83.06 | 15 | 88.24 |
| | 31-40 | 0 | 0 | 2 | 22.22 | 4 | 6.15 | 3 | 5.08 | 2 | 11.76 |
| | 41-50 | 0 | 0 | 0 | 0 | 1 | 1.54 | 2 | 3.39 | 0 | 0 |
| | 51-60 | 0 | 0 | 0 | 0 | 2 | 3.08 | 4 | 6.78 | 0 | 0 |
| | over 60 | 0 | 0 | 0 | 0 | 1 | 1.54 | 1 | 1.69 | 0 | 0 |
| Smoking frequency | Yes | 0 | 0 | 4 | 44.45 | 24 | 36.92 | 18 | 30.51 | 5 | 29.41 |
| | Occasionally | 0 | 0 | 5 | 55.55 | 8 | 12.31 | 2 | 3.39 | 3 | 17.65 |
| | No | 1 | 100 | 0 | 0 | 33 | 50.77 | 39 | 66.10 | 9 | 52.94 |
| Body weight | Underweight | 0 | 0 | 0 | 0 | 3 | 4.62 | 3 | 5.08 | 1 | 5.88 |
| | Normal | 1 | 100 | 8 | 88.89 | 53 | 81.54 | 49 | 83.05 | 15 | 88.24 |
| | Overweight | 0 | 0 | 1 | 11.11 | 9 | 13.84 | 7 | 11.87 | 1 | 5.88 |
| Frequency of physical activity | Daily | 0 | 0 | 3 | 33.33 | 19 | 29.23 | 14 | 23.72 | 6 | 35.29 |
| | Weekly | 1 | 100 | 5 | 55.56 | 12 | 18.46 | 12 | 20.38 | 4 | 23.53 |
| | Occasionally | 0 | 0 | 1 | 11.11 | 22 | 33.85 | 28 | 47.45 | 6 | 35.30 |
| | Monthly | 0 | 0 | 0 | 0 | 8 | 12.31 | 2 | 3.38 | 0 | 0 |
| | Never | 0 | 0 | 0 | 0 | 4 | 6.15 | 3 | 5.07 | 1 | 5.88 |
| Food poisoning | Yes | 0 | 0 | 2 | 22.22 | 15 | 23.08 | 31 | 52.55 | 7 | 41.18 |
| | No | 1 | 100 | 7 | 77.78 | 50 | 76.92 | 28 | 47.45 | 10 | 58.82 |

Even if they are not interested in doing frequent physical activities for health maintenance, people are careful about what they eat. For example, 43.59% of those who do physical activity occasionally chose to read the ingredient list very often. Of those who experienced food poisoning, 62.86% said they always read the ingredient list (table 3).

Significance testing for the variable: frequency of reading label information.

We used the chi-square test of association to test the relationship between the two variables, both measured on the categorical scale.

Before moving on to the actual test, it was useful to create a framework to synthetically represent the values of the two variables. What is called a correspondence table is obtained. This is a correspondence table for two nominal variables, one of which is represented by the gender/food poisoning and the other by the levels of the measurement scale (A - Never; B - Very rarely; C - Sometimes; D - Very often; E - Always).

Determination of statistical decision criteria in any of the variants addressed:

- Choose $\alpha=0.05$ (P=95%), for significant differences.
- GL=degree of freedom = (2-1)*(5-1) = 4
- the tabular $\chi 2$ value for these conditions is 9.49.

Table 3. Reading frequency of ingredients

| Specification | | Never | | Rarely | | Sometimes | | Very often | | Ever | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | N=10 | 6.62% | N=20 | 13.25% | N=47 | 31.13% | N=39 | 25.82% | N=35 | 23.18% |
| Gender | Female | 9 | 90 | 8 | 40 | 31 | 65.96 | 32 | 82.05 | 24 | 68.57 |
| | Male | 1 | 10 | 12 | 60 | 16 | 34.04 | 7 | 17.95 | 11 | 31.43 |
| Domicile | Urban | 9 | 90 | 11 | 55 | 30 | 63.83 | 28 | 71.80 | 20 | 57.14 |
| | Rural | 1 | 10 | 9 | 45 | 17 | 36.17 | 11 | 28.20 | 15 | 42.86 |
| Level of education | Secondary school | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | High school studies | 1 | 10 | 5 | 25 | 12 | 25.53 | 11 | 28.21 | 11 | 31.43 |
| | Professional studies | 0 | 0 | 1 | 5 | 2 | 4.26 | 0 | 0 | 3 | 8.57 |
| | University studies | 9 | 90 | 14 | 70 | 32 | 68.08 | 27 | 69.23 | 15 | 42.86 |
| | Postgraduate studies | 0 | 0 | 0 | 0 | 1 | 2.13 | 1 | 2.56 | 6 | 17.14 |
| Individual monthly income (RON) | Less than 1500 | 9 | 90 | 6 | 30 | 19 | 40.43 | 24 | 61.54 | 11 | 31.44 |
| | 1501-3000 | 1 | 10 | 6 | 30 | 11 | 23.40 | 9 | 23.08 | 9 | 25.72 |
| | 3001-5000 | 0 | 0 | 5 | 25 | 8 | 17.02 | 4 | 10.26 | 2 | 5.71 |
| | 50001-8000 | 0 | 0 | 0 | 0 | 3 | 6.38 | 0 | 0 | 7 | 20 |
| | 8000-10000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 5.71 |
| | More than 10000 | 0 | 0 | 1 | 5 | 1 | 2.13 | 0 | 0 | 2 | 5.71 |
| | other | 0 | 0 | 2 | 10 | 5 | 10.64 | 2 | 5.12 | 2 | 5.71 |
| Age | 20-30 | 10 | 100 | 19 | 95 | 40 | 85.11 | 35 | 88.74 | 25 | 71.43 |
| | 31-40 | 0 | 0 | 1 | 5 | 4 | 8.51 | 3 | 7.69 | 3 | 8.57 |
| | 41-50 | 0 | 0 | 0 | 0 | 2 | 4.25 | 0 | 0 | 1 | 2.86 |
| | 51-60 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2.56 | 5 | 14.28 |
| | over 60 | 0 | 0 | 0 | 0 | 1 | 2.13 | 0 | 0 | 1 | 2.86 |
| Smoking frequency | Yes | 5 | 50 | 8 | 40 | 13 | 27.66 | 11 | 28.21 | 14 | 40 |
| | Occasionally | 0 | 0 | 4 | 20 | 6 | 12.77 | 3 | 7.69 | 5 | 14.29 |
| | No | 5 | 50 | 8 | 40 | 28 | 59.57 | 25 | 64.10 | 16 | 45.71 |
| Body weight | Underweight | 0 | 0 | 3 | 15 | 2 | 4.25 | 1 | 2.56 | 1 | 2.86 |
| | Normal | 7 | 70 | 16 | 80 | 43 | 91.50 | 32 | 82.05 | 28 | 80 |
| | Overweight | 3 | 30 | 1 | 5 | 2 | 4.25 | 6 | 15.38 | 6 | 17.14 |
| Frequency of physical activity | Daily | 1 | 10 | 9 | 45 | 15 | 31.91 | 9 | 23.07 | 8 | 22.86 |
| | Weekly | 3 | 30 | 3 | 15 | 8 | 17.02 | 9 | 23.08 | 11 | 31.43 |
| | Occasionally | 5 | 50 | 6 | 30 | 17 | 36.17 | 17 | 43.59 | 12 | 34.29 |
| | Monthly | 0 | 0 | 2 | 10 | 3 | 6.38 | 3 | 7.69 | 2 | 5.71 |
| | Never | 1 | 10 | 0 | 0 | 4 | 8.51 | 1 | 2.56 | 2 | 5.71 |
| Food poisoning | Yes | 4 | 40 | 5 | 25 | 13 | 27.66 | 11 | 28.21 | 22 | 62.86 |
| | No | 6 | 60 | 15 | 75 | 34 | 72.34 | 28 | 71.79 | 13 | 37.14 |

**a. Research problem**: We want to find out if there are significant differences between female and male individuals in the frequency of reading label information.

The values in the cells represent the number of cases (observed frequencies) that correspond to combinations of the two variables (Table 4.a). The column totals express the number of individuals who chose a particular level of the scale, regardless of gender, and the row totals represent the number of individuals correspond-ding to each gender. At the intersection of the two totals we find the overall total of the research subjects (N = 151)

*Research Hypothesis (HA):* The frequency distribution of reading label information depends on whether the gender is "female" or "male".

*Null hypothesis ($H_0$):* The choice of levels A, B, C, D, E is unrelated to the variable gender.

The calculated $\chi 2$ value is smaller than the tabular $\chi 2$, in which case the null hypothesis is accepted, i.e. the choice of scale levels in the item is not influenced by the gender variable in terms of the frequency of reading food label information (Table 4.b).

Table 4.a. Correspondence table for gender characteristic

| Specification | Never | Rarely | Sometimes | Very often | Ever | Total columns |
|---|---|---|---|---|---|---|
| **Gender** | | | | | | |
| Female | 5 | 14 | 42 | 23 | 20 | **104** |
| Male | 4 | 10 | 19 | 7 | 7 | **47** |
| **Total lines** | **9** | **24** | **61** | **30** | **27** | **151** |

Table 4.b Testing the significance of differences between the two sexes in the frequency of reading labels

| Gender | Observed frequency $f_O$ | Expected frequency $f_A$ | $f_O - f_A$ | $(f_O - f_A)^2$ | $\frac{(f_O - f_A)^2}{f_A}$ |
|---|---|---|---|---|---|
| Female A* | 5 | 6,1983 | -1,1983 | 1,435923 | 0,231663987 |
| Female B* | 14 | 16,5288 | -2,5288 | 6,394829 | 0,386890121 |
| Female C* | 42 | 42,0107 | -0,0107 | 0,000114 | 0,000002713 |
| Female D* | 23 | 20,661 | 2,339 | 5,470921 | 0,264794589 |
| Female E* | 20 | 18,5949 | 1,4051 | 1,974306 | 0,106174597 |
| Male A* | 4 | 2,8017 | 1,1983 | 1,435923 | 0,512518432 |
| Male B* | 10 | 7,4712 | 2,5288 | 6,394829 | 0,855930699 |
| Male C* | 19 | 18,9893 | 0,0107 | 0,000114 | 0,00000600 |
| Male D* | 7 | 9,339 | -2,339 | 5,470921 | 0,585814434 |
| Male E* | 7 | 8,4051 | -1,4051 | 1,974306 | 0,234893816 |
| **Total columns** | **151** | **151** | **0** | | **3,178689429** |

*A - Never; B - Very rarely; C - Sometimes; D - Very often; E - Always;

**b. Research problem:** To test whether there are significant differences between people who have experienced food poisoning and those who have not, given the levels of the measurement scale (A, B, C, D, E) in terms of frequency of reading label information (Table 5.a.).

At the intersection of the two totals in the correspondence table, we find the overall total of research subjects (N = 151). The column totals express the number of individuals who chose a certain level of the scale, regardless of whether they had food poisoning or not, and the row totals represent the number of individuals corresponding to the two response options: 'yes' or 'no'.

*Research hypothesis ($H_A$):* The frequency distribution of reading label information depends on the two categories of people.

*Null hypothesis ($H_0$):* The choice of levels A, B, C, D, E is not related to the variable food poisoning.

In this case the calculated $\chi 2$ value is higher than the tabulated one, in which case we reject the null hypothesis and accept the research hypothesis (Table 5.b). This highlights that people who have suffered from food poisoning are more interested in the information provided by producers on food labels.

Table 5.a. Correlation table for the characteristic food poisoning

| Specification | Never | Rarely | Sometimes | Very often | Ever | Total columns |
|---|---|---|---|---|---|---|
| **Food poisoning** | | | | | | |
| Yes | 5 | 7 | 17 | 10 | 16 | **55** |
| No | 4 | 17 | 44 | 20 | 11 | **96** |
| **Total lines** | **9** | **24** | **61** | **30** | **27** | **151** |

Table 5.b. Testing the significance of differences between the two individuals in the frequency of reading labels

| Food poisoning | Observed frequency $f_O$ | Expected frequency $f_A$ | $f_O - f_A$ | $(f_O - f_A)^2$ | $\frac{(f_O - f_A)^2}{f_A}$ |
|---|---|---|---|---|---|
| Yes A* | 5 | 3,2778 | 1,7222 | 2,965973 | 0,904866935 |
| Yes B* | 7 | 8,7408 | -1,7408 | 3,030385 | 0,346694197 |
| Yes C* | 17 | 22,2162 | -5,2162 | 27,20874 | 1,22472531 |
| Yes D* | 10 | 10,926 | -0,926 | 0,857476 | 0,078480322 |
| Yes E* | 16 | 9,8334 | 6,1666 | 38,02696 | 3,867121805 |
| No A* | 4 | 5,7222 | -1,7222 | 2,965973 | 0,518327364 |
| No B* | 17 | 15,2592 | 1,7408 | 3,030385 | 0,198593939 |
| N0 C* | 44 | 38,7838 | 5,2162 | 27,20874 | 0,701549163 |
| No D* | 20 | 19,074 | 0,926 | 0,857476 | 0,044955227 |
| No E* | 11 | 17,1666 | -6,1666 | 38,02696 | 2,215171062 |
| **Total lines** | **151** | **151** | **0** | | **10,10048533** |

*A - Never; B - Very rarely; C - Sometimes; D - Very often; E - Always

## CONCLUSIONS

The frequency of reading label information is influenced by young, urban people and the number of years spent in university education. Frequency of reading label information is influenced by people who are physically active on a daily basis.

Most people in this category are female, and people who are occasionally physically active are not so interested in reading the information on the label.

It seems that when consumers do read this information, they are most interested in the list of ingredients, substances causing allergies or intolerance, the date of minimum durability or the use-by date.

Females attach more importance to the information that is required by law to be included on the label than males.

Half of the respondents consider that products marketed in Romania are of poorer quality than those marketed in other EU countries.

We thus observe that a decrease in confidence in the information on the label can lead (according to the secondary data sources mentioned in the paper) to a decrease in the frequency of reading the information on the label and, ultimately, can negatively influence consumer health.

An important element in informing consumers is the perception of beneficial food innovations. This study shows that people aged between 31 and 60 are the least confident about the benefits of technological innovations.

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# RESEARCH ON THE SAFETY AND RESILIENCE OF THE MEAT AND MEAT PRODUCTS SECTOR IN ROMANIA

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***Abstract***

*The FAO defines food security as "the direct access of all people to the food they need to fulfill their vital functions and to lead a healthy and active life". In order to ensure the security and resilience of the meat and meat products sector in Romania, in the current geopolitical context, this sector must contribute substantially to providing food for the internal population, for refugees and for those who cross the country. Through resilience, Romania aims, through the meat and meat products sector, to prepare for what it means to think, plan and exercise in order to absorb, return and then adapt existing productive capacities to adverse and disruptive events. The aim of the paper is to analyze the security and organizational resilience of the meat and meat products sector in Romania.*

***Key words:*** *authorized production capacity, food security, livestock, resilience.*

## INTRODUCTION

Food security and resilience are closely linked to the emergence of a new world, dominated by risks and threats. In the face of various risks: natural disasters, pandemics, zoonoses, damaged infrastructure, war, etc., leading factors recognize that risks and threats cannot be avoided, so food security can never be fully ensured.

As a result, increasing the resilience of the meat and meat products sector requires measures to prevent, deter and protect humans and animals from these threats, through thinking, planning and exercise, to absorb, return and then adapt their sources and resources for adverse and disruptive events.

The purpose of this paper is to show whether the meat and meat products sector in Romania can ensure food security and resilience for the internal population, for refugees and for those who cross the country.

## MATERIALS AND METHODS

The research was conducted based on data taken from the *National Agency for Veterinary Sanitary and Food Safety (ANSVSA)*, the *County Agricultural Directorates (DAJ)* and data from the *National Institute of Statistics (INS)* for the period 2020-2021.

The data taken from the DAJ provided information on the number of animals destined for the meat sector (number of heads of animals / year and estimated meat production provided by them tons / year) on each county, economic development regions and at national level.

The data from ANSVSA were processed regarding the authorized slaughtering and processing capacities per animal / year and tons of meat / year, on each county, economic development regions and at national level.

With the help of data published by the NIS, the proportion and capacity of farms to ensure the need for meat production by raising animals and the capacity of authorized economic operators in the animal slaughtering and meat processing sector to ensure the need for meat and meat products on head of population, county, economic development regions and at national level. The data obtained from the statistical processing were then compared with similar statistical data published at Community level.

## RESULTS AND DISCUSSIONS

From the data contained in *Table 1*, an uneven distribution of the number of animals raised in the development regions can be observed. The highest number of cattle recorded is found in the northern region of Romania in both the eastern part (over 155 thousand heads) and the western part (over 135 thousand heads), followed by the central region (about 99 thousand heads), the southern region (about 79 thousand heads), the other regions having a number of animals under 50 thousand heads (DAJ).

Table 1. Number of animals for meat production by geographical region and at national level for the period 2020-2021 (heads / year)

| Specification | N-E | S-E | S | Bucharest Ilfov | S-V | V | Center | N-V | TOTAL |
|---|---|---|---|---|---|---|---|---|---|
| Cattle | 155,812 | 47,959 | 78,640 | 490 | 25,426 | 48,184 | 98,503 | 135,181 | **590,198** |
| Pigs | 628,763 | 384,408 | 725,284 | 4,739 | 260,788 | 1,749,486 | 427,653 | 793,052 | **4,974,173** |
| Sheep | 805,284 | 1,486,158 | 972,005 | 25,404 | 452,207 | 1,268,961 | 1,187,405 | 1,492,649 | **7,690,073** |
| Goats | 141,192 | 212,306 | 207,026 | 8,510 | 297,374 | 54,703 | 85,987 | 100,861 | **1,107,959** |

At the national level, a number of 4,974 thousand pig heads are increased, the highest number being registered in the Western region (1,749,486 heads) followed by the North-West region (793,052 heads), the Southern region (725,284). heads) and the Northeast region (628,763 heads).

The number of sheep and goats registered at national level was 8,798,032 heads, except for the Bucharest Ilfov regions and the South-West region, all other regions approaching or exceeding the figure of 1 million heads exploited.

Making an overall analysis we can see that animal species are grown in specific areas, with tradition and in which natural and economic conditions help them in the efficient use of food resources.

Thus, beef cattle are raised predominantly in hilly and mountainous areas where natural pasture predominates in their food. Pigs are maintained in cereal areas, and sheep and goats are spread, quite evenly, throughout Romania, they efficiently capitalize on forage resources inaccessible to other species.

Table 2. Meat production estimated on the basis of the number of animals slaughtered in the direction of meat production for the period 2020 – 2021 (tonnes / year)

| Specification | N-E | S-E | S | Bucharest Ilfov | S-V | V | Center | N-V | TOTAL |
|---|---|---|---|---|---|---|---|---|---|
| Cattle | 24929.92 | 7673.44 | 12582.88 | 78.4 | 4068.16 | 7709.44 | 15760.48 | 21628.96 | **94431.68** |
| Pigs | 34896.35 | 21334.64 | 40253.26 | 263.01 | 14473.73 | 97096.47 | 23734.74 | 44014.39 | **276066.6** |
| Sheep | 10871.33 | 20063.13 | 13122.07 | 342.95 | 6104.79 | 17130.97 | 16029.97 | 20150.76 | **103815.99** |
| Goats | 1764.9 | 2653.83 | 2587.83 | 106.38 | 3717.18 | 683.79 | 1074.84 | 1260.76 | **13849.49** |
| **Total** | **72462.5** | **51725.04** | **68546.03** | **790.74** | **28363.86** | **122620.67** | **56600.03** | **87054.87** | **488163.75** |

Meat production estimated on the basis of the number of animals farmed in the direction of meat production is shown in Table 2.

It is directly correlated with the number of animals presented in Table 1 and is unevenly distributed at national level.

From the study carried out on the statistically processed data on authorized slaughtering capacities by regions and at national level we can say that all regions of economic development have an authorized slaughtering capacity over the number of animals raised and exploited in the direction of meat production except Bucharest-Ilfov economic development region, where only pig slaughterhouses are authorized (ANSVSA).

Table 3. Authorized capacity of slaughterhouses (no animals / year)

| Specification | N-E | S-E | S | Bucharest-Ilfov | S-V | V | Center | N-V | TOTAL |
|---|---|---|---|---|---|---|---|---|---|
| Cattle | 449462 | 92061 | 250920 | 0 | 86359 | 48580 | 110562 | 194431 | **1232375** |
| Pigs | 996596 | 900658 | 1801680 | 98900 | 307969 | 2201940 | 871622 | 974575 | **8153940** |
| Sheep | 735244 | 764600 | 1331595 | 0 | 136410 | 609600 | 617253 | 558430 | **4753132** |
| Goats | 582860 | 405200 | 982120 | 0 | 84970 | 90000 | 525200 | 403673 | **3074023** |
| Solipeds | 154320 | 18500 | 50260 | 0 | 7680 | 26000 | 248000 | 34940 | **539700** |

For sheep and goat species, the authorized slaughter capacity is reduced by half compared to the number of animals raised and exploited for meat production, except for the economic development region Bucharest-Ilfov where these units are missing (ANSVSA).

Table 4. Authorized slaughterhouse capacity (tons of meat / year)

| Specification | N-E | S-E | S | Bucharest-Ilfov | S-V | V | Center | N-V | TOTAL |
|---|---|---|---|---|---|---|---|---|---|
| Cattle | 71914 | 14730 | 40147 | 0 | 13817 | 7773 | 17690 | 31109 | **197180** |
| Pigs | 159455 | 144105 | 288269 | 15824 | 49275 | 352310 | 139460 | 155932 | **1304630** |
| Sheep | 117639 | 122336 | 213055 | 0 | 21826 | 97536 | 98760 | 89349 | **760501** |
| Goats | 93258 | 64832 | 157139 | 0 | 13595 | 14400 | 84032 | 64588 | **491844** |
| Solipeds | 24691 | 2960 | 8042 | 0 | 1229 | 4160 | 39680 | 5590 | **86352** |

The authorized slaughtering capacity expressed in tonnes of meat / year corresponds as a characterization to the data presented above in terms of slaughterhouse authorization expressed in number of head of animals / year (ANSVSA) (Table 4).

Table 5. The estimated amount of meat resulted, according to the capitalization of production animals (kg / inhabitant / year)

| Specification | N-E | S-E | S | Bucharest-Ilfov | S-V | V | Center | N-V | TOTAL |
|---|---|---|---|---|---|---|---|---|---|
| Cattle | 7.55 | 3.01 | 4.01 | 0.03 | 1.96 | 4.22 | 6.68 | 8.32 | **4.69** |
| Pigs | 10.57 | 8.38 | 12.83 | 0.12 | 6.97 | 53.11 | 10.05 | 16.93 | **13.72** |
| Sheep | 3.29 | 7.88 | 4.18 | 0.15 | 2.94 | 9.37 | 6.79 | 7.75 | **5.16** |
| Goats | 0.53 | 1.04 | 0.83 | 0.05 | 1.79 | 0.37 | 0.46 | 0.48 | **0.69** |

From the data presented in Table 5 it can be seen that the species that contributes the most meat in the diet of the population based on the capitalization of animal production is the pig species, followed by cattle, sheep and goats.

The pig species by geographical regions, the largest amount of meat provided per capita is the West region, with 53.11 kg, followed by the North-West region with 16.93 kg, the South region with 12.83 kg, the North-West region. East, with 10.57 kg, the Center region with 10.05 kg, the rest of the regions contributing with values recorded below 10 kg, at national level the pigs provide 13.72 kg of meat per inhabitant.

Sheep and goat species contribute less than 10 kg of meat per capita in all development regions. The regions that provide the largest amount of meat are: the West region with 9.74 kg / inhabitant, the South-East region with 8.92 kg / inhabitant, the North-West Region with 8.23 kg / inhabitant and the Center region with 7.25 kg / inhabitant. The other regions contribute with values below approx. 5 kg / inhabitant.

The goat species contributes less than 2 kg of meat per inhabitant. At the national level, the two sheep and goat species contribute a contribution of 5.84 kg of meat per inhabitant.

Table 6. Estimated quantity of meat, including bird and soliped species, based on authorized slaughter capacity (kg / inhabitant / year)

| Specification | N-E | S-E | S | Bucharest-Ilfov | S-V | V | Center | N-V | TOTAL |
|---|---|---|---|---|---|---|---|---|---|
| Cattle | 21.78 | 5.79 | 12.80 | 0.00 | 6.66 | 4.25 | 7.49 | 11.96 | **9.80** |
| Pigs | 16.75 | 19.63 | 31.88 | 2.42 | 8.23 | 66.84 | 20.49 | 20.80 | **22.49** |
| Sheep | 3.01 | 4.05 | 5.73 | 0.00 | 0.89 | 4.50 | 3.53 | 2.90 | **3.19** |
| Goats | 2.21 | 1.99 | 3.91 | 0.00 | 0.51 | 0.62 | 2.78 | 1.94 | **1.91** |
| Solipeds | 7.48 | 1.16 | 2.56 | 0.00 | 0.59 | 2.28 | 16.81 | 2.15 | **4.29** |
| Birds | 45.62 | 59.93 | 77.18 | 0.00 | 22.14 | 20.62 | 47.25 | 28.80 | **40.52** |
| **Total** | **96.84** | **92.56** | **134.07** | **2.42** | **39.03** | **99.11** | **98.35** | **68.56** | **82.20** |

The bovine species, in 2021, provided at national level 4.69 kg of meat per capita, the regions with the sector more oriented towards the direction of meat exploitation are found in the northern, central and western areas (the North-West region provides 8.32 kg of meat per capita, the North-West region contributes 7.55 kg of meat / inhabitant, the Central region with 6.68 kg of meat / inhabitant and the West region with 4.22 kg / inhabitant). The rest of the regions contribute with quantities below the threshold of 4.00 kg / inhabitant.

Table 6 shows the estimated values for meat quantities, including bird and soliped species, resulting from the authorized slaughter capacities in kg / inhabitant.

With the help of the data taken from the INS, regarding the number of inhabitants per counties (2011), the capacity to ensure the quantity of meat was established according to the designed, authorized capacity of the economic agents.

From the statistically processed data we obtained the following results:

- for the bovine species, the quantity of meat estimated on the basis of the authorized slaughtering capacity per capita, at national level, is **9.8 kg meat / inhabitant/year**. The highest values are recorded in the northern part of Romania, respectively the economic development regions North-West (21.78 kg), South (12.80 kg) and North-West (11.96 kg) and the București-Ilfov region has no contribution (0 kg);
- in the case of pigs, the estimated amount of meat based on the authorized slaughter capacity per capita is **22.49 kg / inhabitant / year** at national level. By geographical regions, the highest value, of 66.84 kg, is found in the western economic region, followed by the southern region (31.88 kg), the northwestern region (20.80 kg), the central region (20,49 Kg), and the smallest is only 2.42 Kg in the Bucharest-Ilfov region;
- for sheep and goats, the estimated amount of meat at national level is **5.10 kg / inhabitant / year,** the highest estimated amount of meat per capita / year being registered in the South economic region. The lowest values are recorded in the Bucharest-Ilfov (0 kg) and South-West (1.40 kg) regions.
- However, in addition to the need for meat, there is also the sector of breeding and exploitation of birds and solipeds.
- From the breeding and exploitation of birds, the quantity of meat obtained as a result of the authorization of the breeding and slaughtering units ensures **40.52 kg / inhabitant / year**. The highest quantities were recorded in the economic regions of the South (77.18 kg), South-East (59.93 kg), North-East (45.62 kg) and the Central region (47.25 kg). The rest of the regions provide less than 30 kg of poultry / head of meat / year.
- Meat from solipeds, the estimated quantity according to the authorized slaughter capacity is 5.36 kg / capita / year at national level.

## CONCLUSIONS

From the statistically processed data, it can be seen that the number of animals with economic value raised and exploited at national level in the direction of meat production is insufficient to ensure the meat needs of the inhabitants of Romania.

Food security and resilience at national level is not achievable on the basis of national production (animal husbandry and exploitation) because the estimated and achieved quantities are smaller than necessary.

At the national level there are economic regions where animals are raised in very small numbers, which makes the estimated amount of meat insufficient to be taken into account.

It should be noted, however, that the data studied were represented only by the number of animals raised and farmed within the profile units, respectively by the capacity of the authorized slaughtering and cutting units. It was not possible to estimate the number of animals raised and capitalized at the level of households.

The population of Romania to which the level of meat production was reported is the one established at the 2011 census. The current socio-demographic situation was not taken into account, in which a very large number of people (of the order of several million) arrived in Romania or which are in transit.

Analyzing the situation at national level, by development regions, the resilience program cannot be applied because no geographical area of development can support the neighboring areas, therefore it is necessary to apply prevention, deterrence and protection measures.

Increasing the production of the meat and meat products sector can only be achieved by creating and implementing sustainable agricultural policies.

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# THE INFLUENCE OF TEMPERATURE ON THE STABILITY OF REFERENCE MATERIALS

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***Abstract***

*The paper presents the influence of temperature on the stability of the reference material, wheat flour matrix, MR001F-IBA. Experimentally, the stability of the candidate reference material units MR001F-IBA were evaluated in different storage conditions using a temperature of 4°C and, respectively, a range between 25-30°C degrees. The 3 batches of candidate reference material evaluated were produced according to the requirements of the ISO 17034: 2017 "General requirements for the competence of reference material producers". The results obtained by testing the MR001F-IBA reference material units according to the ISO 2171: 2007 standard indicate that there are no differences in the ash content in the analysed samples. The results are interpreted statistically according to the recommendations from the ISO 35: 2017 guide, these substantiating the information necessary for the MR001F - IBA certification. The purpose of the paper was to establish the conditions in storage, transport of MR001F-IBA for ash content.*

***Key words****: ash content, reference material, wheat flour, stability.*

## INTRODUCTION

The agri-food system has developed rapidly in recent decades due to the technology that has allowed the rapid transportation of products globally. Along with the agri-food sector development, many problems related to transport, storage and most importantly food safety have surfaced.

Because food safety plays a pivotal role in our current society the necessity of improving the monitoring of agri-food products and enhancing the quality of measurements in laboratory testing had become a major concern across member states of the European Union.

To support food safety on the ongrowing agri-food market, the European Union imposed regulations and developed programs to support the fight against food fraud.

The projects, under the guidance of the E.U., had supported and encouraged the development and production of reference materials which are essential for calibration, method validation, estimation of measurement uncertainty, for quality assurance, quality control and to ensure trasability.

After analysing specific databases and the catalogues of companies which are reference materials producers, it was noticed that there is a small number of reference materials regarding the wheat flour.

Wheat flour is an essential component in human nutrition, and it is being produced in huge quantities around the globe.

The European Union is being ranked as the second largest consumer which strongly suggests that there is a need to develop a reference material, with specific parameters for testing the quality of wheat flour.

Ash content is one of the major indicators for the quality and use of flour, which is essentially a characteristic that determines the purity of wheat flour. The ash obtained from flour consists of mineral compounds of phosphorus, potassium, calcium, magnesium, iron, zinc and copper, being a source of concentrated and insoluble fibre (Czaja et al., 2020).

Indirectly, ash is an indicator of mill performance as it shows the degree of contamination of bran flour, which represents the outer layer of wheat grain consisting of pericarp and aleurone.

For most baking applications, bakers are looking for excellent quality flour with high protein levels and the highest purity in terms of endosperm content (Carson & Edwards, 2009).
Ash is a measure of mineral content and is used to grade flour into different varieties. For example, whole wheat flour has a higher ash level than white flour. By quantifying ash levels during processing, flour millers can maximize extraction efficiencies and optimize blending (https://assets.thermofisher.com/TFS-Assets/MSD/Application-Notes/AN52269-wheat-flour-protein-moisture-ash-analysis-ft-nir.pdf.)
The determination of the amount of ash contained in wheat flour is necessary for its classification (Table 1).

Table 1. Types of wheat flour

| Type of wheat flour | Ash content % (max) | Application |
|---|---|---|
| Type 000 | 0.48 | Used for puffs and light pastries. It is a fine flour with a low protein content, about 9%. |
| Type 480 | 0.48 | It is also used for the preparation of pastries. |
| Type 550 | 0.55 | A harder flour than the first two, usually used for almost any kind of dough. |
| Type 650 | 0.65 | It has a higher gluten content which makes it used for doughs that need leavening(fermentation). It has a high protein content of 12-14%. |
| Type 1250 | 1.25 | Brown bread contains the largest proportion, over 50%, of wholemeal wheat flour type 1250. |
| Type 1350 | 1.35 | Brown bread containing the highest proportion, over 50%, wholemeal wheat flour type 1350. |
| Type 1750 | 1.75 | Contains wheat graham flour, meaning wholemeal or dietary flour type 1750 with the addition of wheat bran. |

## MATERIALS AND METHODS

In the ISO 17034: 2016 "General requirements for the competence of reference material producers" the reference material is defined as a sufficiently homogeneous and stable material, with one or more assigned properties, considered suitable for the intended use (https://www.iso.org/standard/29357.html).
Therefore, the definition shows that homogeneity and stability are two characteristics that play an important role in the development and production of reference materials.
The assessment of stability for a reference material implies that the stability should be assessed for each assigned property to determine the risk factors to which it may have been exposed, like temperature, humidity, or light, that may influence or degrade these properties over a predetermined time period.
ISO Guide 35: 2017 "Reference materials - Guidance for characterization and assessment of homogeneity and stability" approaches in greater detail the assessment of the stability of the reference material.
The guide ISO 35: 2017 describes two types of stability that are relevant when a reference material is produced. The long-term stability which is corelated with the behaviour of RM in the producer's facility when it is stored under the specified conditions. The second type of stability described is the short-term stability or transport stability which requires testing under the reasonable transport condition (https://www.iso.org/ standard/60281.html).
The stability of the reference material is determined by analysing the values of the certified parameters in the samples of units stored at the recommended temperatures for the reference material, assuming that there is no change in the composition of the reference material at this temperature (Rutkowska et al., 2020)
The candidate reference material used in the experimental stability study was produced within the National Research and Development Institute for Food Bioresources - IBA Bucharest, which followed the production process according to ISO 17034: 2017 meaning that the same raw material was used, under the same conditions, following the same process. A first step in the production process of the candidate reference material involves homogenization, which is made by using the electromagnetic sieving equipment BA 300 N, for-which a single sieve, certified ISO 3310, was used with a ⌀ of 1 mm.
The sample divider Retch PT100 with the vibrating splitter DR100 is used for portioning

and packaging. The sample divider distributes equal fractions of the base material to each unit of the candidate reference material, which can be considered an additional homogenisation.
The units of candidate reference material are sealed with an aluminium foil, preventing moisture transfer, using an electromagnetic induction equipment with forced cooling to avoid the risk of degrading the matrix by exposing it to high temperatures.
Three batches were produced, the first one was used in the feasibility study, the other two, presented in this paper, were used to assess the homogeneity and stability for the reference material.

## RESULTS AND DISCUSSIONS

The ash content for the wheat flour used in the production of MRC, was determined by using the method described in ISO 2171: 2007 "Cereals, pulses and by-products - Determination of ash yield by incineration" (https://www.iso.org/standard/37264.html).
For applying the method described in ISO 2171: 2007 to determine the ash content of wheat flour it is necessary to prepare the crucibles suitable for use at 550°C by drying them in an oven at a temperature of 130°C for a period of 90 minutes. After drying, the crucibles are removed from the oven and placed in the desiccator to cool, then weighed with the precision of 0.1 g. The preparation of the working sample is carried out by weighing between 4.9 g to 5.1 g of sample for incineration at 550°C. It is permitted to use between 2 g and 3 g sample for low density products. The sample must be evenly distributed without being compacted in the crucible.
After preparing the sample, the crucible is placed at the entrance to the furnace, for the sample to reach the incineration temperature for combustion, ethanol was added, as is it is permitted in the case of incineration at 550°C (Figure 1).
When the sample has finished burning it should be placed in the oven for a period of at least 4 hours.

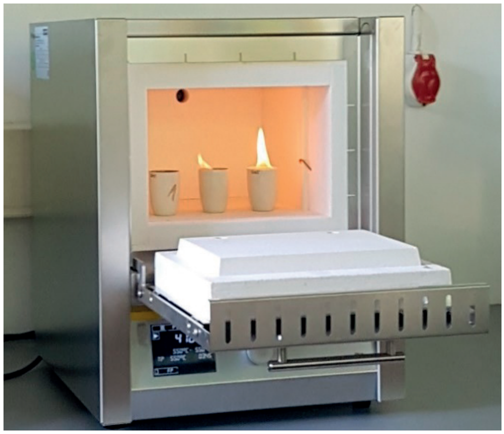

Figure 1. Sample combustion in the furnace

When the incineration is complete, the crucibles are removed from the oven and quickly placed in the desiccator for cooling, between 60 to 90 minutes (Figure 2).
It must be recalled that ash has a high degree of water absorption. Therefore, as soon as the samples reach room temperature, they must be weighed with a precision 0.1 g.

Figure 2. Incinerated sapless in the desiccator

The results obtained following the analysis of the candidate reference material units on the ash content of wheat flour, using the method described in ISO 2171: 2007 for each point in time, are presented in Table 2.

Table 2 Results obtained following the analysis of the candidate reference material units

| Crt. | T0 | | | T1 | T2 | T3 | T4 |
|---|---|---|---|---|---|---|---|
| Sample | L2.1 | L2.9 | L2.10 | L2.15 | L2.2 | L2.3 | L2.4 |
| 1. | 0.54 | 0.52 | 0.52 | 0.54 | 0.52 | 0.50 | 0.56 |
| 2. | 0.50 | 0.50 | 0.55 | 0.56 | 0.53 | 0.53 | 0.53 |
| 3. | 0.55 | 0.52 | 0.54 | 0.53 | 0.50 | 0.55 | 0.53 |
| Average | 0.53 | 0.51 | 0.54 | 0.54 | 0.52 | 0.53 | 0.54 |
| Standard deviation | 0.026 | 0.012 | 0.015 | 0.014 | 0.015 | 0.025 | 0.017 |

A one-way ANOVA was performed to compare the ash content from the analysed units from which it can be observed that there are no significant differences between the averages of the analysed groups. The results are presented in Table 3.

Table 3. One-way ANOVA results for the second batch

| | DF | Sum of Square | Mean Square | F Statistic | P-value |
|---|---|---|---|---|---|
| Group | 6 | 0.002362 | 0.000394 | 1.117 | 0.401041 |
| Residual | 14 | 0.004933 | 0.000352 | NaN | NaN |
| Total | 20 | 0.007295 | 0.000746 | | |

Since the p-value is 0.4010, being higher than the confidence level ($\alpha = 0.05$), and F-statistic does not exceed the critical value, we cannot reject the null hypothesis, which states that the averages of the 7 candidate reference material units analysed are equal, as we can observe re in figure 3.

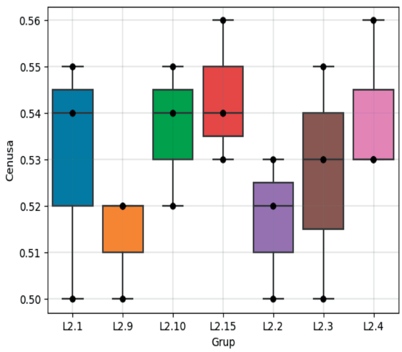


Figure 3. Graphic representation of ANOVA results

The third batch was produced from the same raw material as the first two batches. From the specialised literature and the results obtained from the first two batches, which were analised and provided enough data, that it can be presumed that the ash content is sufficently homogeneus and stable across every batch produced (Figure 4).

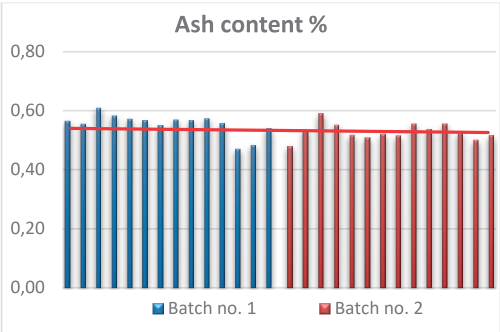


Figure 4. Ash content result for first and second batch

It was decided that for this lot the interval at which the ash content is determined to increase.

In order to determine the optimal storage conditions for the candidate reference material, the third batch was randomly divided into two groups to be stored under different environmental conditions.

The first group being stored at a temperature of 4°C, and the second group at a temperature ranging between 25°C and 30°C.

Table 4 shows the results obtained at $T_0$ and $T_1$ for the ash content in the third batch.

For the interpretation of the data, the analysis of the variance was also chosen to verify if there are significant differences between the 4 samples.

Table 4. Results obtained at T0 and T1 for the ash content in the third batch

| Crt. | T0 | | T1 | |
|---|---|---|---|---|
| Sample | L3.10$^{H}$ | L3.2$^{F}$ | L3.5$^{H}$ | L3.3$^{F}$ |
| 1 | 0.51 | 0.51 | 0.56 | 0.51 |
| 2 | 0.48 | 0.49 | 0.55 | 0.54 |
| 3 | 0.52 | 0.53 | 0.54 | 0.52 |
| Average | 0.51 | 0.51 | 0.55 | 0.52 |
| Standard Deviation | 0.0110 | 0.0204 | 0.01221 | 0.0168 |
| *H – temperature range between 25 – 30°C<br>*F – stable temperature 4°C | | | | |

After examining the results obtained from the analysis of variance it can be observed that the value of p is higher than α, and statistic F does not exceed the value of critical F, which is 3.376 (Table 5).

Table 5. One-way ANOVA results for the 3rd batch

| | DF | Sum of Square | Mean Square | F - value | P-value |
|---|---|---|---|---|---|
| Group (between groups) | 6 | 0.002362 | 0.000394 | 1.117 | 0.401041 |
| Residual (within groups) | 14 | 0.004933 | 0.000352 | NaN | NaN |
| Total | 20 | 0.007295 | 0.000746 | | |

The data shows that the null hypothesis cannot be rejected. This means that there are no significant differences between the averages of the 4 groups analysed.

Figure 5 shows graphically the results of the analysis of variance for the 3rd batch on ash content.

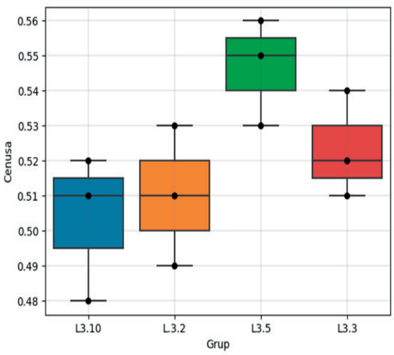


Figure 5. Graphic representation of ANOVA results for the 3rd batch

## CONCLUSIONS

This study has shown that values of the ash content do not vary within and between batches produced from the same raw material.

The research has also shown that the temperature does not influence the analyte studied in the wheat flour matrix under the conditions of the presented experiment. In this context, both the storage and the transport of MR001F - IBA can be done in the temperature range 4°C - 30°C, which leads to the reduction of energy consumption in storage and transport. The study also provides the information needed for MR001F-IBA certification.

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# THE INFLUENCE OF FOOD MATRIX IN THE DEVELOPMENT OF REFERENCE MATERIALS

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***Abstract***

*The paper presents the influence of the raw material (matrix) in the homogeneity and stability assessment of two batches of candidate reference material MR001F - IBA produced at an interval of 30 days and evaluated for three months. The statistical interpretation of the moisture content of wheat flour type 650 indicates the need to carry out feasibility studies for each batch made under the same processing conditions. The feasibility study carried out followed the implementation of the recommendations from the ISO 35: 2017 guide regarding the risk in the stability of the reference material produced in successive batches. Stability has been assessed according to section 8.3.2.1 - Classical stability studies - Repeatability conditions of measurement from the ISO 35:2017 guide.*

***Key words***: *homogeneity, reference material, stability, wheat flour.*

## INTRODUCTION

The most developed industry, at European level, is the agri-food industry, with cereal processing being the most important sector. The annual amount of produced flour is around 35 million tons of flour, obtained through processing over 50 million tons of wheat, rye and oats.
Among cereals, wheat has the potential to contain the highest quantity of gluten, which is what creates the elasticity and strength of the dough and influences the texture of pastries.
It has been shown that the temperature in storage affects the qualities attributed to wheat flour. Unfortunately, only a few studies have been conducted to evaluate the influence of storage conditions on the properties of flour (Sujitha et al., 2018).
Wheat flour is the most important product in the milling industry, its quality can be defined as the set of organoleptic, physical, chemical and rheological characteristics imposed by the processing requirements for the purpose for which it is intended.
The purpose of flour is different depending on the industry in which it will be processed: bread and bakery products, pasta, etc. (Dabija, 2016).
The importance of the quality of wheat flour is due to the fact that it is a biological material that can be obtained from different sources that can vary significantly and can influence its characteristics.
The quality of wheat flour is closely related to moisture, having a special importance since a lower moisture content means a higher number of solid components (protein, starch, fat, sugar, ash) speculated in the bakery industry but also due to the fact that high humidity is a risk factor for microbial and fungal development and has a significant effect on protein and raw fats. However, it has been observed that it does not have a significant effect on the fiber and ash content (Nasir et al., 2003).
The moisture content of wheat flour is also of particular importance in determining the shelf life: the lower the moisture content, the longer the shelf life.

Appropriate methods and procedures for laboratory testing need to be used for quality control and assurance, as this sector of the agri-food industry is a key point in food safety.
Given that food safety is a major priority for the European Union (EU), it has invested research programs to improve the quality of analytical measurements (physical, chemical and biological) by developing reference materials (Maier et al., 1997).
The role of reference materials in the quality of analytical results in laboratory testing has received increasing attention in recent years, especially in the agri-food industry where they are being used for method validation, calibration, QA and QC, trasability.
The quality of measurements have an important role in technological and socio-economic development, thus supporting the development of trade and monitoring the quality of products and services and allows the optimization of decisions in monitoring food safety (Rychlik et al., 2018).
Reference materials are defined as sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process (International Organization for Standardization, 2016). The definition highlights two important characteristics of a reference material: homogeneity and stability.

## MATERIALS AND METHODS

The production of a reference material requires, according to standard 17034:2017 (Standardization, 2016), an experimental stability study to determine whether the value of the properties for which the candidate reference material was characterized varies or degrades due to the environmental conditions under which was exposed. Conditions such as: temperature, humidity and light.
In order to test the stability of a reference material, sampling units are randomly extracted, an exception being made at an early stage in the development of a reference material, when it is possible to produce experimental batches of small size, subjected to treatment processes, when necessary and packaging, which may be used in whole or part for stability testing.
The stability of the reference material is determined by analyzing the values of the certified parameters in the samples of units stored at the recommended temperatures for the reference material, assuming that there is no change in the composition of the reference material at this temperature (Rutkowska et al., 2020).
The experimental stability study was performed on two batches of the candidate reference material produced within the National Research and Development Institute for Food Bioresources - IBA Bucharest, from the same raw material, under the same conditions, following the same process of production. Given that wheat flour is a sufficiently homogeneous natural matrix and that the parameter selected for the characterization of CRM obtained, moisture, respectively, the processing requires only homogenization and packaging under normal conditions (without inert gas).
For the preparation of a batch of candidate reference material, the homogenization was done using digital electromagnetic sieving equipment, with a single sieve, certified ISO 3310, while ensuring that the grain of wheat flour complies with ISO 712: 2009.
Packaging and portioning MR001F - IBA is performed using the Retsch Sample Divider


Figure 1. MR001F - IBA Candidate reference material

PT100 with vibrating splitter DR 100. Due to the fact that the device divides the sample so that the composition of each fraction of the sample corresponds exactly to that of the used material, an additional homogenization is being performed. The sealing of the vial was through electromagnetic induction and forced cooling using the automatic equipment LX6000A to eliminate the risk of heating of the matix.

The finished product was packaged in 250 g or 500 g bottles. After that, it is stored under predetermined storage conditions. It will be tested in experimental studies of homogeneity and stability according to ISO 17034:2009.

Figure 2. Batch produced for feasibility study

The moisture content of wheat flour used to produce the candidate reference material MR001F - IBA Wheat Flour was determined by using the reference method described in ISO 712:2009. Candidate reference material units were randomly selected and sampled for triplicate analysis. Approximately 5 g of type 650 flour with an accuracy of 0.001 g were weighed for each sample and placed in a metal capsule with lid, dried and weighed beforehand. After weighing, the capsules are placed in the oven at 130° C for 90 minutes.

Figure 3. Samples inside oven.

After 90 minutes, the capsules are removed and placed in the desiccator for 30-45 minutes for cooling, followed by the final weighing using the analytical balance.

Humidity is calculated as a percentage using the formula $W_{H2O} = \left(1 - \frac{m_1}{m_0}\right) \times 100$, where $m_0$ is the mass of the working sample, and $m_1$ is the mass of the working sample after drying.

Figure 4. Final weighing of the candidate reference material

**RESEARCH AND DISCUSSIONS**

Using the method described above, six samples from the first batch of candidate reference material were analysed. The first three unites of candidate reference material MR001F - IBA Wheat Flour, were randomly selected when the first batch was produced, for the initial analysis (also called $T_0$). They were included in the homogeneity study; the rest of the samples have been-analysed at one-month interval.

The experimental study design used is a classical stability study described in ISO Guide 35:2017 (International Organization for Standardization, 2017).

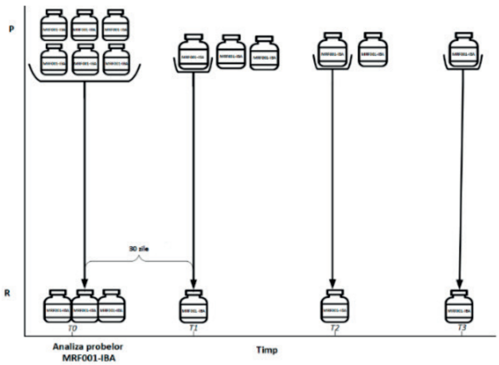


Figure 5. Representation of a classical stability study

**Assessing moisture content on the first batch**

The results of the experimental stability study for the first batch are presented in Table 1.

To determine whether there are significant differences in humidity between the six units of analyzed CRM, a one-way analysis of variance was applied. The results are presented in Table 2.

Table 1. Moisture content results for the first batch

| Crt. | T0 | | | T1 | T2 | T3 |
|---|---|---|---|---|---|---|
| Sample | L2.1 | L2.9 | L2.10 | L2.2 | L2.3 | L2.4 |
| 1. | 10.12 | 10.14 | 10.15 | 10.18 | 10.21 | 10.22 |
| 2. | 10.17 | 10.11 | 10.18 | 10.24 | 10.26 | 10.19 |
| 3. | 10.14 | 10.13 | 10.14 | 10.20 | 10.24 | 10.18 |
| Average | 10.14 | 10.13 | 10.15 | 10.21 | 10.24 | 10.20 |
| SD | 0.025 | 0.015 | 0.021 | 0.031 | 0.025 | 0.021 |

Table 2. One-way ANOVA result for the first batch

| | DF | Sum of Square | Mean Square | F | P-value |
|---|---|---|---|---|---|
| Group | 5 | 0.026711 | 0.005342 | 9.713131 | 0.000671 |
| Residual | 12 | 0.0066 | 0.00055 | NaN | NaN |
| Total | 17 | 0.333133 | 0.0019595 | | |

The results indicate a statistically significant difference in humidity averages in the analyzed CRM samples. From Table 2 we can observe that F-value exceeds the value of F critical, and the p value is 0.000671, which means that p is less than the significance level α which is equal to 0.05, indicating a confidence level of 95%.

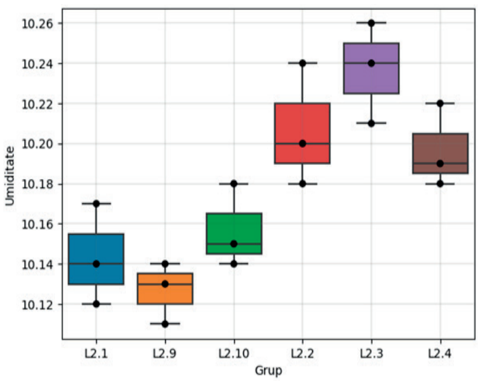


Figure 6. ANOVA chart for the first

Thus, it can be deduced that the null hypothesis is rejected, resulting in the fact that there are significant differences between the averages of the 6 groups, presented in chart (Figure 6).
In order to determine exactly where the differences are located in the ANOVA analysis, it was performed a post-hoc test, called Tukey HSD, which is a multiple comparison procedure, also called the significantly honest difference test.
Table 3 shows the values resulting from the application of the Tukey HSD test, which shows that there are significant differences between certain groups.

Table 3. Tukey HDS results

| Group 1 | Group 2 | Mean Diff | Crit Diff | P-adj | Reject |
|---|---|---|---|---|---|
| L2.1 | L2.10 | 0.0133 | 0.0643 | 0.9 | F |
| L2.1 | L2.2 | 0.0633 | 0.0643 | 0.0546 | F |
| L2.1 | L2.3 | 0.0933 | 0.0643 | 0.004 | T |
| L2.1 | L2.4 | 0.0533 | 0.0643 | 0.1278 | F |
| L2.1 | L2.9 | 0.0167 | 0.0643 | 0.9 | F |
| L2.10 | L2.2 | 0.05 | 0.0643 | 0.1679 | F |
| L2.10 | L2.3 | 0.08 | 0.0643 | 0.0126 | T |
| L2.10 | L2.4 | 0.04 | 0.0643 | 0.3538 | F |
| L2.10 | L2.9 | 0.03 | 0.0643 | 0.6203 | F |
| L2.2 | L2.3 | 0.03 | 0.0643 | 0.6203 | F |
| L2.2 | L2.4 | 0.01 | 0.0643 | 0.9 | F |
| L2.2 | L2.9 | 0.08 | 0.0643 | 0.0126 | T |
| L2.3 | L2.4 | 0.04 | 0.0643 | 0.3538 | F |
| L2.3 | L2.9 | 0.11 | 0.0643 | 0.001 | T |
| L2.4 | L2.9 | 0.07 | 0.0643 | 0.0304 | T |
| Reject F – False<br>Reject T – True | | | | | |

In the case of these groups, in addition to the fact that the absolute difference of the means exceeds the critical difference, both presented in Table 3, we can also observe that the value of p is less than 0.05, rejecting the null hypothesis which states that there is no significant difference between the two compared means.
The chart in Figure 7 shows the comparison between the absolute difference between groups averages and the critical difference.

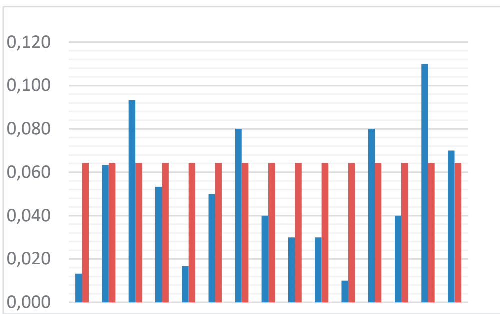


Figure 7. The difference between the absolute mean of the groups and the critical difference

Table 4. Moisture content results for the second batch

| Crt. | T0 | | | T1 | | | T2 | |
|---|---|---|---|---|---|---|---|---|
| Sample | L3.2 | L.3.12 | L3.10 | L.3.14 | L.3.3 | L3.5 | L.3.6 | L3.8 |
| 1. | 11.22 | 11.06 | 11.19 | 11.04 | 11.13 | 11.25 | 11.37 | 11.28 |
| 2. | 10.91 | 10.96 | 11.02 | 10.96 | 11.41 | 11.10 | 11.38 | 11.27 |
| 3. | 11.40 | 10.96 | 11.08 | 10.96 | 11.25 | 11.31 | 11.35 | 11.30 |
| Average | 11.18 | 10.99 | 11.10 | 10.99 | 11.26 | 11.22 | 11.37 | 11.28 |
| SD | 0.248 | 0.058 | 0.086 | 0.046 | 0.140 | 0.108 | 0.015 | 0.015 |

**Assessing moisture content on the second batch**

Table 4 shows the values obtained from the determinations made on the second batch produced. As in the case of the first batch, for $T_0$, three samples were randomly selected for analysis, being used in the homogeneity study. The analysis of the following samples, for $T_1$ and $T_2$, were also performed at an interval of 30 days.
The one-way ANOVA analysis was also used for this batch results to determine whether there were significant differences between the group means. The results obtained are presented in Table 5.

Table 5. One-way ANOVA result for the second batch

| | DF | Sum of Square | Mean Square | F Statistic | P-value |
|---|---|---|---|---|---|
| Group | 7 | 0.398667 | 0.056952 | 4.288852 | 0.007557 |
| Residual | 16 | 0.212467 | 0.013279 | NaN | NaN |
| Total | 23 | 0.611134 | 0.070231 | | |

From these results, as in the case of the first batch, there can be observed significant differences between the 8 compared groups, highlighted by the graphical representation shown in Figure 8.

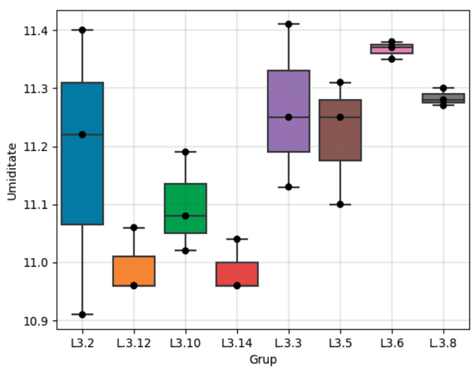


Figure 8. ANOVA chart for second batch

It is observed in Table 5 that F-value exceeds the critical F, as in the previous case and the value of p is well below the limit of 0.05, respectively 0.0075.

## CONCLUSIONS

From the statistical interpretation of the results, it can be concluded that there is a need to carry out homogeneity and stability studies for each new batch produced even if the raw (starting) material is the same. The difference in results is determined by the moisture content of the raw material unprocessed and unpackaged for production.
The interpretation of the statistical data, obtainned from the homogeneity studies and, respecttively, the stability studies, were performed taking into account the test performance obtainned within the MR laboratory from INCD IBA Bucharest.


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# ASPECTS REGARDING THE PRODUCTION AND THE HYGIENE-SANITARY CONTROL OF THE DORNA SWISS CHEESE

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***Abstract***

*The pastures and hayfields from the Dorna area are characterized by rich floristic biodiversity, which favors the production of organic milk with high content of bioactive principles and propionic bacteria, specific to the production of a local Swiss cheese. The Dorna Swiss cheese is a very demanding product that requires particular knowledge to process Emmental cheeses. In this paper, we have analyzed the current state of some traditional procedures, specific to the Dorna Swiss cheese production. Moreover, the present research documents and describes a procedure characteristic of the area, which consists of the selection, verification, and processing of the raw material, until obtaining the finished product. The processing is based on obtaining "wheels" of cheese (12-13 kg) from a mixture of raw milk (60%) with pasteurized milk (40%) at a temperature of 70°C, by pressing and salting, followed by ripening in three rooms, differentiated by the time interval, temperature and humidity provided. The entire procedure lasts for 60-70 days, throughout this interval the evolution of temperature and humidity being monitored. All these characteristics of biodiversity and processing give the Swiss cheese the characteristics of a highly appreciated assortment of Emmental cheeses.*

***Key words****: Dorna Swiss cheese, Emmental cheeses, mountain biodiversity.*

## INTRODUCTION

As is well known, Emmental cheeses originate in the region of the same name in the canton of Bern, Switzerland. Their production is recorded in very old documents (from 1542), following that in the 11$^{th}$ century to gain ground, and from 2002 to obtain the certification of product with protected origin (PDO) (Bisig et al., 2010). Different types of Emmental cheeses, generically considered traditional products, are produced in considerable quantities in many European countries, such as France, Austria, Germany, Finland, Ireland (Bisig et al., 2010). Emmental cheese or Swiss cheese, called in our country "şvaiţer", is produced mainly in the Dorna area. The mountainous land of Dorna is characterized by wild pastures, with diverse flora and rich in bioactive components, specific to soils poor in manganese and iron. It favors the obtaining of high-quality milk, rich in bioactive elements, lactic and propionic bacteria, which have a special impact on the production of Swiss cheese. The mountainous area of Dorna, located at an altitude of 800-1800 meters, is favorable for obtaining milk of special quality and implicitly of superior cheeses (Necula et al., 2021). The quality of the mountain geo-climatic conditions, which can influence the health and well-being of the animals, as well as the physical and microbiological composition of the milk also have a special contribution to the quality of Swiss cheese and other traditional dairy products (Ognean et al., 2012; Someșan et al., 2013). In this context, the major impact is on the origin and microbial load of milk, which mainly influences the sensory characteristics of cheeses obtained under such conditions (Lafarge, 2004; Ognean et al., 2008). To those mentioned, we can add the action of the mechanical factors for extracting the whey, the ripening conditions specific to this type of cheese, which also influence the quality of the Swiss cheese. The use of raw milk, as well as the feeding specificity of lactating cows in mountain areas, which are mainly based on good quality hay and the exclusion of silage and other processed feed, are largely found in the achievement of the quality standards of the Swiss cheese. This paper focuses on updating the knowledge about obtaining and processing milk for the preparation of the Dorna Swiss cheese, specific to some mountainous areas in Bucovina. The paper also includes own research on the processing of the Dorna Swiss cheese, going through the main technological stages: the mixture of raw milk (60%) with pasteurized milk (40%), at a temperature of 70°C; cheese "wheels" ripening (12-13 kg), by successive storage at three different temperatures, totaling 60-80 days.

## MATERIALS AND METHODS

Documentations and investigations were carried out on Emmental type cheeses, materialized by presenting and analyzing the technological process of obtaining the Swiss cheese in a commercial company from Șara Dornei municipality, during six months of 2021.

**Data on the commercial company under study.** It should be noted that this company is licensed according to the rules in force (Law 84/1998) and it is registered under the trademark Dany Lily/21.11.2019 "Călimani". The company also has the authorization to use the term "mountain product". In the Dorna area, cattle from Maramureş Brown (Brună de Maramureș) and Pinzgau of Transylvania breeds (black variety) and their crossbreeds predominate, followed by the Romanian Spotted (Bălțată Românească) breed and its crossbreeds. The owner of this micro-farm owns a herd of 35 lactating cows, including the breeds Maramureș Brown and Pinzgau of Transylvania. In addition, this company also processes the milk taken from other 40 micro-farms and 4 commercial farms in the area. Milk is received at the unit headquarters where samples are taken from each can and analyzes are made with the Eco Milk device in terms of fat, protein content, respectively density levels, cryoscopic point, and acidity. The antibiotic content is also determined with the Biokom system. Samples of milk with high acidity are targeted for other types of products, and those with antibiotic content are returned to farmers.

**Investigation of water, milk, and cheese samples.** The procedure for collecting and storing milk is essential for obtaining a quality Swiss cheese, in the sense that the duration of the collection must not exceed 24 hours after milking, and the processing and ripening time must be at least 60-80 days. According to the public health strategies, the veterinary health authority (ANSVSA Suceava) collects monthly and tests microbiologically samples of water, milk, and cheese.

The water analysis consisted of the evaluation of the load with coliform bacteria and *E. coli*, respectively with intestinal enterococci and with *Clostridium perfringens*. Under current regulations, the investigation of milk consisted in the determination of total bacteria count (TBC) and somatic cells count (SCC), and of the cheese in the evaluation of the degree of contamination with coagulase-positive staphylococci. Simultaneously, we randomly collected samples of milk from the cans selected for processing the Swiss cheese, and the samples (no = 20) were investigated at the Milk Quality Laboratory of the Cluj-Napoca Foundation, using an advanced set of tests for raw milk. Those tests were focused on determining the main compositional and hygienic indicators (Table 1). Thus, with the help of the Milkoscan system, the current set of physicochemical parameters of milk was determined, of which in our paper, we attributed relevance to the evolution of the fat content, total protein, and lactose, as well as the evolution of acidity. At the same time, the main hygienic-sanitary indices were determined: TBC (germs/mL) with the BactoScan automatic system and SCC (Cel./mL) with the Fossomatic automatic system. The data obtained were statistically analyzed, by using the GraphPad, InStat, and Microsoft Excel programs, which allowed the calculation of the main statistical parameters.

**The processing procedure of the Dorna Swiss cheese**. Ensuring the specific sensory qualities of the Swiss cheese requires that the milk which is used to meet the quality parameters and strictly follow the specific technological procedures (Berdagué et al., 1990). The study company processes 2000-2400 L of milk daily, from which the sample required for the Swiss cheese is selected first to immediately filter it through five or six layers of gauze. Basically, out of the total amount of milk collected, the unit uses 500 L for processing the Swiss cheese and the rest for other products.

The processing is done in double-walled stainless steel cauldrons, with a capacity of 500 L, following the next steps (Figure 1):

- Of the 500 L, 200 L are heated to a temperature of 30-35$^{0}$C and they are normalized by mixing with other 300 L, at the fat of 3.5%;
- Subsequently, 20% normalized milk is mixed with 20% raw milk and pasteurized at 70 $^{0}$C for one minute, thus destroying a good part of the natural flora;
- In the double-walled stainless steel cauldron, there is 60% whole raw milk (300 L), which is passed through the cream separator to clean the impurities. It is then mixed with pasteurized milk and brought to a temperature of 32°C, by mechanical stirring;
- Afterward, the rennet is added, about 10 g per 500 L of milk, and after about 30 minutes, coagulation occurs, following a uniformity with a spoon of special shape used in the preparation of cheeses;
- The content from the bottom of the cauldron is brought to the surface to even out the temperature of the curd. Then, it is cut into small pieces, up to the size of a rice grain, a process that takes about 15-20 minutes depending on the consistency of the curd and the acidity (in winter, the acidity being lower, the amount of curd used must be higher than 10 g/500 L);
- The previous stage is followed by mechanical stirring for at least 30 minutes for good dehydration and drying of the grain, until a suitable consistency;
- The homogenization is then stopped and about 25-30% of the whey is released, after which it is switched to the second heating at 54-56$^{0}$C (in summer, the heating is done only at 52-53$^{0}$C), for 30-40 minutes (this new heating is also called scalding);
- The next step consists in the mechanical stirring to dry the curd beans, about 40-60 minutes, during which the technologist usually does the manual test, by squeezing the grain in the palm; if it spreads, the stirring process for dehydration and drying must be continued, and if it does not dissolve in "rice grains" it means that the process is over. At this point, the stirring process is ended and the content is allowed to settle, forming a compact mass, which is removed from the cauldron in three parts. It is then put in the press and three "wheels" of cheese are obtained, of 12-14 kg. On the same day it is pressed several times to drain the whey;
- The next day, the cheese wheels are removed from the press and the edges are cleaned, and then, for 24 hours they are turned on both sides about 5-6 times for the drying stage;
- After another 48 hours of drying, liquid brining (20-23%) follows, by immersion for 48 hours;
- Then the cheese is removed and left to dry, following its placement on the shelf in the first room to continue the drying process for 7-10 days, at a temperature of 18-20$^{0}$C and relative humidity of 80-82%. During this period, the cheese is turned on both sides every day, and after about 10 days it is transferred to the second room, also called the warm room, where the fermentation takes place at 22-24$^{0}$C and humidity of 82-85%, for 60-70 days.
- Finally, it is transferred to the third room in the cold, in which the cheese is kept at a temperature of 4-5$^{0}$C until delivery.

In the first and second rooms, the cheese wheels are removed and ventilated, respectively washed with brine twice a week to prevent the formation of mold, after which it is left to dry for a few hours and then put back on the shelf. At the same time, the shelves for cheese maturing are washed and cleaned with brine. As the company under study works with small sizes of cheese wheels, the obtained Swiss cheese requires a minimum ripening period and can be marketed for human consumption after 70 days.

## RESULTS AND DISCUSSIONS

The analyzes revealed minor oscillations regarding the compositional parameters and markers for monitoring the health and biologically active potential of milk.

These are presented in Table 1 and Figure 1, and the obtained results confirmed the inclusion of the recorded values within the national and European standards on milk quality and health.

We have found average normal levels of the total dry matter (DM) (13.21%) and non-fat dry matter (NDM) (8.70%).

The average total protein content was also within the normal limits of 3.56%, with oscillations between 3.40% and 3.73% (Table 1 and Figure 1A), in which casein reached the average level of 2.77%.

The average fat content (4.39%) was also within the standard values, with oscillations ranging from 3.10% to 5.94% (Table 1 and Figure 1A).

The average lactose concentration was 4.38%, with oscillations between 4.16% and 4.68% (Table 1 and Figure 1A).

The acidity of the milk was in line with the product standards, the pH values being 6.55 (6.48-6.62) (Table 1).

Table 1. The average values of the main physical-chemical and hygienic-sanitary parameters of the processed milk from a company located in the Dorna area

| **Sample code** | **Fat (g/100g)** | **Protein (g/100g)** | **Casein (g/100g)** | **Lactose (g/100g)** | **Non-fat dry matter (g/100g)** | **Total dry matter (g/100g)** | **pH** | **SCC/ml x1000** | **TBC/ml x1000** |
|---|---|---|---|---|---|---|---|---|---|
| | FIL IDF 141C: 2000 | FIL IDF 141C: 2000 | Milkoscan | FIL IDF 141C: 2000 | Milkoscan / Lactoscop | Milkoscan | Milkoscan | SR EN ISO 13366-2:2007 | Bactoscan FC |
| 8907363954 | 4.75 | 3.64 | 2.87 | 4.58 | 9.03 | 13.88 | 6.60 | 24.12 | 6.02 |
| 8907363955 | 3.10 | 3.40 | 2.60 | 4.20 | 8.28 | 11.54 | 6.50 | 22.12 | 6.68 |
| 8907363956 | 3.27 | 3.40 | 2.61 | 4.20 | 8.29 | 11.72 | 6.48 | 18.10 | 7.87 |
| 8907363957 | 3.38 | 3.41 | 2.62 | 4.19 | 8.29 | 11.82 | 6.49 | 10.16 | 9.85 |
| 8907363958 | 5.63 | 3.63 | 2.87 | 4.46 | 8.92 | 14.64 | 6.56 | 38.78 | 7.62 |
| 8907363959 | 5.94 | 3.58 | 2.87 | 4.60 | 9.05 | 15.06 | 6.58 | 22.70 | 7.50 |
| 8907363960 | 4.96 | 3.49 | 2.75 | 4.39 | 8.66 | 13.74 | 6.54 | 9.19 | 9.46 |
| 8907363961 | 3.27 | 3.41 | 2.62 | 4.20 | 8.29 | 11.71 | 6.49 | 8.12 | 9.23 |
| 8907363962 | 5.27 | 3.67 | 2.85 | 4.27 | 8.71 | 14.07 | 6.54 | 39.71 | 2.71 |
| 8907363963 | 4.62 | 3.64 | 2.87 | 4.62 | 9.08 | 13.79 | 6.60 | 20.15 | 6.60 |
| 8907363964 | 3.29 | 3.41 | 2.61 | 4.19 | 8.29 | 11.73 | 6.50 | 11.10 | 7.44 |
| 8907363965 | 3.73 | 3.54 | 2.71 | 4.20 | 8.43 | 12.30 | 6.52 | 6.13 | 5.44 |
| 8907363966 | 4.76 | 3.73 | 2.87 | 4.29 | 8.76 | 13.62 | 6.56 | 34.95 | 4.15 |
| 8907363967 | 4.35 | 3.67 | 2.87 | 4.53 | 9.00 | 13.43 | 6.59 | 3.97 | 6.10 |
| 8907363968 | 4.11 | 3.65 | 2.87 | 4.68 | 9.15 | 13.35 | 6.62 | 13.48 | 4.05 |
| 8907363969 | 4.80 | 3.73 | 2.85 | 4.16 | 8.62 | 13.52 | 6.53 | 27.31 | 4.48 |
| 8907363970 | 4.61 | 3.50 | 2.74 | 4.38 | 8.64 | 13.38 | 6.54 | 30.08 | 3.67 |
| 8907363971 | 5.17 | 3.53 | 2.78 | 4.43 | 8.76 | 14.04 | 6.54 | 5.20 | 1.06 |
| 8907363972 | 4.08 | 3.54 | 2.77 | 4.50 | 8.82 | 13.03 | 6.55 | 7.16 | 4.59 |
| 8907363973 | 4.76 | 3.65 | 2.86 | 4.53 | 8.97 | 13.82 | 6.59 | 30.71 | 7.12 |
| **AVERAGE** | **4.39** | **3.56** | **2.77** | **4.38** | **8.70** | **13.21** | **6.55** | **19.16** | **6.08** |

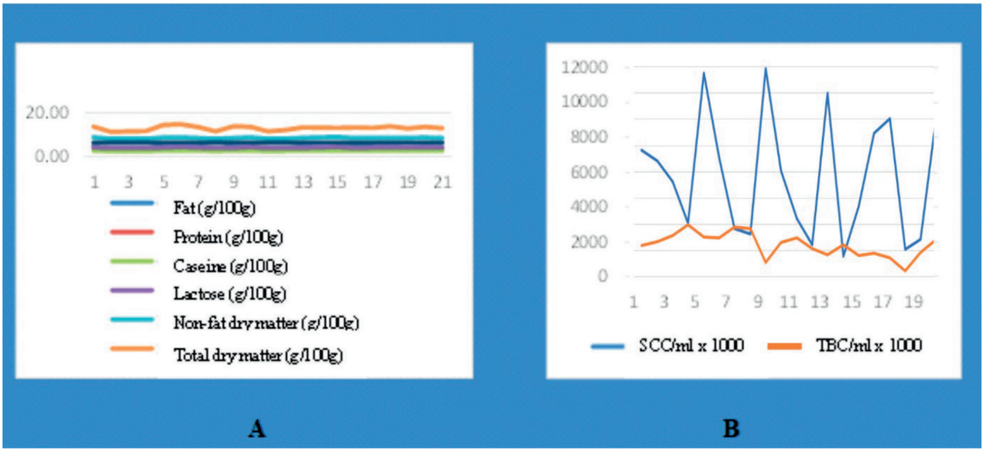


Figure 1. Evolution of the main physical-chemical (A) and hygienic-sanitary parameters of milk (B)

The processing procedure of the Dorna Swiss cheese has already been presented, and regarding the obtained results we note that the technological flow correlates the traditional knowledge and practices confirmed over time, with the current ones specific to Emmental cheeses. We also mention that the producer strictly complied with the criteria for milk selection (Figure 2) and the processing steps adopted (Figure 3), which have ensured the obtaining of a high-quality Swiss cheese, as shown in Figure 4.

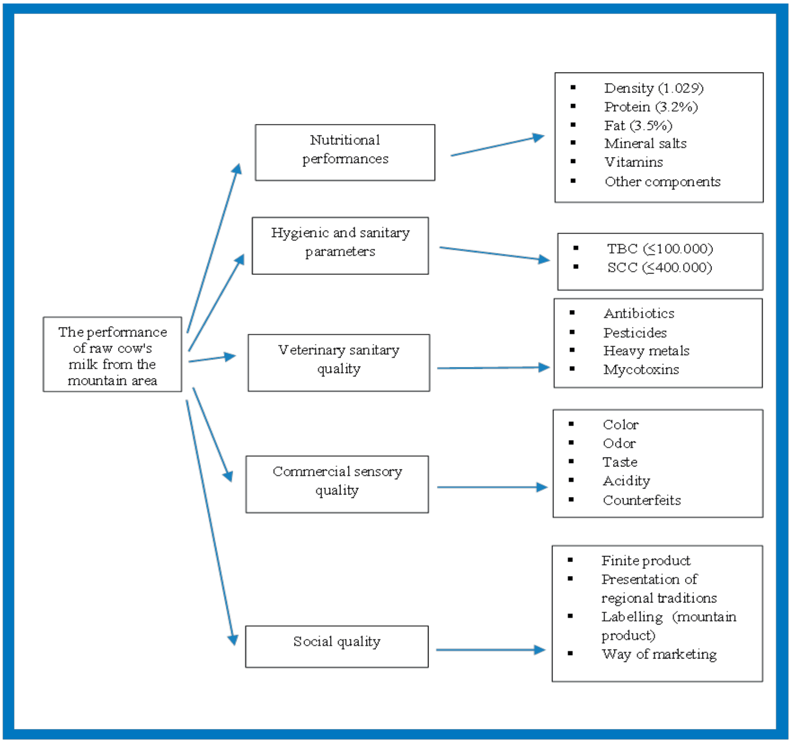


Figure 2. Basic criteria used in the selection of milk for processing the Dorna Swiss cheese, including compositional, hygienic-sanitary, sensory and social parameters

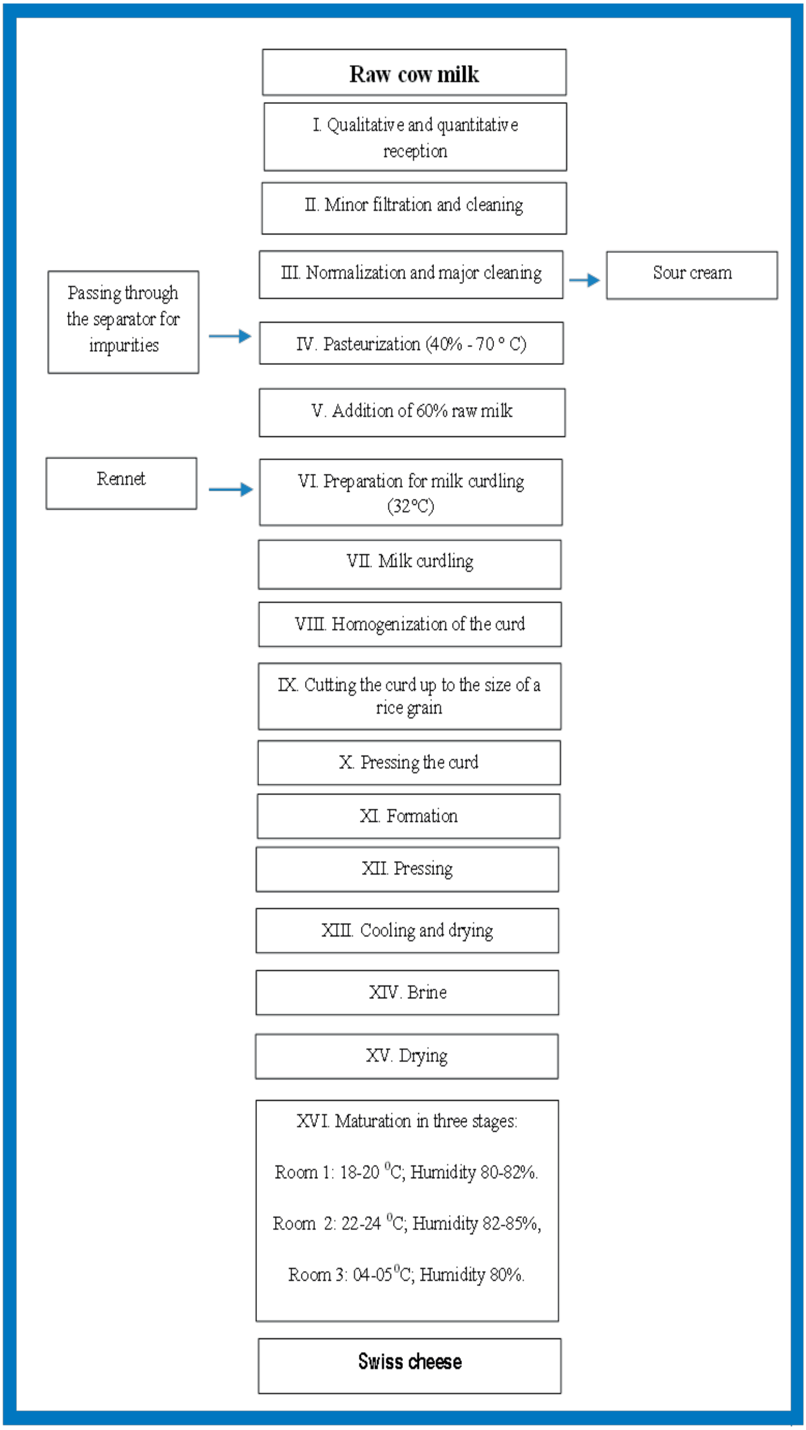


Figure 3. The stages of the technological flow used in the preparation of the Dorna Swiss cheese, including collecting the milk in cans and then reuniting it in double-walled stainless steel cauldrons; carrying out the processing procedures up to the finite product


Figure 4. Detailed pictures concerning the main stages of the technological flow of processing the Dorna Swiss cheese

Milk and dairy products play an important role in human nutrition, being considered a good source of essential biologically active nutrients. There is a wide variety of dairy products, especially cheeses, which sum up the organoleptic, physicochemical, and microbiological characteristics necessary to satisfy the needs and preferences of consumers. From this point of view, there are many assortments of cheeses, including those of the Emmental type, produced including in our country, which is characterized by specificity, differentiation, and classification. Based on these considerations, dairy products are classified into: conventional dairy products, obtained according to conventional recipes and technologies; traditional dairy products, produced in a certain geographical area, using specific recipes; organic dairy products obtained from raw materials from organic farming. This classification responds to the growing need to diversify dairy products and increase food quality and safety standards. All these categories of products have a common characteristic given by the raw material, which must include milk of the best compositional and hygienic-sanitary quality. The presented arguments fully justify the need for new research in this segment of the food industry, to evaluate the compositional and hygienic-sanitary parameters of the milk obtained in the conditions of a mountainous area. The diversity and composition of mountain pastures play a special role in the milk quality through their botanical composition. In this regard, Falchero et al. (2010) demonstrated the role and variation of fatty acids in milk and cheese obtained from cows that are grazed on mountain pastures. Another study has shown that the floristic diversity of mountain pastures is associated with environmental factors that influence the sensory characteristics of raw milk cheeses (De Noni and Battelli, 2008). In the case of cheeses in general and of the Emmental type in particular, the appearance, composition, texture, aroma, and taste are completed in the final stage of ripening (Kongo and Malcata, 2016; Mietton et al., 2018). An important role in the ripening of the Swiss cheese is played by maintaining an optimal temperature and humidity in the ripening and storage spaces (Ozturkoghe-Budak et al., 2017). The famous holes of this type of cheese are created by propionic bacteria, which during ripening consume lactic acid and release carbon dioxide. This forms the gas bubbles that form the well-known holes (Fröhlich-Wyder et al., 2017).

## CONCLUSIONS

Regarding the investigated milk samples, the total protein content showed mild variations (3.40-3.73%), while the fat content revealed important variations (3.10-5.94%). Moderate oscillations were recorded in lactose content and other compositional parameters.

The main hygienic-sanitary parameters, monitored for the evaluation of milk and mammary gland health, also showed low mean values, with variations of SCC (3.97-39.71 Cel./mL x 1000) and TBC (1.06-9.85 germs/mL x 1000). Overall, the compositional and hygienic parameters of milk did not show variations with a major impact on the producer, processor, and consumer.

Swiss cheese is part of the Emmental cheese category and it is specific to the Dorna area. This type of cheese is made only from milk obtained from mountain areas (natural meadows, hay), without the addition of juicy fodder such as silage. The ripening period is much longer than in any other type of cheese, which in time gives superior qualities from an organoleptic point of view. We consider that the main characteristics of Swiss cheese are given by the unique taste, aroma, and texture and especially by the abundance of the well-known holes, which distinguishes it from any other assortment of cheese.

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# OBTAINING AN ASSORTMENT OF FRESH CHEESE BY COAGULATION WITH LETTUCE (*LACTUCA SATIVA)* EXTRACT

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***Abstract***

*The aim of this work was to evaluate the milk-clotting potential of lettuce (Lactuca sativa). Two extracts from this plant were obtained, by dissolving in a cold water, first, by a simple aqueous extraction and the second, by a concentration of simple extract. These two extracts were compared in their milk-clotting activity with a commercial animal rennet, chymosin. The analysis consisted in the characterization of raw cow's milk used by physico-chemical and microbiological analyses and characterization of the fresh cheese curd obtained by physico-chemical, microbiological and sensory analyses. The yield of the process was calculated in each of the three cases and a comparison between them was performed. The obtained results revealed a good milk-clotting activity for both tested plant rennets.*

***Key words****: cheesemaking, Lactuca sativa, milk-clotting activity, plant rennet.*

## INTRODUCTION

Milk-clotting is the main stage of cheese production. It is made with milk-clotting enzymes that are prepared by proteolytic enzymes, this being the oldest application of them known for thousands of years.

The researches targeting the milk-clotting with the aid of plant coagulants has shown a growing interest in the industry of milk and dairy products, due to the easy availability of raw materials and simple extraction processes (Shah et. al., 2014). Another argument in the use of rennets obtained from plant sources is that the use of vegetable proteases in the process of obtaining cheeses promotes greater acceptability of this range of products from people with a vegetarian diet, to which are added certain benefits represented by the fact that they can improve their nutritional intake with various bioactive compounds from plant sources used in the process of milk coagulation.

The aim of this study was to evaluate the milk-clotting potential of lettuce (*Lactuca sativa*) and the possibility of using extracts obtained from this plant in dairy products such as cow's milk fresh cheeses. Leaves of lettuce have been indicated as a source of milk-clotting enzymes, which can substitute animal rennet (Derso & Dagnew, 2019).

Lettuce belongs to *Asteraceae (Compositae)* botanical family. The origin of the lettuce is quite controversial, most hypotheses claim that the appearance of the current form involved four species of European origin: *Lactuca sativa, L. saligna, L. serriola* and *L. virosa* (Kesseli et al., 1991). It has been cultivated since ancient times by the Egyptians, Greeks and Romans and is also popularly called lettuce. Lettuce is an annual plant with a short growing season. It is cultivated for its leaves and heads, which are eaten mostly fresh. The heads contain large amounts of vitamins (C, A, K, B complex), mineral salts (720 mg per 100 g, of which 234 mg potassium, 37 mg calcium, 24 mg phosphorus, 11 mg magnesium, the rest being iron and zinc) (Burzo et al., 2005), as well as significant amounts of sugars, polyphenols and cellulose. It is a low-calorie vegetable, being recommended in all diets.

The consumption of lettuce reduces the risk of heart disease, cancer and cataracts. Lettuce is very rich in vegetable fibers, which can significantly reduce cholesterol and prevent constipation. Also, it can induce a feeling of satiety much faster and thus help to lose weight or maintain weight within optimal limits. Lettuce is a remineralizing, purifying, emollient vegetable (Pârvu, 2006). The leaves can be eaten fresh, as salad, in early spring and autumn. The

storage temperature is around 1°C and the shelf life is usually two weeks (Lagunovschi-Luchian, 2014).
According with Shah et al. (2014), a serine protease named lettucine from *Lactuca sativa*, were identified with milk-clotting activity.

## MATERIALS AND METHODS

In order to perform the experiments, a series of materials were used to make the curd obtained from a plant source, such as cow's milk, salad extracts, as well as laboratory reagents, which were used to perform physico-chemical analyses of the curd samples, whey, but also of the raw milk, in two repetitions for each.

### Obtaining the plant extract

A comparative study was performed, in the simulation of the process of obtaining fresh cheeses, two extracts from *Lactuca sativa*, obtained as a crude extracts by dissolving the shredded and grinding plant in distilled water at cold: the first extract was obtained, as a supernatant, by filtration and centrifugation of the plant-water mix and, in the case of the second extract, the supernatant-base was concentrated by precipitation with ammonium sulphate, followed by centrifugal separation and dissolution of the precipitate in pH 5.5 citrate buffer solution. Ammonium sulphate precipitation of proteins is a widely used technique in enzyme purification, which takes advantages of the desolvation effect caused by high concentrations of salts (Duarte et al., 2009)
The preparation of cold plant extracts that were used as sources of vegetable curd in the coagulation of cow's milk in the process of fresh cheese production is shown schematically in figure 1 and figure 2, which represent the working protocol to obtain the simple crude extract of *Lactuca sativa,* at cold, respectively the working protocol to obtain the concentrated crude extract of *Lactuca sativa,* at cold.
In relation to a standardized product, the coagulation of milk with chymosin, the animal milk-clotting enzyme frequently used in industrial processes, was used as control sample. The commercial product used was CHY-MAX, manufactured by CHR.HANSEN.

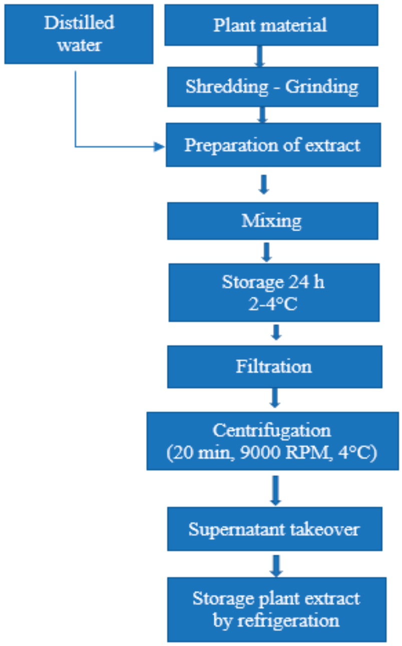


Figure 1. Working protocol used to obtain *Lactuca sativa* simple extract

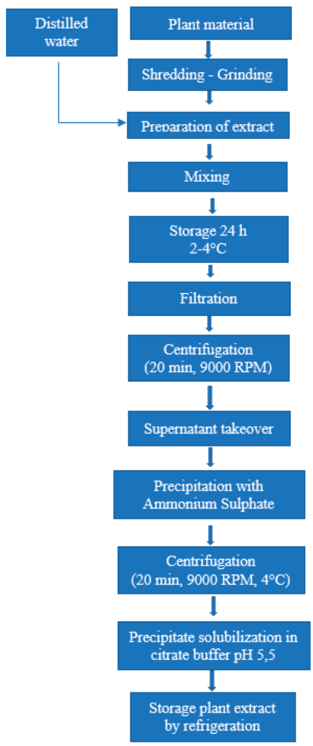


Figure 2. Working protocol used to obtain *Lactuca sativa* concentrated extract

**Physico-chemical and microbiological analyses of raw milk**

The raw milk was purchased from a free-market Farm Dispenser.

**Physico-chemical analysis**

**Determination of the physico-chemical properties of raw milk using the Ekomilk device**

The Ekomilk device is an automatic, fast and specially designed laboratory equipment used for the analysis of the main physico-chemical quality parameters of cow's, sheep's, goat's or buffalo's milk.

The Ekomilk device provides a large number of measurements, using ultrasound technology, and a 50 ml of milk is required to analyse all parameters. The milk samples must have a temperature between 5-35°C, the measurement time is 90 seconds, and the device analyses the fat, dry matter, density, protein and water added to the milk.

**Determination of pH**

Before performing the pH meter measurements, the instrument must be calibrated using standard buffer solutions. To determine the pH of the milk sample, the beaker was filled 2/3 full, with milk and the electrode was completely immersed. The value is read 15-20 seconds after immersion.

**Determination of ionic calcium in milk by complexometric method**

Calcium ion dosing is done by complexing with the disodium salt of ethylenediaminetetraacetic acid ($Na_2H_2EDTA$) or complexon III, in the presence of murexid as an indicator, (Campeanu et al., 1993).

**Dosing of milk casein**

The determination of casein is based on the principle of its precipitation at isoelectric pH (4.6 for cow's milk).

It is very important not to exceed the isoelectric pH value, as casein is resolubilized and the casein content of the sample is calculated from the amount of sodium hydroxide which participated in the complete solubilization of the casein (Campeanu & al., 1993, Căpriţă & Căpriţă, 2008).

**Dosing of reducing carbohydrates by the Schoorl method**

The Schoorl method can directly dose soluble reducing carbohydrates based on the redox reaction with Fehling's reagent. The amount of directly reducing carbohydrates in the sample is deduced from the tables which establish a correlation between the volume of sodium thiosulphate used in the titration and the directly reducing carbohydrates (Vasu et al., 1985).

**Microbiological analysis**

For the raw milk samples, microbiological analyses were performed on specific culture media.

**Total aerobic count**

This parameter refers to the quantification of the number of microorganisms that grow in aerobiosis, at 30°C, after incubation 72h ± 3h.

According to REGULATION (EC) NO. 853/2004 of 29 April 2004 laying down specific hygiene rules for on the hygiene of foodstuffs, the maximum Plate Count at 30 °C allowed by law is 100.000 CFU / ml for raw milk from dispenser.

**Coliform bacteria**

A sample is seeding using the technique of incorporating the inoculum into a Mac Conkey agar culture medium to count coliform bacteria. *Staphylococcus* can multiply in milk at high temperatures and produces enterotoxins retained by casein and found in cheeses made from contaminated milk. The culture media used for *Staphilococcus* determination was Manitol.

**Total combined yeasts and molds count (TYMC)**

Samples are performed according to the preparation standards specific to each matrix which provide for the preparation of samples for analysis, initial suspension and decimal dilutions for the microbiological examination, also complying with the provisions of SR EN ISO 7218: 2007 / A1: 2014.

Yeasts and molds can contaminate machinery and storage facilities in dairy plants.

**Testing of milk-clotting activity**

The research aim was to simulate the technological process of obtaining fresh curd cheeses (see Figure 3) and to establish the coagulation yield using two extracts of *Lactuca*

*sativa*, by comparison with the coagulation yield obtained with a commercial rennet based on chymosin.
The following simulation steps were as follows: (a) to pasteurize raw cow's milk, at 72°C, for 20 seconds and, immediately after, to cool it down to 35°C; (b) the milk-clotting enzyme is added to the pasteurized and cooled milk and it follows an incubation of 16 h at 30°C, stage in which the milk is coagulated; (c) after processing of the curd by cutting and stirring, light heating to 35°C, to favor the separation of the whey; (d) the last step is to separate the whey curd and evaluate the samples obtained (Figure 3).
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The notations used for the curd samples thus obtained were:
P1 - sample of milk + 0.025% chymosin CHY-MAX;
P2 - sample of milk + 10% simple aqueous extract of *Lactuca sativa*;
P3 - sample of milk + 0.81 % concentrated aqueous extract of *Lactuca sativa*;

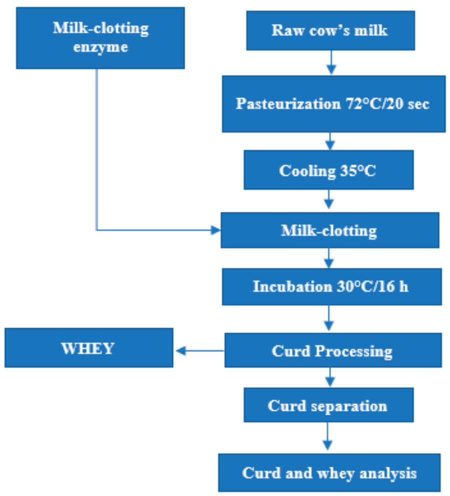


Figure 3. Working protocol used to obtain milk curd with salad extracts

**Determination of yield**
The calculation of the yield when the quantities of milk, raw material and cheese are known is obtained by the following formula:

$$R\ (\%) = (100 \times CB) / CL$$

In which:
CB - the amount of cheese (curd) obtained, in grams;
CL - the amount of coagulated milk, in liters.

**Physico-chemical analysis of the curd**

**Determination of the water activity index (aw)**
The curd sample is inserted into the thermostatic boxes of the determination device aw.
After 30 minutes of incubation, the sample is placed in the reading chamber of the device. When the aw value stabilizes, the values are read on the device screen.

**Determination of total dry matter**
Determination of dry matter by thermobalance is a fast and reliable method of determining the moisture content using the thermogravimetric principle. Thermogravimetry consists of weighing the sample before and after heating to determine the moisture content from the mass difference.
The sample is prepared at the time of the measurement. This prevents the exchange of moisture with the environment. The sample is evenly distributed in a thin layer on the weighing pan to obtain reproducible results. If the sample is applied unevenly, it will cause unhomogeneities in the heat distribution in the heated sample, resulting in incomplete drying or extending the measurement time.

**Microbiological analysis of the curd**
For the fresh cheese curd obtained, microbiological analyzes were performed on specific culture media.

**Total aerobic count**
This parameter refers to the quantification of the number of microorganisms that grow in aerobiosis, at 30 °C, after incubation 72 h ± 3 h.
According to REGULATION (EC) NO. 853/2004 of 29 April 2004 laying down specific hygiene rules for on the hygiene of foodstuffs, the maximum Plate Count at 30 °C allowed by law is minim $10^4$ CFU/g – maxim $10^5$ CFU/g for cheeses from raw milk.
Microbiological analyzes for the fresh cheese curd obtained, were performed on specific culture media, as described in 2.2.2 section.

**Organoleptic analysis of the curd**
The methods of sensory analysis used consist of the method of organoleptic analysis performed with a group of panelists and the method of color determination with a spectrocolorimeter.
*The method principle*. In order to perform the sensory analysis of cheese samples, certain

quality indicators are monitored, such as: appearance, color, texture, smell and aroma of the samples. The scoring system has been performed using the method by comparison with a unit scoring scale, from 1 to 5. The number of points awarded to each quality indicator is awarded on the scale described in Table 1.

Table 1. 5-point scale for assessing the quality of the curd (source: Banu, 2007)

| Quality appreciation step | Points number | General description of the degree of appreciation |
|---|---|---|
| Excellent | 5 | Excellent quality |
| Very good | 4 | Quality in full compliance with the specifics of the product |
| Good | 3 | Good quality, appropriate |
| Satisfactory | 2 | The product has slight defects that can be accepted |
| Unsatisfactory | 1 | The product has obvious, multiple and systematic defects |
| Altered | 0 | The product has significant defects and can no longer be consumed |

Next, the average score (Pm), which represents the arithmetic mean of the results of the evaluation by points of a sensory characteristic and the weighted average score (Pmp) assigned to each sensory characteristic, have been calculated using the relation below:

Pmp = Pm x fp or Pmp = Pm x fi x ft

In which:

fp - the weighting factor that represents the product between fi and ft;

fi - the factor of importance, which indicates the extent to which each sensory characteristic participates in the quality of the product (the sum of the factors of importance is equal to 1, and the values of the factors of importance are established for each product in the standards of sensory analysis);

ft - the transformation factor with the help of which one passes from the 5-point scale to the 20-point scale, in order to establish the quality of the product. The transformation factor is equal to 4.

Finally, the total average score (Pmt) has been calculated by summing the values of the weighted average scores from all sensory characteristics. The total average score is expressed to one decimal place (Banu, 2007).

Each quality indicator has its own weight in relation to the other indicators. The individual scores given to each sensory characteristic are recorded in the summary sheets, which are presented in Table 1.

### 2.7. Determination of color using a spectrocolorimeter

A HunterLab MiniScanTM XE Plus spectrocolorimeter was used to measure the color of the samples, for which the working conditions were: Geometry of the device: 45º / 0º; Viewing area: LAV; Illuminant: D65; Observer: 10º; Color system: CIELAB’76.

## RESULTS AND DISCUSSIONS

Coagulation of milk with vegetable milk-clotting enzymes is essential in the cheesemaking process. Previously, several plant sources (*Taraxacum officinale, Rumex acetosa, Lactuca sativa, Urtica dioica*) were used to study the coagulation capacity of milk (Nitu et al., 2021). These plants are found in the spontaneous and cultivated flora of Romania, so they were harvested and used in the form of aqueous extracts. When testing a potential replacement for animal rennet, it is particularly important to perform a milk coagulation test.

From the plant sources studied in the previous article, lettuce (*Lactuca sativa*) presented the best premises to be used as a substitute for commercial animal rennet.

### Results of physico-chemical determinations of cow's milk used as raw material

Table 2 shows the results of the physico-chemical analyses obtained for the cow's milk sample, using Ekomilk device and pH-meter, and also analyses obtained for the raw material milk sample, in terms of casein content, ionic calcium content and lactose content.

Table 2. Results of physico-chemical analysis of cow's milk used as raw material

| Sample | Analyzed parameters | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | pH | Fat (%) | Non-fat dry mater (%) | Density (g/cm³) | Water added (%) | Protein (%) | Casein (g/100 ml) | Ionic calcium (mg/100 ml) | Lactose (g/100 ml) |
| Raw cow milk | 6.7±0.1 | 3.66±0.1 | 8.2±0.1 | 1.028±0.001 | 0±0.1 | 3.2±0.1 | 2.87±0.01 | 326.6±0.1 | 4.56±0.1 |
| Reference values* | 6.4-6.7 | 3.5±0.1 | 8-8.5 | 1.027-1.033 | 0 | 3-3.2 | - | - | - |

*State Standard STAS 143-84 on the quality of raw cow’s milk

The results show that the raw milk used in the experiments is of a good quality, which is in line with current standards.
The determined casein content is optimal, as it must be at least 80% of the total protein content. At a protein content of 3.2 g / 100 ml milk, as shown in Table 2, the casein content should be at least 2.56 g / 100 ml milk. The result obtained, 2.87 g casein / 100 ml milk gives the auspices of a good coagulation yield, casein representing the substrate of the curd.
The ionic calcium content represents the ability of milk to form a three-dimensional structure during coagulation, through calcium bridges. According to Walstra et al., 2006, the minimum ionic calcium content is 120 mg / 100 ml. The determined calcium ion content, 326.6 mg / 100 ml, indicates a high potential for the three-dimensional structure of the raw milk used.
The determined lactose content is within the normal content of cow's milk 4.2-4.6 g / 100 ml (Walstra et al., 2006) and will influence the use of whey resulted from the cheesemaking process, as a substrate. for obtaining probiotic biomass.

**Microbiological analysis**

The results of the quantification of microbiological parameters are summarized in Table 3.

Table 3 Results of microbiological analyzes in the milk sample (CFU / mL)

| Microbiological indicator | Total number of aerobic germs (NTG) | TYMC (total combined yeasts and molds count) | ***Staphylococcus*** | Coliforms bacteria |
|---|---|---|---|---|
| Value | $4.3 \times 10^7$ | $4.9 \times 10^5$ | $4.3 \times 10^1$ | $2 \times 10^5$ |

Visual aspects of the results from the microbiological analyzes can be observed in Figure 4.

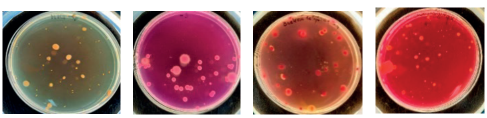

Figure 4. On-plate aspects of the results of microbiological analyses in raw milk (from left to right: NTG, TYMC fungi, coliform bacteria, staphylococci)

The results of the microbiological analyses, presented in Figure 4 and centralized in Table 6, give an overview of the quality of the raw milk.
Thus, regarding the Plate Count, the result obtained, 4.3 x 107 CFU / mL is higher than the one provided in REGULATION (EC) NO. 853/2004 of 29 April 2004 which establish specific hygiene rules for on the hygiene of foodstuffs, of 100.000 CFU / mL for raw milk, possible causes being faulty handling in the vending machine supply, quality of packaging provided; by using pasteurization in the treatment of milk intended to obtain the fresh cheese curd, the pathogenic germs will be eliminated.
The result obtained for the total number of yeasts and filamentous fungi (TYMC), $4.9 \times 10^5$ CFU / mL, indicates a contamination of raw milk with yeasts and molds, potentially obtained from the contact of milk with the atmosphere; these microorganisms are destroyed by a properly applied pasteurization regime.
It is observed that staphylococci were also detected, $4.3 \times 10^1$ CFU / mL, but no confirmatory tests were performed; their presence indicates, in principle, that the sample is not compliant, but by pasteurization these microorganisms will be destroyed.
The total number of coliform bacteria determined, $2 \times 10^5$ CFU / mL is an indicator of the degree of hygiene in which the milk was obtained and handled, the raw milk being non-compliant; these bacteria are also destroyed by a pasteurization regime.

**Testing of milk-clotting activity**

According to Lo Piero et al., (2002), lettucine from *Lactuca sativa* has the highest milk-clotting activity at 50°C. For these experimental tests, the decision to have the incubation for coagulation at 30°C was taken considering the intended comparison with the commercial animal rennet.
After coagulation and processing of the curd, they led to the following quantitative results, in Table 5. It is observed that in the case of the two salad extracts, the amount of curd obtained is higher by 45%, which represents a significant increase, which is also reflected in the calculation of yield. A first expression of the yield is expressed also in Table 5.
It is observed that the most efficient coagulation yield is obtained when using the concentrated lettuce extract, being approximately 23% higher than the yield obtained when using the animal

rennet and 45% higher than the yield obtained when using the gross lettuce extract.

## Physico-chemical characterization of the curd

### Determining water activity

The results of the determinations are presented in Table 4.

It is known that a lower value of the water activity index leads to an increase in the shelf life of the finished product. The results obtained from the determination of the water activity index of the cow's milk curd with the addition of animal rennet and the vegetable coagulants obtained from *Lactuca sativa* are presented in the Table 4.

Table 4. Total dry matter and water activity of the curd samples obtained

| | P1 | P2 | P3 |
|---|---|---|---|
| Total dry matter, % | 41.925 | 44.248 | 35.838 |
| Water activity, aw | 0.990 | 0.985 | 0.975 |

The sample of milk with animal rennet, registered the highest value of the water activity index, respectively 0.990. Also, sample 2 of milk with simple extract of *Lactuca sativa* registered a close value of 0.985, and sample 3 of milk with concentrated extract of *Lactuca sativa* had the lowest value of the water activity index, 0.975. Therefore, the data obtained for the three samples are comparable.

### Determination of total dry matter

The fresh cheese curd resulting from the three cases of the experimental determinations was recorded with the total dry matter contents shown in Table 4.

The resulting curd quantities, with the dry matter content described above, were recalculated to a standard 40% fresh cheese dry matter content. The physical yield of the cheeses will be recalculated in the Table 5, resulting in a recalculated standard yield.

Table 5. Quantities of cheeses obtained in the tests – Physical and Recalculated standard yield

| Data/Sample | P1 | P2 | P3 |
|---|---|---|---|
| CB, kg | 0.295 | 0.500 | 0.725 |
| CL, liters | 2 | 4 | 4 |
| Physical yield, Rphy, % | 14.75 | 12.5 | 18.125 |
| Total Dry Matter, % | 41.925 | 44.248 | 35.838 |
| CB Standard Recalculated at 40% dry matter kg | 0.309 | 0.553 | 0.650 |
| Recalculated yield, Rrec, % | 15.460 | 13.828 | 16.239 |

The results obtained show that fresh cheese obtained by coagulation with concentrated extract of *Lactuca sativa* had the highest yield, being 5% higher than the yield of fresh cheese by coagulation with commercial rennet CHY-MAX and 17.5% higher than the yield obtaining cheese by coagulation with simple extract of *Lactuca sativa.*

The addition of the source of concentrated vegetable coagulant enzyme from *Lactuca sativa* in cow's milk leads to the lowest value of the water activity index, which implies an increase in the shelf life of the finished product. The curd sample obtained with the addition of simple extract from *Lactuca sativa* also reached a lower value of the aw index than the curd obtained from the milk sample in which a quantity of chymosin was added, which recorded the highest value. of the water activity index.

Following the determination of the dry matter for the curd samples obtained, it was found that the milk sample in which the concentrated extract of *Lactuca sativa* was added had the lowest dry matter content and therefore the lowest energy value.

## Microbiological characterization of the curd

The curd samples were analysed 5 days after obtaining, being kept in refrigeration conditions. The values of the microbiological parameters of the three samples are detailed in Table 6.

Table 6. Values analysed in curd samples

| Sample/ Microbiological indicator | Total number of aerobic germs (NTG) | ***Staphylococcus*** | Coliforms bacteria |
|---|---|---|---|
| P1 Milk + chymosin | $6.75 \times 10^{7}$ | 0 | $2.15 \times 10^{6}$ |
| P2 Milk + simple extract of lettuce | $2.8 \times 10^{10}$ | 0 | $2.92 \times 10^{9}$ |
| P3 Milk + Concentrated extract of lettuce | $7.60 \times 10^{9}$ | 0 | $6.7 \times 10^{8}$ |

Visual aspects of the microbiological analysis can be seen in Figure 5 for Aerobic colony count, respectively Figure 6 for coliforms.

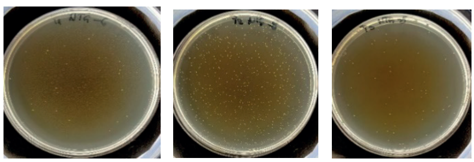

Figure 5. Visual aspects of Aerobic colony count analysis in different curd samples (P1, P2 P3)

The results of the microbiological analyzes performed on the curd samples obtained give an overview of the fresh cheese manufacturing process and storage conditions.
In the case of aerobic colony count it is observed that by the addition of the curd of vegetal origin the total load is higher by 2-3 logarithmic units in relation to the milk coagulated with enzyme of animal origin.

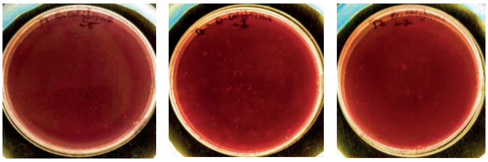

Figure 6. Visual aspects of coliform analysis in different curd samples (P1, P2 P3)

Staphylococci were not present in any of the s analysed sample, thus the samples being compliant. In the case of total coliform bacteria it is observed that the addition of plant extract increases the contamination by 2-3 logarithmic units.
The source of the contamination may be due to the defective microbiological quality of the plant extract and the inadequate handling of the material. It is recommended that this extract also undergo sterilizing filtration so that microbiological indicators are kept within the limits of admissibility.
The results obtained following the microbiological analyses indicates a contamination of the three curd samples after at least 5 days of storage in refrigeration conditions, which indicated the highest values in the case of sample P2, coagulated with simple *Lactuca sativa* extract, followed by those of sample P3, coagulated with concentrated extract of *Lactuca sativa*.
The explanation of this phenomenon lies reside even in the process of concentration, by precipitation and centrifugation, by removing the supernatant can be removed some of the microbiological contaminants.
The next concern in the process of obtaining plant coagulant extracts should be the treatments applied in order to remove contaminants.

**Organoleptic characterization of the curd**
Cheesemaking is a more complex process that involves concentrating protein along with a variable fraction of fat and minerals, eliminating a significant amount of water and lactose (Costin, 2003).
Sensory determination of the samples of milk coagulated with milk-clotting enzyme from the same plant source, *Lactuca sativa*, obtained simply or concentrated, by comparison with the sample of milk coagulated with commercial chymosin, consisted in the use of two methods, described in the materials and methods section, respectively of scoring quality indicators according to sensory analysis and determination of the colour of the curd samples using the Hunterlab spectrocolorimeter.
The sensory analysis was performed by a sensory panel of 20 people (staff and students from the Faculty of Biotechnology). The characterization of the tasting team is described in Table 7. It could be noticed that the panel consisted of people of different ages (between 10 and 55 years old). and the females were predominant (70%); meanwhile, in the group 35% were smokers.

Table 7. Characterization of the sensory panel

| Gender | | Female | Male |
|---|---|---|---|
| Ratio, % | | 70,00 | 30,00 |
| Smokers, % | | 42.86 | 16,67 |
| Age, % | 0-20 years | 57,14 | 16,67 |
| | 20-30 years | 14,29 | 16,67 |
| | 40-50 years | 14,29 | 33,33 |
| | >50 years | 14,29 | 33,33 |

The sensory analysis performed on the appearance of the fresh cheese curd, which should have a well-shaped shape. The analysis of the consistency or texture of the curd is intended to be elastic, compact, and the colour is characteristic, white and glossy, almost uniform in the same container. The smell of the analysed samples must be pleasant, specific to fermentation, without foreign odour. The appearance of the analysed whey should be clear or slightly opalescent.
The sensory description of the curd and whey samples resulting from the experiments is presented in Table 8.

Table 8. Sensory description of curd and whey samples

| | | | |
|---|---|---|---|
| Sample P1 | | | |
| Characteristics | Curd | Appearance | Firm consistency, with the appearance of porcelain in the section, easy expulsion of whey, without gas bubbles |
| | | Colour | White |
| | | Taste | Pleasant, specific |
| | | Smell | Pleasant, specific |
| | Whey | Appearance | Clear, slightly opalescent |
| | | Colour | Yellow-greenish |
| | | Taste | Sour, pleasant |
| | | Smell | Pleasant, specific |
| Sample P2 | | | |
| Characteristics | Curd | Appearance | Friable consistency, sandy appearance, compact, without whey expulsion, gas bubbles in section |
| | | Colour | greenish white |
| | | Taste | Pleasant, specific, slightly sour |
| | | Smell | Pleasant, specific |
| | Whey | Appearance | Opalescent, fat separation on the surface |
| | | Colour | Yellowish |
| | | Taste | Sour, pleasant |
| | | Smell | Pleasant, specific |
| Sample P3 | | | |
| Characteristics | Curd | Appearance | Suitable consistency, slightly soft, slightly sandy appearance, with whey expulsion, rare gas bubbles in section |
| | | Colour | Greenish white |
| | | Taste | Pleasant, specific, slightly sour |
| | | Smell | Pleasant, specific |
| | Whey | Appearance | Slightly opalescent, slightly greasy on the surface |
| | | Colour | Yellowish |
| | | Taste | Sour, pleasant |
| | | Smell | Pleasant, specific |

The centralized results of Total average score (Pmt), following the processing of the data obtained from the tasting team are presented in Figure 7.

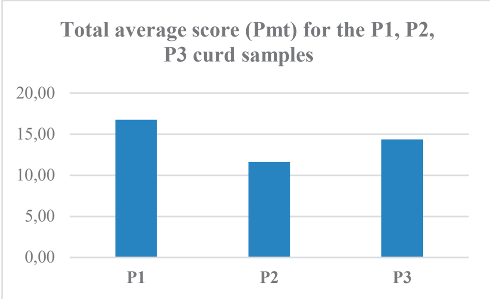


Figure7. Graphical representation of Total average score (Pmt) for the P1, P2, P3 CURD SAMPLES

The results obtained from the sensory analysis of the tasted curd samples suggest that P1 sample of milk with chymosin was preferred, being the sample that obtained the highest average total score, respectively 16.74.
This was followed by P3 milk sample with concentrated *Lactuca sativa* extract which obtained a total average score of 14.36 and P2 milk sample with simple *Lactuca sativa* extract, which obtained the average total score of 11.62. The degree of organoleptic appreciation of the fresh cheese curd obtained with concentrated extract of *Lactuca sativa* is quite close to that obtained with commercial rennet.

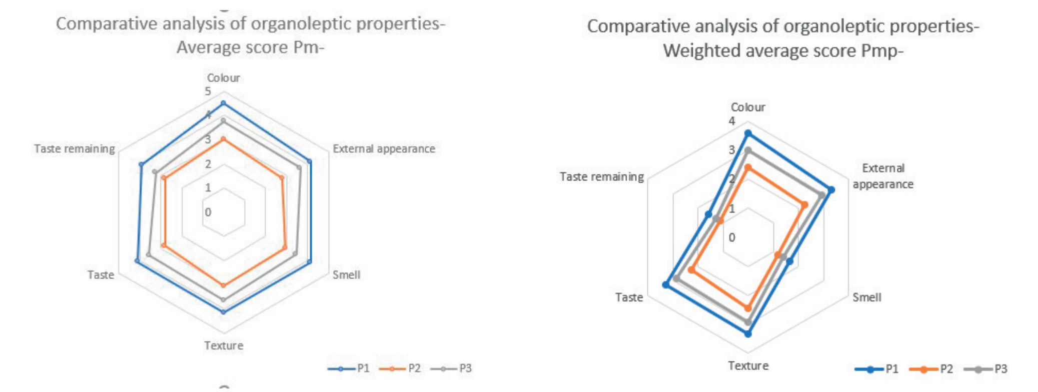


Figure 8. Graphical representation of the sensory characteristics of the curd (Pm-Average score and Pmp-weighted average score)

From the analysis of the graphical representations of the average scores and the weighted average scores obtained after the tasting session of the three samples of fresh cheese curd (Figure 8), it is observed the quite clear delimitation of the preferences for sample P1 (milk coagulated with chymosin), closely followed by sample P3 (coagulated milk with concentrated *Lactuca sativa* extract), sample P2 (coagulated milk with simple *Lactuca sativa* extract) being on the last place in the tasters' preferences.

**Determination of colour using a spectrocolorimeter**

The samples of fresh cheese curd obtained in the experimental determinations were analysed, both immediately after obtaining and at 5 days.

The results obtained from the colour determinations, with the help of the HunterLab spectrocolorimeter, of the curd samples, respectively the numerical values are presented in graphical form in Figure 9.

As can be seen, the colours of the analysed curd samples are very close, located grouped in the yellow spectrum of the analysis.

Following the physico-chemical determinations carried out on the raw cow's milk used as raw material for the preparation of the curd, a very good quality and freshness was found, and the values recorded were within the limits provided by the standards in force.

The curd obtained from milk treated with concentrated extract of *Lactuca sativa* had a higher production yield than the curd obtained from milk treated with commercial rennet chymosin. The lowest yield was obtained in the case of the curd obtained from milk treated with simple extract of *Lactuca sativa*.

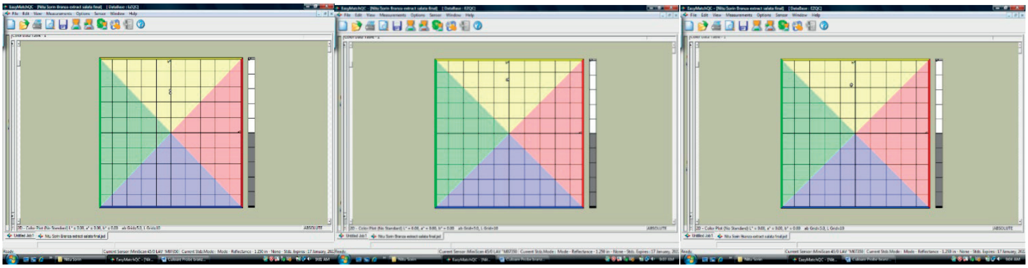

Figure 9. Graphical representation of colorimetric analyses for curd samples analysed at baseline and after 5 days (a-P1; b-P2; c-P3); From left to right: a. Sample P1 - initial (top point), after 5 days (bottom point); b. Sample P2 - initial (top point), after 5 days (bottom point); c. Sample P3 - initial (top point), after 5 days (bottom point)

## CONCLUSIONS

The data obtained from the physico-chemical analyses of the raw milk and the final product aswaell as the results from organoleptic analyzes of the curd samples with the addition of *Lactuca sativa* plant extracts, obtained differently, led to promising results in terms of quality and preference of the finished products. The same cannot be said for microbiological indicators, as milk was not compliant. Pasteurization can solve these microbiological issues.

In the milk sample in which a simple extract of *Lactuca sativa* was added, a very well separated curd was formed from the whey, with the highest dry matter content of the three curd samples obtained.

Regarding the yields obtained from the technological simulations performed, in the first phase the physical yield was expressed, by reporting the quantities of physical curd obtained to the volumes of milk raw material used.

The recalculated standard manufacturing yield, performed at 40% dry matter, is an important indicator of the milk-clotting capacity of the plant enzymatic extracts, providing a more accurate picture of the efficiency. For instance, the curd obtained from milk treated with concentrated *Lactuca sativa* extract had a 5% higher manufacturing yield than the curd obtained from milk treated with commercial rennet-chymosin. The lowest yield was obtained in the case of the curd obtained from milk treated with simple extract of *Lactuca sativa,* less than 17.5% than that obtained with concentrated extract of *Lactuca sativa.*

The comparative analysis of the organoleptic properties of the three curd samples clearly indicates the preferences of the tasting team, the P1 sample being preferred to the P3 sample in terms of all sensory characteristics. The P2 test was on the last place in the preferences of the tasting team in terms of all the sensory characteristics.

The studied *Lactuca sativa* plant extracts have shown that they have the potential to coagulate cow's milk and can be used successfully as a milk-clotting enzyme from the vegetable source in the process of obtaining fresh cheeses.

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# RESEARCH ON THE INFLUENCE OF LACTATION STAGE ON GOAT'S MILK CHARACTERISTICS

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***Abstract***

*Goat's milk is a very important food for human nutrition and is also an important raw material for a whole series of dairy products. This paper aims to investigate the qualitative variations of goat's milk during the lactation period. For this purpose, milk samples at different times of the lactation period were collected and analysed, such as: density, total dry matter, percentage of fat, protein substances and lactose. The obtained results, compared to the second month of lactation, showed significant decreases (p<0.05) in milk production with 9.9% in the fourth month and with 42.84% in the eighth month. Significant decreases were also obtained in the case of milk density with 0.38% in the fourth month and in the case of milk total dry matter with 9.53% in the fourth month and with 10.42% in the sixth month. Regarding the percentage of fat, significant decreases were observed (p<0.05) for the fourth months (with 18.68%) and for the sixth month (with 20.94%), compared to the second month of lactation. Significant decreases (p<0.05) in milk protein were also observed in the fourth months (with 10.68%), in the sixth month (with 9.50%) and in the eighth month (with 5.7%), compared to the second month. Regarding the percentage of lactose, significant increases were observed (p<0.05) for the fourth months (with 0.91%) and for the eighth month (with 1.37%).*

***Key words***: *fat, goat, lactose, milk, proteins.*

## INTRODUCTION

Goat breeding is an activity with a tradition in Romania country, meeting in the past mainly in peasant households. Today, due to the growing consumer interest in goat's milk and dairy products from goat's milk obtained in proper hygiene conditions (Răducuță, 2011; Gonciarov et al., 2004; Oprea et al., 2019; Petcu et al., 2020), goat farming is intensive, with an increasing trend in the number of farms. Goat's milk is superior to cow's milk due to its nutritional, toning, antirachitic, antianemic and anti-infective effects (Oprea et al., 2020; Petcu et al., 2021). Because fat globules are very fine, goat's milk is easily absorbed by intestinal villi with higher digestibility (Savu & Petcu, 2002). Compared to cow's milk, goat's milk is richer in minerals and has a high content of vitamin A, and is especially indicated in the diet of children and the elderly (Petcu et al., 2020). As goats rarely get tuberculosis, goat's milk can be consumed raw, thus maintaining its maximum effectiveness in proteins, lipids, sugars, vitamins, enzymes and mineral salts.

Due to its varied and rich chemical composition, milk provides the body with the substances necessary for the metabolic processes that take place in the body (Răducuță, 2004; Răducuță et al., 2015; Oprea et al., 2019).

Particular attention must also be paid to food safety, from obtaining to packaging of dairy products (Visoescu et al., 2015; Petcu, 2006).

Goat's milk is considered superior to cow's milk because it is easier to digest (its consumption rarely causes pathological conditions such as allergies and digestive intolerance), and is also richer in minerals and trace elements (Savu & Petcu, 2002). Organoleptically, goat's milk is easily recognizable by its light white colour, as well as by the fact that it does not need to be homogenized, because the fats it contains are evenly dispersed. Goat's milk is also characterized by its sweet taste and characteristic odour (Savu & Petcu, 2002).

The quality of goat's milk is influenced by several factors, among which we can mention: breed, age, health (general health and udder health), gestation, feeding regime (Răducuță et al., 2015). Also, the quality of milk may vary depending on the season in which the calving take place, this being directly influenced by the onset oestrus (Douhard et al., 2013; Bociu et al., 2015; Ghiță & Cotor, 2019).

At present, the consumption of goat's milk is a much healthier alternative to the consumption of cow's milk (especially when it is fresh and organic), offering a wide variety of health benefits (Escareño et al., 2012). The aim of this research is to evaluate the quantitative and qualitative evolution of goat milk production, during a lactation stages.

## MATERIALS AND METHODS

In our study, we wanted to observe how the quantity and quality of goat's milk changes during lactation period. For this purpose, we harvested milk samples every first day of the month of lactation that interested us, in order to obtain data on the quality of goat's milk at the beginning, middle (in two moments) and at the end of the lactation period. In this way, the milk samples used in our determinations were harvested in the 2nd, 4th, 6th and 8th months of lactation, taking into account the following parameters: quantity, milk density, the percentage of fat, total dry matter, the percentage of protein and the percentage of lactose. The milk samples harvested were rapidly refrigerated at 4°C and transported immediately to the laboratory for processing.

The milk quantity was determined using a graded cylinder. The milk density was determined with a lactodensimeter.

The percentage of fat was determined using the acid-butyrometric method (Gerber method).

The determination of the total dry matter was made using the following formula, were:

$$S.U.T. = (1,2xG) + 266,5\,\frac{D-1}{D} + 0,5$$

G - the percentage of fat;

D - the density of milk at 20°C;

1.2 and 266.5 – coefficients;

0.5 - correction factor for the density determined at 20°C.

The determination of the percentage of protein substances was done by the titration method.

Lactose was determined using the potassium ferricyanide method (Savu & Petcu, 2002).

The biological material was represented by a herd of 10 goats (*Carpathian* breed), from which a total 40 samples were collected. These animals were individualized and benefited from normal maintenance conditions.

During the winter, the goats were kept in the shelter and fed with dry fodder (coarse) and feed concentrates, and during the warm season, they were taken to grassland and fed with grass and concentrates (in the morning and in the evening). Comparisons on the statistical relevance of differences between obtained values were processed using the t-Student test.

## RESULTS AND DISCUSSIONS

The results obtained will be presented in the form of Table 1 and figures as follows: determination of milk quantity, determination of milk density, determination of total dry matter, determination of fat percentage, determination of protein percentage and determination of lactose percentage during lactation period.

The values obtained will be presented in the form of average values.

Comparisons are made with the values recorded in the second month (beginning of lactation).

Changing the food regime by increasing the amount of green mass in the diet, explains the high milk production during the summer, while its decrease causes a decrease in the amount of milk during the autumn season. Another explanation for the change in milk production is the intervention of neuro-hormonal changes that occurred with the decrease of daylight (Codreanu, 2020).

The results regarding the quantity obtained in the four experimental lactation periods are presented in Table 1 and Figure 1.

Comparisons (values recorded in lactation months 4, 6 and 8) were made with values recorded in the 2nd lactation month).

Analysing the data presented in Table 1 and Figure 1, it is observed that the highest amount of milk is obtained in the fourth month (middle of lactation), it is maintained at a high level until the sixth month, after which it decreases (end of lactation). The results indicate a significant decrease in milk production in the sixth month (9.9%) and eighth month (42.84%), compared to second month of lactation.

Table 1. The values of the parameters analysed during the lactation period (mean and standard deviation)

| Parameter | 2nd month of lactation | 4th month of lactation | 6th month of lactation | 8th month of lactation |
|---|---|---|---|---|
| **Quantity (ml)** | 1974.67 ±84.482 | 2180.69 ±102.240 | 1779.15 ±73.821 | 1128.2 ±64.521 |
| **Density** | 1.033 ±0.002 | 1.029* ±0.003 | 1.031 ±0.002 | 1.032 ±0.003 |
| **Total dry matter** | 13.42 ±1.42 | 12.14* ±1.86 | 12.02* ±2.11 | 13.28 ±1.24 |
| **Fat (%)** | 4.87 ±0.55 | 3.96* ±0.64 | 3.85* ±0.82 | 4.94 ±1.22 |
| **Protein (%)** | 4.21 ±0.14 | 3.76* ±0.74 | 3.81* ±0.42 | 3.97* ±1.11 |
| **Lactose (%)** | 4.35 ±0.28 | 4.39* ±0.42 | 4.37 ±0.31 | 4.41* ±0.53 |

*P<0.05

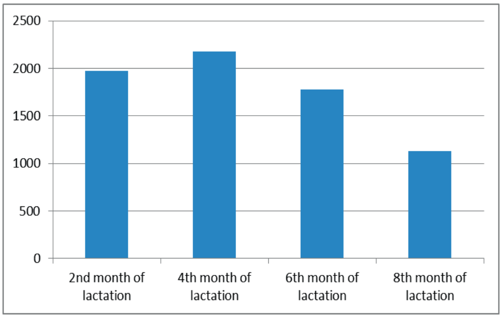


Figure 1. Variation of the average of milk quantity in the four experimental moments

These results are explained by the change in diet (increasing the amount of green mass in the diet, explains the large production of milk during the summer, while its decrease causes a decrease in the amount of milk). Another explanation (Codreanu, 2018) is represented by the intervention of neuro-hormonal changes that appeared with the decrease of the light day duration.

The results regarding the **milk density** obtained in the four experimental lactation periods are presented in Table 1 and Figure 2.

Table 1 shows that the highest value of milk density was recorded in the second month (the beginning of lactation) and the lowest value was recorded in the fourth month (middle of lactation), while in the sixth months and eighth (towards the end of lactation), the value of milk density was between the two limits.

The results indicate a significant decrease (p <0.05) in milk density in the fourth month of lactation (0.38%), compared to the second month of lactation.

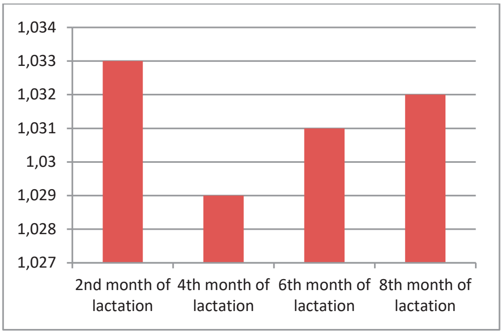


Figure 2. Variation of the average density value of milk in the four experimental moments

This observation can be explained by changing the food regime, feeding with green fodder, causing an increase in the milk quantity, to the detriment of the decrease in its density, a fact also reported in the literature consulted (Cotor et al., 2011).

For the **total dry matter** over the four experimental lactation periods, the results obtained are presented in Table 1 and Figure 3.

Analysing the results obtained, it was observed that the highest percentage of total dry matter is obtained at the beginning (13.42%) and the end of lactation (13.28%), while in the middle of lactation this percentage is lower (varies between 12.14 % and 12.02%).

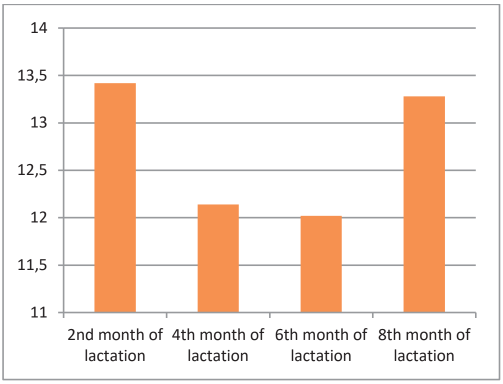


Figure 3. Variation of the percentage of total dry matter in the four experimental moments

The results indicate a significant decrease (p<0.05) in the total dry matter of milk in the fourth months (9.53%) and sixth months (10.42%), compared to the second month of lactation. These results are similar to those found in the literature consulted (Antunac et al., 2001; Cotor et al., 2015) and are due to the feeding of animals during the summer, which in the case of our group corresponds to the middle of the lactation period.

The results regarding **the percentage of milk fat** in the four experimental lactation periods are presented in Table 1 and Figure 4.

Analysing the data, it was observed that the percentage of fat had the highest value towards the end of lactation (4.94%) and at the beginning of lactation (4.87%), while the lowest values were recorded in the middle of the lactation period (3.96% and 3.85%).

The results obtained indicate a significant decrease ($p<0.05$) in milk fat in the fourth months (18.68%) and sixth months (20.94%), compared to the second month.

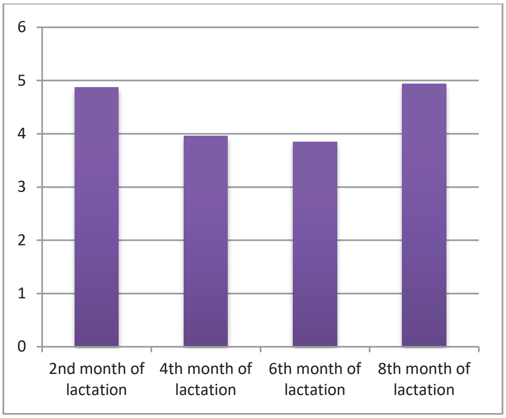


Figure 4. Variation of fat percentage in the four experimental moments

The explanation of these results is represented by the consumption of coarse fodder, being known that their consumption influences the concentration of fatty acids in milk (Cotor et al., 2009; Codreanu, 2018).

The results on **the percentage of milk protein** in the four experimental lactation periods are presented in Table 1 and Figure 5.

According to the results obtained, it was observed that the highest percentage of protein is recorded at the beginning of the lactation period (4.21%), while in the other lactation periods, the percentage of milk protein is less than 4% (3.76% and 3.81%, in the middle of the lactation period, respectively 3.97%, at the end of the lactation period).

The results obtained by us, indicate a significant decrease ($p<0.05$) of milk protein in the fourth months (10.68%), in the sixth months (9.50%) and in the eighth months (5.7%), compared to the second month of the lactation period, fact explained by the functional status of the mammary gland (Cotor et al., 2012) and the feeding regime of the studied periods (Bălăceanu et al., 2017).

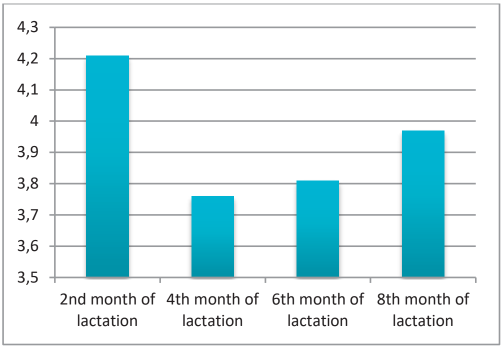


Figure 5. Variation of protein percentage in the four experimental moments

Regarding the **percentage of lactose in milk** in the four experimental lactation periods, the results obtained are presented in Table 1 and Figure 6.

Analysing the data obtained, we notice that its percentage varied quite a bit, with values between 4.35% (at the beginning of the lactation period) and 4.41% (at the end of the lactation period).

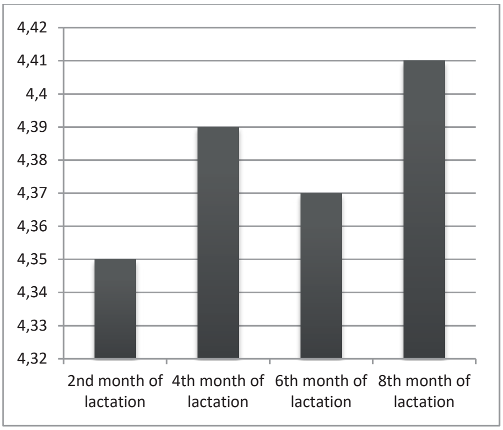


Figure 6. Variation of the percentage of milk lactose in the four experimental moments

The results indicate a significant increase ($p<0.05$) in the milk lactose in the fourth month (0.91%) and eighth month (1.37%), compared to the second month. These data do not coincide with the results obtained by other researchers in the field, constituting a particular aspect, resulting from the specific conditions in which the experimental protocols in our work were conducted.

## CONCLUSIONS

Milk production marked a significant decrease in the sixth month (9.9%) and eighth month (42.84%) compared to the second month.

Milk density decreased significantly in the fourth month (0.38%), compared to the second month of lactation. The total dry matter in milk registered a significant decrease in the fourth (9.53%) and sixth months (10.42%), compared to the second month of lactation. The percentage of fat in goat's milk decreased significantly in the fourth (18.68%) and sixth months (20.94%), compared to the second month of lactation. The percentage of milk proteins showed a significant decrease in the fourth month (10.68%), sixth month (9.50%) and eighth month (5.7%), compared to the second month of lactation. The percentage of lactose in goat's milk marked a significant increase in the fourth (0.91%) and eighth months (1.37%), compared to the second month of lactation.

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# ANTIBIOTIC RESIDUES IN MILK AND ASSESSMENT OF HUMAN HEALTH RISK IN ROMANIA

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***Abstract***

*Antimicrobial resistance is expected to make many more victims in the future than cancer, which requires constant, rigorous and thorough control of all factors that contribute to the growth of this particularly worrying phenomenon. Animal products and in particular milk, especially the one obtained in the households of the population, could be especially contaminated with antibiotic residues. The present paper aims at the qualitative and quantitative screening of 284 samples sold by individual producers from the entire territory of Romania, in a period of 3 months (January-March this year). The qualitative test was a microbiological test in tubes with spores of Geobacillus stearothermophilus. 6.33% (n = 18) of the samples confirmed positively in the qualitative test, the quantitative test performed using chromatography showed the presence of beta-lactams and tetracycline in quantities exceeding the maximum residue limit (MRL), (21, 50 µg / kg milk for penicillin and on average 115 µg / kg milk for oxytetracyclines). Among aminoglycosides, gentamicin was on average 294.5 µg / kg milk far exceeding the MRL.*

***Key words****: antibiotic residues, health, milk, Romania.*

## INTRODUCTION

Even though antibiotics have been banned from being used as growth promoters in animals, certain conditions still require such medications. Excessive and inappropriate use even for such conditions causes a transmission of these drug residues in organs, muscles and other animal products such as eggs and milk. This phenomenon contributes to the spread of antimicrobial resistance, a worrying and burdensome phenomenon of health systems around the world (Bassetti et al., 2020). Antibiotic residues in food have been reported in several recent studies (Tiseo et al., 2020; Treiber et al., 2021). Eggs (Mbodi et al., 2014; Nonga, et al.,2010), fish (Wang et al., 2019, Gaspar,et al., 2019), meat (Al-Ghamdi et al., 2000; Er et al., 2013; Ezenduka, 2019; Monger et al., 2021), seafood and especially milk (Ondieki et al., 2017; Pogurschi et al., 2015; Prado et al., 2015; Vragović et al., 2011) from various parts of the world have been reported as foods in which antibiotic residues were found. Milk, in particular, is a food consumed by vulnerable age groups such as children and the elderly. In addition, milk is also a source of protein and minerals essential for childhood and old age. Moreover, milk has an affordable price, which makes it present in today's diet almost daily or several times a day. All these considerations lead to the conclusion: milk placed on the market must be a safe product, without contaminants and drug residues, especially antibiotic residues. Previous research has shown the presence of different groups of antibiotics in milk in a well-defined legal context. All these considerations, together with the major health risks of antibiotic residues, make the monitoring of the hygienic quality of milk continuous and the monitoring systems to be correlated and upgraded periodically according to the reported determinations. The eating habits of the Romanian population are constantly changing (Iordachescu et al., 2020), and the source of acquisition has become increasingly important. Family producers, suppliers of agri-food products are in the preferences of the modern consumer. However, the lack of monitoring of their products has negative consequences on health; this is unfortunately known by few consumers. The present study presents the results of the determinations of the presence of antibiotics in milk marketed by Romanian individual producers in the first 3 months of 2022.

## MATERIALS AND METHODS

Out of a total of 300 milk samples collected from individual producers, 284 samples were subjected to determinations. The rest of the samples did not present an optimal condition to be analysed, probably due to the conditions during the transport to the laboratory. 250 samples tested negative for qualitative testing for antibiotics. A number of 16 tests had uncertain results, being false positive, which is why they were excluded from the following determinations. Only 18 samples were confirmed positive. The period analysed in this study was January - March 2022.

*The qualitative screening*

The qualitative screening of antibiotics was based on their inhibitor action against the in vitro growth of the bacterium *G. stearothermophilus* at 64 ± $0.5^{0}$C. In the determination tube containing spores of the bacterium *G. stearothermophilus* preheated to the mentioned temperature 1 ml of milk sample was placed. The sample was left at the room temperature for 20 minutes for the possible present antibiotic to action. The tube with the milk sample to be analysed was reintroduced in incubator at 64 ± $0.5^{0}$C for 3 - 3.5 hours for the second incubation. The colour differences between the milk sample tube and the control tubes represented the confirmations of the presence of antibiotics in the analysed milk samples (Figure 1).

*The qualitative screening*
*Equipment and reagents*

The equipment used were: Boeco centrifuge C-28A (Germany), ABI Digital Vortex - Genie 2 vortex mixer (USA), Elmasonic S 50 R ultrasonic bath (Germany), Heidolph Rotary Evaporator Laborota 4000 (Germany), Kern Analytical balance ABJ 220-4NM (Germany), Agilent 1260 Infinity II LC System (Germany), B 30 Water purification system ADRONA (Latvia).

Figure 1. Positive confirmed milk samples

The chromatographic grade reagents were: methanol (Honeywell Burdick & Jackson, USA), acetonitrile (Baker, Mexico), and triethylamine (Vetec, Brazil). The grade reagents were citric acid (Reagen, Brazil), EDTA disodium salt (Labsynth, Brazil), oxalic acid crystal (Dinamica, Brazil), anhydrous dibasic sodium phosphate (Nuclear, Brazil), and trichloroacetic acid (Reagen, Brazil). Ultrapure water for solutions preparation was obtained in the laboratory using B 30 Water purification system ADRONA (Latvia).

The study focused on the main groups of antibiotics that are widely used in both dairy and human medicine – B-lactams (Penicillin), Tetracycline (Oxytetracycline) and Aminoglycosides (Gentamicin). The operating procedures for storage and handling of the standards have been followed according to the manufacturer's instructions. Stock solutions were prepared by dissolving standards in methanol by using class A glassware (Final volume 25 ml) so that effective concentration remained more than 100 µg/mL. Standard solutions of different concentrations were stored in a deep freezer at -18°C. For preparation of standard solutions, the maximum residue limits (MRLs) prescribed by European Union Commission (EU, 2010) and Codex Alimentarius Commission of WHO (Codex, 2015) for all antibiotics were considered. Based on these MRL values, a linearity range (50, 100, 150, 200, 250 µg/kg) was selected to cover the lowest MRLs for all the analyte molecules.

*LC instrumentation and condition*
Agilent, 1260 Infinity II LC system used to determine milk antibiotics was equipped with: 1260 vialsampler and 1260 Flexible pump connected to C18 column (Poroshell 3.0 mm x 100 mm porosity 2,7 um) housed in 1260 MCT column oven with ELSD 1260 Infinity detector was used throughout the experiment. The system was controlled by Open Lab CDS 2.6 Software. The working technique was adapted to determine the three antibiotics studied - penicillin, oxytetracycline and gentamicin at sub MRL levels. The method was validated for specificity, precision, recovery and linearity. The extracted samples were centrifuged for 20 minutes at 3000 rpm in Eppendorf tube followed by filtration using 0.2 nm MFS filters. The final extracted samples were set to run in the LC system described above.

*Risk assessment*
To assess the risks associated with the presence of the three antibiotics determined in milk consumed the following equations were used in accordance with Juan et al., 2010 and Rahman et al., 2021.

$$\text{Hazard Quotient} = \frac{\text{EDI}}{\text{ADI}}, \text{ where}$$

*EDI- Estimated Daily Intake*
*ADI- Acceptable Daily Intake*

$$\text{EDI} = \frac{\text{(concentration of residue as µg/ kg) x (daily intake of food in kg/person)}}{\text{Adult body weight (60 kg)}}$$

The mean level of antibiotic concentrations in milk sample was calculated, then, the value of the mean concentration and average daily milk consumed based on 60 kg body weight were taken into consideration. The ADI values set by Codex Alimentarius Commission (2015) are: 3µg/kg BW/day for oxytetracycline, 4 µg/kg BW/day for gentamicin and 30µg/person/day for penicillin. According to data provides by National Institute of Public Health the daily milk consumption in Romania, was in 2020 109, 0 ml/man/day and 113, 2 ml/woman/day (https://insp.gov.ro/RAPORT-Sanatate-Mediu-2020.pdf).
If the Hazard Quotient value is higher than 1 then the likelihood of harm is stated but it is not the statistical probabilities of occurrence. A negligible hazard is when the value of the hazard quotient is less or equal to 1.

## RESULTS AND DISCUSSIONS

In the present study, three of the most widely used classes of antibiotics used in both animals and humans were considered (Figure 2).

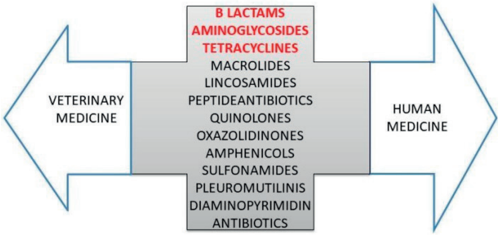


Figure 2. Classes of antibiotics used in human and veterinary medicine in Europe

284 of the milk samples subjected to qualitative screening, 6,34% (n=18) were positive, 88,03% (n=250) were negative and 5,63% (n=16) had false positive results, which is why they were removed from subsequent determinations. Table 1 and Figure 3 show the proportion of each sample category.

Table 1. The percentage distribution of tested milk samples

| Specification | n | % |
|---|---|---|
| Milk samples | 284 | 100 |
| Positive milk samples | 18 | 6,34 |
| False positive milk samples | 16 | 5,63 |
| Negative milk samples | 250 | 88,03 |

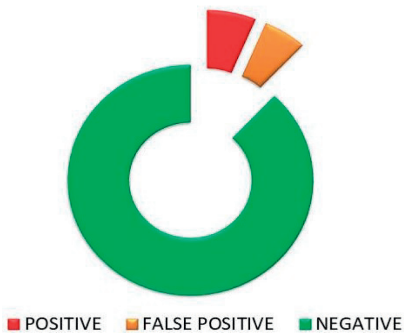


Figure 3. The percentage distribution of tested milk samples

After the successful completion of the qualitative testing, the samples tested positive for the three (Penicillin, Oxytetracycline and

Gentamicin) antibiotics were subjected to quantitative determinations. The overall occurrence of antibiotics residues is presented in Table 2.

Table 2. The overall occurrence of antibiotics residues in positive samples milk

| Positive samples milk n=18 | | |
|---|---|---|
| Penicillin | Oxytetracycline | Gentamicin |
| n=5 | n=9 | n=4 |
| 27,77% | 50,00% | 22,23% |

Oxytetracycline is the antibiotic present in half of the positive samples (n=9), while the milk samples tested positive for penicillin represented a little more than a quarter of the total samples (27,77%).

Gentamicin was tested positive for a number of 4 samples, which represents 22% of the total positive samples (Figure 4).

These results are similar to those obtained in 2015 by Pogurschi et al., in the metropolitan area of the Romanian capital, where 28.37% of the samples were contaminated with beta-lactams, a group to which Penicillin belongs and 71.43% of the samples were reported contaminated with tetracycline, group to which oxytetracycline belongs.

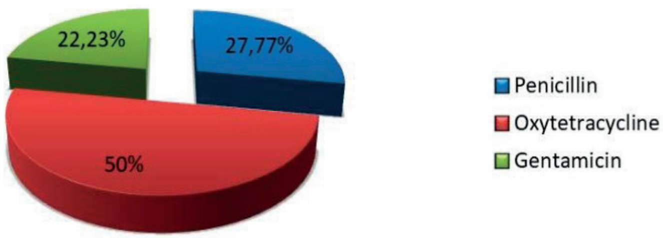


Figure 4. The proportion of samples tested positive for the three antibiotics studied

The total number of positive samples is lower when comparing to others (Rahman et al., 2021) but the difference is not significant, respectively 6.34% compared to 7%. Zanella et al., 2010 reported significantly high presence of tertacyclines in milk, 18,5% (n=48) of the samples being contaminated with tetracyclines.

The results of the quantitative test performed using Agilent 1260 Infinity II LC System are shown in Table 3.

Table 3. Mean concentration of antibiotics residues in milk

| Group of antimicrobials | Analytes | Mean Conc.[a] (μg / kg) | MRL value[b] (μg / kg) |
|---|---|---|---|
| B-lactams | Penicillin | 21. 50 | 4 |
| Tetracycline | Oxytetracycline | 115.00 | 100 |
| Aminoglycosides | Gentamicin | 294.5 | 100 |

[a] The average concentration of positive samples
b MRL value was collected from EU regulation no.37/2010

As can be seen from the data presented in Table 3, the mean Penicillin concentration (21, 50 μg/kg) was more than 5.37 times higher than the MRL level, while Gentamicin (294.5 μg/kg) was more than 2.95 times higher than the MRL level.

The level of oxytretracycline, the antibiotic detected in 50% of the milk samples, exceeded the MRL values, but this excess is a small one, 1,15%. The amount of antibiotics detected in these samples may be due to the fact that the milk from individual producers does not undergo heat treatments. Milk treatments, such as pasteurization, can reduce the final milk content of residues.

Pasteurized milk, therefore subjected to a heat treatment, contains fewer contaminants. This is confirmed by several researchers including Rasooli et al., 2014, in whose study of 432 pasteurized milk samples only 1.62% (n=7) contained tetracycline residues above MRL.

In order to assess the risk through the intake of raw analysed milk in the present study, the (EDIs) for consumers were calculated. Based on the mean value of residues in analysed milk samples and the daily milk consumption in Romania, reported in 2020, the risk of dietary exposure to penicillin, oxytetracycline and gentamicin through the milk consumed has been assessed. The results are presented in Table 4. The lowest EDI value was calculated for penicillin.

Table 4. Evaluation of risk assessment

| Antibiotic | EDI (µg /kg/day) | | ADI (µg/kg/day) | | Hazard Quotient | |
|---|---|---|---|---|---|---|
| | Woman | Man | Woman | Man | Woman | Man |
| Penicillin | 0.041 | 0.039 | 200 | | 0.00021 | 0.00019 |
| Oxytetracycline | 0.217 | 0.208 | 30 | | 0.0072 | 0.0069 |
| Gentamicin | 0.555 | 0.535 | 30 | | 0.0018 | 0.0178 |

The highest individual calculated EDI was obtained for gentamicin, followed by oxytetracycline. For the three studied antibiotics, the Hazard Quotient is less than 1, which is why it can be concluded that the detected levels of residues in milk could not be considered as a public health issue with regards to these veterinary antimicrobial substances.

## CONCLUSIONS

The methodology used to determine antibiotics residues in milk was in accordance with the protocol recommended by the U.E. legislation, which requires two steps: qualitative microbiological procedure and quantitative procedure for confirmation of the results obtained in the first stage. The most common contaminant was oxytetracycline, an antibiotic whose residues was found in half of the confirmed positive samples. Residues measured for gentamicin were far above the maximum residue limits (MRLs) set by law. The Hazard Quotient calculated for all three antibiotics was less than 1. This indicates that the milk marketed by individual producers in Romania, in average, contains low levels of veterinary drugs, and therefore, it could be considered as safe for human consumption. Regarding the milk consumption, this should be doubled in order to reach 200 ml / person / day, an amount that can be considered acceptable as part of a healthy diet and lifestyle.

## ACKNOWLEDGEMENTS

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# REMARKS ON CONSUMER AWARENESS OF FOOD ADDITIVES IN CHILDREN FOOD PRODUCTS

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***Abstract***

*Food additives (commonly known as E) are substances that are not normally consumed as a stand-alone food and are not used as characteristic food ingredients with or without nutritional value. The aim of the paper was to observe the presence of food additives in some products, such as cold cuts and spreads, more frequently used in children's food, which have high visibility on the shelf, due to the attractive packaging, but also advertising messages in the media. The method applied was the observation of components from the label of products for children and the determination of those that are harmful to human consumption. A questionnaire was also developed to determine consumer opinion. This study was conducted in the Bucharest area, in three large shopping centres. Sodium nitrite (E 250) is found in all foods for children under study. The parents have the obligation to choose the right nutrition for their children. 52.9% of adults admitted that they did not read the label of the food they bought. It is necessary to continuously educate consumers about potentially dangerous ingredients, added to food, reading the label, to determine their presence in the chosen product on the shelf, giving up buying food on the principle of quantity or reduced price to the detriment of quality.*

***Key words***: *children, consumer, food additives, food products.*

## INTRODUCTION

Understanding consumer profile is important for any company that wishes to successfully operate on a market. Understanding the way globalization influences this profile is equally important, especially for agri-food markets. The one in Romania is particularly interesting from this point of view because it has entered this global economic context during the last 20 years. Under its influence and amid the economic and social changes of this period, the local agri-food consumer has shifted from providing the minimum necessary staple goods to phenomena such as preferential consumption, acquiring wide distribution areas or agri-food selection underpinned by individual preferences (Marin et al., 2019). Nevertheless, against consumer profile in developed countries, fundamental differences still exist (Burda, 2009).

The Romanian food market is still in the process of integration into the European Union market both as a regulation and as specific phenomena for consumption and the consumer. From this point of view, one problem is the transformation that took place in the profile of the Romanian consumer of food goods, before and after Romania's accession to the European Union. Romania wants to equalize the living standards of developed countries in the European Union, so the evaluation standard of the measure of transformation of consumption and purchasing behavior is the way it has evolved and characterizes the profile of the consumer of food in these countries. Strongly influenced by the American lifestyle, the new generations of consumers impose specific behaviors regarding food consumption (Pascaud, 1995)

Food additives (commonly known as E) are any natural or chemical substance that is not consumed as a food in itself and is not used as a constituent ingredient of a food, whether or not it has nutritional value and is intentionally added, for a technological purpose (including organoleptic changes) during the production, processing, preparation, treatment, packaging, wrapping, transport, storage, or other modification applied to a food, becoming a component or affecting the characteristics of the food in one way or another (Regulation EC no 1333/2008).

Food preservatives, which are widely used in food products, affect human health. Their effect varies according to age and health status. The most used preservatives are chemicals such as sodium nitrite, $NaNO_2$, which is used in meats and fish as an antimicrobial and preservative. Unfortunately, despite its powerful preservative efficiency, $NaNO_2$ has various worrisome hazardous effects on human health and safety (Attia et al., 2021)

Children are the most important audience when it comes to healthy eating. According to the World Health Organization (WHO), the adequate nutrition of infants is essential to ensure the growth and optimal development of children and achieve better health throughout life, including prevention of overweight, obesity, and diet-related non-communicable diseases (WHO, 2019). A balanced diet is essential for their normal growth and development. At this age they form and define their eating habits that they will follow throughout their lives. Proper nutrition promotes better health and a lower susceptibility to disease. It also contributes to cognitive development and school success.

## MATERIALS AND METHODS

The working method applied was the study of labels and the observation of food additives presence in some products, such as cold cuts and spreads, more commonly used in children's food, due to the high visibility on the shelf, due to the attractive packaging, but also to advertising messages in the media.

In order to determine a specific eating behavior, a questionnaire was also developed, addressed to those who bought these products

This study was conducted in the Bucharest area, in three large shopping centres.

## RESULTS AND DISCUSSIONS

Sodium nitrite (E 250) was found in all foods for children under study (Martinel Sausages and Martinel Spreadable cream produced by The Family Butchers România SRL (Reinert); Mini Chichen Sausages and Pepe Spreadable cream produced by Perutnina Ptuj – Slovenia).

E250 is a food additive approved by the European Union (EU) and used as an antibacterial synthetic preservative in food products, as well as a colour fixative.

It is specifically effective at inhibiting the botulism-causing bacteria, *Clostridium botulinum.*

Over the years, sodium nitrite has raised some concerns about its safety in foods, but it remains in use. Studies in the 1990s indicated some adverse effects of sodium nitrite, for instance the potential to cause childhood leukemia and brain cancers (Abdollahi & Khaksar, 2014).

The amount of nitrite permitted in meat products is heavily regulated because at high levels it can be toxic (Barbut, 2017)

As a result, in recent years consumer and regulatory pressure has mounted to reduce the levels of nitrite in processed meats (Austin, 2014)

The main concern with sodium nitrite is the interaction with proteins in food that lead to the formation of nitrosamines, which are considered carcinogenic, when meat products containing E250 are exposed to high temperatures (Boerema & Broda, 2004).

Ascorbic acid (vitamin C, E300), tocopherol (vitamin E) or erythorbic acid (E315) prevent the formation of nitrosamines, which is why they are used together with sodium nitrite.

The responsibility for proper nutrition of children lies with the parents, who have the obligation to choose the right nutrition for their children.

In the questionnaire, the adults received various questions in order to establish a profile of the consumer.

In each store were interviewed 5 people each day, in an interval of 7 days, a total of 35 people / store resulting in a total number of 105 people per activity.

Only those who are parents were selected, for data processing.

The interviewees were asked questions related to sex, age, education and average monthly income (to establish social and financial status) (Figures 1-6).

As shown in Figure 1, the majority of people who answered the questions were female, respectively 68.8%.

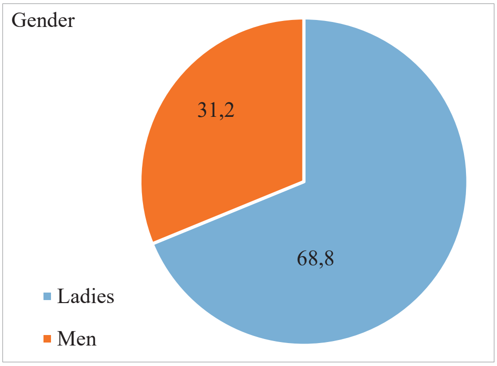


Figure 1. Consumers gender

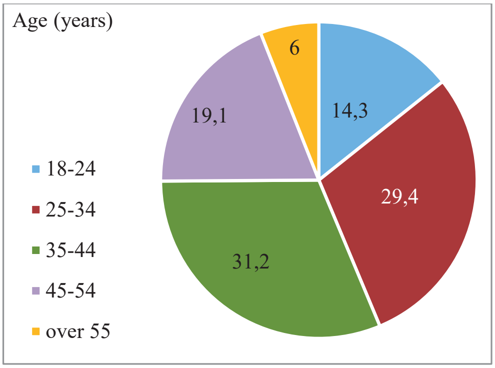


Figure 2. Consumers age

Various age categories were included from 18 to over 55 years old and here it can be seen that most were between 35-44 years with a percentage of 31.2%, the next age category being between 25-34 years, here we have a percentage of 29.4%, the lowest percentage was the age category over 55 years (6%).

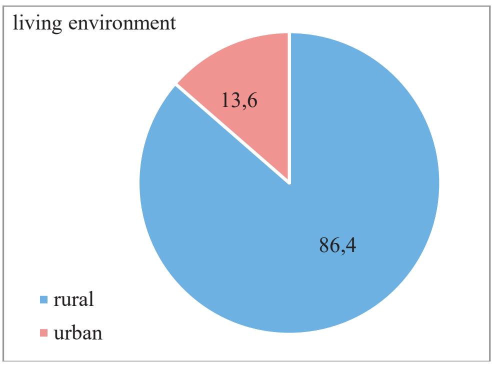


Figure 3. Consumers living environment

1 of the 3 supermarkets where the questionnaires were applied was located on the suburb of Bucharest, some of the buyers being from Ilfov County.
However, those in urban areas were visibly the majority compared to those in rural areas with a majority of 86.4% compared to 13.6%.

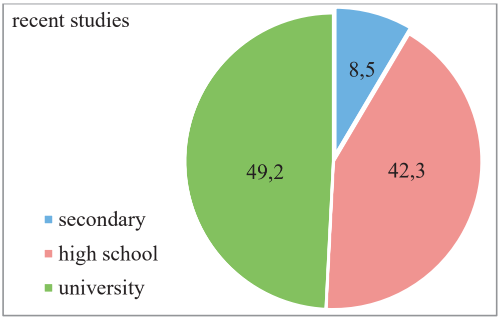


Figure 4. Consumers last studies graduated

Regarding the graduated studies, the majority were those with university studies with a percentage of 49.2%, being closely followed by those with high school studies with 42.5%.
Those with high school education are in a much lower percentage (8.5%).

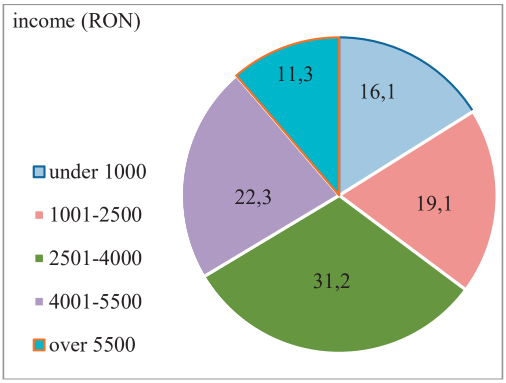


Figure 5. Consumers income

The income category between RON 2501-4000 represented the highest percentage (31.2%).
The lowest percentages were for the categories of incomes below 1000 RON (16.1%) and over 5500 RON (11.3%).

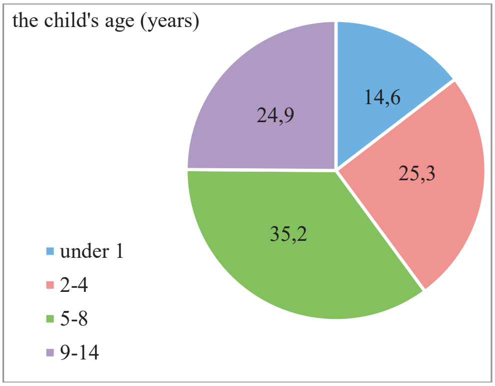


Figure 6. The age of the consumer's child

Most people on the shelves with food for children said that their child's age is between 5-8 years, this category having the highest percentage of 35.2%.

The interviewees were also asked questions related to age of the child/children, frequency of purchase of the products studied, interest in the information on the label. and awareness of the presence of food additives in products, confidence in these products, interest in purchasing organic (organic) products (establishing eating behavior and level of education in this regard – Figures 7-14).

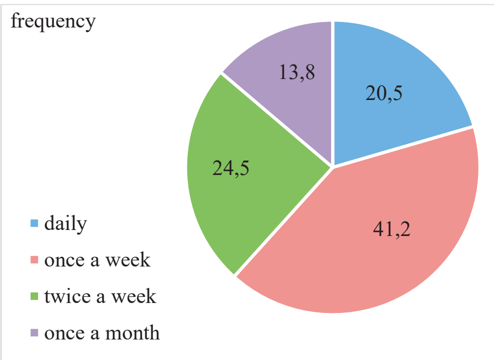


Figure 7. Frequency of purchasing the products under study

The rate for the purchase of food for children is 41.2% for those who buy once a week, 20.5% for those who buy daily, 24.5% for those who buy twice a week and 13.8% for those who buy once a month.

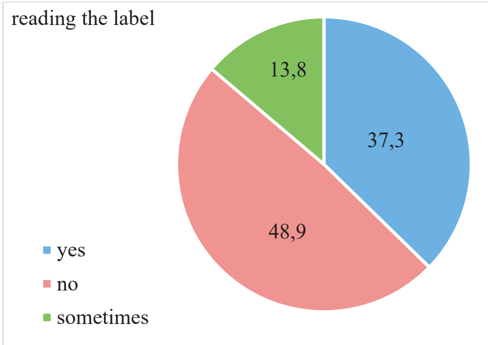


Figure 8. Do you read the product label

Asked if they read the product label before purchasing a baby food, most of the answers were negative, with a high percentage of 48.9%, compared to 37.3% and 13.8% who read it sometimes.

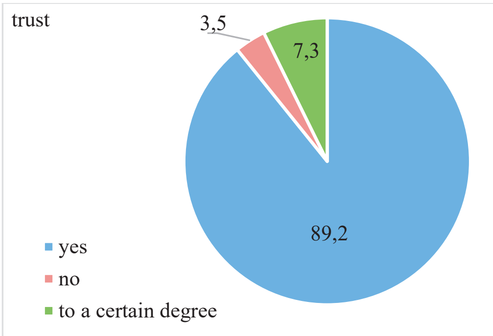


Figure 9. Do you trust the food you buy?

According to figure 9, those who buy food for children trust 89.2% of it, 3.5% do not trust it completely and 7.3% trust it to some extent.

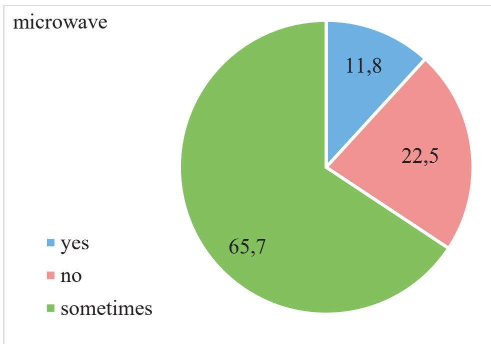


Figure 10. Do you buy organic products for children?

Regarding the purchase of organic food products, the majority of parents (65.7%) answered that they occasionally buy these products for their children, 22.5 do not buy and 11.8 do so frequently.

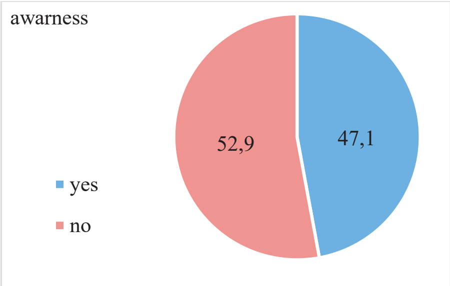


Figure 11. Are you aware of the presence of food additives in children products?

Figure 11 shows that 52.9% of people are unaware of food additives in baby products.

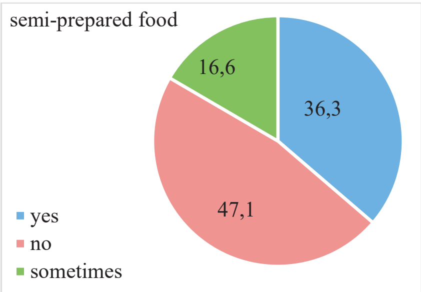


Figure 12. Do you buy semi-prepared foods more often than fresh ones?

Most parents prepare food at home, using raw materials and fresh products (47.1%), but a fairly high percentage (36.3), have a habit of buying semi-prepared products to use in the family's diet. Only 16.6% do not usually buy semi-prepared foods.

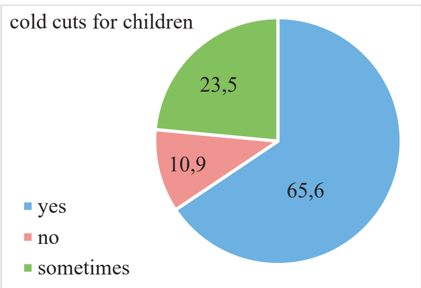


Figure 13. Do you buy cold cuts for children?

More and more parents choose to introduce cold cuts to the diet of young children (70.6%), which is worrying because their number is much higher than those who do not do so (10.9%) or those who do it occasionally (23.5%).

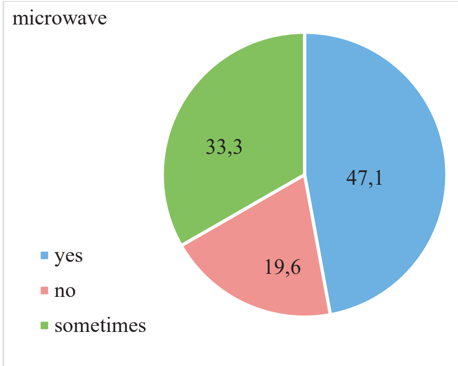


Figure 14. Do you heat food for children in the microwave?

47.1% of parents choose to heat the food to be served to their children in the microwave, 19.6% do not do it and 33.3% do it occasionally.

## CONCLUSIONS

Children are the most important audience when it comes to healthy eating. A balanced diet is essential for their normal growth and development. At this age they form and define their eating habits that they will follow throughout their lives.

Proper nutrition promotes better health and a lower susceptibility to disease. It also contributes to cognitive development and school success.

Worryingly, there are many shoppers who are unaware of the potentially dangerous ingredients in the foods they feed their children. The high confidence in the products intended for children is due to the mentality that these products are much better monitored and verified by the competent sanitary-veterinary control bodies. Consumers sometimes make the mistake of placing the responsibility of the food authorities, and do not practice the personal verification of the respective producer.

It is necessary to continuously educate consumers about potentially dangerous ingredients, added to food, reading the label, to determine their presence in the chosen product on the shelf, giving up buying food on the principle of quantity or reduced price to the detriment of quality.

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# THE CHARACTERISTICS OF COWSKIN GELATIN PRODUCED FROM CURING ACETIC ACID CONCENTRATION

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***Abstract***

*Gelatin is a denaturalized protein that is derived from collagen by acidic or alkaline hydrolysis and is an important functional biopolymer that has a very broad application in many industrial fields. This research was aimed to determine the effect of acetic acid concentration on characteristics of cowskin gelatin. The experiment used Completely Randomized Design (CRD) with two factors and three replicates of treatment. The first factor was concentration of acetic acid solution, consisted of (2.5, 5 and 7.5%. The second factor was soaking time in acetic acid (12, 24 and 36 hours). The result showed that concentration acetic acid solution had no significant effect (P>0.05) on the viscosity value but had significant effect (P<0.01) on the gel strength, yield, protein content and sensory test of cowskin gelatin. The soaking time had no significant effect (P>0.05) on the yields, gel strength, viscosity and protein content of gelatin. It was concluded that the best characteristics of cowskin gelatin was produced from curing 5% acetic acid concentration with soaking time 36 hours due the gel strength value, viscosity, yield and protein content of gelatin which are optimal and sensory test acceptable by panelists.*

***Key words****: acetic acid, cowskin, gelatin,*

## INTRODUCTION

Gelatin is a denaturalized protein that is derived from collagen and is an important functional biopolymer that has a very broad application in many industrial fields. Gelatin is a protein of animal origin, that can be obtained from collagen by acidic or alkaline hydrolysis. Its functional properties depend on processing conditions as well as the raw material (Liu & Guo, 2008, Sompie et al., 2018, Sompie et al., 2019).

Animal age also influences the protein content of skin collagen, which increases with animal age, protein collagen and fibrous collagen growing stronger (Ockerman & Hansen, 2000). Gelatin production require a curing step to improve its quality. Curing materials from the group of acids have been widely applied in gelatin production (Pranoto et al., 2006; Said et al., 2012).

The use of cowhide as raw material for extraction gelatin (Sugihartono, 2014) and gelatin from Bali cowskin by using lime solution immersion (Mokoolang et al., 2019) has been studied but the effect of acetic acid concentration and curing time to produce gelatin from cowskin was limited information. Thus, this research was conducted to study the effect of combination between different concentration acetic acid solution and soaking time on characteristics of cowskin gelatin.

## MATERIALS AND METHODS

Gelatine was prepared by the acid extraction method (Ockerman & Hansen, 2000). Acetic acid ($CH_3COOH$ 0.5M) concentrations of 2.5%, 5% and 7.5% (v/v) were used for hydrolysis. First, the clean cowskin was cut into small pieces and then soaked in a lime solution of $Ca(OH)_2$ for 48 hours, after that, it was washed with running water until clean, then soaked in several concentrations of acetic acid solution, 2.5%, 5% and 7.5% as a treatment. The raw material was soaked at different time immersion of acetic acid solution 12 hours, 24 hours and 36 hours. After soaking samples were neutralized to pH 6, weighed and extracted. The extraction process was performed on three steps (each step for 3 hours), the first step at $50^0$C, second step at $55^0$C and then at $60^0$C. Solubilized gelatin was separated from residual skin fragments by filtration through a nylon filter. The extracted gelatin was concentrated at $70^0$C for 5 hours and it was stored in the refrigerator 5-$10^0$C for 30 minutes, then dried at $60^0C$ for 24-36 hours until the gelatin sheet solid. Gelatin sheets were milled and packaged in vacuum plastic and stored in a desiccator for subsequent process.

**Method of analysis**

**Gel strength** was determined with a Universal Testing Machine (Zwick/Z.0,5). Gelatin solution 6,67% w/v (6,67 grams to 100 ml distilled water) was heated at $60^0C$ to dissolve the particles. Solution in the container Ø5 cm and height 6 cm was stored at $5^0C$ for 16-18 hours. Gelatin was placed at the bottom of the plunger (Ø=13mm). Measurement was conducted at the temperature of $10^0C$ and the speed 10 mm/min as deep as 4 mm was used as plunger. The value of gel strength (g Bloom) uses the formula = 20 + 2,86 x $10^{-3}$D, where D = F/G x 980; F = height chart before fracture; G = constant (0,07)

**Viscosity** was measured by gelatin powder dissolved in distilled water at a temperature of 40°C with a solution concentration of 6.67%. The values were measured by Stromer Viscosimeter Behlin CSR-10. The results obtained were expressed in centipoise according to the method Gomez

**The yield** is obtained from the comparison between the weight of dry gelatin produced with fresh skin weight.

Yield = weight of gelatin / weight of fresh skin x 100%

**Protein content** FOSS Kjeltec 2200 was used to determine protein content. A total of 0,5 g of sample + ¼ bussino tablet + 12 ml $H_2SO_4$ was concentrated in the destruction of the tube FOSS at ± $410^0C$ for 1 hour. The result of destruction was distilled with thio-NaOH 40% + $H_3BO_4$ 4% + BCGMR indicators. A total of 150 ml was distilled in Erlenmeyer glass and titrated with 0,099 N HCl until the colour changed from blue to pink. Five points fifty-five was used as the conversion factor of gelatin protein. The protein content (%) was calculated using the formula (ml HCL–ml Blanko) x N HCL x 14,0008 x 100 x 5,55)/g sample x 1000.

## RESULTS AND DISCUSSIONS

**Gel Strength**

Gel strength of gelatin is very important on physical properties of gelatin. The average gel strength of cowskin gelatin is displayed in Table 1. Statistical analysis indicated that the soaking time in acetic acid gave highly significant effect ($P<0.01$) while the level concentration of acetic acid and their interaction had no significant effect ($P>0,05$) on cowskin gelatin. The value of gel strength tended to decrease with increasing level of acetic acid concentration. Gel strength values from cowskin gelatin was ranged 73.31 - 78.30 g Bloom, that in line with the criteria of ISO 75-300 g Bloom (Said et al, 2011). The presence of hydroxyproline caused the stability of the hydrogen bonds between water molecules and free hydroxyl groups of amino acids in gelatin, it is very important for gel strength. The gel formation of a stable condition that ability of a free chain to form a lot of crosslinking (Sompie et al., 2019).

**Viscosity**

The average viscosity of cowskin gelatin is displayed in Table 1. Statistical analysis indicated that the interaction between concentration of acetic acid and soaking time had no significant effect ($P>0,05$) on cowskin gelatin. The value of viscosity tended to decrease with the increasing of acetic acid concentration. This is because the viscosity of gelatin is directly proportional to the gel strength that was not significantly different between treatments (Said et al., 2001). Sompie et al. (2020) explained that viscosity is affected by molecular weight and amino acid chain length. Increased concentrations of acetic acid in the gelatin production process can reduce the viscosity. The curing material has been breaking the peptide bonds of amino acids into short-chain molecule so that its viscosity decrease. Viscosity values from cowskin gelatin was ranged 6.03 to 7.27 cP. It values is included in the ISO range 2.0 to 7.5 cP (Said et al., 2011).

**Protein Content**

Gelatin is a collagen protein, a group derived from the structural proteins and extracellular matrix and produced in large quantities (Said et al, 2011; Sompie et al., 2015**).** Statistical analysis indicated that the soaking time of acetic acid had highly significant effect ($P<0.01$) on protein content of cowskin gelatin, while the concentration of acetic acid and the interaction between these two different factors had not significant effect ($P> 0.05$) on levels of protein gelatin. Duncan test results showed that protein content of gelatin from cowskin had a tended to increase with age increasing. According to Swatland (1984), age at slaughtering influences the content of collagen in the skin, increasing age increased the level of collagen protein. Protein content from cowskin gelatin ranged 86.14 to 89.04 %. That it was not different with protein content from chicken leg skin ranged 83-90 % and commercial gelatin, 91, 63% (Hasdar, 2012; Said et al., 2011).

**Yield**

Results shows that the acetic acid concentration in cowskin gelatin had no significant effect (P< 0.05) to the yield value. This means that the yield of cowskin gelatin was the same for all three concentrations of acetic acid. There was no effect of acetic acid concentration on gelatin yield because of the concentration range used in this study was only 2.5 percent different, so although there was an increase in yield for each treatment, the increase was very small. The yield value is influenced by the concentration of the acetic acid solution. The increasing yield is related to a large amount of collagen converted and transformed to gelatin (Kolodziejska et al., 2008). Total yield value is affected by the concentration of the acetic acid solution used in immersion. The increase in yield is related to amount of collagen converted to gelatin (Sompie et al., 2019).

Table 1. The Characteristics of cowskin gelatin

| Parameters | Acetic acid (%) | Soaking time (hours) + Sd | | | Average |
|---|---|---|---|---|---|
| | | 12 | 24 | 36 | |
| Gel strength (g Bloom) | 2.5 | 78.30±0.02 | 75.10±0.02 | 73.31±0.03 | 75.57±0.13 |
| | 5 | 75.08±0.40 | 74.18±0.10 | 75.04±0.21 | 74.76±0.02 |
| | 7.5 | 76.20±0.12 | 74.02±0.02 | 74.04±0.12 | 74.75±0.03 |
| | Average | 76.52±0.02$^{c}$ | 74.43±0.01$^{d}$ | 74.13±0.02$^{d}$ | |
| Viscosity (cP) | 2.5 | 7.21±0.01 | 7.24±0.01 | 7.27±0.01 | 7.24±0.32$^{a}$ |
| | 5 | 6.16±0.06 | 7.17±0.04 | 7.18±0.06 | 6.83±0.22$^{b}$ |
| | 7.5 | 6,03±0.07 | 6.06±0.07 | 6,86±0.07 | 6.32±0.14$^{a}$ |
| | Average | 6.47±0.05$^{c}$ | 6.82±0.53$^{c}$ | 7.10±0.34$^{d}$ | |
| Yield (%) | 2.5 | 12.22±0.21 | 13.04±0.20 | 11.25±0.11 | 12.17±0.21 |
| | 5 | 12.16±0.03 | 12.36±0.33 | 12.06±0.23 | 12.16±0.13 |
| | 7.5 | 12.03±0.07 | 12.01±0.12 | 13.03±0.02 | 12.19±0.04 |
| | Average | 12.13±0.05 | 12.47±0.21 | 12.11±0.25 | |
| Protein content (%) | 2.5 | 88.04±0.57 | 89.04±0.57 | 89.04±0.57 | 88.70±0.12$^{b}$ |
| | 5 | 88.50±0.07 | 88.30±0.07 | 88.50±0.07 | 88.62±0.31$^{ab}$ |
| | 7.5 | 88.14±0.23 | 87.14±0.23 | 86.14±0.23 | 87.13±0.02$^{a}$ |
| | Average | 88.23±0.12$^{c}$ | 88.16±2.27$^{c}$ | 87.89±3.02$^{d}$ | |

Different letters in the same row same row and column indicated the significant differences (P<0.05)
Sd = standard deviation

Figure 1. Comparison of physical appearance of cowskin gelatin using acetic acid 2.5, 5% and 7.5%

**Sensory Test**

The sensory test is one of the assessment factors besides the physical and chemical properties of a product and have a high relevance to the quality of the product because it is directly related to consumer tastes. Based on the results of the researcher's subjective identification of the physical appearance of the product.

The figure 1 above showed that physically the main difference of gelatin produced using acid curing material with base differs in colour and texture of granule while the shape and odour are relatively the same. Comparison of visual appearance of cowskin gelatin products produced by using acetic acid curing concentration (2.5, 5 and 7.5%). Sensory test has high relevance to product quality because it is directly related to consumer tastes (Said, 2011). Humans are panelists who can sometimes be affected by physical and mental conditions, so panelists become bored and degrade their sensitivity. Gelatin is uncolored to yellowish or light brown and have no odour and tastes. Gelatin produced either by acid or base process is not much different from the quality standard required by SNI, shaped sheets, pieces, have a coarse or fine powder texture

## CONCLUSIONS

It was concluded that the best characteristics of cowskin gelatin was for that produced from curing with 5% acetic acid concentration for 36 hours soaking time; this is due to its better gel strength value, better viscosity, yield and protein content of gelatin which are optimal and sensory test acceptable by panelists.

## ACKNOWLEDGEMENTS

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# THE ENRICHMENT OF BREAD WITH ALGAE SPECIES

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***Abstract***

*The goal of this paper was to highliht the use of some algal species in bread fortification. Using data fom scientific literature, two directions were followed: highlighting the role and nutritional value of algae species; the analisys of potential benefits of the bread fortification. Food enrichment consists in the incorporation of food resources, rich in proteins, desired lipids or micronutrients, in a widely consumed and accessible basic food, to improve its nutritional balance. Bread, which is a high carbohydrate food, is habitually traditionally consumed with almost all foods in our country. White bread contains 35-43% moisture, 6-16% proteins, 45-58% carbohydrates, 0.5-1.5% lipids, 0.5-1.5% ash, and 1-1.5% salt, and 100 gram bread has approximately 250-270 calories. Nutritional value of the traditional bread types are increased by adding them foods additives such as walnuts, grapes, and sunflower seeds. Also, the micro and macroalgas are valuable sources for the bread fortification. The algal biomass shows promising qualities as a novel source of protein for bread. Compared to conventional bread, the average nutritive quality of most of the breads enriched with algae was superior, or at least equal. According with the analized data all the enriched breads presented a good global acceptability, even though the lower levels of the algae contents were more appreciated for color.*

***Key words:*** *algas, bread, health, nutritive value.*

## INTRODUCTION

In our days, humans seek alternative nutritional sources and tendency to natural and additive-free food. Bread is considered a staple food worldwide because of its nutritive value, low price and its simplicity of use (Kuijsten et al., 2005). The developed countries such as United States of America, Japan, England, Germany, and Norway benefit from the nutritional richness of microalgae (Becker, 1994).
The food industry consumes large amounts of algae known to provide a wide variety of nutrients. The characteristics of these algae combined with the great potential for cultivation have drawn the attention of industries to their exploration. Algae are easily obtained on moist land or aquatic surfaces, freshwater or saltwater media and are an important source of essential compounds for human nutrition (Rocha et al., 2007).
One of the most important algae types cultured today is *Spirulina platensis*. It has a high biomass productivity in hot and sunny climates (Richmond, 1986). Spirulina which is a microalgae was proposed in human food by several scientists and nutritionists thanks to its exceptional nutritional qualities. It is considered as unconventional food resource which can contain until 70% of proteins (Benahmed-Djilali, 2012), is rich in vitamin B12 (193 μg/100 g) and gamma linoleic acid and a source of calcium and iron (1043.62 and 338.76 mg/100 g) (Cifferi, 1983; Richmond, 2004; Morsy et al., 2014).
Spirulina has a special value among the other algae types. It is the a lack of cellulose in its cell wall, contains high amount of iron, which makes it important in anemia disease. Also, *Spirulina* is a natural resource rich in GLA (approximately 1% of its dry weight) (Takeuchi 1978). It is an energy supplement
for elderly people. In Japan, 73% of people aged over 50 eat *Spirulina*. 10 grams of Spirulina contains only 36 calories (Seshadri, et al., 1992). *Spirulina platensis* is digestible because 86% of

its cell wall is composed of digestible polysaccharide (Li & Qi, 1997).
In nature, Spirulina platensis is found intensively in alkaline waters. Macroalgal biomass can be produced in culture tanks under controlled conditions, or collected from natural environments. (Ereno, 2010).
It appears as microalgae of the hope which will have a leading role to be played to take up the challenge of food selfsufficiency and to serve as remedy with certain diseases which affect in particular developing countries (Fox, 1999).
There are several applications of spirulina in the human food such as instant noodles for children (Xu, 1993); drinks (Zeng & Liang, 1995); tablets (Yamaguchi, 1997) and couscous (Doumandji et al., 2012). Most fortifications, on cereal-based food, were devoted to legumes seeds such as bean, soya (Dhingra, 2002) and sesame (ElAdawy, 1995).
In the prospect of valuation and exploitation of the algae species, the main goal of this paper was to analyze and evaluate the effect of the incorporation of this algas on the nutritive value of bread based of physical, chemical and sensory parameters.
The goal of this paper was to highliht the use of some algal species in bread fortification.

## MATERIALS AND METHODS

### Biological materials

The biological materials used in these studies were:
The biological materials used in these studies were:
- (a) an isolated autochthonous microalgae spirulina (*Arthrospira fusiformis*), as small green dry pellets, which was collected in farm located in southern of Algeria (Tamanrasset) (Yaiche et al., 2014);
- (b) *Spirulina* (*Arthrospira*) *platensis* (Burcu et al., 2016);
- (c) *Spirulina platensis* (Figueira et al., 2011);
- (d) *Cladophora* spp. and *Ulva* spp. (Menezes et al., 2015).

The composition of algas is very important because, according to Schwochow & Zanbon (2007) the composition may vary by time of year that it is collected. In Table 1 are shown the main characteristics of the algae species used in the analized papers, acording with the authors presentation.

Table 1. Characterization of the biomass of algae applied in the formulation of the breads

| Determinations | *Cladophora* spp. *and Ulva* spp.[d] | *Spirulina platensis*[b] |
|---|---|---|
| Moisture [%] | 7.0 ± 0.19 | 6.7 |
| Ash [%] d.b. | 26.3 ±1.04 | - |
| Protein [%] d.b. | 8.7 ± 2.42 | 86.0 |
| Fat [%] d.b. | 1.1 ± 0.28 | 3.3 |
| Fiber [%] d.b. | 3.9 ± 0.62 | - |
| Carbohydrates [%] d.b. | 60.0 ± 2.87 | - |
| Caloric value [kcal $100g^{-1}$] | 168.9 ± 3.52 | |
| Digestibility [%] | 45.0 ± 1.36 | 84 |
| Carotenoids [$\mu g.g^{-1}$] | 378.6 ± 0.02 | |

Values are means ± SD of analyses performed in triplicate; d.b: dry basis.

The lipid content in algae is low, ranging from 1-5%, *Ulva lactuca* also shows high levels of omega 3 and 6. The mineral fraction of some algae represent 36% of total dry weight. (Patarra, 2008). The fiber content varies with the algal species analyzed, with an average of 30 - 40% dry weight (Tabarsa et al., 2012).
Spirulina contains 50-70% protein, 20% carbohydrate, 5% lipid, 7% minerals and 3 to 6% moisture. Therefore, unlike the proteins obtained from meat and dairy products, it is a protein resource which is low lipid, low-calorie, and cholesterol-free (Richmond, 2004).

### Preparation of spirulina enriched bread samples

The analyzed data on the fortification of bread with algae species were varied, as types of algae and as percentages of participation of algae in the formulation of bread. Thus, the percentage of participation varied between 1 and 10%, with eight different levels.
The (micro and macro) algae species levels from bread, the main characteristics evaluated and the authors were shown in Table 2.

Table 2 The algae species levels from bread and the main characteristics evaluated

| Biomass of the algal in bread, [%] | The characteristics evaluated | Researchers |
|---|---|---|
| *Spirulina* *(Arthrospira fusiformis)* 0, 1 and 3 | - chemical composition (protein, fat, crude fiber, carbohydrates, ash); the specific volume; the acceptability | [(a)] Yaiche et al., 2014 |
| *Spirulina platensis* 0, 10 | - nutrient composition, (protein, lipid, Ca, Mg and Iron); volatile compounds; microbiological and sensory properties; | [(b)] Burcu Ak et al., 2016 |
| *Spirulina platensis* 0, 2, 3, 4, 5 | - nutritional quality; the specific volume, crumb hardness; crumb color | [(c)] Figueira et al., 2011 |
| *Cladophora* spp. and *Ulva* spp. 0, 2.5, 5.0 and 7.5 | - nutrient composition and caloric value; technological properties; sensory evaluation. | [(d)] Menezes et al., 2015 |

## RESULTS AND DISCUSSIONS

**a. Physicals characteristics**.

The physical characteristics of control and enriched breads are presented in Table 3.

Table 3. Physical characteristics of control and enriched breads

| Paper | Biomass of the algal in bread [%] | The specific volume ($cm^3 g^{-1}$) | Hardness (g) | |
|---|---|---|---|---|
| | | | Fresh | 24 h |
| a | 0 | 2,27 | - | - |
| | 1 | 2,19 | - | - |
| | 3 | 2,14 | - | - |
| c | 0 | 3.11 | 215.05 | 431.71 |
| | 2 | 3.03 | 308.48 | 337.07 |
| | 3 | 3.09 | 289.24 | 288.91 |
| | 4 | 3.10 | 277.39 | 300.49 |
| | 5 | 2.43 | 460.01 | 377.45 |
| d | 0 | 3.75 | 252.72 | 469.38 |
| | 2.5 | 3.50 | 254.99 | 414.99 |
| | 5.0 | 3.74 | 386.73 | 615.10 |
| | 7.5 | 3.54 | 232.95 | 502.15 |

Yaiche et al. (2014) noticed slight differences in ***the specific volumes*** of the the enriched breads. The specific volumes decreased with the increasing of the algae content in the bread.

The data varied between 2.14 $cm^3 g^{-1}$, for bread with 1% Spirulina (*Arthrospira fusiformis*) and 3.74 $cm^3 g^{-1}$, for bread with 5% *Cladophora* spp. and *Ulva* spp.

According to Figueira et al. (2011), the specific volume was not affected by the addition of up to 4% of alga, but a decrease of 22% in the volume of the bread were noted with the addition of 5%.

From all the macroalgal biomass added in the evaluated samples, the formulation with 5.0% ($w.w^{-1}$) was the one with the highest specific volume. (Menezes et al., 2015)

The incorporation of the algae species, which are wealth in the proteins, in the dough of bread result in an increase of the availabality of proteins for the enzyme, and causes the formation of the crosslinks between proteins. This fact makes the dough rigid and prevents expanding of gas, and respectively reduction of the specific volumes of breads (Moore et al., 2006).

The data for ***densities*** varied between 0.44 and 0.47 g $cm^{-3}$. The values of densities were very closed density remaining almost unchanged. The addition of spirulina does not cause changes in the bread density. Furthermore, the density is inversely related to specific volume. The average thickness crust of control and enriched breads present values going from 0.13 to 0.15 cm. They present no significant difference (Yaiche et al., 2014).

The ***crumb hardness*** was not affected by the addition of up to 4% of alga, but an increase of 113% in crumb hardness of the bread was noted with the addition of 5% (Figueira et al., 2011).

The addition of macroalgal biomass had no significant difference in the measured hardness of fresh bread and after 24 hours of baking except for the fresh bread enriched with 5% ($w.w^{-1}$) biomass, which was different from the control, presenting greater hardness (Menezes et al., 2015).

Figueira (2011) found that the hardness of the breads was unchanged with the addition of up to 4% ($w.w^{-1}$) of algae, the hardness only increased when 5% ($w.w^{-1}$) of algae (based on flour) was added.

**b. Nutritional and sensory analysis**

The enrichment by spirulina improved the nutritional quality of bread (Figure 1), a better increase on the rate of **proteins** was obtained for the bread with a rate of 3%. The value was estimated at 9.98%. For the rest of nutriments (fat, crude fiber, carbohydrates) the values remain almost unchanged (Yaiche et al., 2014).

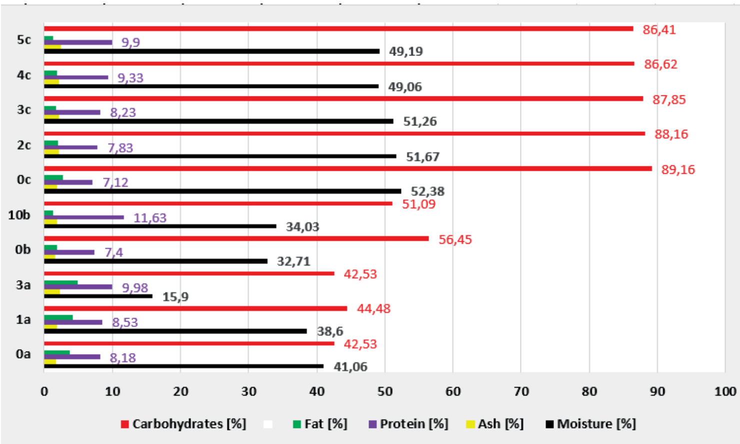


Figure 1. The nutritional quality of bread

Figueira et al. (2011) have shown that the addition of *Spirulina* resulted in products with improved nutritional quality, with a significant increase of 39.04% in the protein content as well as of some essential amino acids (threonine, methionine, isoleucine and leucine), when compared to bread without the addition of the alga. A smaller, but significant protein increase was found by Menezes et al. (2015). In this case, the increase in the concentration of macroalgal biomass in the bread resulted in a protein increase from 16.5 to 18.5% (w.w$^{-1}$). The content of protein reported by Burcu et al. (2016) was 7.40% when microalgal was not added, respectively 11.63% for 10% microalgal content.

According to Figueira (2011), **the lipid** content of bread enriched with *Spirulina,* with samples spiked with 2, 3, 4 and 5% (w.w$^{-1}$), ranged from 1.24 to 2.81% (w.w$^{-1}$) and the lipid content was reduced with the addition of *Spirulina,* this occurred probably due to the smaller amount of this components in the algae biomass. Menezes et al. (2015) reported for lipid concentration values ranging from 7.0 to 17.0% (w.w$^{-1}$), with the increase in biomass there was a decrease in the lipid content, by about 5% (w.$^{w-1}$). Also, Burcu et al. (2016) presented a significant decrese of lipid content, from 1.91% to 1.36%, when 10% microalgal content was added.

Burcu et al. (2016) noticed an increase for the **moisture**, from 32.71 to 34.03%, when the *Spirulina platensis* content was raised from 0 to 10%. Oppositelly, all other authors highlited that the moisture content gradually decreased with the increase of spirulina level added. Both, Yaiche et al. (2014) and Menezes et al. (2015) showed small differences in water contents of the analyzed samples. According to Yaiche et al. (2014), the moisture content gradually decreased with the increase of spirulina level added, revealed significant ($p$>0.05) difference. The registered values were 41.06, 38.60 and 15.90% for 0, 1 and 3% microalgae contents. The decrease is due probably to the addition of the spirulina in their dried form.

Figueira et al. (2011) reported that the incorporation of spirulina in bread formulation improve **ash** (total mineral content) level. The data reported by (Menezes et al., 2015) were ranging from 1.92 to 2.52%, with the increase in biomass there was a slightly increase in the ash content. The ash content between all the breads did not have any significant difference, except for the bread formulation with 7.5% (w.w$^{-1}$), which differed significantly due to the mineral content (2.5%) present in macroalgae. Also, Yaiche et al. (2014) reported an improvement for the ash in the bread, the values are 1.86 and 2.31% for 1 and 3% enriched bread, respectively. The amount of improvement was estimated at 31.81% for bread enriched with 3% of spirulina.

The reported data for calcium, magnesium and iron are presented in Figure 2.

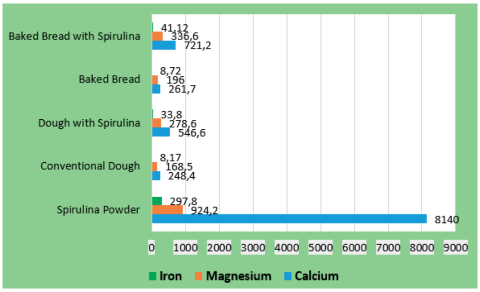


Figure 2. Calcium, magnesium and iron contents of *Spirulina,* dough, and breads (ppm)

Although all the cited authors analyzed the content of total substances, only Burcu et al. (2016) highlighted several mineral elements, namely ***calcium, magnesium and iron***.

The highest values in calcium (8140 ppm), magnesium (924.2 ppm), and iron (297.8 ppm) were detected in *Spirulina.* The lowest values were identified in conventional bread dough and in baked bread without *Spirulina.* The amounts of calcium, magnesium and iron in conventional bread was much lower than those of the bread with *Spirulina*. Approximately 5 times more iron was detected in baked bread with *Spirulina*.

Figueira et al. (2011) reported that the addition of spirulina decrease slight the ***carbohydrate*** content of bread. Also, Yaiche et al. (2014) found no change in carbohydrates values between control and enriched breads. On the other hand, Menezes et al. (2015) showed that the carbohydrate content was 65% (w.w$^{-1}$) on average, reduced with addition of macroalgae, all breads differed significantly from the control bread.

In relation to the attributes ***color, odor and consistency***, there was no significant difference at 95% level of significance between the bread formulations containing 2.5, 5.0 and 7.5% (w.w$^{-1}$) of macroalgal biomass (Menezes et al., 2015). Yaiche et al. (2014) noticed that all enriched bread presented a good global acceptability. Anyway, the bread supplemented by 1% of spirulina seem to have more preferred color, bread enriched with 3% being the least appreciated. Also, Figueira et al. (2011), who evaluated breads containing 3% and 5% (w.w$^{-1}$) of *Spirulina platensis*, showed that was no significant difference between the breads of the different concentrations of microalgal biomass. Burcu et al. (2016) were reported that *Spirulina* addition in the proportion of 1% or 4% made no difference.

## CONCLUSIONS

The algal biomass can be produced in a controlled environment, or collected in natural environments Adding spirulina in bakery products is a useful strategy to increase the consumption of proteins in human diet. The algal biomass shows promising qualities as a novel source of protein for bread. Compared to conventional bread, the average nutritive quality of most of the breads enriched with algae was superior, or at least equal. According with the analized data all the enriched breads presented a good global acceptability, even though the lower levels of the algae contents were more appreciated for color.

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# WILD LIFE MANAGEMENT, FISHERY AND AQUACULTURE

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# STUDY REGARDING THE EVOLUTION OF WILD BOAR IN ROMANIA - DOBROGEA AREA, BETWEEN 2018 - 2021

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***Abstract***

*The aim of this study is to reveal the massive involution of wild boar herds in Romania and especially in Dobrogea area. Everybody knows about the effects of the African swine fever on wild boar herds, but no one talks about the impact it has on the environment. In the current conditions, there would have been the possibility to intervene and to populate with animals raised in these hunting complexes. The study focused on the Dobrogea area because it was the first and most affected area of the country. We analyze the official data from national evaluation of sedentary game in Dobrogea area, more exactly Constanta and Tulcea County. Hunting territories in these two counties are managed by National Forest Authority, county associations of hunters and other associations for conservation of biodiversity and management of hunting territories. We analyze wild boar real effective between 2018 and 2021 by counties, by sexes, and in comparison with optimal effective (maximal number of individuals who can leave in a hunting area, without causing damage to the agricultural fields or in the forest).*

***Key words****: African fever, hunting, wild boar.*

## INTRODUCTION

The characteristics of the species have made the wild boar widespread in Romania. The wild boar is a forest animal, but in the studied area it lives and feeds with pleasure in the reeds and on the plains by the lakes and on the natural channels of the Danube Delta and not only.

The African swine fever has greatly affected the wild boar populations from Romania, and especially the counties from the south of the country. The most affected area was Dobrogea, more precisely the territories of Constanta and Tulcea counties. A very important factor in the late detection and spread of this disease was the prohibition of hunting in the Danube Delta Biosphere Reservation. Lack of control of population of sedentary game species, especially of predators, absence of observations that normally are helpfully for management of hunting areas, and ignorance of biosphere reserve administrators by lack of organization of actions to control raptors and maintain ecological balance, led to the rapid spread of the disease, the consequences being those that we will present in this paper. Although there are many non-governmental organizations that advocate for environmentalists, teaching us about bears or duck species, but none of these organizations have noticed the negative impact of reducing wild boar numbers to near extinction.

## MATERIALS AND METHODS

We analyze the official data from national evaluation of sedentary game in Dobrogea area, more exactly Constanta County and Tulcea County. Hunting territories in these two counties are managed by National Forest Authority, county associations of hunters and other associations for conservation of biodiversity and management of hunting territories, and Danube Delta Biosphere Reserve. Within this reservation area no hunting can be organized except by the administrator of the protected area). We analyse wild board effective between 2018 and 2021. We use some statistics like average, standard deviation, error of average, and variability coefficient in order to have a better overview of the situation. The data was collected from Ministry of Environment which is the national authority for conservation of biodiversity and hunting.

## RESULTS AND DISCUSSIONS

In table 1 is presented the evolution, or better the involution, of wild boar effective in Constanta and Tulcea Counties and for all Dobrogea region. The situation is catastrophic between 2018 and 2019. The most affected County is Tulcea, the situation being presented much more clear in figure 1. Constanta County (figure 2) was also affected but not at the same level as Tulcea County.
At the level of Constanta county, the number of wild boar population decreased by 78.31% (957 individuals from which 721 was hunted), while at the level of Tulcea county the decrease was slightly smaller, of only 76.82% (1074 individuals from which 735 was hunted).

Table 1. Evolution of wild boar population in Dobrogea Region, between 2018 - 2021

| ***County/ area*** | ***Year*** | ***Evaluated population*** | ***Hunted*** | ***Optimal effective*** |
|---|---|---|---|---|
| Constanta County | 2018 | 1222 | 721 | 285 |
| | 2019 | 265 | 150 | 285 |
| | 2020 | 261 | 205 | 285 |
| | 2021 | 370 | 303 | 285 |
| | **X** | **529.5** | **344.75** | **285** |
| | **stdev** | 464.42 | 258.69 | 0 |
| | **Sx** | 268.13 | 149.36 | 0 |
| | **CV%** | 87.71 | 75.04 | 0 |
| Tulcea County | 2018 | 1398 | 735 | 647 |
| | 2019 | 324 | 187 | 475 |
| | 2020 | 262 | 195 | 475 |
| | 2021 | 298 | 42 | 475 |
| | **X** | 570.50 | 289.75 | 518.00 |
| | **stdev** | 552.25 | 305.05 | 86.00 |
| | **Sx** | 318.84 | 176.12 | 49.65 |
| | **CV%** | 96.80 | 105.28 | 16.60 |
| Dobrogea area | 2018 | 2620.00 | **1456** | **932** |
| | 2019 | 589.00 | **337** | **760** |
| | 2020 | 523.00 | **400** | **760** |
| | 2021 | 668 | 345 | 760 |
| | **X** | 1100.00 | 634.50 | 803.00 |
| | **stdev** | 1015.07 | 548.38 | 86.00 |
| | **Sx** | 586.05 | 316.61 | 49.65 |
| | **CV%** | 92.28 | 86.43 | 10.71 |

From statistical point of view Tulcea County was less affected by the African swine fever because 9.93% from this decrease of wild boar effective was due to the African swine fever, compared to situation from Constanta County (24.66% from total loss was due to the epidemic action).
We mention that during this period no cases of poaching or other accidents were reported. It is true, however, that this momentum overlaps with the outbreak of the epidemic. At the level of the Dobrogea region, the losses represented 77.52% (2031 individuals), and from these 28.31% was founded dead due to the action of same disease.

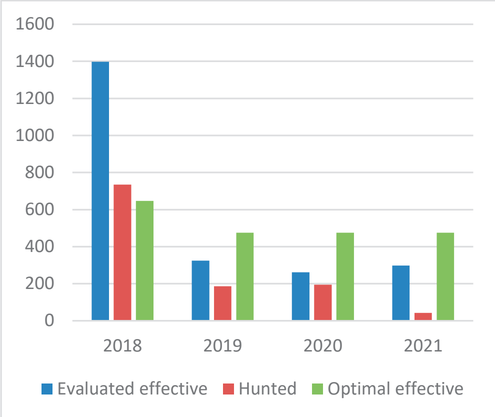


Figure 1. Wild boar dynamic in Tulcea County

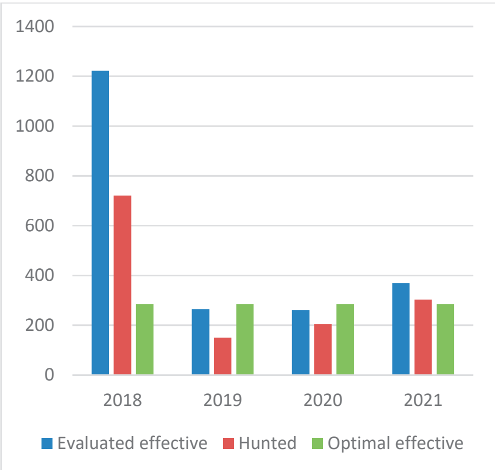


Figure 2. Wild boar dynamic in Constanta County

In 2019-2020 we start with an evaluated effecttive of only 586 wild boars, in entire Dobrogea Region (15588 km$^2$, from which 7104 km$^2$ for Constanta County and 8484 km$^2$ in Tulcea County), which means only 22.48% from the evaluated effective in 2018. This means that the most affected individuals was piglets and youths but also the reproductive nucleus. Only in this way can be explained such a decreasing of populations.

The situation isn't looks so bad if we will report to the evaluated effective from 2019. In period 2019 - 2020, the decreasing of wild boar effective was 11.21% in Dobrogea, from which only 1.51% in Constanta County, but 19.14% in Tulcea County. Number of hunted wild boars, in this hunting season represent 57.22% from evaluated effective which is a very important share. This situation can be explained by the measures taken by the authorities authorized to fight with the epidemic, the National Sanitary Veterinary Agency, one of these measures aiming at the complete hunting of wild boars in disease outbreaks.

We must also draw attention to the fact that in 2020 the optimal effective of wild boar, at the level of Tulcea county, was modified, decreasing by 172 heads, which represents 26.58% of the optimal effective of 2018. This decrease of the optimal effective was made without changes in the ecological diagnosis keys for wild boar (Tables 2 and 3), and in the conditions in which, in the Danube Delta Biosphere Reserve (4178 $km^2$) hunting has not been practiced for quite a long time and the natural conditions have not changed. It is impossible for us to believe that the factors that influence the creditworthiness of the hunting fields have experienced such a large depreciation and on a relatively small area of the county in order to determine the calculation of an optimal number so diminished.

Regarding 2020 - 2021 period we record, for the first time after few years, a small evolution of wild boar effective evaluated in 2021. The most important evolution was recorded In Constanta County where the evaluated effective number has increasing with 41.76% (109 individuals). In Tulcea County these increasing represent only 13.74% (36 individuals). At the Dobrogea Region level this increase was 27.72% (145 individuals). The situation looks good if we refer only to the percentage values of this increase of population. It is gratifying that we have an upward trend, but if we translate this increase into the actual number of individuals with which the population has grown, we realize that this increase is almost insignificant.

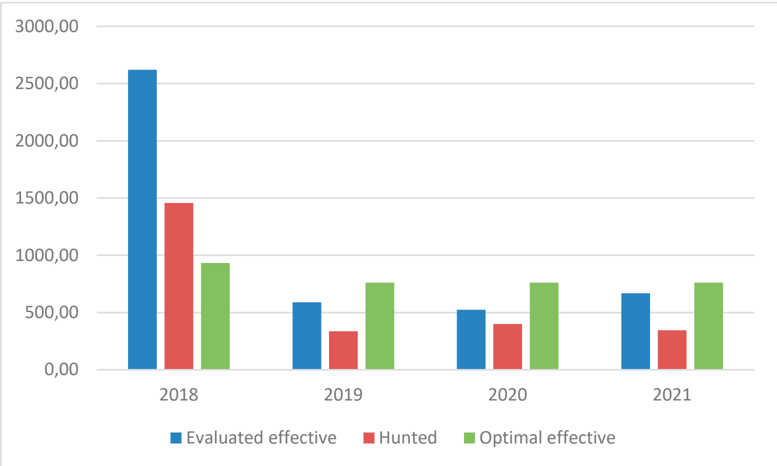


Figure 3. Wild boar dynamics in Dobrogea Region, between 2018 - 2021

Table 2. The ecological diagnosis keys for wild boar - abiotic and biotic factors

| No. | The environmental factor | Station specifics | Score | Station specifics | Score | Station specifics | Score | Station specifics | Score |
|---|---|---|---|---|---|---|---|---|---|
| A. ABIOTIC FACTORS - 200 points | | | | | | | | | |
| 1 | Average altitude | 0-400 m | 30 | 401-800 m | 20 | 801-1200 m | 10 | >1200 | 0 |
| 2 | Average temp. in calving period | >$3^0$C | 50 | 2.1-$3^0$C | 45 | 1-$2^0$C | 30 | <$1^0$C | 15 |
| 3 | Precipitation during calving | <50 mm | 30 | 51-65 mm | 20 | 66-70 mm | 10 | >70 mm | 0 |
| 4 | The average thickness of the snow cover and the size of the snow cover period | Snow layer thickness <10cm; snowy period <30 days | 60 | Snow thickness 10-20cm; snowy period 31-40 days | 40 | Snow thickness 21-30cm; snowy period 41-60 days | 20 | Snow layer thickness <10cm; snowy period> 60 days | 0 |
| 5 | Hydrographic network | Uniformly distributed, accessible | 30 | Accessible on> 50% of the area | 20 | Accessible on 20-50% of the area | 10 | Accessible on <20% of the area | 0 |
| B. BIOTIC FACTORS - 250 points | | | | | | | | | |
| 1 | Percentage of afforestation | >50% of area | 50 | 31-50% of the area | 35 | 10-30% of the area | 20 | <10% of the area | 5 |
| 2 | The share of age classes | Classes I, V, VI on> 40% of the forest area | 20 | Classes I, V, VI on 35-40% of the forest area | 15 | Classes I, V, VI on 30-34% of the forest area | 10 | Classes I, V, VI on <30% of the forest area | 5 |
| 3 | Forest formations | >50% quercete and hillside | 30 | 20-50% quercete and hill stalks | 20 | Beech and beech softwood mixtures | 10 | Pure sprouts | 5 |
| 4 | Undergrowth | On > 0.7 of the forest area | 30 | On 0.4-0.7 from the forest area | 20 | On 0.1-0.3 of the forest area | 10 | On <0.1 of the forest area | 5 |
| 5 | Agricultural crops | From > 8 species ha% | 20 | of 6-8 species / 100 ha | 15 | from 3-5 species / 100 ha | 10 | of <3 species / 100 ha | 5 |
| 6 | Vegetation outside the forest floor | Protective curtains, reeds, fences, brambles on> 10% of the surface of the hunting ground | 50 | Protective curtains, reeds, ditches, brambles on 7-10% of the surface of the hunting ground | 35 | Protection curtains, reeds, ditches, brambles on 3-6% of the surface of the hunting ground | 20 | Protection curtains, reeds, ditches, brambles on <3% of the surface of the hunting ground | 5 |
| 7 | Biomass accessible in winter (bulbs, rhizomes, etc.) | > 200 kg/ha | 50 | 151-200 kg/ha | 35 | 100-150 kg/ha | 20 | <100 kg/ha | 5 |

Table 3. The ecological diagnosis keys for wild boar - cynegetic management and negative anthropic factors

| No. | *The environmental factor* | *Station specifics* | *Score* | *Station specifics* | *Score* | *Station specifics* | *Score* | *Station specifics* | *Score* |
|---|---|---|---|---|---|---|---|---|---|
| **C. Factors of cynegetic management** | | | | | | | | | |
| 1 | Land for winter feeding | > 5 ha/1000 ha from the surface | 70 | 2.1-5 ha‰ from surface | 50 | 1-2 ha‰ from surface | 30 | > 5 ha‰. from surface | |
| 2 | Winter-fed food and distribution | Over the amount of instructions; evenly distributed over the snowy period, in the wintering territory | 80 | The amount of instruction; evenly distributed over the snowy period, in the wintering territory | 60 | The amount of instructions; distributed over> 50% of the wintering territory | 40 | Amount of instructio; distributed over <50% of the wintering territory | |
| 3 | Numerical ratio of natural predators / wild boar | > 1:30 | 70 | 1: 20 -1: 30 | 50 | 1: 10-1: 19 | 30 | <1.10 | |
| 4 | Wandering dogs /1000 ha | It does not exists | 80 | 1-2 /1000 ha | 40 | 3-4/1000 ha | 20 | > 4 specimens / 1000 ha | |
| **D. NEGATIVE ANTHROPIC FACTORS** | | | | | | | | | |
| 1 | Grazing | It is not practiced | 90 | It is practiced on <20% of the surface | 60 | It is practiced on 21-30% of the area | 30 | It is practiced on> 30% of the area | 0 |
| 2 | Poaching | There are no cases discovered | 90 | There is 1 case discovered | 60 | There are 2 cases discovered | 30 | There are> 2 cases discovered | 0 |
| 3 | Raising domestic pigs | In closed premises; domestic pigs are vaccinated | 35 | In closed premises; domestic pigs are not vaccinated | 20 | Accidental presence in the forest; domestic pigs are vaccinated | 10 | They feed on the productive surface of the hunting field, being unvaccin. | 0 |
| 4 | The density of the road network | < 1 km / km$^2$ | 35 | 1-1.5 km / km$^2$ | 25 | 1.6-2 km / km$^2$ | 15 | > 2 km / km$^2$ | 5 |

## CONCLUSIONS

Following the analyzes performed and presented previously, we issued the following conclusions and recommendations:

***Conclusions:*** Situation of wild boar population in Dobrogea Region is a little bit more special than in the rest of the country. In Tulcea County we have an area of over 4000 km$^2$, Biosphere Reservation "Danube Delta" in which the hunting it was prohibited. Even if at present moment the problem of hunting resumes, in this area, in some species of mammals, it is hard to believe that the administrator of this protected natural area has the technical and organizational capacity to do so. In this situation we will continue to have an upward trend of the golden jackal population and a downward trend of other mammals. The presence of these raptors in large numbers will only increase the spread of the disease.

To hunt all wild boars from hunting fields it is impossible, even if some authorities want it.

The numerical evolution of the wild boar population, at the level of the studied area, is insignificant, even if their percentage expression shows a rosier situation.

The script modification of the optimal wild boar effective, in Tulcea county, was made without having a logic, a scientific basis.

Perhaps the most serious problem is the ignorance of the authorities and of the civil society regarding the impact that the "disappearance" of the wild boar has on the environment.

Recommendations:

- Prohibition of wild boar hunting in the affected areas and in the surrounding areas;
- Rapid intervention on predatory species in order to maintain the ecological balance but especially to limit the spread of the disease;
- Reanalysis of the keys to the diagnosis of hunting funds;
- Population with wild boars from profile farms;
- Limitation of the extraction quota;
- The popularization of the impact that this drastic decrease, of the wild boar herd, has on the environment.

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# COMPARATIVE STUDY ON THE GROWTH AND DEVELOPMENT OF THYME AND BASIL HERBS IN AQUAPONIC SYSTEM AND HYDROPONIC SYSTEM

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***Abstract***

*This experiment aimed to compare the growth and development of two aromatic herbs, thyme (Thymus vulgaris) and basil (Ocimum basilicum var Aristotle) in an aquaponic system with Carassius auratus, versus a hydroponic system. The experiment took place at the pilot system from University Dunărea de Jos, Galați, Faculty of Food Science and Engineering. The system consists of six rearing units for fish and twelve units for plants (filled with substrate light expanded clay aggregate L.E.C.A.), led lamps for plants with purple light (36 W), biological and mechanical filters, and pumps for water recirculation. Three rearing units were populated with Carassius auratus at a stocking density of 20.93± 0.11 kg $m^{-3}$ and the other three were left without fish. For the hydroponic treatment, a nutritive solution was added daily to support plant growth. Plants units were populated with seedlings of thyme and basil. The physico-chemical parameters of water were measured twice per week during the trial. At the end of the trial, the fresh weight of the plants was measured, and it was concluded that the productivity of the plants was higher in the aquaponics units comparing the hydroponic units.*

***Keywords****: aromatic herbs, Integrated aquaculture, nutrient supplementation, plant productivity.*

## INTRODUCTION

The world's population is continuously growing, that`s because in the future satisfying consumers' demands only by classic agriculture will lead to more intense competition for natural resources. Thus, the adoption of technologies for the sustainable farming system can release environmental pressure, reduce gas emissions and implicitly reduce their contribution to environmental impact (Schwitzguébel & Wang, 2007; Kloas et al., 2015; Rizal et al., 2018). Lately, hydroponic and aquaponic farming became popular and are the most innovative methods in the agricultural sector. Generally, hydroponics refers to the production of plants without soil, only with the addition of nutrient solutions to support growth (Pantanella, 2008; Panda et al., 2016). Aquaponics combines hydroponics and aquaculture (fish culture), plants being grown together with aquatic species in a soilless water-based system (Rakocy et al., 2006; Timmons and Ebeling, 2013). In an aquaponic system, fish waste supplies the nutrients for plant growth. Both growing systems are suitable for urban areas, highly productive, and can address the shortage of land concerning growing demand for food production (Medina et al., 2016). In this context, aquaponics can be a sustainable farming technique, because offers many advantages in terms of environmental impact (Ghamkhar et al., 2022), recycling nutrients (Graber and Junge et al., 2009), and reducing water consumption (Rakocy & Hargreaves, 1993; Turcios & Papenbrock, 2014; Yigit et al., 2016) being at the same time more profitable, because both plant (vegetables) and animal (fish) production are obtained. Aromatic herbs (e. g. basil, thyme, coriander, spearmint, sage, etc.) are among the most used in aquaponics, mainly because have a fast harvest rate, and due to the higher applicability of these plants in various fields (cosmetics, medicinal, or food industry), making them the most economically important herbs worldwide. Thyme (*Thymus vulgaris*) and basil (*Ocimum basilicum* var. *Aristotle*) are between the most appreciated aromatic plants by consumers as culinary herbs, but also with multiple uses in the pharmaceutical, cosmetic industry, or the food industry for meat bio-preservation (Mihailović et al., 2013; Grespan et al., 2014; Fratianni et al., 2010; Sandulachi et al., 2021).

In this context, this study aimed to compare the growth performances of basil (*O. basilicum* var. Aristotle) and thyme (*Thymus vulgaris*) in an aquaponic system with *Carassius auratus,* versus a hydroponic system, with the addition of plant fertilizers. The plant and fish growth performance were analyzed and discussed together with the physicochemical parameters of water.

## MATERIALS AND METHODS

*Design and components of the aquaponics/hydroponic system*. The study was conducted for 45 days at the Aquaponics unit of the Research Center for Modelling Recirculating Aquaculture Systems (MoRAS) of Faculty of Food Science and Engineering, Dunărea de Jos, University of Galați, Romania. The system consists of six rearing units for fish (1) and twelve units for plants (5), led lamps (6) with purple light, biological filter (4), mechanical filter (5), and pumps for water recirculation (2) (Figure 1).

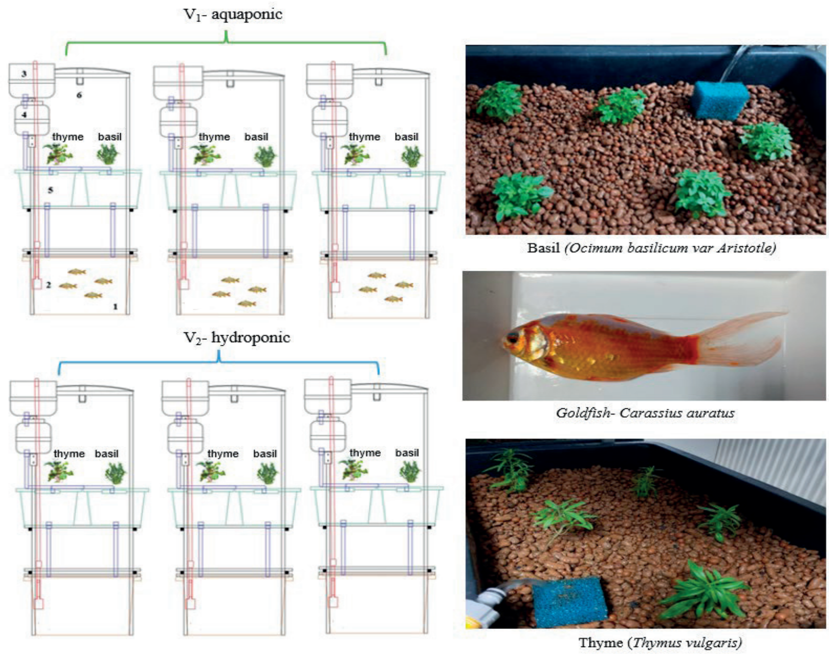


Figure 1. The scheme of the aquaponic and hydroponic systems
1-fish rearing unit; 2- recirculation pump; 3- mechanical filter; 4- biological filter; 5- aquaponic units; 6- led lamp

The LED lamps – pink color (36 Watts, model YQ-88012-36, China) were located at 0.8-meter above the plant's units to maintain the optimal illumination for the growing and were scheduled to work for 12 hours per day.

From each fish tank water was recirculated to the plant's units using a submersible pump (Aqua Zonic Evo E05, EVO PUMPS, Latvia), with a flow rate of 3000 liters per hour. The pump is submerged in the fish rearing unit from where it pumps the water up through a polypropylene pipe into the mechanical filtration unit. From the mechanical filter, the water moves gravitationally through the rest of the system. From the mechanical filtration unit to the biological filtration unit, through distribution pipes to the two plant units (of each module), and from there back into the fish rearing unit (Figure 1). The mechanical filter uses sponges of two different densities, housed inside a plastic container, while the biological filtration consists of hundreds of small plastic media beads on which naturally-occurring bacteria (mainly *Nitrosomonas* sp. and *Nitrobacter* sp.) can develop. Weekly, 1 % of rearing water from the aquaponics system was replaced to remove settable solids. Also, weekly freshwater was added to compensate for its evaporation.

*Experimental design.* Two treatments in three replicates per experimental variant were performed. Therefore, three rearing units (V1-aquaponic) were populated with *Carassius auratus* (mean weight of 62.95±0.35 g) at a stocking density of 20.93± 0.11 kg $m^{-3}$ and the other three were left without fish (V2-hydroponic). For the hydroponic treatment, a nutritive solution (0.27% Fe, 0.07% Mn, 0.05% Zn, 0.04% B, 0.009% Cu, and 0.006% Mo) was added daily to support plant growth (2 mL solution/L water).

Fish were fed manually with extruded pellets (34 % protein, 15 % lipids, 1.4 % crude fibre, 6.80 % ash). The daily feed ratio was adjusted to 2% from fish biomass. The daily feeding amount was divided into three meals (at 8:00 AM, 12:00 PM, and 5:00 PM). Plants units were populated with seedlings of thyme (*Thymus vulgaris*) and basil (*Ocimum basilicum* var. *Aristotle*), at a density of five plants per tank (0.17 plants/$m^2$). Plants units were filled with lightweight expanded clay aggregate (LECA).

*Water quality.* During the trial, the temperature (°C), dissolved oxygen (mg $L^{-1}$), and pH (pH units) were daily monitored, with the Pro1020 (YSI Incorporated, USA), pH-meter WTW (InoLab pH 7110, Xylem Analytics, Germany), while the nitrogen compounds: nitrates, nitrites, and ammonium concentration (mg $L^{-1}$), were measured twice per week, using Merck kits and the Merck Spectroquant Nova 400 spectrophotometer (Merck Chemicals GmbH, Germany). The sampling points of the water samples were established as follows: after mechanical and biological filtration (M+B), after evacuation of water from thyme growing units (T), after evacuation of water from basil growing units (B), and from the fish basins (F), respectively from the hydroponic units (H). The luminous intensity (lx) was measured with TESTO 545 light meter (Testo Co. United States) once per week.

*Determinations of nitrogen, phosphorus, and chlorophylls.* After weighing and measuring, the plants were oven-dried at 60ºC until constant weight, and then, their dry biomasses were determined. From dry plants, we determinate the nitrogen, according to the Dumas method by combustion of dry samples at 1100°C (Primacs SNC 100, Skalar Analytical B.V., The Netherlands). The nitrite and nitrate levels in plants were determined using the Griess method (STAS 9065:2002).

The amount of phosphorus was determined colorimetric using the San$^{series}$ of Automated Wet Chemistry Analyzers (SAN$^{series}$ Skalar Analytical B.V., The Netherlands). The method of phosphate determination is based on the reaction of ammonium hepta molybdate and potassium antimony (III) oxide tartrate in an acidic medium with diluted solutions of phosphate to form an antimony-phospho-molybdate complex. This complex is reduced by L(+) ascorbic acid to an intensely blue-coloured complex which is measured spectrometrically at 660 nm. The chlorophyll content indexes were determined. Chlorophylls were extracted from the tissues of leaves with ethanol according to the protocol of Castle et al. (2011). Absorbance was measured at adequate wavelengths characteristic for chlorophyll a, b, and total chlorophyll respectively 649 and 665 nm.

*Growth parameters.* At the beginning of the experiment, and the end, basil and thyme seedlings were measured for plant height (cm), plant weight (g), and roots height (cm). Fish growth parameters, including initial weight (g), final weight (g), weight gain (g), initial biomass (g), final biomass (g), feed conversion ratio (FCR), specific growth rate (SGR), and protein efficiency ratio (PER) were calculated:

- Weight Gain (WG) = Final Weight (Wt) (g) – Initial Weight (W0) (g);
- Food Conversion Ratio (FCR) = fish feed quantity (g)/WG (g) (g/g);
- Specific Growth Rate (SGR) = (ln Wt − ln W0)/t × 100 (% BW/day), where t- duration of the experiment;
- Protein efficiency ratio (PER) = Total weight gain (W) / amount of protein fed (g).

*Statistical analysis*. All collected data were analysed with the SPSS 21.0 (SPSS Company Inc., Chicago, IL. USA) statistical software package and Microsoft Excel. Statistically significant differences were reported at $p<0.05$.

## RESULTS AND DISCUSSIONS

*Water*. The daily values of temperature, dissolved oxygen, and pH are presented in Figure 2. During the experimental period, water temperature registered a mean value of 23.03±4.31°C in the aquaponic system respectively 23.33±3.36°C in the hydroponic system, with no statistical differences ($p>0.05$). Regarding the dissolved oxygen, significantly higher values were registered in the hydroponic system (8.79±0.12 mg $L^{-1}$) in comparison with the aquaponic system (7.47±0.14 mg $L^{-1}$). The pH values were statistically lower ($p<0.05$) in the hydroponic system (6.75±0.24) in comparison with the aquaponic system (7.12±0.31).

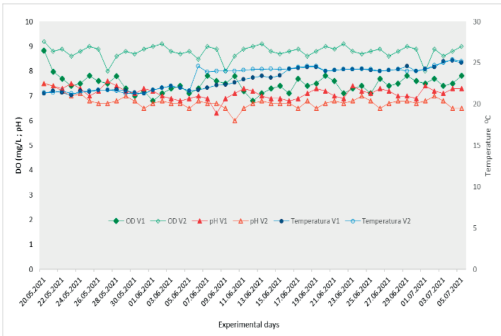


Figure 2. Daily values of temperature, pH, and dissolved oxygen during the experimental period

To obtain higher production of plants and fish in aquaponics systems plant and fish species must correspond as closely as possible for water temperature and pH requirements. For basil and thyme, the optimum temperature is reported between 20-25°C (Somerville et al., 2014; Abdelrazzaq et al., 2016), while the pH values must be between 6 and 8. According to some authors (Dong et al., 2001), high-temperature levels have a negative impact on the roots because reduce the absorption of N and inhibit the assimilation of K and P.

Also, for optimum growth, it is necessary to ensure plants higher nitrogen concentrations. In the Figures 2, 3, 4 and 5 are presented the minimum, mean and maximum values during the whole experimental period.

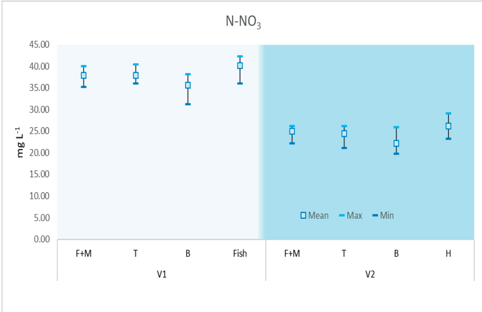


Figure 3. Values of nitrate during the experimental period

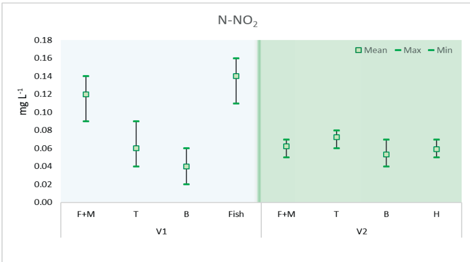


Figure 4. Values of nitrite during the experimental period

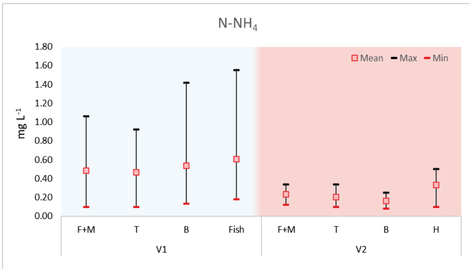


Figure 5. Values of ammonium during the experimental period

Significant higher ($p<0.05$) concentration of nitrogen compounds was recorded in the aquaponic system. The average $N\text{-}NO_3^-$, $N\text{-}NO_2^-$ and $N\text{-}NH_4^+$ concentrations in the effluent water after removal from the plant units, showed a better removal rate capacity in the case of basil in comparation with thyme. Also, during the experimental trial the nitrate concentration gradually increase, and at the end of the experiment we registered a higher nitrate concentration comparing with the beginning of the experiment. Nitrogen is the element that is required in the greatest amounts by several crops (Lavres et al., 2005), principally because nitrogen contributes in the synthesis of proteins. The ammonium ($N\text{-}NH_4^+$) represents the major source of inorganic nitrogen used for growth plants (Vaillant et al. 2004), while the concentration of nitrate is relatively harmless, and it is the preferred form of nitrogen for plant growing (Rakocy et al., 2006). Concentration of nitrogen compounds were found to be in nontoxic range for fish (Eding & Kamstra, 2002; Timmons & Ebeling, 2013), but in the recommended values to sustain plants life in the aquaponic system (Bittsanszky et al., 2016; Mulay & Reddy, 2021). According to some authors nitrate ($NO_3$) is not toxic to fish even at high concentrations of up to 150-300 mg $L^{-1}$ (Graber & Junge, 2009; Yildiz et al., 2017). Wongkiew et al. (2017) reported nitrate values around 16-50 mg $L^{-1}$ in an aquaponic system using Common carp (*Cyprinus carpio*) and Pak choi (*Brassica chinensis*), respectively values of 26.7-54.7 mg $L^{-1}$ for Tilapia (*Oreochromis* sp.) and Basil (*Ocimum basilicum*).

*Plants.* Plant productivity is shown in Table 1. No significant differences ($p>0.05$) in the plants' weight, plant biomass, total heights, and root length were registered at the initial moment when the aquaponic and hydroponic systems were populated. After 45-days, the obtained results indicate a significant ($p<0.05$) higher plant biomass in the aquaponic system, both in the case of thyme and basil. From Table 1 it could be seen that the thyme and basil weight, total fresh biomass, and the total heights from the aquaponic system are significantly higher than those from the hydroponic system. In terms of plant roots, the statistical analysis revealed that the thyme roots from the aquaponic system (15.13±4.77 cm/plant) are significantly lower ($p<0.05$) compared to those from the hydroponic system (26.31±15.63 cm/plant), while no significant differences ($p>0.05$) were not highlighted between the lengths of the basil roots.

Table 1. Plant growth data in an aquaponic system vs. hydroponic system

| Plant | Growing method | Experimental moment | Plant weight (g/plant) | Plant biomass (g) | Total heights (cm/plant) | Root length (cm/plant) |
|---|---|---|---|---|---|---|
| Thyme | Aquaponic | Initial | 4.02±0.33 | 20.11±1.33 | 12.66±1.98 | 8.53±1.06 |
| | | Final | 49.13±24.76 | 245.64±2.18 | 48.21±16.11 | 15.13±4.77 |
| | Hydroponic | Initial | 3.63±0.78 | 18.14±1.45 | 11.95±2.56 | 7.91±1.40 |
| | | Final | 28.44±8.75 | 142.21±43.73 | 29.41±19.11 | 26.31±15.63 |
| Basil | Aquaponic | Initial | 6.48±0.96 | 32.38±1.42 | 9.55±1.15 | 1.06±0.72 |
| | | Final | 50.67±9.09 | 253.36±3.22 | 48.84±12.11 | 16.98±4.21 |
| | Hydroponic | Initial | 6.12±0.61 | 30.61±3.06 | 10.15±0.64 | 7.63±0.35 |
| | | Final | 24.32±9.62 | 121.62±32.94 | 45.18±11.91 | 17.67±2.87 |

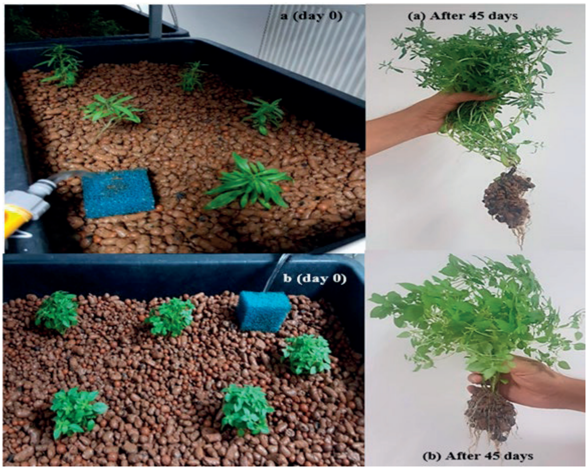

Figure 6. Thyme (a) and basil (b) cultivated in LECA substrate
(Day 0 of cultivation and after 45-days)

Fish growth performance is presented in Table 2. Survival rate was 98.33±2.36 % in experimental fish during the trial. The final individual weight of fish (mean±SD) was 82.75±5.56 g, with no significant differences ($p<0.05$) between the triplicates. The feed conversion ratio (FCR) was 1.15±0.07 and the specific growth rate (SGR) was 0.61±0.01 % $day^{-1}$.

Table 2. Fish growth performance at the end of the trail

| Parameters | V1 |
|---|---|
| Initial weight (g) | 62.95±0.35 |
| Final weight (g) | 82.75±5.56 |
| Initial length (cm) | 11.5±1.09 |
| Final length (cm) | 12.8±3.01 |
| Individual weight gain (g/fish) | 18.80±1.70 |
| Food Conversion Ratio (g/g) | 1.15±0.07 |
| Specific Growth Rate (g $day^{-1}$) | 0.61±0.01 |
| Protein efficiency ratio | 2.56±0.09 |
| Survival rate (%) | 98.33±2.36 |

Table 3 presents the values of the dry matter content (%), Chlorophyll A and B, total nitrogen (%), nitrite (mg/kg), nitrate (mg/kg), and phosphorous (mg/kg) content from leaf plants.

Table 3. Plant analysis at the end of the trial

| Parameter | Plant | V1 | V2 |
|---|---|---|---|
| Dry matter (%) | Thyme | 15.14±1.39 | 17±4.87 |
| | Basil | 8.09±0.98 | 10.6±2.58 |
| Chlorophyll A ($\mu g \times g^{-1}$) | Thyme | 0.23±0.01 | 0.19±0.03 |
| | Basil | 0.35±0.03 | 0.29±0.03 |
| Chlorophyll B ($\mu g \times g^{-1}$) | Thyme | 2.23±0.02 | 2.06±0.24 |
| | Basil | 2.25±0.11 | 2.18±0.06 |
| Total Nitrogen (%) | Thyme | 5.59±1.2 | 5.80±0.60 |
| | Basil | 5.79±0.19 | 5.61±1.63 |
| Nitrite (mg/kg) | Thyme | 0.56±0.06 | 0.58±0.13 |
| | Basil | 0.32±0.02 | 0.36±0.09 |
| Nitrate (mg/kg) | Thyme | 4.32±0.37 | 4.27±0.15 |
| | Basil | 2.85±0.33 | 2.35±0.18 |
| Phosphorous (mg/kg) | Thyme | 2.12±0.36 | 2.04±0.83 |
| | Basil | 2.45±1.31 | 2.32±0.14 |

Significant higher ($p<0.05$) values of Chlorophyll A were recorded in the case of the aquaponic system, both in the case of thyme and basil. Also, higher values of Chlorophyll B were obtained in the case of the aquaponic system, but the values are not statistically different ($p>0.05$). The chlorophyll content of leaf provides valuable information about the physiological and the nutrient status of plants (Filella et al., 1995; Gitelson et al., 2008). In our experiment, the chlorophyll content was higher in the case of basil. The results obtained by us are contradictory with those obtained by Saha et al. (2016) and Rakocy and Hagreves (1993), who did not find any differences in chlorophyll content from basil and lettuce crop grown under aquaponic and hydroponic systems. According to Gang et al. (1992), the differential chlorophyll accumulation can be explained most probably with the higher biomass growth is positively correlated with the basil biomass growth.

The values of total nitrogen content (%) were higher in the case of the aquaponic system, but the differences were not statistically different ($p>0.05$). Also, although the total nitrogen content (%) was higher in the case of basil, no significant ($p>0.05$) differences were recorded in comparison with thyme.

Regarding the nitrite and nitrate values (mg/kg) recorded significant ($p<0.05$) higher values were recorded for thyme, with no significant ($p>0.05$) differences between the two growing systems, while the values of phosphorous (mg/kg) registered no significant differences ($p>0.05$) between the experimental plants and growing systems. According to Madar et al. (2019), higher nitrate and nitrite content in leafy vegetables result in lower quality. However, the values obtained in our experiment do not exceed the acceptable upper limits of nitrates and nitrites set up in Ordinance no. 438/2002.

## CONCLUSIONS

In this experiment, we compare the growth of thyme and basil in two soilless cultures, aquaponic and hydroponic. Both aquaponic and hydroponic systems can produce significant crop with limited water and without soil. From our results, we can conclude that the production of thyme and basil is more profitable in the aquaponic system. The use of wastewater with the aquaponic system is more favorable for basil and thyme growing since also a profitable fish production is obtained.

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# THE INFLUENCE OF THE POPULATION DENSITY ON THE DEVELOPMENT OF THE SPECIES *SANDER LUCIOPERCA* (LINNAEUS, 1758) IN THE POSTEMBRYONIC PERIOD

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***Abstract***

*The pikeperch (Sander lucioperca, L. - 1758) is one of the freshwater species recently introduced in intensive aquaculture. In the last decade there have been made great efforts in the direction of developing the intensive culture of this species. The population density is a very important technological parameter for fish growth, in all stages of development and is specific to the species, age and technology applied, the most difficult to achieve and with the most significant losses being recorded in the post-embryonic period. Therefore, the growth of the pikepech in the post-embryonic period was experienced in "Evos" type fiberglass pools, in three experimental versions, with three different population densities like: V1 - 1000 ex./basin, V2 - 2000 ex./basin and V3 - 3000 ex./basin. There were two critical moments in the post-embryonic development period: the first was at the beginning of exogenous feeding time, and the second during the swelling of the gas bladder. The experiments were performed at S.C.D.P Nucet, in triplicate, during three growing seasons (2018, 2019 and 2020). The best results were obtained in V1 version, where the survival rate was 69.5% in 2020, the average individual growth rate in 2019 was 1.555 g/ex., and the Fulton coefficient was between 0.93 and 1.13.*

***Key words***: *density, fry, pikeperch, survival weight.*

## INTRODUCTION

The pikeperch (*Sander lucioperca* L.), is a species of interest to fish farmers due to its marketing qualities (Jankowska et al., 2003) and rapid growth (Terlecki, 1955; Nagiec´ 1961; Steffens, 1986), as well as for fishermen (Wołos et al., 1998; Bninska and Wołos, 2001) and is present in most of continental waters on the Romanian territory (Dobrotă et al., 2021). The results of studies conducted in several European countries, including Germany, France and Finland, indicated that one of the main obstacles in the growth and development of the pikeperch culture is in the post-embryonic development period (Hilge and Steffens, 1996). Data from the speciality literature on the methods of the pikeperch intensive breeding larvae are very limited and none of the available reports on this subject analyze the impact of population density on the development of this species (Schlumpberger & Schmidt, 1980; Steffens, 1986; Ruuhijärvi et al., 1991; Hilge & Steffens, 1996; Ruuhijärvi & Hyvärinen, 1996; Mamcarz et al., 1997). After hatching at 18-21 days old, the pikeperch can be fed with mixed feed (*Artemia* sp. and artificial feed), followed exclusively by artificial feed (Szkudlarek, 2004) up to commercial size (Zakes et al., 2000).

The post-embryonic stage, in fish in general, and in pikeperch in particular, is an absolutely necessary stage in the producing process of a one summer old juveniles.

We are disscusing about bringing the fry to the independent larvae stage, which are very fragile immediately after hatching (skin respiration due to the absence of gills, and the inability to feed on exogenous organisms due to the absence of the mouth) and vulnerability to bioaggressors. Szkudlarek & Zakęś (2002) studied and mentioned the effect of population density on the survival and growth performance of pikeperch larvae under controlled growth conditions.

The intensive growth techniques of the American pikeperch (*Stizostedion vitreum*) have been developed and experimented for a long time, and in the opinion of Molnar et al. (2004), they can be applied at least in part to the European one (*Sander lucioperca* L.).

A team of researchers from Hungary (Molnar et al., 2004) conducted an extensive experiment to evaluate the effect of accustoming pikeperch larvae with additional feed and the effects of population density on feeding strategy and behavior.

## MATERIALS AND METHODS

The experimental works took place in the years 2018, 2019 and 2020 at the Research and Development Station for Fish Farming of Nucet. In order to carry out the experimental works in the post-embryonic period in the intensive system of pikeperch larvae, the population density was the variable factor. The degree of repeatability was 3 times, and as a feeding modality, mixed feeding was adopted. The works for the development of the pikeperch in the post-embryonic period were carried out in the pilot installation for growing in fiberglass tubs, with a useful volume of 1,000 liters ("Evos" basins), installed in the Incubation Station no. 1 (Figure 1). The "Evos" type basins are round in shape and are fed through an external pipe with free fall, and the evacuation is done centrally, with the creation of a circular current. The optimum height of the water layer is between 0.40-0.65 m. The baths tubs were supplied with a water quantity of 7-15 l/min, depending on the temperature.

Figure 1. Pilot installation ("Evos" type basin) for raisingand the development of pikeperch larvae in the post-embryonic period

Experimental development works in the post-embryonic period in intensive system of pikeperch larvae, were carried out according to the following working methodology: establishing experimental versions; preparation of the pilot station for the population; larval population; feeding; monitoring the environmental conditions into the growth modules ("Evos" type basins); monitoring the development and health of the larvae; fishing the pikeperch larvae at the end of the growing period, establishing the value of specific indices and discussing the results.

From the specific literature data, the population density of such growth modules in the post-embryonic period depends primarily on the degree of intensification, with a gap of variation from 1-10 exemplares.

The working methodology took into account the following aspects:

- three experimental variant were established in terms of population density;
- "Evos" type pools/experimental variant: 3;
- degree of repeatability: 3;
- feed type: in three stages, natural food in the live state, mixed (live food/fodder) and only with fodder, all three stages well determined in number of days;
- administration: *ad libitum*, only during the day (12 hours), with permanent food consumption control and three-day monitoring of the main physico-chemical parameters of technological water (temperature, oxygen content, pH, organic matter, etc.);
- duration of the post embryonic period: 40 days.
- the experimental version regarding the population density were (Table 1 and Figure 2):
  - variant I: 1000 ex./basin;
  - variant II: 2000 ex./basin;
  - variant III: 3000 ex./basin.

In the post-embryonic pikeperch development stage, in all experimental variants, feeding strategy was adopted and implemented for a 30-40 days period as follows:

- pikeperch larvae were fed both live food and fodder;
- the first 10 days, the larvae feeding was done with live food (*Artemia salina nauplii*) obtained from directed culture within S.C.D.P. Nucet;
- after the 10 days of live food administration, mixed feeding (live food + fodder, in equal percentages, with a 60% of crude protein content) was changed to a 10 days period, a time in which pikeperch larvae are starting to get used to consume both fodder and live food, the live food ration being reduced daily, so that at the end of the 10 days, it represented 10-15% of the total fodder;

Table 1. Population of the "Evos" baths with 7-8 days old larvae

| Nr. crt | Experimental Variant | Basin Volume (l) | Ex. / basin | Nr. basine | Ex. total/year |
|---|---|---|---|---|---|
| 1 | Variant 1 (V1) | 1000 | 1000 | 3 | 3000 |
| 2 | Variant 2 (V2) | 1000 | 2000 | 3 | 6000 |
| 3 | Variant 3 (V3) | 1000 | 3000 | 3 | 9000 |

- almost 20 days of feeding exclusively with fodder, the live food being administered sporadically, once every three days for 5 days, an then, only with fodder, until the age when the growth parameters were reached and the appearance of the cannibalism phenomenon was observed (40 days);
- the feed daily rations, in the form of live food, mixed food (live food + fodder) or only fodder, were established according to the mass of consumers evaluated at 5 days for each experimental variant;
- in all feeding stages, both live food and fodder were mixed or separately administered, in 1-2 hours intervals according to the *ad libitum* system;
- the feed amount was gradually increased as the young fish gained weight and their numerical approximation at the date of the "control fishing".

## RESULTS AND DISCUSSIONS

The experimental works of postembryonic development of the larvae in the intensive system were carried out in three experimental variants, in which the variable factor was the population density. They took place over 3 years, in the period 2018-2019-2020 in almost identical conditions, both technically and technologically. The biotechnological indicator on the number of larvae per experimental variant being dependent primarily on the population density is obvious that at a higher population density also the number of larvae is higher or vice versa even in conditions where the population density also influenced the percentage survival.

Analyzing the data on the larvae growth and development in the post-embryonic period (Tables 2 and 3) by the following biotechnological indicators: average mass (W med./ex., Figure 3), total length (TL), survival rate (Figure 4) and the Fulton coefficient (Figure 5) the following are found:

Figure 2. The 7-8 day old pikeperch larvae population

*Average mass (W) - g/ex.*

- the pikeperch larvae highest average mass after 40 days was obtained in the experimental version V2 in 2019 (1.555 g/ex.), and the lowest was obtained in the V3 version (2018 of 0.821 g/ex);
- in 2018 the highest average weight was obtained in variant V1 (1.414 g/ex.), the lowest in V3 variant (0.821 g/ex.), and in variant V2 the average weight (1.078 g/ex.) was obtained;
- in 2019 the highest average weight was obtained in variant V1 (1.555 g/ex.), the lowest in variant V2 (0.969 g/ex.), and in variant V3 the average weight (0.792 g/ex) was obtained;
- in 2020 the highest average weight was obtained in variant V1 (1.489 g/ex.), the lowest in variant V3 (0.880 g/ex.), and in variant V2 the average weight (1.110 g/ex.) was obtained;
- in conclusion, in all study years, the highest average weight was obtained in variant V1, followed by variant V2, and the lowest average weight in variant V3.

*Total length (TL) - mm/ex.*

- the highest average total length of pike larvae after 40 days was obtained in the experimental variant V1 in 2019 (55.0 mm/ex.), and the smallest was obtained in the variant V3 (41 mm/ex.) in the year of 2019;
- in 2018, the highest total length was obtained in variant V1 (50 mm/ex.), the smallest in variant V3 (42.0 mm/ex.), and in variant V2 the average total length of 45 mm/ex. was obtained;
- in 2019, the highest total length was obtained in variant V1 (55 mm/ex.), the smallest in variant V3 (41 mm/ex.), and in variant V2 the average total length of 44 mm/ex was obtained;

Table 2. The values of the biotechnological indicators for the development of the pikeperch by variants/years in the post-embryonic period in the years 2018, 2019 and 2020

| Year | Variant | Basin | No. ex. populated | No. ex. achieved | Average W (g) | Average L (mm) | Sv (%) |
|---|---|---|---|---|---|---|---|
| 2018 | V1 | B1 | 1000 | 638 | 1.236 | 48 | 63.8 |
| | | B2 | 1000 | 681 | 1.682 | 54 | 68.1 |
| | | B3 | 1000 | 696 | 1.325 | 49 | 69.6 |
| | V2 | B4 | 2000 | 986 | 1.206 | 48 | 49.3 |
| | | B5 | 2000 | 1036 | 1.065 | 45 | 51.8 |
| | | B6 | 2000 | 1148 | 0.964 | 43 | 57.4 |
| | V3 | B7 | 3000 | 1422 | 0.947 | 44 | 47.4 |
| | | B8 | 3000 | 1228 | 0.702 | 40 | 40.9 |
| | | B9 | 3000 | 1331 | 0.815 | 42 | 44.4 |
| 2019 | V1 | B1 | 1000 | 642 | 1.715 | 58 | 64.2 |
| | | B2 | 1000 | 675 | 1.523 | 54 | 67.5 |
| | | B3 | 1000 | 593 | 1.428 | 52 | 59.3 |
| | V2 | B4 | 2000 | 1059 | 1.052 | 47 | 53.0 |
| | | B5 | 2000 | 1078 | 0.928 | 43 | 53.9 |
| | | B6 | 2000 | 1111 | 0.926 | 43 | 55.6 |
| | V3 | B7 | 3000 | 1442 | 0.712 | 40 | 48.1 |
| | | B8 | 3000 | 1387 | 0.865 | 42 | 46.2 |
| | | B9 | 3000 | 1566 | 0.798 | 41 | 52.2 |
| 2020 | V1 | B1 | 1000 | 682 | 1.68 | 56 | 68.2 |
| | | B2 | 1000 | 656 | 1.328 | 51 | 65.6 |
| | | B3 | 1000 | 748 | 1.46 | 55 | 74.8 |
| | V2 | B4 | 2000 | 1170 | 1.296 | 49 | 58.5 |
| | | B5 | 2000 | 1221 | 0.928 | 43 | 61.1 |
| | | B6 | 2000 | 1040 | 1.105 | 44 | 52.0 |
| | V3 | B7 | 3000 | 1535 | 0.767 | 42 | 51.2 |
| | | B8 | 3000 | 1407 | 0.988 | 44 | 46.9 |
| | | B9 | 3000 | 1632 | 0.886 | 43 | 54.4 |

Table 3. Average results on experimental variants

| Year | Variant | Basin | Average survival. (%) | Average W/variant (g) | Average Total Lenght / variant (mm) | Fulton Coeficient |
|---|---|---|---|---|---|---|
| 2018 | V1 | B1+B2+B3 | 67.2 | 1.414 | 50 | 1.130 |
| | V2 | B4+B5+B6 | 52.8 | 1.078 | 45 | 1.180 |
| | V3 | B7+B8+B9 | 44.2 | 0.821 | 42 | 1.090 |
| 2019 | V1 | B1+B2+B3 | 63.7 | 1.555 | 55 | 0.930 |
| | V2 | B4+B5+B6 | 54.1 | 0.969 | 44 | 1.130 |
| | V3 | B7+B8+B9 | 48.8 | 0.792 | 41 | 1.140 |
| 2020 | V1 | B1+B2+B3 | 69.5 | 1.489 | 54 | 0.945 |
| | V2 | B4+B5+B6 | 57.2 | 1.11 | 45 | 1.210 |
| | V3 | B7+B8+B9 | 50.8 | 0.88 | 43 | 1.110 |

- in 2020, the highest total length was obtained in variant V1 (54 mm/ex.), the shortest in variant V3 (43 mm/ex.), and in variant V2 the average total length of 45 mm/ex was obtained;
- in conclusion, in all the study years, the highest average total length was obtained in variant V1, followed by variant V2, and the lowest average total length in V3 variant.

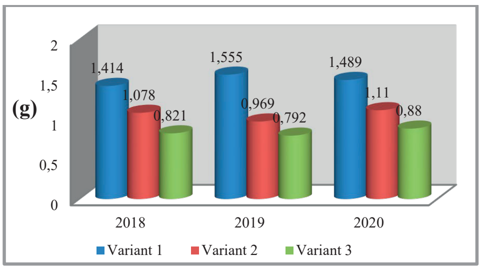



Figure 3. Average weight variation

Survival rate (%)

- after 40 days of post-embryonic growth, the highest survival rate was obtained in the experimental variant V1 in 2020 (69.5%), and the lowest was obtained in the variant V3 in 2018 of 44.2%;
- in 2018, the highest percentage of survival was obtained in variant V1 (67.2%), the lowest in variant V3 (44.2%), and in variant V2 a survival rate of 52.8% was obtained;

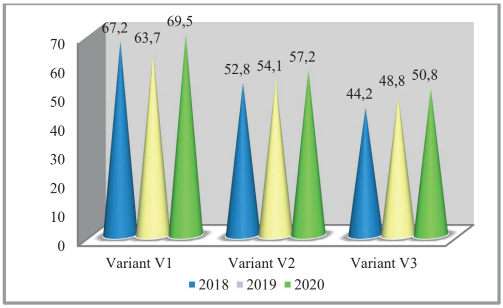



Figure 4. Survival rate variation

- in 2019, the highest survival rate was obtained in variant V1 (63.7%), the lowest in variant V3 (48.8%), and in variant V2 a survival rate of 54.1% was obtained;
- in 2020, the highest survival rate was obtained in variant V1 (69.5%), the lowest in variant V3 (50.8%), and in variant V2 a survival rate of 57.2% was obtained;
- in conclusion, in all the study years, the highest average survival percentage was obtained in variant V1, and the lowest in variant V3.

The Fulton coefficient also had similar values in all experimental variants, falling in the range 0.930 - 1.210, as follows:

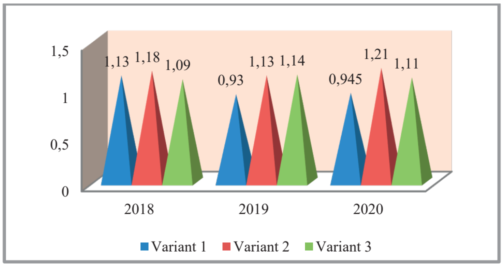



Figure 5. Fulton coefficient variation

- in 2018 the highest Fulton coefficient was obtained in variant V1 (1.130), the lowest in variant V3 1 (1.090), and in variant V2 it was 1.180;
- in 2019 the highest Fulton coefficient was obtained in variant V3 (1.140), the lowest in variant V1 (0.093), and in variant V2 it was 1.130;
- in 2020 the highest Fulton coefficient was obtained in variant V2 (1.210), the lowest in variant V1 (0.945), and in variant V3 it was 1.110.

The observations and data on the growth and development of lark larvae in the post-embryonic period in an intensive system in the "Evos" type basins, of the morpho-physiological characteristics observed macro and microscopically are:

I. At the age of 7-10 days (figure 6 a):

- existing yolk sac but with small dimensions;
- gill buds without being covered by lids;
- transparent body through which the brain and the primordia of the spine are observed;
- the larvae swim lightly.

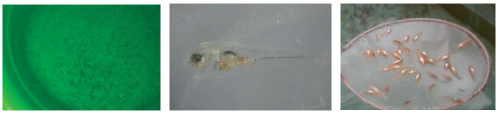
II. At the age of 17-18 days (figure 6 b):

- less transparent body with a tendency to colorate to yellowish, with pigment spots that cover almost entirely the surface of the body;
- well-developed mouth located in the ventral position;
- teeth are observable on the lower jaw in the incipient phase of formation;
- the intestine formed from the larval stage has a more accentuated curvature in the ventral area of the stomach;
- the larvae swim easily in the mass of the water in search of food;
- food consisting mainly of supplementary food and juvenile forms of plankton (algae, rotifers, cladocerans, less adult forms, organisms that have fallen into the water with the supply water from the settling tank through the filtration system).

III. At the age of 27-28 days (figure 6 c):

- the body is no longer transparent, the pigment spots of brown color cover almost the entire surface of the body;
- the fins are almost entirely formed, which allows the larvae to swim quickly in search of food;
- the stomach is well individualized;

▪ intestinal contents that highlight the presence of live food and additional feed and traces of cladocere from the water of the settling tank.



(a) (b) (c)

Figure 6. Post embrionic larvae of 7-10 days old (a), pikeperch larvae of 17-18 day sold (b), pikeperch larvae of 27-28 days old (c)

<u>*IV. At the age of 40 days*</u> (figure 7):
▪ body shape similar to that of the adult;
▪ specific color (green-gray back, less often yellow-gray, the sides are gray-silver, with darker stripes, arranged transversely, the abdominal region with a lighter color);
▪ body completely covered with scales;
▪ alert swimming specific to predatory fish species;
▪ tendency to manifest cannibalism phenomenon;
▪ the stomach content reveals the presence of the feed in proportion of 90% in different phases of digestibility and nd the remaining 10% live food.

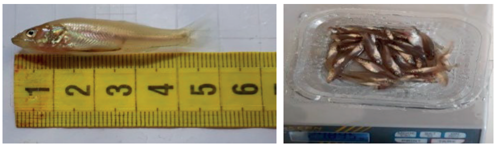


Figure 7. Pikeperch larvae at the end of the post-embryonic period

## CONCLUSIONS

The pikeperch (*Sander lucioperca* L. - 1758) is one of the freshwater species recently introduced in intensive aquaculture.
Therefore, the growth of the pikepech in the post-embryonic period was experienced in "Evos" type fiberglass pools and there were two critical moments in the post-embryonic development period: the first was at the beginning of exogenous feeding time, and the second during the swelling of the gas bladder.
The best results were obtained in the version with 1000 ex./basin, where the survival rate was 69.5% in 2020, the average individual growth rate in 2019 was 1.555 g/ex., and the Fulton coefficient was between 0.93-1.13.

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# EXPERIMENTAL RESULTS REGARDING THE GROWTH OF PIKEPERCH (*SANDER LUCIOPERCA* - LINNE, 1758) IN THE FIRST YEAR IN PONDS

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***Abstract***

*The pikeperch (Sander lucioperca, L.-1758) is a valuable fish species, with a high demand among human consumers, due to its superior nutritional and organoleptic characteristics such as: white flesh, soft texture, lack of intramuscular bones and pleasant taste. One of the main challenges of the pikeperch rearing technology is encountered during the first summer of the production cycle. The main desideratum during this period is to obtain large quantities of fingerlings per unit area, with the highest possible survival rate and low production costs, under the conditions specific to the rearing units. Therefore, the aim of the present study was to apply 2 different feeding regimes for the rearing of one summer old pikeperch in earthen ponds, as it follows: V1- with pelleted fish feed and V2- with live fish food. Thus, the experiments were performed at S.C.D.P. Nucet during three different rearing seasons (2018, 2019 and 2020), in triplicate. The best results were obtained in variant V2 (live food administration) and the followed indicators were survival rate, individual growth rate and production per unit area.*

***Key words***: *earthen ponds, fish feed, live food, productivity, Sander lucioperca.*

## INTRODUCTION

The development of aquaculture depends on the introduction of new species in the rearing technologies, as well as on the success of obtaining fingerlings for further stocking in ponds. The pikeperch (*Sander lucioperca*, Linnaeus, 1758) is a very active and energetic predatory fish in its natural environment, but extremely sensitive in aquaculture farms (Dobrotă et al., 2021). It is a new species in aquaculture and it is produced in Europe in extensive systems, in large earthen ponds. This method was applied in order to maintain a balanced fish population, since no fish feeds are administered and fish nutritional requirements were achieved by the natural productivity of ponds (Falahatkar & Javid Rahmdel, 2021). Nowadays, the pikeperch is used forthe biological control against fish with no economic value and other undesirable aquatic species in cyprinid polyculture ponds, to increase production (Falahatkar et al., 2018). The aforementioned specie can live in freshwater, brackish water and saltwater, but generally prefers freshwater systems such as rivers and lakes (Zakes, 2009).

Pikeperch rearing in monoculture technologies have been carried out in recirculating aquaculture systems (RAS) since the beginning of the 21st century in Western Europe (Teletchea & Fontaine, 2014). Therefore, it is considered a relatively new approach in the aquaculture industry. According to FAO, the Czech Republic, Hungary, Romania, Poland, Ukraine, Denmark, the Netherlands and Tunisia are the largest industrial producers of pikeperch (FAO, 2020).
To support the development of rearing technologies for this valuable fish species, the European Commission set up a comprehensive program in 2005 in partnership with 11 other EU Member States to improve breeding, hatching, larval, fry and fingerling rearing (Kucharczyk et al., 2007).
However, despite recent development sachieved in the fish feed production sector, specifically intended for the rearing of various one-summer old fish species, live food remains the main food used for the initial stages of fish nutrition (Chiorean et al., 2009). The main challenges in rearing pikeperch are the high mortality rates and failure to adapt to artificial feeds. A significant share of the pikeperch mortalities can

result from the lack of adequate food, when the shift from live food to artificial feed is made. The 45-day-old larvae are unable to recognize or ingest artificial food particles, as the behaviour of live food in the water column includes active movement, which is a crucial factor in attracting the larvae's attention (Xu et al., 2003).

**The aim of the study** is to grow the pikeperch (*Sander lucioperca*) in the first summer, in monoculture, by administering live food or fodder. The performance indices followed are: the quantity of biological material obtained per unit of area, the survival rate, the average weight and the feed conversion coefficient, under the specific conditions of the aquaculture units in Romania. The ability of the species to adapt to feeding on granulated feed when growing in the first summer has been considered.

## MATERIALS AND METHODS

The researches within the present study were carried out in the period 2018-2019-2020 at the Fish Culture Research and Development Station Nucet, Romania. The experimental ponds are located in the river bed of the Ilfov creek, downstream of the Ilfoveni accumulation dam. For the rearing of one summer old pikeperch, the material base was represented by six earthen ponds, with an area of 1000 square meters each. The inlet and outletare done individually for each pond, through monk-type installations. The depth of the rearing ponds is between 0.8-2.0 m. The water inlet was made from a common inlet channel and at the monks' grills metal sieve with the eye of 4 mm was installed, in order to prevent other species of fish from entering the pond. Since the supply channel was common, the physico-chemical water parameters were similar inall experimental ponds. Before stocking, the ponds were drained and disinfected with calcium hypochlorite, more intensely in the wet areas.

The fish stocking was made with 50-day-old pikeperch (Figure 1 a-e.), with average weights between 1.823-2.850 g in two variants, in triplicate, as follows:

1. Variant V1, using a stocking density of 750 specimens/pond, where pelleted fish feed was administered, in ponds B1, B2 and B3;
2. Variant V2, using a stocking density of 750 specimens/pond, where live food was administered, in ponds B4, B5 and B6.

In 2018, the stocking was made on June 5$^{th}$ and the feeding period was undertaken until October 7$^{th}$, resulting in a number of 124 days of feeding (on Sunday no feed was administered). Also, the fish harvesting was carried out on October 24$^{th}$.
In 2019 the stocking was made on June 9$^{th}$ and the feeding period was undertaken until October 6$^{th}$, resulting in a number of 119 days of feeding (on Sunday no feed was administered). Fish harvesting was carried out at October 16$^{th}$.
In 2020, the stocking was made on June 11$^{th}$ and the feeding period was undertaken until October 9$^{th}$, resulting in a number of 120 days of feeding (on Sunday no feed was administered). Fish harvesting was carried out at October 14$^{th}$.

Figure 1 (a-e). Fish biometrics and pond stocking

In variant V1, in all the study years, for the feeding of the biological material, pelleted fish feed "Aqua Start 0.7" was administered, with a variable granulation of 0.6-0.8 mm, especially for fingerlings of 1-5 g. The aforementioned fish feed was obtained by applying a microencapsulation technology, in order to protect nutrients and to maintain water quality, and at the same time, possess a high nutritional value, with high stability in water. The crude protein was 55% and the feed was administered in the first 30 days after stocking. After 30 days, the biological material was fed with "Aqua Start 1", which has the same characteristics as "Aqua Start 0.7", except for the feed size (0.9-1.1 mm) and the crude protein (57%).

In variant V2, before the pond flooding, barley was sown to form a vegetable bed in order to facilitate the reproduction of the crucian carp. During the summer, 10% of the pond surfaces of B4, B5 and B6, was used for aquatic vegetation development. After flooding, the pond was stocked with 50 kg of crucian carp, with an average weight between 15-80 g/ex. For the feeding of the crucian carp, approximately 1000 kg of feed was administered in each pond. The feed had the following ingredients: 35% corn, 15% wheat, 17% soybean meal, 25% sunflower meal, 5% fish meal, 3% vitaminized calcium. The feed ingredients were ground and mixed to result a crude protein of 25.5%. This variant was foreseen so that the crucian carp offspring will serve as live food for the pikeperch.

**Calculations.** During the experiments, measurements (TL ± 1 mm) and weighing of the total and individual fish biomass were performed. For individual biometric measurements, 200 specimens of pikeperch were taken from each growth unit (W ± 1 g) and the following parameters were calculated:

a) The production per unit of area (kg/ha) =

$$= \frac{\text{quantity of biomass obtained (kg)}}{\text{unit of area (ha)}};$$

b) Fulton coefficient K = $(W*100)/l^3$ (Pojoga, 1977), where:

W- individual weight (g);

l - standard length (cm).

c) Feed Conversion Coefficient (FCR)

$$= \frac{\text{quantity of managed feed (kg)}}{\text{obtained fish biomass (kg)} - \text{stocked fish biomass (kg)}}$$

In the V2 version, where the live food was administered, at the FCR 's calculation, in order to obtain an increase of 1 kg of pike-perch, 2 kg of crucian carp (*Carassius gibelio*) are required, and to obtain a growth increase of 1 kg of crucian carp were administered 3 kg of feed. In order to make individual biometric measurements, the pike-perchs were anesthetized with 2-phenoxyethanol in order to reduce the stress at handling.

**Statistical data processing.** The results, of groth and developement parameters, of the experimental groups were statistically analyzed using descriptive statistics and ANOVA One Way test. The programs used were Microsoft Excell (Office 2010) and SPSS Statistics 20.0 for Windows. The results were presented as mean±standard deviation.

## RESULTS AND DISCUSSIONS

During the experimental period, the water physico-chemical parameters were monitored. The obtained results were compared to the optimal values according to the “Norm on the classification of surface water quality”, correlated with the data from the specialized literature for waters destinated for fish use (OMMGA no. 161/2006) (Table 1).

During the rearing cycle of one summer old fish, the supply of the necessary food, both in terms of quantity and quality, is a decisive factor in achieving superior growth rates (Kozloski et al., 2018)..

The nutrition of the fingerlings is of particular importance in the fish feeding process, especially during the first summer.

Thus, during this period, the natural or artificial food which the fingerlings will consume, must ensure a balance in terms of the necessary elements (proteins, lipids, carbohydrates, vitamins, macro- and micro-elements), otherwise, serious damage in the further development of the fish body will be registered, manifested through the significant decrease of growth, survival rate and welfare (Zakes et al., 2003).

As well, besides the relationship between food needs and larval weight, other abiotic factors such as water temperature and dissolved oxygen concentration must be taken into account when calculating the daily feeding ratio and when establishing the administration protocol (Cadar, 1984).

In both experimental variants (V1 and V2), the feeding began the day after fish stocking. Feed was administered daily, in four rounds, in each pond. The quantities administered during the rearing cycle were according to Table 2.

Table 1. Water physico-chemical parameters during the experimental period

| *No.* | *Analysed parameter* | | *Measurement unit* | *Registered valued* | | |
|---|---|---|---|---|---|---|
| | | | | *Inlet* | *Ponds* | *Optimum values* |
| | | | | *Mean values for the studied years* | | |
| 1 | pH | | pH units | 7.1 | 7.6 | 7-7.8 |
| 2 | Alkalinity | | mg/l | 168 | 174 | 200-400 |
| 3 | Calcium ($Ca^{2+}$) | | mg/l | 34.1 | 41.4 | 90-120 |
| 4 | Magnesium ($Mg^{2+}$) | | mg/l | 19.8 | 22.6 | 10-40 |
| 5 | $Ca^{2+}$/ $Mg^{2+}$ | | mg/l | 1.7 | 1.8 | 5 |
| 6 | Organic matter | | mg $KMnO_4$/l | 19 | 22.3 | 20-60 |
| 7 | Oxygen | | mg/l | 8.2 | 7.1 | 5-12 |
| 8 | Ammonia ($NH^+_3$) | | mg/l | - | - | - |
| 9 | Nitrates ($NO^-_3$) | | mg/l | - | 0.17 | 2.5-4 |
| 10 | Nitrites ($NO^-_2$) | | mg/l | 0.003 | 0.003 | 0.03 |
| 11 | Phosphates ($PO^{3-}_4$) | | mg/l | - | 0.06 | 0.05-1.5 |
| 12 | Chloride | $Cl^-$ | mg/l | 9.32 | 9.39 | 30 |
| | | NaCl | mg/l | 12.14 | 14.56 | 20 |
| 13 | Ammonium ($NH^+_4$) | | mg/l | - | 0.015 | 0.5-1 |
| 14 | Total hardness | | ($^0$D) | 13.5 | 14.6 | 12 |

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Table 2. Fish feed administration, detailed on months

| **Experimental variant** | **Month** | **June** | **July** | **August** | **September** | **TOTAL** |
|---|---|---|---|---|---|---|
| V1, V2 | Quantity (%) | 10 | 25 | 35 | 30 | 100 |

In variant V1, the amount of feed administered was different from one day to the next and from one pond to another, depending on the appetite of the fish material. In terms of the frequency, the meals were administered twice a day, in the morning at 9 and in the afternoon at 14:30. Due to its floatability, the consumed amount could be observed and thus allowing the *ad-libitum* administration.

In variant V2, the administration of the feed was carried out in a single step, during the morning at 9 o'clock. The amount of feed administered in this variant was the same for all the ponds during the whole experimental period, 1000 kg/basin respectively.

Once every 15 days, control fishing was carried out, in order to determine the growth rate and health status.

The ruler and centimetre were used for measurements and electronic scales were used for weight determination (Figure 2 a-b and Figure 3 a-b).

The main biotechnological indicators calculated within the present study, in the intensive rearing system, for the pikeperch were as it follows:

- Quantity per unit area;
- Individual weight (W g/ex);
- Sv survival rate (%).

Figure 2 (a-b). Harvest fishing

Figure 3 (a-b). Harvest fishing

The values of the biotechnological indicators for the rearing of pikeperch one summer old fingerlings are presented in Tables 3 and 4.

Table 3. Biotechnological indicators for the rearing of one summer old pikeperch, during the experimental period

| Year | Variant | Pond | STOCKING No. of specimen | STOCKING Mean weight (g/ex) | STOCKING Quantity (kg) | Sv (%) | PRODUCTION Nr. Ex. | PRODUCTION Mean weight (g/ex) | PRODUCTION Quantity (kg) | Kg/ha | Fish feed (kg) | Conversion coefficient | Fulton coefficient |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2018 | V1 | B1 | 750 | 1.929 | 1.447 | 67 | 503 | 156 | 78 | 784 | 112 | 1.46 | 1.08 |
| | | B2 | 750 | 1.823 | 1.367 | 71 | 533 | 127 | 68 | 676 | 84 | 1.27 | 0.91 |
| | | B3 | 750 | 1.967 | 1.475 | 74 | 555 | 121 | 67 | 672 | 90 | 1.37 | 0.92 |
| | V2 | B4 | 750 | 2.562 | 1.922 | 91 | 683 | 269 | 184 | 1836 | 1000 | 1.52 | 1.13 |
| | | B5 | 750 | 2.85 | 2.138 | 92 | 690 | 265 | 183 | 1829 | 1000 | 1.5 | 1.17 |
| | | B6 | 750 | 2.624 | 1.968 | 84 | 630 | 335 | 211 | 2111 | 1000 | 1.37 | 1.01 |
| 2019 | V1 | B1 | 750 | 1.983 | 1.487 | 69 | 517 | 135 | 70 | 699 | 88 | 1.29 | 0.88 |
| | | B2 | 750 | 2.141 | 1.606 | 63 | 473 | 141 | 67 | 666 | 76 | 1.17 | 0.98 |
| | | B3 | 750 | 1.945 | 1.459 | 59 | 442 | 152 | 67 | 673 | 93 | 1.41 | 1.06 |
| | V2 | B4 | 750 | 2.234 | 1.676 | 89 | 668 | 312 | 208 | 2083 | 1000 | 1.4 | 1.26 |
| | | B5 | 750 | 2.456 | 1.842 | 92 | 690 | 296 | 204 | 2042 | 1000 | 1.38 | 1.13 |
| | | B6 | 750 | 2.235 | 1.676 | 93 | 698 | 285 | 199 | 1988 | 1000 | 1.42 | 1.14 |
| 2020 | V1 | B1 | 750 | 1.894 | 1.421 | 68 | 510 | 138 | 70 | 704 | 93 | 1.35 | 1.03 |
| | | B2 | 750 | 2.236 | 1.677 | 67 | 503 | 167 | 84 | 839 | 104 | 1.26 | 0.91 |
| | | B3 | 750 | 2.045 | 1.534 | 62 | 465 | 145 | 67 | 674 | 86 | 1.31 | 1.01 |
| | V2 | B4 | 750 | 2.486 | 1.865 | 89 | 668 | 296 | 198 | 1976 | 1000 | 1.4 | 1.21 |
| | | B5 | 750 | 2.563 | 1.922 | 88 | 660 | 321 | 212 | 2119 | 1000 | 1.37 | 1.29 |
| | | B6 | 750 | 2.554 | 1.916 | 95 | 713 | 284 | 202 | 2024 | 1000 | 1.41 | 1.2 |

Table 4. The results (average) obtained for the biological indicators in experimental variants

| Year | Variant | Sv (%) | W mean (g) | Quantity (kg) | kg/ha | Conversion coefficient | Fulton coefficient |
|---|---|---|---|---|---|---|---|
| 2018 | V1 | 70.7 | 135 | 71 | 711 | 1.36 | 0.97 |
| | V2 | 89.0 | 290 | 192 | 1925 | 1.46 | 1.10 |
| 2019 | V1 | 63.7 | 143 | 68 | 679 | 1.29 | 0.97 |
| | V2 | 91.3 | 298 | 204 | 2038 | 1.40 | 1.18 |
| 2020 | V1 | 65.7 | 150 | 74 | 739 | 1.31 | 0.98 |
| | V2 | 90.7 | 300 | 204 | 2039 | 1.39 | 1.23 |

***1. Quantity per unit area***

The best production obtained was in 2020 in variant V2, pond B5, 212 kg/pond (2119 kg/ha) respectively, and the lowest in 2019 in variant V1, pond B2, 67 kg/pond (666 kg/ha) respectively.

In all the study years, the highest quantity obtained was in variant V2 (Figure 4), being from 2.7 to 3 times higher than variant V1, as it follows:

- in 2018 the average quantity obtained in variant V2 was 192 kg (1920 kg/ha) and in variant V1 71 Kg (710 kg/ ha), which is 2.7 times higher compared to variant V1;
- in 2019 the average quantity obtained in variant V2 was 204 kg (2040 kg/ha) and in variant V1 68 Kg (680 kg/ha), which is 3.0 times higher compared to variant V1;
- in 2020 the average quantity obtained in variant V2 was 204 kg (2040 kg/ha) and in variant V1 74 Kg (740 kg/ha), which is 3.0 times higher compared to variant V1.

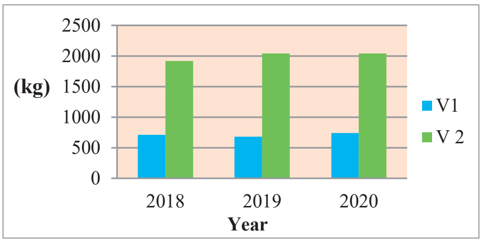


Figure 4. Quantity variation reported to the surface area

*2. Average weight*

The highest average weight was registered in 2018 in variant V2, pond B6 (335 g/specimen) and the lowest in 2018 in variant V1, pond B3, 9221 g/specimen).

During the whole experimental period, the highest average weight was obtained in variant V2 (Figure 5), being from 2.0 to 2.2 times higher compared to variant V1, as it follows:

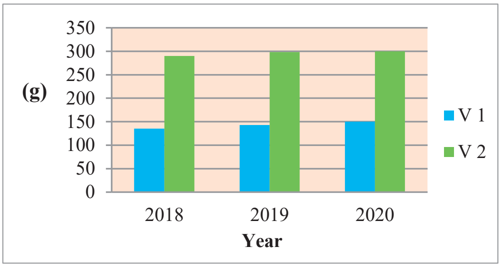


Figure 5. Variation of fish average weight

- in 2018 the average weight obtained in variant V2 was 290 g/specimen and in variant V1 was 135 g/specimen, which is 2.2 times higher compared to variant V1;
- in 2019 the average weight obtained in variant V2 was 298 g/specimen and in variant V1 of 143 g/specimen, which is 2.1 times higher compared to variant V1;
- in 2020 the average weight obtained in variant V2 was 300 g/specimen and in variant V1 was 150 g/specimen, which is 2.0 times higher compared to variant V1.

*3. Survival rate*

The highest survival rate was registered in 2020 in variant V2, pond B6, 95% respectively and the lowest was registered in 2019 in variant V1, pond B3, 59% respectively.

In all the study years, the highest survival rate was obtained in variant V2 (Figure 6), ranging from 1.3 to 1.4 times higher than in variant V1, as it follows:

- in 2018 the average survival percentage obtained in variant V2 was 89.0% and in variant V1 was 70.7%, which is 1.3 times higher compared to variant V1;
- in 2019 the average survival percentage obtained in variant V2 was 91.3% and in variant V1 was 63.7%, which is 1.4 times higher compared to variant V1;
- in 2020 the average survival rate obtained in variant V2 was 90.7% and in variant V1 was 65.7%, which is 1.4 times higher compared to variant V1.

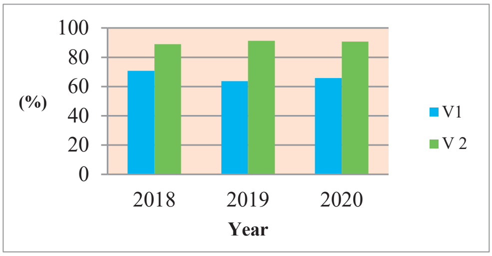


Figure 6. The variation of the survival rate

***The conversion coefficient*** had similar values in both experimental variants, falling in variant V1 within the range 1.29-1.36 and in variant V2 within the range 1.39-1.46. When calculating this coefficient in variant V2, the transformation of the administered feed into live food and live food in the amount of pikeperch obtained was taken into account.

***The Fulton coefficient*** also had similar values in both experimental variants, falling in variant V1 within the range 0.97 - 0.98 and in variant V2 within the range 1.10 - 1.23.

It was observed that the highest values were registered in variant V2 in terms of production per unit area, average weight and survival rate. In terms of conversion rate and Fulton coefficient, the values were also similar. This is due to the fact that the pikeperch mainly prefers live food and consumes it with pleasure, being a complete, attractive food, intended for this predatory species. It can be stated that pelleted fish feed is available at any time, which gives it an advantage from this point of view.

## CONCLUSIONS

The observed indices such as: the quantity obtained per unit area, the survival rate, the average weight and the conversion coefficient were higher in variant V2 (administration of live food) compared to variant V1, where the biological material was fed with granulated fodder. Good results were also obtained in the V1 version, and it can be stated that the pikeperch has adapted and consumed the granulated fodder, which that the fish farmers can easily procure and store.

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# OBSERVATION ON THE FEEDING BEHAVIOR OF ORPHANED BABY RED SQUIRRELS *SCIURUS VULGARIS* RAISED IN CAPTIVITY BETWEEN 3 AND 12 WEEKS

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***Abstract***

*The feeding habits of a three orphaned red squirrel Sciurus vulgaris were observed during spring, in Western part of Romania, Arad county. Observations on the behavior of squirrel baby were made from 3 weeks to 12 weeks, when the squirrels were released into the wild. The baby squirrels were found fallen from the tree, together with the nest. At about 3 - 4 weeks of age, the feeding takes place 9 times a day, at 6 weeks of age, the number of feeds begins to decrease, is the period when diversification begins by introducing some seeds and nuts in the diet. In terms of weight, the baby weighed about 50 g when they were found, and will reach a weight of 100 g in week 8, and a weight of about 200 grams at about 10 weeks. The paper provides information regarding feeding pattern the first day after finding an orphaned baby squirrel until release into the wild.*

***Key words****: captivity, feeding behaviour, Sciurus vulgaris.*

## INTRODUCTION

The Eurasian red squirrel (*Sciurus vulgaris*) is present in both deciduous and coniferous habitats (Stachura et al., 2004; Shar et al., 2008), in primeval stands (Wauters et al., 1992; Shuttleworth, 2000) as well as in small woodlots (Wauters, 1997). It can also be found in suburban and urban areas, including city parks (Babin ́ska-Werka & Z ́ółw, 2008). Its diet varies with season (Magris & Gurnell, 2002; Fericean, 2017), but also with habitat type (Bosch & Lurz, 2012; Shuttleworth, 2000) and includes a wide variety such as plants seeds and fungi, as well as animal matter (Bosch & Lurz, 2012).

There is no more controversial topic than the diet of wild animals baby, including squirrel baby. Some caretakers insist that raising baby squirrel could only be successful with Esbilac Powder Milk Replacer for puppies. There are many brands of formulas and recipes. Some mix moisturizers with formulas instead of water, others add colostrum powder, organic egg yolks, honey, glucose, vitamins and minerals powders, rodent powder or cream.

Not having access to existing milk formulas and studying cow's milk that contains less than half the protein, fat and nutrients needed for a healthy squirrel's chick to be even harder to digest, causing electrolyte loss through diarrhea and dehydration of the animal we tried to we use goat's milk together with calcium and vitamin D3 supplements, to avoid diarrhea we also used probiotics, administered together with milk.

## MATERIALS AND METHODS

The baby squirrel (Figure 1) was found in a hilly area in the in Western part of Romania, Arad county, fallen from the tree together with the nest and their mother was found dead near the nest. The chicks were about 3 weeks old.

Figure 1. The squirrel nest

Observations on squirrel baby (*Sciurus vulgaris*) regarding the feeding behavior were made by using our own milk formula consisting of goat's milk, egg yolk and honey (Table 1).

Table 1. Squirrel milk formula (original)

| No | Ingredients | % |
|---|---|---|
| 1. | Goat milk | 65% |
| 2. | Egg yolk | 35% |
| 3. | Honey | 5% |

The liquid was fed using 5 ml pipettes, for 4-7 week old baby squirrel and a 2.5 ml syringe for the following weeks (Figure 2).

Figure 2. The feeding of baby squirrel

For the proper functioning of the intestinal flora, we administered probiotics together with the milk during a feeding session. To supplement the necessary nutrients for the harmonious development of muscles and skeleton, were administered 1 ml of vitamin D drops and 1 ml of calcium every three days.

## RESULTS AND DISCUSSIONS

On the first day (evening) were administered sachets with re-hydration salts for babies. They contain the mineral salts needed to keep the baby away from dehydrating. The contents were administered with a pipette, at the corner of the mouth, but due to the stress he went through, he had a hard time learning to suck. The administration should be done very slowly so that the baby does not aspirate the contents. Aspiration of food or fluids causes pneumonia *ab ingestis*, one of the most common causes of death in infants.

After consuming about 5-10% of their body weight from the re-hydration formula, should be stimulated to urinate and defecate because they cannot do it on their own.

According to some authors, most of the babies found are dehydrated. These are some signs of dehydration: pale gums, gray, dry mouth, sunken eyes, whites around the eyes that present, rough, prickly fur, dry and scaly skin. Some caretakers use the "tent test" to check for dehydration.

After the baby was warmed and hydrated, milk mixed with such re-hydration salts was gradually introduced (Table 2).

Table 2. Feeding pattern the first day after finding an orphaned

| | |
|---|---|
| Feeding 1 | 100% moisturizing liquid |
| Feeding 2 | 60% moisturizing liquid<br>40% milk |
| Feeding 3 | 40% moisturizing liquid<br>60% milk |
| Feeding 4 | 25% moisturizing liquid<br>75% milk |
| Feeding 5 | 100% milk |

The adult of *Sciurus vulgaris* has a very varied diet deciduous and coniferous seeds dominate its diet when available. The growth of squirrel populations is related to the abundance and annual changes in tree seeds (Wauters et al., 2008).

Factors influencing body development, reproduction and survival during cold periods are correlated with tree seed production (Bertolino et al., 2004). If the seeds are depleted, the squirrels move to other locations (Lurz et al., 2000; Wauters et al., 2005).

Spring staple food is supplemented with shoots, buds, and flowers, and mushrooms are eaten in summer and autumn. In winter, feeding time is reduced due to high-energy feeding (pine or deciduous seeds) that allow squirrels to meet their requirements in just a few hours. During the spring, the diet is lower in calories and the time spent searching for food is longer (Gryz et al., 2015; Wauters et al., 2008).

Research by Lee (2002) has shown that squirrels are very selective in food, with a preference for food. Seeds of certain species of conifers compared to others and are present on some trees more than others (Molinari et al., 2006).

At about 3-4 weeks of age, the feeding takes place 8 times a day, with a frequency of 2 hours, and at night it is fed only once, depends on the over al health of the squirrel baby. A sickly squirrel will need more attention. At this age, baby squirrel begins to open their eyes and the lower front teeth appear. The reddish fur on the

body continues to grow as the tail hair grows longer. The white fur begins to form on the neck and belly.
At about 4 - 5 weeks of age, the frequency of feeding increases to 3 hours, and the interval between feeding at night is 6 hours. Their behavior is very slow in the next few days they continue to eat and will return to sleep immediately.
At about 5 to 6 weeks of age, the number of feeds decreases to 7, and at night the interval between feeds is up to 8 hours. Solid foods were offered, but were unsuccessful.
At about 6 to 7 weeks of age, the teeth develop, they can hold the food in the front paws, but the tail cannot lift it (Figure 3). The number of feeds begins to decrease, is the period when diversification begins by introducing some seeds and nuts in the diet. Walnuts are the first food accepted, and after a few days they prefer seeds. During this period, they refuse to eat any fruits or vegetables.
They are also starting to become more active and trying to climb different surfaces. They can grind hard food.

Figure 3. The tail position at 6 weeks

At about 7 to 8 weeks of age, the frequency of consuming milk is reduced to 4 hours, the food being supplemented with small snacks of seeds and nuts, as well as corn cereals.
At about 8 to 9 weeks of age, the frequency of consuming milk decreases to 4 -5 hours a day, fruits and vegetables being accepted in the diet, strawberries being the favorite fruits. Begin to lift the tail up during feeding or other activities (Figure 4)

Figure 4. The tail position at 8 weeks

At about 9 to 10 weeks of age, the number of milk feeds decreases to 3, the food being supplemented with seeds, fruits and vegetables.
At about 10 to 11 weeks of age, consuming milk takes place twice a day, with a 12-hour break between. It is very important to pay attention to them in order to develop the fighting skills that are essential in the wild for their survival. He begins to make different sounds, expressing his joy, curiosity or nervousness.
At about 11 to 12 weeks of age, the amount of milk consumed decreases up to once a day.
At 12 weeks squirrels have an active behavior of about 10 hours each day, the beginning and end of the activity corresponding to sunrise and sunset. This behavior was similar to that observed by Wauters (2000) and Gryz (2009).
In the case of additional feeding, hazelnuts were the most important food source (Shuttleworth, 2000).
As for the amount of milk consumed, it was 5 ml every 4 weeks, increasing in week 12 to 16 ml (Figure 5).
At about 5 to 6 weeks of age the amount of milk gradually increased to 7 ml, and the frequency of feeding should be gradually reduced to 6-7 meals a day, 2.5-3 hours away from 8-10 hours between feedings at night.

Table 3. Number and frequency of feeding (3 - 12 weeks)

| Age | Number of feeds | Frequency | Type |
|---|---|---|---|
| 3-4 weeks | 8 | *2* hours + 1 night | *Milk* |
| 4-5 weeks | 8 | 2.5 - 3 hours at night sometimes | *Milk* |
| 5 to 6 weeks | 7 | 3 hours - not at night | *Milk* |
| 6 to 7 weeks | 6 | 3 hours - not at night | *Milk / start of diversification* |
| 7 to 8 weeks | 5 | 3-4 hours - not at night | *Milk / solid food* |
| 8 to 9 weeks | 4 | 4-5 hours - not at night | *Milk / solid food* |
| 9 to 10 weeks | 3 | 6 hours - not at night | *Milk / solid food* |
| 10 to 11 weeks | 2 | 12 hours - not at night | *Milk / solid food* |
| 11 to 12 weeks | 2 | *12 hours - not at night* | *Milk / solid food* |
| 11 to 12 weeks | 1 | once a day | *Milk / solid food* |

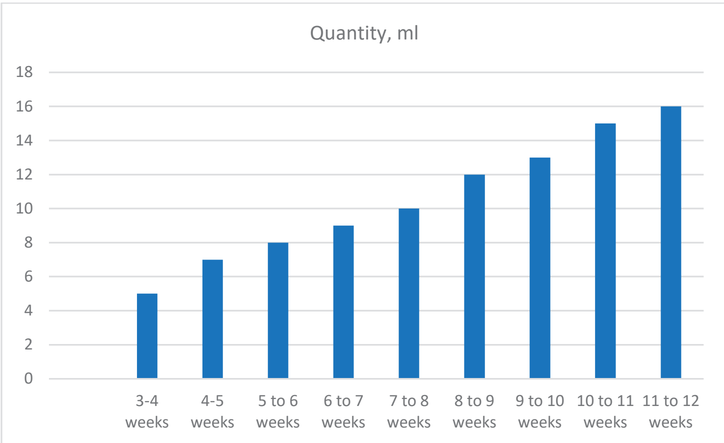


Figure 5. The amount of milk consumed 4 to 12 weeks

In terms of weight, the baby weighed about 50 g when they were found, and will reach a weight of 100 g in week 8, and a weight of about 200 grams at about 10 weeks. A squirrel weighing 100 grams should be fed every 4-5 hours (Figure 6).

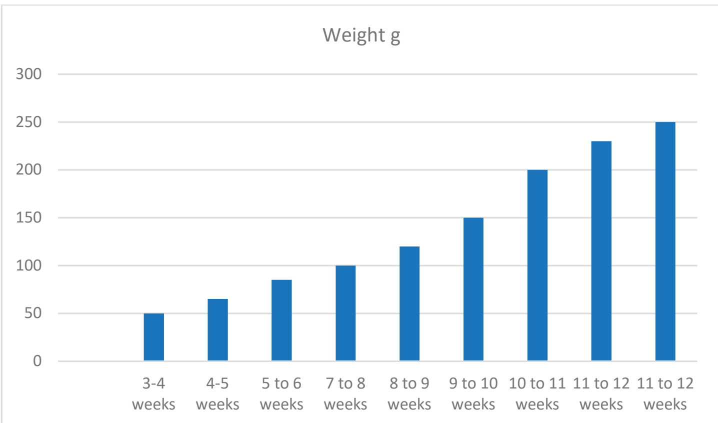


Figure 6. Baby squirrel weight 4 to 12 weeks

In some cases, squirrels may ingest small amounts of bark. The bark is sometimes removed to reach at of cambial tissue. Some tree species are preferred for example spruce, Scots pine, beech, spruce, silver birch (Wauters, 2000).

Squirrels also feed on the mycelium of fungi that grow under the bark (Holm, 1990). Occasionally are introduced into the diet other plant substances like lichens and mosses (Sulkava & Nyholm, 1987). Nevertheless, animal matter does not play an important role in the diet of squirrels. Very rarely they may prey on eggs or even young of birds (Shuttleworth, 1996).
Sometimes the soil can be digested by squirrels in response to mineral requirements (Holm, 1990; Shuttleworth, 1996).
Due to the fact that squirrels have managed to adapt very well to the environments populated by humans, it can be said that they have become the rodents with which people are most familiar. In some very large cities, squirrels may even be the only wild mammals that humans have a chance to see (Wauters et al., 2005).
The European squirrel (*Sciurus vulgaris*) or the native squirrel, as it is called in our country is present in Great Britain, Europe and Asia, and is considerably smaller, measuring between 19-23 cm, with a tail between 15-20 cm. The fur color of squirrel changes with the seasons, ranging from reddish to black, with tufts of fur on the ears; but all European squirrels have white bellies. They normally can live up to 3 years in the wild and up to 10 years in captivity (Bosch, 2012).
Peeled walnuts offered in squirrels in parks were eaten regardless of the season in most cases. The squirrels hid the reserves and consumed them in the following days.

## CONCLUSIONS

We can conclude that the use of our own milk formula consisting of goat's milk, egg yolk and honey had a beneficial effect on the growth and development of baby squirrel.
At about 3 - 4 weeks of age, the feeding takes place 9 times a day, at 6 weeks of age, the number of feeds begins to decrease, is the period when diversification begins by introducing some seeds and nuts in the diet.
In terms of weight, the baby weighed about 50 g when they at week 4, and will reach a weight of 100 g in week 8, and a weight of about 200 grams at about 10 weeks.

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# BIODIVERSITY AND STRUCTURE OF THE HELMINTH COMMUNITIES OF *CARASSIUS GIBELIO* (BLOCH, 1782) FROM THE TUNDZHA RIVER, BULGARIA

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***Abstract***

*In 2021, ecologoparasitological research was done based on the helminths and helminth communities of Prussian carp (Carassius gibelio (Bloch, 1782)) from the freshwater ecosystem of the Tundzha River, Aegean Water Basin. As a result of the examined twenty-one specimens of Prussian carp, three taxa of helminths were found: Nicolla skrjabini (Iwanitzky, 1928) Dollfus, 1960; Pomphorhynchus laevis (Müller, 1776) Porta, 1908; Contracaecum sp. The dominant structure of the helminth communities was determined. N. skrjabini is a core species for helminth communities of C. gibelio (P% = 23.81). New data on the helminth communities of the Prussian carp from the studied area of the freshwater ecosystem are presented. The basic ecological indices of the helminth populations and communities were determined. The bioindication role of the established helminth species as well as an assessment of the ecological status of the studied biocenoses was presented.*

***Key words***: *bioindication, Carassius gibelio, helminth communities, river Tundzha.*

## INTRODUCTION

After the Danube and Iskar rivers, the Tundzha River is the third largest river in Bulgaria (390 km) and the Maritsa River's largest tributary, Aegean Water Basin. The river springs from the Balkan Mountain (2083 m altitude) and flows into the Maritsa River before Edirne (32 m altitude). The river ecosystem and its adjacent territories are distinguished by great biological diversity, including rich ichthyofauna, related to the declaration of a number of protected areas. Helminths and helminth communities are elements of biodiversity. In most cases, helminths are characterized by complex life cycles involving intermediate hosts. Therefore, the invasion indices with them reflect the integrity of food chains and the state of ecosystems in general. *Carassius gibelio* (Bloch, 1782) has been the subject of helminthological research by various authors in a number of countries (Shukerova, 2005; Koyun & Altunl, 2007; Cojocaru, 2010; Őktener, 2014; İnnal et al.,2020; Stroe et al., 2021). In Bulgaria, the Prussian carp from the Tundzha River Basin has been the subject of ecologo-helminthological research by a few authors (Grupcheva & Nedeva, 1999; Chunchukova & Kirin, 2021). The study presents data on the endohelminths and helminth communities of Prussian carp from the Tundzha River (middle section) and discusses the condition of the communities from the studied part of the river.

## MATERIALS AND METHODS

In 2021, twenty-one specimens of Prussian carp from the Tundzha River were examined for helminths. The examined fish were caught by angling according to permission from the Ministry of Agriculture, Food and Forestry of the Republic of Bulgaria. According to Froese & Pauly (Eds.) (2020), the fish's scientific name was present. The fish were collected in the section of the river located between the Balkan Mountain and the Mountain range Sredna Gora, Central Southern Bulgaria (42°33′12″N, and 25°38′21″ E; 309 m). The helminthological study was carried out according to Petrochenko (1956); Zashev & Margaritov (1966); Bauer (Ed.) (1987); Moravec (2013). Helminth specimens were fixed in 70% of ethyl alcohol. Species diversity was determined on permanent slides according to the method of staining with alum carmine (Dubinina, 1948) and on temporary slides carried out by the methods of Moravec (2013) and Petrochenko (1956). Helminth community structure was analysed on the two levels: on the level of component community (prevalence (P%); mean intensity (MI) for the determined species) and on the level of infracommunity (total number of fish species; total and mean number of fish specimens; Brillouin's diversity index (HB)). In the component community, the found species were divided into three groups: core species (P% > 20), component species (P% > 10) and accidental species (P% < 10), according to the criteria of Magurran (1988); Bush et al. (1997) and Kennedy (1997). The obtained results were statistically processed using Statistica 10 (StatSoft Inc., 2011) and MS Exel (Microsoft 2010).

## RESULTS AND DISCUSSIONS

### Characteristics of the studied fish species

*Carassius gibelio* (Bloch, 1782) (Cyprinidae) is freshwater, benthopelagic and brackish fish species. The Prussian carp is considered to be an omnivorous species. It feeds mainly on worms, insect larvae, and even small fish during the cold months. During the summer months, the main food is plant food. The Prussian carp grows best in gullies, swamps, mortuaries, old riverbeds, micro-dams, gravel pits, lower slow rivers and large dams. It is characterized by a specific method of reproduction unique to it (Froese & Pauly, 2020; Karapetkova & Zhivkov, 2006). *C. gibelio* has an unclear conservation status on a European scale as a native or non-native species. The species is defined as not endangered in the International Red Book (LC; IUCN). The Prussian carp is not a protected species on the territory of Bulgaria. The species is widespread in the country.

### Helminths and helminth community structure

In 2021, as a result of the ecologoparasitological examinations of 21 specimens of *C. gibelio* from the Tundja River, three taxa of endohelminths was established: Nicolla skrjabini (Iwanitzky, 1928) Dollfus, 1960; *Pomphorhynchus laevis* (Müller, 1776) Porta, 1908 and *Contracaecum* sp., larvae, belonging to three classes, three orders, three families and three genera (Table 1).

***Nicolla skrjabini* (Iwanitzky, 1928) Dollfus, 1960** is an intestinal parasite of many species of fish from Cyprinidae, Percidae, Gobiidae, Cobitidae, Siluridae, Gadidae, Esocidae, Acipenseridae, Salmonidae. The development cycle of the species is carried out with the participation of two types of intermediate hosts.

Table 1. Biodiversity and ecological indices of helminths and helminth communities of *Carassius gibelio* from the Tundja River

| *Silurus glanis* ($N^1 = 21$) Helminth species | $n^2$ | $p^3$ | $P\%^4$ | $MI^5$ (min-max) |
|---|---|---|---|---|
| **Class** Trematoda Rudolphi, 1808 **Order** Plagiorchiida La Rue, 1957 **Family** Opecoelidae Ozaki, 1925 **Genus***Nicolla* Wisniewski, 1944 | | | | |
| *Nicolla skrjabini* (Iwanitzky, 1928) Dollfus, 1960 | 5 | 11 | 23,81 | 2,2 (1-5) |
| **Class** Acanthocephala Rudolphi, 1808 **Order** Echinorhynchida Southwell & Macfie, 1925 **Family** Pomphorhynchidae Yamaguti, 1939 **Genus** *Pomphorhynchus* Monticelli, 1905 | | | | |
| *Pomphorhynchus laevis* (Zoega in Müller, 1776) Porta, 1908 | 2 | 5 | 9,52 | 2,5 1-3 |
| **Class** Nematoda Rudolphi, 1808 **Order** Ascaridida Skrjabin et Schulz, 1940 **Family** Anisakidae Skrjabin et Karokhin, 1945 **Genus** *Contracaecum* Railliet et Henry, 1912 | | | | |
| *Contracaecum* sp., larvae | 1 | 2 | 4,76 | 2,0 2 |

Legend: $^1$N = total number of examined fish specimens.
$^2$n = total number of infected fish specimens.
$^3$p = total number of helminth specimens.
$^4$P% = prevalence.
$^5$MI = mean intensity.

The first intermediate host is the snail *Lithoglyphus naticoides* (Pfeiffer, 1828) (Class Gastropoda). Sporocysts of parasites are localized in the liver, gonads and gills. The second intermediate hosts are crustaceans *Gammarus balcanicus* Schäferna, 1923 (Class Malacostraca) with localization of metacercariae in the back muscles and limbs (Bauer, 1987; Kakacheva-Avramova, 1983). In Bulgaria, *N. skrjabini* (as *Crowcrocoecum skrjabini*) is reported as endohelminth species of *Gobio gobio* (Linnaeus, 1758); *Pelecus cultratus* (Linnaeus, 1758); *Cyprinus carpio* Linnaeus, 1758; *Sabanejewia bulgarica* (Drenski, 1928) (*Cobitis bulgarica*); *Silurus glanis* Linnaeus, 1758; *Sander lucioperca* (Linnaeus, 1758) (*Lucioperca lucioperca*); *Perca fluviatilis* Linnaeus, 1758; *Zingel zingel* (Linnaeus, 1766) (*Aspro zingel*); *Gymnocephalus cernua* (Linnaeus, 1758) (*Acerina cernus*); *G. schraester* (Linnaeus, 1758) (*A. schraester*); *Neogobius melanostomus* (Pallas, 1814) (*Gobius cephalarges contructor*); *Neogobius fluviatilis* (Pallas, 1814) (*G. fluviatilis*) of the Danube River (Margaritov, 1966); of *Salmo trutta fario* Linnaeus, 1758 of the Chuprenska River (Kakacheva-Avramova, 1969); of *C. carpio* of Fisheries Belene (Margaritov, 1975, 1976, 1992). *N. skrjabini* is reported also of *Acipenser ruthenus* Linnaeus, 1758; *P. fluviatilis*; *S. lucioperca*; *S. volgensis* (Gmelin, 1789); *G. schraetser*; *Z. zingel*; *Z. streber*; *C. carpio*; *Carassius carassius* (Linnaeus, 1758); *Abramis brama* (Linnaeus, 1758); *Ballerus ballerus* (Linnaeus, 1758) (*Abramis ballerus*); *Blicca bjoerkna* (Linnaeus, 1758); *Leuciscus aspius* (Linnaeus, 1758) (*Aspius aspius*); *P. cultratus*; *G. gobio*; *S. bulgarica* (*C. bulgarica*); *Gobio gobio* (Linnaeus, 1758) (*Gobio fluviatilis*); *Neogobius melanostomus* (Pallas, 1814) (*Gobio cephalarges*); *Proterorhinus marmoratus* (Pallas, 1814) (*Proterorinchus marmoratus*) of the Danube River (Kakacheva-Avramova et al., 1978); of *Salmo trutta fario* Linnaeus, 1758 of the rivers Trigradska and Vacha (Kakacheva-Avramova & Menkova, 1978); of *S. t. fario* of the rivers Chuprenska, Trigradska, Vacha, Shirokolashka (Kakacheva-Avramova & Menkova, 1978); of *Alburnus alburnus* (Linaneus, 1758) of the Danube River (Chunchukova et al., 2019); of *A. alburnus* of the Danube River (Zaharieva & Kirin, 2020a); of *A. brama* of the Tundja River (Kirin & Chunchukova, 2021); of *A. alburnus* and *A. brama* of the Danube River (Zaharieva & Zaharieva, 2021); of *Vimba vimba* (Linnaeus, 1758) of the Danube River (Zaharieva & Kirin, 2021), etc.

***Pomphorhynchus laevis* (Müller, 1776)** develops as a marita in a lot of freshwater fish species of Cyprinidae, Salmonidae, Percidae, Siluridae, etc. The developmental cycle isrelated to the participation of an intermediate host *Gammarus pulex* (Linnaeus, 1758) (Bauer, 1987; Kakacheva–Avramova, 1983). *G. pulex* is a bioindicator forx–β–mesosaprobity as well as relatively tolerant forms (Group C) in terms of environmental conditions in habitats (Belkinova et al., 2013). Smallfish species of Cyprinidae have been established as reservoir hosts. The species was reported of *Sq. cephalus* of the Iskar River, of *B. barbus* of the Danube River (Margaritov, 1959); of *A. ruthenus*, *G. gobio*, *B. barbus*, *A. alburnus*, *B. bjoerkna*, *P. cultratus*, *C. gibelio*, *C. carpio*, *S. bulgarica*, *Silurus glanis* Linnaeus, 1758, *S. lucioperca*, *Z. zingel*, *Z. streber*, *G. cernua*, *G. schraetser*, *P. constructor*, *G. gobio*, *Benthophilus stellatus* (Sauvage, 1874) of the Danube River (Matgaritov, 1966); of *Chondrostoma nasus* (Linnaeus, 1758) and *Phohinus phoxinus* from rivers Ogosta and Nishava (Kakacheva-Avramova, 1969); of *B. cyclolepis* in Bulgaria of the Tundzha River (Kakacheva–Avramova, 1972); of *A. ruthenus*, *A. güldenstädtii* Brandt & Ratzeburg, 1833, *Salmo labrax* Pallas, 1814, *Alosa immaculata* Bennet, 1835 (*Alosa pontica* Bennet, 1835), *Anguilla anguilla* Linnaeus, 1758,

*C. carpio*, *C. gibelio*, *V. vimba*, *A. brama*, *Ballerus sapa* (Pallas, 1814) (*Abramis sapa*), *Ballerus ballerus* (Linnaeus, 1758) (*Abramis ballerus*), *P. cultratus*, *A. alburnus*, *B. bjoerkna*, *G. gobio*, *Romanogobio albipinnatus* (Lukasch, 1933) (*G. albipinatus*), *B. barbus*, *Ch. nasus*, *L. idus*, *Scardinius erythrophthalmus* (Linnaeus, 1758), *Sq. cephalus*, *Leuciscus aspius* (Linnaeus, 1758) (*Aspius aspius*), *Ctenopharyngodon idella* (Valenciennes, 1844), *Proterorhynus marmoratus* (Pallas, 1814), *S. glanis*, *Lota lota* (Linnaeus, 1758), *Esox lucius* Linnaeus, 1758, *S. lucioperca*, *S. volgense*, *P. fluviatilis*, *G. cernua*, *G. schraester*, *Z. zingel*, *Z. streber*, *Ponticola kessleri* (Günther, 1861) (*Gobius kessleri*), *Lepomis gibbosus* (Linnaeus, 1758), *G. gobio*, *B. stellatus* of the Danube River (Kakacheva-Avramova et al., 1978); of *B. barbus* from rivers Struma, Zheleznitsa, Gradevska, of *A. bipunctatus* from rivers Zheleznitsa and Gradevska, of *Sq. cephalus* of the Struma River (Kakacheva-Avramova & Menkova, 1981); of *C. carpio* and *S. lucioperca* (Nedeva & Grupcheva, 1996); of *C. gibelio* of Reservoir Zhrebchevo (Grupcheva & Nedeva, 1999); of *Sq. cephalus* of the Danube River (Cakis et al., 2004); of *P. fluviatilis* of the Arda River (Kirin, 2005); of *P. fluviatilis* of the Adra River (Kirin, 2005); of *A. brama*, *B. sapa*, *A. ruthenus*, *A. alburnus*, *A. immaculata*, *B. barbus*, *C. gibelio*, *E. lucius*, *G. schraester*, *Sq. cephalus*, *P. cultratus*, *S. lucioperca*, *Sc. erythrophthalmus*, *S. glanis*, *Z. zingel* of the Danube River (Atanasov, 2012); of *Sq. sephalus* of the Tunja River (Kirin et al., 2013); of *B. barbus* of the Danube River (Chunchukova & Kirin, 2018); of *A. alburnus* of the Danube River (Chunchukova et al., 2019); of *Sq. orpheus* of the Stryama River (Kirin et al., 2019); of *A.*

*brama* of the Danube River (Chunchukova & Kirin, 2020); of *B. cyclolepis* and *Sq. orpheus* of the Topolnitsa River (Chunchukova et al., 2020); of *V. vimba* of the Danube River (Zaharieva & Kirin, 2021), etc. ***Contacaecum* sp.** is reported of *A. alburnus* (Chunchukova et al., 2019);of *Ch. nasus* (Zaharieva & Zaharieva, 2020a, b; Zaharieva & Kirin, 2020a, b; Zaharieva & Kirin, 2021, respectively;) of the Danube River; of *Scardinius erythrophthalmus* (Linnaeus, 1758) of the Maritsa River (Chunchukova et al., 2019), etc.

**Component community**

The presented helminth taxa were found in 8 of the studied twenty-one Prussians carp specimens (30.09%). Prevalence (P%), mean intensity (MI) and rank were determined for each taxon. *N. skrjabini* (P%=23.81) is a core species of the endohelminth communities of *C. gibelio* from the Tundzha River. The other two species are accidental ($P\%_{P.lavis}$=9.52; $P\%_{Contr.sp.}$=4.76). *P. laevis*is distinguished with the highest mean intensity (MI=2.5), followed by *N. skrjabini* and *Contracaecum* sp. (MI=2.2 and MI=2.0, respectively). Only two specimens of *Contracaecum* sp. was fixed in the infected specimen of *C. gibelio*. *Contracaecum* sp. is an allogenic species. *N. skrjabini* and *Contracaecum* sp. *P. laevis* are autogenic species. Therefore, the established taxa are generalists for the helminth communities of *C. gibelio* from the Tundzha River, Bulgaria (Table 1).

**Infracommunity.** A total of thirteen examined specimens of *C. gibelio* are free of helminths (61.90%). In this study detected no mixed invasion. The maximum number of parasites found in a single specimen by the host is five (*N. skrjabini*). The average number of all endohelminth specimens is low (0.86±1.35), as well as the value of Brillouin's diversity index (HB) (Table 2).

Table 2. Infracommunity data

| Number of helminth species | | |
|---|---|---|
| Number of infected fish | 13 | 8 |
| Number of helminth species | 0 | 1 |
| Number of helminth specimens | | |
| Total number | 18 | |
| Mean±SD | 0,86±1,35 | |
| Range | 1-5 | |
| Mean HB±SD | 0,41±1,49 | |

A total of seven endohelminth taxa of Prussian carp were reported in Bulgaria. According to the study, only three taxa were reported (42.86%). In the country, *P. laevis* was reported in previous studies as endohelminths of *C. gibelio*.

Detected specimens of the genus *Contraceaceum* and *N. skrjabini* have not been identified. Research on Prussians carp parasites are mainly related to the Danube and Tundja River Basins (Tables 1, 3).

Table 3. Endohelminths of *Carassius gibelio* from freshwater ecosystems of Bulgaria

| **Species diversity** | **Authors** | **Freshwater ecosystems (Biotopes)** |
|---|---|---|
| **Trematoda** | | |
| Trematoda sp. metacercaria | Grupcheva, Nedeva, 1999 | reservoir Zhrebchevo, Tundja River Basin |
| Trematoda sp. cysts | Grupcheva, Nedeva, 1999 | reservoir Zhrebchevo, Tundja River Basin |
| **Cestoda** | | |
| *Cysticercus Paradilepis scolecina* (Weld, 1855) | Grupcheva, Nedeva, 1999 | reservoir Zhrebchevo, Tundja River Basin |
| **Acanthocephala** | | |
| *Pomphorhynchus laevis* (Müller, 1776) | Margaritov, 1966 | river Danube |
| | Kakacheva, Margaritov, Grupcheva, 1978 | river Danube (t. Svishov, t. Ruse, t. Vidin, t. Lom t. Tutrakan) |
| | Atanasov, 2012 | river Danube (v. Archar, v. Dobri dol, t. Svishov, v. Botevo, v. Gomotarci, v. Vardim, v. Novo selo, v. Simeonovo, t. Kozloduj) |
| *Acanthocephalus anguillae* (Müller, 1780) | Atanasov, 2012 | river Danube (v. Archar, t. Svishov, v. Vardim,) |
| | Chunchukova, Kirin (2021) | river Tundja |
| **Nematoda** | | |
| *Raphidascaris acus* (Bloch, 1799), larvae | Shukerova, 2005 | Biosphere Reserve Srebarna |
| *Contracaecum microcephalum* (Rudolphi, 1809), larvae | Shukerova, 2005 | Biosphere Reserve Srebarna |

## CONCLUSIONS

The study presents the first data on the helminths and helminth communities of the *C. gibelio* of the Tundzja River, middle section. Of the three found helminth species, *N. skrjabini* is a core species, and the other two are accidental species for the helminth communities of *C. gibelio*. Only *Contracaecum* sp. is an allogenic species in communities. The values of the prevalence and mean intensity are closely related to the intensity of the intermediate host populations and food chains' integrity.

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# LENGTH-WEIGHT RELATIONSHIPS AND FULTON CONDITION FACTOR (K) OF FRESHWATER FISH SPECIES FROM THE RUSCOVA RIVER, SPAWNING GROUND OF DANUBE SALMON *HUCHO HUCHO,* LINNAEUS, 1758 (PISCES: SALMONIDAE)

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***Abstract***

*This study is the first reference regarding the length-weight relationships of freshwater fishes inhabiting one of the most important spawning waters of the endemic endangered Danube salmon (Hucho hucho). Fulton condition factor (K) was calculated for 1366 individuals belonging to 16 species from Ruscova River, north of Romania. Length-weight relationships were determined for 1362 specimens from 14 species. The smallest slope value (b) was determined for Romanogobio uranoscopus (b=2.2437) and the highest value for Telestes souffia (b=3.6058). The Danube salmon (Hucho hucho) showed positive allometric growth, having the calculated value of the slope of 3.3879. The mean values of Fulton condition factor (K) for the captured specimens were: Cottus gobio (1.161), Alburnus alburnus (0.3726), Alburnoides bipunctatus (0.8142), Barbus barbus (0.9434), Barbus carpathicus (0.9202), Chondrostoma nasus (0.8867), Romanogobio uranoscopus (0.8196), Phoxinus phoxinus (0.9888), Squalius cephalus (1.137), Telestes souffia (0.898), Barbatula barbatula (0.6693), Eudontomyzon danfordi (0.1293), Hucho hucho (0.8454) and Thymallus thymallus (0.9522).*

***Key words****: allometry, electrofishing, endangered species, ichthyofauna, LWR.*

## INTRODUCTION

The abundance of the Danube salmon *Hucho hucho,* the largest salmonid inhabiting the Danube basin (montane and submontane rivers) is decreasing due to anthropic activities such as pollution, poaching, habitat fragmentation, riverbed regulations, and hydro-power plants (Bănărescu, 1964; Holčik, 1990; Bănăduc, 2008; Bănăduc et al., 2013; Witkowski et al., 2013; Ihuț et al., 2014; Freyhof et al., 2015; Cocan et al., 2020). The knowledge on Danube salmon spawning sites regarding fish composition is crucial for the conservation of the species. Young Danube salmon specimens remain in the spawning tributaries feeding on invertebrates, but when they reach 50-90 mm they start feeding on fish (Holčik, 1990). Bănărescu (1964) stated that juveniles start preying on fish when they are a few months old, especially on common nase (*Chondrostoma nasus*), and feed on insects and larvae only when prey fishes are missing. Šubjak (2013) studied the stomach content of Danube salmon from Slovak rivers during the winter season and mentioned that the species' main food source consisted of brown trout (*Salmo trutta*), rainbow trout (*Oncorhynchus mykiss*), European grayling (*Thymallus thymallus*), European chub (*Squalius cephalus*), common nase (*Chondrostoma nasus*), common barbel (*Barbus barbus*), bream (*Abramis brama*), spirlin (*Alburnoides bipunctatus*), bleak (*Alburnus alburnus*), perch (*Perca fluviatilis*) and frogs

(*Rana* sp.). Habitat alteration affects all the trophic levels (producers = plants; aquatic insects and non-predatory fish = primary consumers; invertebrate consumers = secondary consumers and vertebrate predators = tertiary consumers) (Amila & Suhaila, 2017). In the current study, on Ruscova River, fish species (primary, secondary and tertiary consumers) length-weight relationships and condition factor were analyzed through allometric growth and Fulton condition factor K (Le Cren, 1951, Froese, 2006; Nash et al., 2006; Rawat et al., 2014; Jisr et al., 2018; Borga et al., 2019).

## MATERIALS AND METHODS

### STUDY AREA

Ruscova River (Figure 1) is situated in the Northern part of Romania and it is tributary to Vișeu River, one of the most important habitats of the Danube salmon *Hucho hucho*. It has a total length of 39 km and it crosses four localities: Poienile de sub Munte, Repedea, Ruscova, and Leordina, where it flows in Vișeu River. Ruscova River is considered one of the most important spawning grounds for the Danube salmon, the most enigmatic freshwater fish species of the Salmonidae family. A total number of 15 sampling stations were analysed in terms of fish species composition and length-weight relationships.

### FISH SAMPLING

Fish sampling was carried out from June 2013 to July 2013 by single-pass electrofishing techniques using a SAMUS 725G apparatus powered by 12V and 24 A rechargeable battery (Reid et al., 2009). Each captured fish was photographed, weighed, measured, and released back into the river. Total length (TL) was digitally measured using ToupView software version 3.7 (ToupTek Photonics) based on the fish images taken on laminated graph paper to the nearest 0.1 mm. Wet body weight (BW) was measured using a digital scale to the nearest 1 g (Brosset et al., 2015).

### DATA ANALYSIS

The relationship between total length and body weight (LWR) was estimated by fitting the exponential curve to the data ($BW=aTL^b$, where *BW* is body weight, *TL* the total length, *a* the intercept and *b* the slope) (LeCren, 1951). To detect the strong deviation from isometric growth ($b=3$) 95% confidence intervals of *b* were determined and also determination coefficient $R^2$ was calculated.

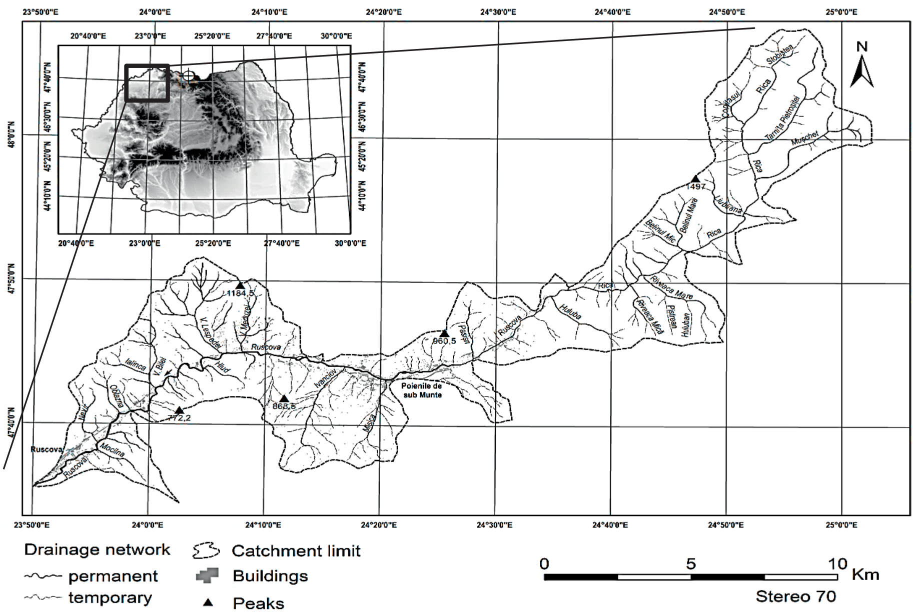


Figure 1. Ruscova River catchment (Source: Cocan et al., 2020)

When the slope value $b$=3, the weight increase is considered isometric. When the value of $b$ is higher than 3, the weight increase is allometric positive, and when $b$ is lower than 3 the weight is allometric negative. Fulton condition factor (K) for each individual was calculated based on the formula:

$$K = \frac{BW \cdot 100}{TL^3}$$

where:
$K$ – Fulton condition factor
$BW$ – wet body weight (g)
$TL$ – total length (cm)

## RESULTS AND DISCUSSIONS

The altitude of the 15 sampling stations ranged from 401 m to 616 m. A total number of 1366 individuals from 16 species and 9 families were sampled (Leuciscidae: *Alburnoides bipunctatus, Alburnus alburnus, Phoxinus phoxinus, Squalius cephalus, Chondrostoma nasus, Telestes souffia*; Cyprinidae: *Barbus barbus, Barbus carpathicus*; Gobionidae: *Romanogobio uranoscipus*; Cottidae: *Cottus gobio*; Cobitidae: *Sabanejewia balcanica*; Nemacheilidae: *Barbatula barbatula*; Lotidae: *Lota lota*; Salmonidae: *Hucho hucho, Thymallus thymallus* and Petromyzontidae: *Eudontomyzon danfordi*) (Figure 2). Allometric growth of the burbot *L. lota* and the Balkan spined loach *S. balcanica* was not calculated in this study because of their small number (1 *L. lota* and 3 *S. balcanica* specimens).

Mean Fulton's condition factor (K) ranged from 0.1293 in the case of *E. danfordi* to 1.1607 for *C. gobio*. The mean value of K for the other species was as follows: *S. cephalus* – 1.1370, *P. phoxinus* – 0.9888, *T. thymallus* – 0.9522, *B. barbus* – 0.9434, *B. carpathicus* – 0.9202, *T. souffia* – 0.8980, *C. nasus* – 0.8867, *H. hucho* – 0.8454, *R. uranoscopus* – 0.8196, *A. bipunctatus* – 0.8142, *A. alburnus* – 0.6726, *B. barbatula* – 0.6693 and *L. lota* – 0.5722, *S. balcanica* – 0.4771 (Figure 3).

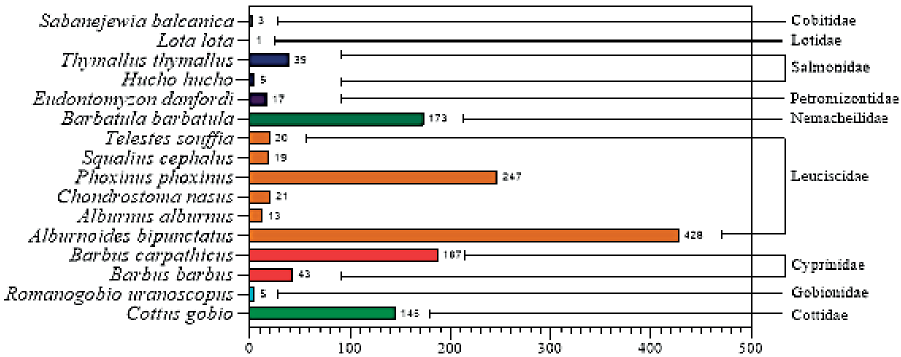


Figure 2. Fish species abundance from Ruscova River

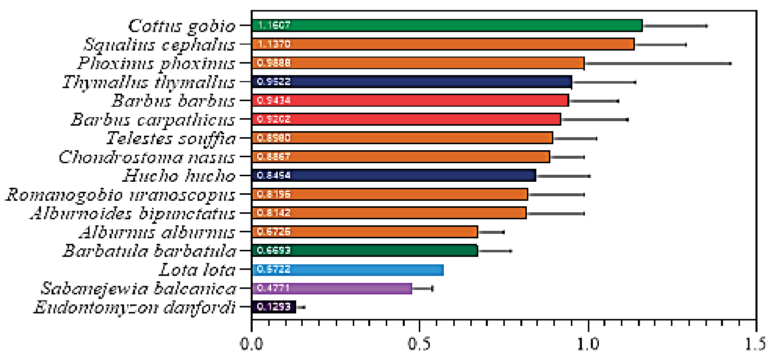


Figure 3. Fulton Condition Factor (K) determined for fish species from Ruscova River

In the case of the analyzed species, the LWRs were significant ($p<0.05$) and the coefficient of determination $R^2$ ranged from 0.638 in the case of *P. phoxinus* to 0.995 in the case of *H. hucho*. In addition, the calculated values of $R^2$ values were larger than 0.90 for 8 species (58%), larger than 0.8 for 4 species (28%), between 0.6 and 0.7 for 2 species (14%). The slope values (*b* values) ranged from 2.2437 for *G. uranoscopus* to 3.6058 for *T. souffia*. The growth type of the studied species showed isometric growth (b=3) in two cases: *C. nasus* and *C. gobio*. The following 6 species showed positive allometric growth (b>3): *A. alburnus, S. cephalus, T. souffia, B. barbus, B. carpathicus* and *H. hucho*. The remaining 6 species showed a negative allometric growth (b<3) type (*A. bipunctatus, P. phoxinus, G. uranoscopus, B. barbatula, T. thymallus* and *E. danfordi*) (Table 1).

The data regarding LWRs in the case of Danube salmon are similar to those of Simonovic et al. (2011) where the *b* values of adult Danube salmon from Drina River (Serbia) ranged from 2.187 to 3.910. It is worth mentioning that the authors used standard length. In terms of Fulton's condition factor K, the same authors obtained values between 1.074 to 1.190, slightly higher than the values from our study caused by the use of standard length. Ratschan (2012) and Treer et al. (2013) mentioned that the size of Danube salmon is dependent on its habitat size. Four out of five captured specimens in this study were small-sized fish (39-92 g). The largest specimen caught had 1150 g. Treer et al. (2013) obtained a condition factor of 1.1559 but for much larger specimens (4.5-18 kg).

Table 1. Length-weight relationship of fish species from Ruscova River, Romania.

| Family | Species | N | Weight (g) Mean ± SD (Min-Max) | Length (cm) Mean ± SD (Min-Max) | Equation $BW=a\ TL^b$ | $R^2$ | S.E. of b (95% C.I. of b) | Growth Type |
|---|---|---|---|---|---|---|---|---|
| Leuciscidae | *Alburnoides bipunctatus* | 428 | 5.22±2.63 (1.00-20.00) | 8.48±1.28 (5.81-13.96) | $BW = 0.0155\ TL^{2.6829}$ | 0.709 | 0.0831 (2.519-2.846) | Allometric (-) |
| | *Alburnus alburnus* | 13 | 11.00±5.86 (4.00-21.00) | 11.41±2.00 (8.37-14.75) | $BW = 0.0042\ TL^{3.1942}$ | 0.968 | 0.1744 (2.810-3.578) | Allometric (+) |
| | *Chondrostoma nasus* | 21 | 99.95±72.80 (23-294) | 21.12±5.58 (13.96-31) | $BW = 0.0096\ TL^{2.9694}$ | 0.974 | 0.1121 (2.735-3.204) | Isometric |
| | *Phoxinus phoxinus* | 247 | 3.58±1.33 (1.00-9.00) | 7.12±0.96 (2.49-10.31) | $BW = 0.0344\ TL^{2.3365}$ | 0.638 | 0.112 (2.115-2.558) | Allometric (-) |
| | *Squalius cephalus* | 19 | 125.47±79.70 (11.00-329.00) | 21.16±4.89 (10.02-30.38) | $BW = 0.0081\ TL^{3.106}$ | 0.97 | 0.132 (2.826-3.386) | Allometric (+) |
| | *Telestes souffia* | 20 | 18.65±11.37 (3.00-42.00) | 12.18±2.22 (8.06-15.58) | $BW = 0.0019\ TL^{3.6058}$ | 0.976 | 0.134 (3.323-3.887) | Allometric (+) |
| Cyprinidae | *Barbus barbus* | 43 | 32.00±31.07 (4.00-194.00) | 13.98±3.71 (7.80-27.68) | $BW = 0.0061\ TL^{3.1605}$ | 0.956 | 0.1063 (2.946-3.375) | Allometric (+) |
| | *Barbus carpathicus* | 187 | 30.99±28.39 (2.00-163.00) | 13.63±4.25 (5.96-24.41) | $BW = 0.0069\ TL^{3.1081}$ | 0.976 | 0.0358 (3.037-3.179) | Allometric (+) |
| Gobionidae | *Gobio uranoscopus* | 5 | 3.80±1.30 (2.00-5.00) | 7.71±1.20 (6.43-9.24) | $BW = 0.0375\ TL^{2.2437}$ | 0.827 | 0.593 (0.356-4.131) | Allometric (-) |
| Cottidae | *Cottus gobio* | 145 | 10.74±4.98 (3.00-30.00) | 9.54±1.40 (6.64-13.41) | $BW = 0.0106\ TL^{3.0348}$ | 0.895 | 0.087 (2.863-3.207) | Isometric |
| Nemacheilidae | *Barbatula barbatula* | 173 | 7.68±2.59 (3.00-19.00) | 10.36±1.20 (7.50-16.89) | $BW = 0.0098\ TL^{2.8311}$ | 0.809 | 0.1052 (2.623-3.038) | Allometric (-) |
| Salmonidae | *Hucho hucho* | 5 | 287.20±482.80 (39.00-1150) | 25.96±11.99 (17.37-47.03) | $BW = 0.0024\ TL^{3.3879}$ | 0.995 | 0.141 (2.937-3.838) | Allometric (+) |
| | *Thymallus thymallus* | 39 | 73.59±50.89 (3.00-195.00) | 18.60±5.53 (5.83-26.87) | $BW = 0.0152\ TL^{2.8193}$ | 0.885 | 0.167 (2.481-3.157) | Allometric (-) |
| Petromyzontidae | *Eudontomyzon danfordi* | 17 | 9.23±4.21 (3.00-17.00) | 18.90±3.43 (13.41-23.02) | $BW = 0.0031\ TL^{2.6877}$ | 0.908 | 0.2208 (2.217-3.158) | Allometric (-) |

## CONCLUSIONS

This paper represents a uniquely comprehensive data set on the length-weight relationship and condition factor of the fish species community from Ruscova River, one of the most important spawning habitats of the endangered Danube salmon.

The growth type of Danube salmon was allometric positive (b=3.3879), while the growth of the second Salmonidae species, *T. thymallus* was allometric negative (b=2.8193). Fulton condition factor of the Danube salmon was K=0.8454.

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# RESEARCH STATE OF *ALOSA IMMACULATA* (BENNETT, 1835) STOCKS FROM ROMANIAN SECTOR OF DANUBE – SHORT OVERVIEW

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***Abstract***

*Shad (Alosa spp.) is an important fish species and has high economic value. Commonly, it is found in the Black Sea basin and is represented by four species and one subspecies: Alosa maeotica, Alosa tanaica, Alosa immaculata, Alosa caspia, which are distributed in the Black Sea. For the Romanian fishery sector, the Pontic shad (Alosa immaculata) is an important fishery resource. The attractiveness of the Pontic shad fishery, both in the sea and especially in the Danube River (during the migration period), depends on the seasonal nature of this activity but also the interest of consumers for these species. Consumers' interest for Pontic shad is due to both the flavor of the meat and its nutritional qualities. According to IUCN, Pontic shad is estimated as a vulnerable fish species, stocks being affected by the construction of the Iron Gates I and II hydroelectrical dams. In this context, this paper aims to present an overview of the current stocks state of the Pontic shad in the context of environmental and exploitation conditions in Romania.*

***Key words****: exploitation, fish catch, migratory fish species, shad.*

## INTRODUCTION

The generally called "shads" include types of fish that differ very little in terms of external appearance. Clupeids are ancient, tertiary forms that inhabited the ancient Sarmatian Sea, which is why among them some are migratory freshwater species (potamodromous Clupeids) and marine migratory species (anadromous Clupeids) (Niculescu-Duvăz, 1959).

Despite their commercial importance, there are many unknown aspects regarding their phylogenetic bonds within the genus *Alosa*, leading to systematic and taxonomic uncertainties, that can make it harder to establish appropriate conservation measures (Faria et al., 2006).

*Alosa* species occur in the northern hemisphere of the Earth, inhabiting the Atlantic Ocean and the Mediterranean, Black, and Caspian Seas.

The Pontic shad is the most widespread species from the *Clupeidae* family that inhabits the Black Sea area (Kottelat & Freyhof, 2007). Along with the *Alosa tanaica* and *Alosa maeotica* species, the Pontic shad migrate for reproduction in the Black Sea and Azov Sea tributary rivers (Kolarov, 1991; Navodaru & Waldman, 2003). If in the past some specimens of Pontic shads migrated up to Budapest (Danube river, 1650 km) (Bănărescu, 1964), nowadays fishes barely reach km 864, migration route distance being shortened by the construction of the Iron Gates II Hydropower Plant (Navodaru & Waldman, 2003).

In Romania, the Pontic shad commercial fishing catch has a commercial value of about 1.5 million euros, with an average annual catch of 200-500 tons. But the bigger is the interest in the exploitation and commercialization of the *Alosa* stocks, the greater the danger of causing a drastic decline of the populations from the Danube and the Black Sea regions. In the last decades, the population of Pontic shads are rapidly declining, according to the IUCN Red List of Threatened Species, *Alosa immaculata* is classified as vulnerable specie (VU) (Freyhof, 2010). Some of the major current threats for this species are represented by overfishing, pollution, climate changes and dams construction, threats that have led to a big reduction of reproduction and feeding in large areas, and implicitly a decrease in shad stocks (Kottelat & Freyhof, 2007).

Numerous studies have highlighted the influence of medial factors and climate change on the migration of Pontic shad and implicitly on commercial catches. For example, Smederevac-Lalić et al. (2018) analyzes the influence of water level oscillations in the Danube river, especially of the spring floods upon the *Alosa immaculata* catches, as a key to predicting future fluctuations in catches and the influence of solar activity on shad stocks. The influence of temperature on the migration of Pontic shad was also underlined, a migration that generally begins during the February – March period, when the water temperature gets close to 5-6°C, reaching maximum potential in April, at 9-13°C water temperatures (Năstase et al., 2018) and ending in June-July when temperatures are within 22-26°C interval (Năvodaru 1996, 1998).
This research aims to generally present the current state of Pontic shad stocks, analyzed in the context of environmental factors and the exploitation state of Romania.

## MATERIALS AND METHODS

The Danube is the most important river in Romania. Targeted areas are parts of the Romanian Danube river that represent a central point of wetland with particular importance for fish populations (Ibănescu et al., 2016). A long large stream, like the Danube, the second river in Europe, is an extremely variable and complex environment. Its multiple uses set up the foundation of numerous arguments for long-term systematic research which can help us understand the seasonal variation of physico-chemical and hydrological parameters, factors that have extremely important consequences on the structure and dynamics of fish communities (Calin Sandu et al., 2013).
Data regarding the hydrological and physical parameters of the water (water level and temperatures) were daily collected from the Galati Lower Danube River Administration website (Administrația Fluvială a Dunării de Jos RA Galați, n.d.) (Figure 1). These data were processed using MS Excel 2021 package.
The dynamics of the reported catches from 2010 to 2021 were presented with the use of the official data obtained from the National Agency for Fisheries and Aquaculture (www.anpa.ro).
The Total Allowable Catch (TAC) data regarding our in-study species were collected from the legislation 2010-2021 TAC Orders.

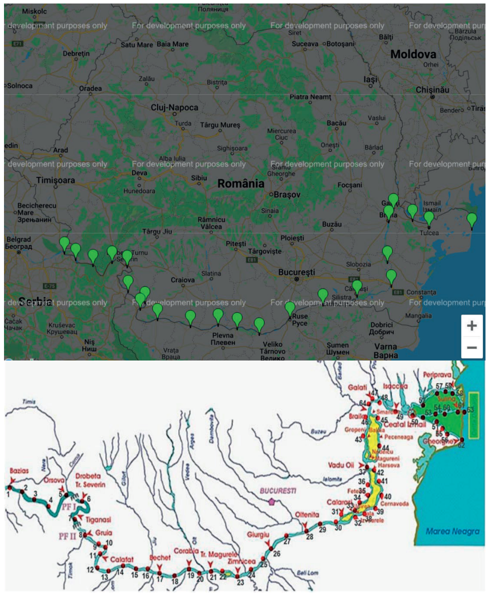


Figure 1. Sampling stations along the Danube river (Galati Lower Danube River Administration, n.d.)

## RESULTS AND DISCUSSIONS

Numerous researches state that upstream migration and reproducing of European *Alosa* spp. are triggered by water temperature, water flows and water levels (Cassou-Leins et al., 2000; Mennesson-Boisneau et al., 2000; Aprahamian et al., 2003; Acolas et al., 2006, Esteves & Andrade, 2008).
Recently, the current research taken over the Danube riverine countries does not sufficiently address the influence of environmental factors on the migration of the Danube shad, nor on the state of stock-exploitation. Ciolac (2004), describes the migration of the Danube shad during the 2000 years and concludes that its migration began in March when the water level was rising and the water temperature stabilized at about 6°C, with a maximum intensity of migration during April, and an ending point in July. Năstase et al. (2018) describe the shad migration as typical and directly influenced by environmental factors, especially the water temperature increase on a side and elevated water levels (spring floods) on the other side,

which in 2016, favored the start of migration earlier than usual, in February, with a peak of reproduction migration in early April and ending time in May, mainly because to the lack of data, given by the fact that the market and catches no longer motivated the fishermen to catch and report this species. A general analysis of the average annual temperatures of Danube River water in 2020-2021 period is presented in Table 1.

Table 1. Temperatures (°C) from the Gruia, Calafat, Giurgiu, Brăila, Tulcea and Sulina stations

| Years | Calculated indices | Registered temperatures (°C) / Stations | | | | | |
|---|---|---|---|---|---|---|---|
| | | Gruia | Calafat | Giurgiu | Brăila | Tulcea | Sulina |
| 2010 | Average±S.D. | 13±7.9 | 13.4±8.2 | 13.7±8.2 | 13.5±8.3 | 13.6±8.4 | 13.5±8.4 |
| | Min. | 1 (Feb/Dec) | 0.8 (Feb) | 0.5 (Feb) | 1 (Feb) | 0.5 (Jan) | 0.5 (Jan) |
| | Max. | 26.5 (Jul) | 27 (Jul) | 27.4 (Jul) | 28 (Aug) | 28 (Aug) | 27 (Aug) |
| 2011 | Average±S.D. | 13.3±8.9 | 13.6±8.8 | 14±8.9 | 13.9±9.1 | 13.8±9.2 | 13.7±9.2 |
| | Min. | 0.5 (Jan) | 1 (Jan) | 1.1 (Jan) | 1 (Jan) | 1 (Jan) | 1 (Jan) |
| | Max. | 25.8 (Jul) | 26.2 (Jul) | 26.8 (Jul) | 28 (Aug) | 28 (Aug) | 28 (Jul) |
| 2012 | Average±S.D. | 13.4±9.1 | 13.8±8.8 | 14.3±9 | 14.4±9.3 | 14.3±9.3 | 14.4±9.4 |
| | Min. | 0.2 (Feb) | 0 (Feb) | 0 (Feb) | 0.1 (Feb) | 0.3 (Feb) | 0.2 (Feb) |
| | Max. | 28 (Jul) | 27.2 (Jul) | 27.8 (Jul) | 29 (Jul) | 28 (Jul) | 28 (Jul/Aug) |
| 2013 | Average±S.D. | 13.1±8.3 | 13.6±8 | 13.6±8.2 | 14±8.4 | 13.8±8.4 | 13.8±8.4 |
| | Min. | 1.3 (Jan) | 3 (Jan/Dec) | 1.7 (Jan) | 1.5 (Jan) | 2 (Jan) | 2 (Jan) |
| | Max. | 26.9 (Aug) | 27 (Aug) | 26.8 (Aug) | 29 (Sep) | 27.5 (Aug) | 27.5 (Aug) |
| 2014 | Average±S.D. | 13.6±7.7 | 14.1±7.3 | 14±7.5 | 14.1±7.8 | 13.9±7.9 | 13.9±8 |
| | Min. | 1 (Feb) | 1.8 (Feb) | 1.4 (Feb) | 1 (Feb) | 1 (Feb) | 1 (Feb) |
| | Max. | 26 (Aug) | 26 (Aug) | 26.5 (Aug) | 27 (Aug) | 27 (Aug) | 27 (Aug) |
| 2015 | Average±S.D. | 13.6±8.5 | 14.4±8.2 | 14.4±8.4 | 14.6 ±8.5 | 14.5±8.5 | 14.4±8.5 |
| | Min. | 1.2 (Jan) | 1 (Jan) | 1.6 (Jan) | 1.5 (Jan) | 1.5 (Jan) | 1.5 (Jan) |
| | Max. | 27.4 (Aug) | 31 (Jul/Aug) | 28 (Aug) | 28 (Aug) | 28,5 (Jul) | 28 (Jul/Aug) |
| 2016 | Average±S.D. | 13.8±8.2 | 14.2±8.3 | 14.3±8.2 | 14.3±8.4 | 14.3±8.5 | 14.3±8.5 |
| | Min. | 1 (Jan) | 1.2 (Jan) | 1.2 (Jan) | 1 (Jan) | 1 (Jan) | 1 (Jan) |
| | Max. | 27.2 (Aug) | 35 (Jul) | 27 (Aug) | 27 (Aug) | 27.2 (Aug) | 27.2 (Aug) |
| 2017 | Average±S.D. | 14±9 | 14 ±9 | 14±9 | 14.2±9.1 | 14.1±9.1 | 14.1±9.1 |
| | Min. | 0 (Jan) | 0 (Jan) | 0 (Jan/Feb) | 0 (Jan) | 0 (Jan/Feb) | 0 (Jan/Feb) |
| | Max. | 28.5 (Aug) | 28.2 (Aug) | 28 (Aug) | 28 (Aug) | 28.2 (Aug) | 28 (Aug) |
| 2018 | Average±S.D. | 14.3 ±9.3 | 14.7±9 | 14.8±9 | 14.6±9 | 14.7±9 | 14.6±9 |
| | Min. | 1 (Mar) | 2 (Jan/Feb/Mar) | 2 (Mar) | 1 (Mar) | 0.4 (Mar) | 0.3 (Mar) |
| | Max. | 27.5 (Aug) | 27.5 (Aug) | 31 (Aug) | 27 (Aug) | 26.8 (Aug) | 26.8 (Aug) |
| 2019 | Average±S.D. | 14.9±8.4 | 15±8.1 | 15.1±8.1 | 15.1±8.2 | 14.9±8.3 | 14.7±8.3 |
| | Min. | 2 (Jan) | 2 (Jan) | 1.8 (Jan) | 1 (Jan) | 1.2 (Jan) | 1.2 (Jan) |
| | Max. | 27.5 (Jul/Aug) | 27 (Aug) | 27.2 (Aug) | 27.5 (Aug) | 27.5 (Aug) | 27.2 (Aug) |
| 2020 | Average±S.D. | 14.5±8.2 | 14.7±8 | 14.9±8 | 14.9±8 | 14.9±8.1 | 14.8±8.1 |
| | Min. | 3 (Jan) | 3 (Jan) | 3.1 (Jan) | 3 (Jan) | 2.6 (Jan) | 2.6 (Jan) |
| | Max. | 27 (Aug) | 26.8 (Aug) | 27 (Aug) | 27 (Aug) | 26.7 (Aug) | 26.7 (Aug) |
| 2021 | Average±S.D. | 14.2±8.2 | 14.1±8.1 | 14.2±8.1 | 14.3±8.2 | 14.2±8.3 | 14.2±8.3 |
| | Min. | 3 (Jan) | 3 (Jan) | 3.4 (Jan) | 3 (Feb) | 2.5 (Feb) | 2.5 (Jan/Feb) |
| | Max. | 29 (Jul) | 28 (Jul/Aug) | 28.5 (Aug) | 28.5 (Jul) | 28 (Jul/Aug) | 28 (Jul/Aug) |

The average annual temperatures of the Danube river water show an increasing trend from year to year, for the 2010-2021 period.

It can be observed that since 2010, for example, in Gruia station, the average annual temperature of the Danube river water increased from 13 ± 7.9°C registered in 2010 to 14.2 ± 8.2°C, in 2021. Also, the minimum and maximum temperature of the Danube river water show increasing trends from year to year, as can be seen in Table 1.

This phenomenon of increasing the average annual temperatures leads to changes in the migration behavior and also in variations regarding the reproduction and pre-development of the *Alosa immaculata* species.

In this regard, researches upon the shad population migration in the context of global climate changes are suitable to be conducted. The water levels of the Danube river in the springtime display significant variations from year to year, but in the 2010-2021 period it can be seen a clear increasing trend as observed in the following graphs (Figures 2-7).

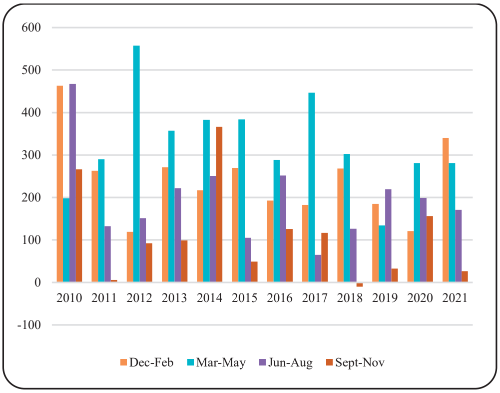


Figure 2. Water level fluctuation at the Gruia station

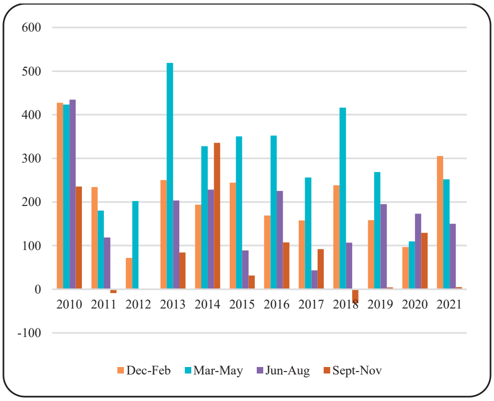


Figure 3. Water level fluctuation at the Calafat station

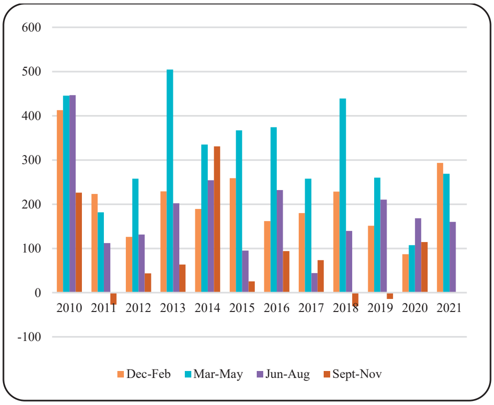


Figure 4. Water level fluctuation at the Giurgiu station

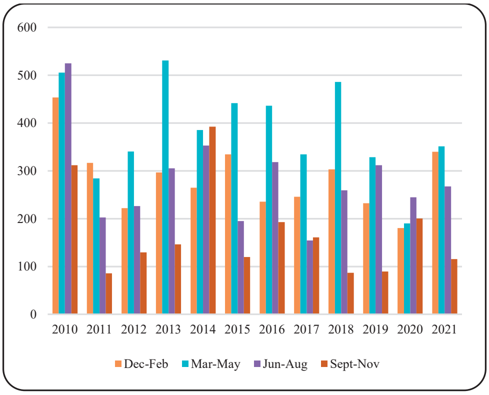


Figure 5. Water level fluctuation at the Brăila station

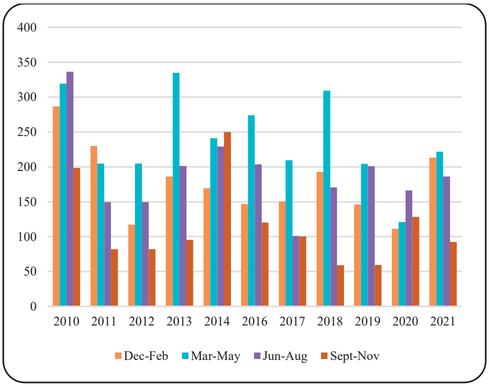


Figure 6. Water level fluctuation at the Tulcea station (*no data available for 2015)

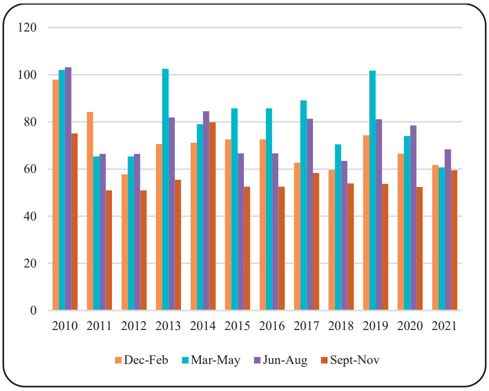


Figure 7. Water level fluctuation at the Sulina station

This phenomenon appears due to abundant rainfall or melting of the ice, specific to the spring season, which influences shad migrations.

Năstase et al. (2018) report substantial catches during the 2016 year, that were registered at the end of February, varying until mid of March, correlated with the increase of the Danube water level from 200 cm to 350 cm. On the other side, a decrease in catches was reported at the same time with the decrease of the water level below 250 cm and the increase in the water temperature. For Romania, Ukraine and Bulgaria, the Danube shad stock supports an important activity of integrated fishing.

The egg spawning takes place between 180 and 500 kilometers of the Danube river. The eggs are pelagic and the larvae swim passively toward the Black Sea (Năvodaru & Năstase, 2014). Shad fishing is estimated at a commercial value of about 1.5 million euros, with average annual catches of 200-500 tons (Mocanu et al., 2020).

In an analysis performed by Ibănescu et al. (2020), on the species structure part of commercial catches in Romania during the 2008-2018 period, it was highlighted that shad represents 10.54% of the total catch of our country. According to Năvodaru and Năstase (2014), *Alosa immaculata* has a cyclical evolution of catches, with minimums or maximums at 10-11 years, for example during the period 1960-1998, the absolute minimum was 200 tons, and the maximum was 2,400 tons.

In the period 2010-2021, the catches varied between a minimum of 174.6 tons in 2015 and a maximum of 634.5 tons in 2019 (Figure 8).

At the same time from the Figure 8 it can be observed that in the 2010-2021 period, there were only 3 years (2016, 2019, 2021) with exceeded catches (related to the total allowable catch orders reports), a fact that indicates that the *Alosa immaculata* specie was overfished.

In the rest of the years, there were reported lower catch values than the calculated total allowable catch. If a comparison of the quantities in the annual orders (TACs) and the quantities reported by fishermen is made, it can be seen that reports are sometimes even lower - less than 50% of the TAC (e.g. 2013, 2018, 2020) therefore, it can be concluded that in this case, we face the non-reporting of shad catches, that are traded illegally (IUU - Illegal, unreported and unregulated fishing).

This trend is also indicated by Ibănescu et al. (2020), in a study that analyzed the dynamics of commercial catches in Romania during the 2008-to 2018 period. It should be noted that recent studies indicate concerning values over the exploitation index of the species *Alosa immaculata* (Mocanu et al., 2021).

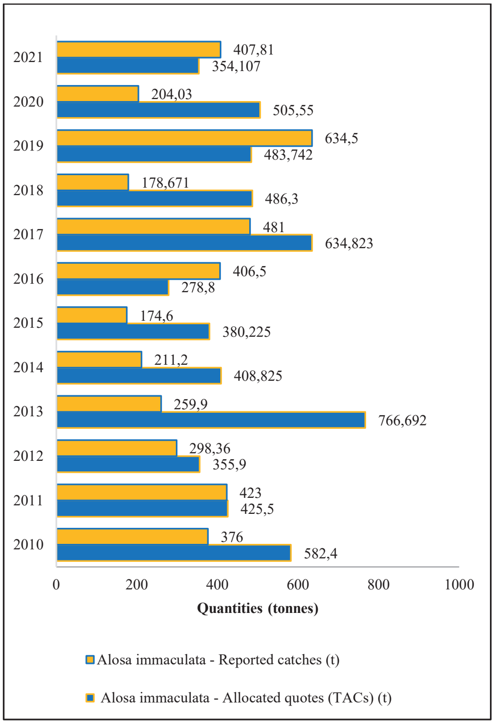


Figure 8. Allocated and reported quotes for *Alosa immaculata*

## CONCLUSIONS

Even though migration, reproduction and the conservation status of Danube shad stocks are currently clearly important, it is obvious that overexploitation is the most concerning cause of stock declining stocks of this species. Regular research that overseers the migratory population of A. *immaculata* in the Danube River must be carried out annually in order to be able to notice and take action in case of occurred changes regarding the conservation status of the species. Additional research is needed to quantify any changes concerning the number of individuals, their age structure and their state of welfare and to validate whether there is an upward trend in abundance. Also, with the help of the obtained annual data resulting from the research, it is necessary to make predictions about the state of the shad stocks and to elaborate a common plan of measures for the protection of the species, at the Danube riverine countries level.

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# ACAROLOGICAL CHARACTERISATION (ACARI: MESOSTIGMATA) OF AN URBAN GREEN AREA IN BUCHAREST, ROMANIA

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***Abstract***

*The objectives of the study were to assess and compare mite communities from the biggest urban area in Bucharest- Morii Lake, in relation to the soil environmental variables (soil and air temperature; soil pH; soil and air moisture content; soil penetration resistance) and the type of habitats/transects (park area, natural area-island, grassland). The study was made in June 2017. For soil fauna, sixty soil samples were collected, using a MacFadyen core. Seventeen mite species were identified, with 55 individuals. We observed that soil and air temperature, air humidity and soil acidity varied highly significantly between the three transects. Soil temperature, soil moisture content, air humidity influenced significantly the structural composition of the mite populations. Certain parameters were used: numerical abundance, dominance, constancy, species diversity and equitability. Using these indices, we demonstrated that the transect T1-park area offered the most favourable conditions, with the least favourable being T2-island. Acarological characterisation of an urban green area in Bucharest, Romania, revealed that, even at the local scale, the type of habitat and environmental variables influenced significantly the structural composition of the mite populations.*

***Key words:*** *environment, habitat, local scale, mite, urban.*

## INTRODUCTION

Various ecological studies in Europe have been conducted during the last thirty years, regarding the soil fauna of urban ecosystems. These studies highlighted that soil invertebrates could constitute valuable bioindicators of the environmental conditions that are specific to anthropised ecosystems, and that could be useful in monitoring programmes. Based on their ecological and biological requirements, different soil groups were used as bioindicators i.e.: nematodes, mites, springtails, enchytraeids, earthworms, isopods, beetles, ants, spiders, chilopods, diplopods, etc. Different urban habitats were investigated, i.e.: parks, urban forests, grasslands, industrial areas, cemeteries, transport routes, recreation areas, open lands, domestic gardens, waste grounds, green areas within housing estates, streetside grass verges and green roofs, and studies were conducted in many European countries: Latvia, Denmark, Poland, Romania, Italy, Bulgaria, Poland, Hungary, Germany, Austria, Switzerland, England, Czechia, etc. (Niedbała et al., 1990; Christian & Szeptycki, 2004; Stoev, 2004, Schrader & Boning, 2006; Vilisics et al., 2008; Minova et al., 2015; Manu et al., 2015; Napierała et al., 2015; Santorufo et al., 2015; Giurgincă et al., 2017; Szlavecz et al., 2018; Tóth & Hornung, 2020; Braschler et al., 2020; Manu et al., 2021).

These studied revealed that urbanisation changes the invertebrate fauna, especially reducing the species richness. Extreme urbanisation (from the urban core areas) also reduces this population parameter, through loss of habitable area for invertebrates or degradation of remaining habitat by many anthropogenic activities e.g pollution or traffic. Moderate levels of urbanisation, especially those in suburban areas, do not have such a drastic impact upon invertebrate diversity, and sometimes even increased species richness has been observed (McIntyre, 2000; McKinney, 2008; Nagy et al., 2018). Urban soils differ from those from other managed ecosystems in terms of heterogeneity, unique organic matter inputs and exposure to past and present anthropogenic activities. Pedogenesis in urban ecosystems is influenced by the activity of bacteria and fungi, but also strongly correlated with invertebrate activity, due especially to their importance in soil organic matter dynamics (Bray & Wickings, 2019). In this context, mites (Acari) represent one of the most important and abundant invertebrate groups. They play an important role in the complex soil ecological systems, being actively involved in the flow of energy, matter and information. Mite research could enrich many different approaches, from zoogeography to ecology, taxonomy and parasitology or even palaeontology (Gwiazdowicz, 2021). Specific habitat and environmental variables will influence the composition of mite communities, being a valuable tool for monitoring environmental quality, including in urban soils, where one particular order has been highlighted as important i.e. predatory mites (Mesostigmata). Focussing only on habitats in urban ecosystems, faunistic and taxonomic studies have occurred in Italy, Hungary, Slovakia, Latvia, Poland, as well as in Romania (Bucharest city), but with little information regarding the ecology of soil mite communities in relation to urban environmental factors (Niedbała et al., 1990; Kontschán et al., 2015; Santorufo et al., 2015; Fendá & Hruzova, 2016; Salmane, 2018; Manu et al., 2021). Detailed studies from Bucharest (Manu et al., 2021) revealed the presence of specific mesostigmatid mite communities (together with differing numerical abundance and species richness) in managed green areas and in unmanaged green areas. In comparison with managed green areas, unmanaged urban habitats were characterised by higher values of community parameters (i.e. Shannon diversity, dominance and equitability), as well as by the highest values of the soil maturity index. Making a comparative analysis of different managed green areas (metropolitan, municipal and district urban parks), the study revealed that the species communities from metropolitan parks were richer than those from district parks. This study demonstrated the important links between mite communities in specifically urban ecosystems that are under anthropogenic pressure, also highlighting that unmanaged urban green areas were "hotspots" of Mesostigmata diversity (Manu et al., 2021). These studies analysed the specificity of mite communities from urban green areas in Bucharest to different management practices. However, the study did not examine what happened at the small scale, focussing on one green area in Bucharest i.e. Morii Lake.

The aims of the present study are: (1) to assess and compare mite communities on three separate areas (transects) from Morii Lake, divided by the lake dam as a barrier; (2) to identify the major soil environmental variables shaping the structure of mite communities; (3) to investigate the taxonomic and compositional response of mites to urban management scenarios and environmental variables.

## MATERIALS AND METHODS

### Study area

The research was conducted in June 2017 in the green area close to Morii Lake, Bucharest (44°27'20"N; 26°01'31"E). Morii Lake is the largest lake in Bucharest, with an area of 246 hectares. After floods in 1972, 1975 and 1979, local administrators from that period decided to build a reservoir, with the purpose of protection against damage from extreme climatic events. Thus, in 1986 Morii Lake appeared on the map of Bucharest city, protecting the capital against floods, and also becoming an important recreation area. Morii Lake provides a constant flow to the Dâmboviţa River in the city. The lake is protected by a dam of 15 m height, situated approximately six kilometres from the centre of Bucharest (Piața Unirii) and located between the Polytechnic University of Bucharest neighbourhood to the east, the Crângași and Giulești districts to the north, and the Militari district to the south (Figure 1) (Nae & Turnock, 2011).

In order to investigate the project objectives, three transects were analysed (T1, T2 and T3), which were well-spaced (approximately 1500 metres) and under different management types (T1= park area; T2= natural area-island; T3= overgrazed grassland). Transect 1 was located at 44°27'14.9"N; 26°02'38.6"E, and 79 metres altitude, on sandy soil. Transect 2 was located at 44°27'59.5"N; 26°01'77.9"E, at 87 metres altitude and with alluvial soil. Transect 3 had an alluvial soil, located at 44°27'35.1"N; 26°01'00.2"E, and at 83 metres altitude. None of the transects were on a slope (Figure 1).

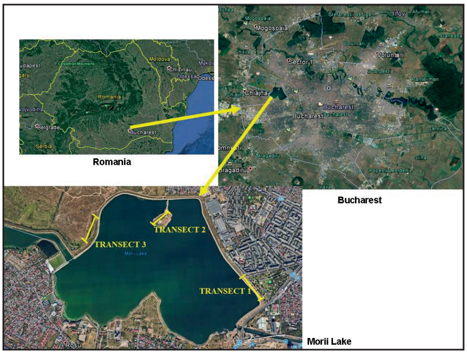


Figure 1. Geographical characterisation of the investigated area at Morii Lake, Bucharest-Romania, in 2017

Characterisation of vegetation type revealed the presence of the following dominant species:

- in T1 (park area): *Platanus x hispanica, Rumex* sp., *Capsella bursa-pastoris, Dactylis glomerata, Trifolium repens, Urtica dioica, Silene latifolia* subsp. *alba*.
- in T2 (island): *Salix babylonica, Lolium perenne, Poa* spp., *Taraxacum* sp., *Cardaria draba, Heracleum sphondylium, Prunus* sp., *Trifolium repens*.
- in T3 (grassland): *Salix babylonica, Prunus* sp., *Capsella bursa-pastoris, Lolium perenne, Poa* spp., *Taraxacum* sp., *Cardaria draba, Heracleum sphondylium, Trifolium repens.*

**Soil fauna**

In April 2017, sixty soil samples were collected, using a MacFadyen soil core (5 cm diameter) to 10 cm depth. The samples were collected randomly (20 samples/transect). For each investigated transect, the sampled area was 200 $m^2$. Mites were extracted with a Berlese-Tullgren funnel, in ethyl alcohol, clarified in lactic acid and identified to species level, using published identification keys (Ghilyarov & Bregetova 1977; Hyatt, 1980; Karg, 1993; Mašán, 2003; Mašán & Fendá, 2004; Mašán, 2007; Mašán et al., 2008; Mašán & Halliday, 2010, 2013; Özbek & Halliday, 2015). Some specimens were mounted on permanent slides. All species were deposited in the collection of the Institute of Biology-Bucharest, Romanian Academy- Research Station Posada. No immature stages were identified, since these were missing from the soil samples.

**Environmental variables**

In total, six environmental variables were quantified: a) within the soil (temperature –$T_{soil}$; acidity-pH; moisture content- $H_{soil}$; penetration resistance- RP); and b) 5 cm above the soil level (temperature - $T_{air}$; air moisture content- $H_{air}$). In total, 60 soil samples were analysed (20 samples/transect) in order to measure these abiotic factors. A digital thermo-hygrometer PCE-310 was used to measure air and soil moisture and temperature. Penetration resistance was determined with a soil penetrometer, Step System GmbH, 41010. The pH was measured with a C532 Jasco Consort pH-meter. Due to the homogeneity of the vegetation cover (especially between samples of each transect), this parameter was not considered further (Figure 2 a, b, c). The average values of environmental variables are presented in table 1.

a. b. c.

Figure 2. The vegetation aspect of each investigated transect, in Morii Lake-Bucharest (a = transect T1-park area; b = transect T2- island; c = transects T3-grassland)

**Data analysis**

The population parameters used in the statistical analysis were: the numerical abundance (number of individuals), dominance (D%), constancy (C%), species diversity (Shannon-Wiener index) and equitability (J index).

The dominance index (D %) was obtained using the formula: D = nA / N x 100, where: nA – number of individuals of species "A" and N – total number of individuals. In terms of this index, the mite communities were grouped as follows: eudominant with D over 10% (D5);

dominant with D between 5.1 and 10% (D4); subdominant with D between 2.1 and 5% (D3); recedent with D between 1.1-2% (D2) and subrecedent with D under 1.1% (D1) (Engelmann, 1978).

The constancy index (C %) was obtained using the formula: C = 100% * pA/P, where: pA = number of samples with species A; P = total number of samples. The mite species were classified in 4 constancy classes: euconstant species with C of 75.1–100% (C4); constant species with C of 50.1–75% (C3); accessory species with C of 25.1–50% (C2); and accidental species with C of 1–25% (C1) (Selvin & Vacca, 2004).

The relationship between the environmental parameters and the number of species was established using canonical correspondence analysis (CCA). CCA is the analysis of the correspondence of a site / species matrix, in which each site gave values for one or more environmental variables. Sorting axes are linear combinations of environmental variables. CCA is thus an example of direct gradient analysis, where the gradient of environmental variables is known a priori and species abundances are considered to be a response to this gradient (Legendre & Legendre, 1998). Eigenvalues for the first two ordination axes are given, indicating their relative importance in explaining the spread in the data. For the environmental parameters, the mean values were evaluated, including the standard error (± SE).

The software also includes standard statistical tests for univariate data, such as the ANOVA test. This analysis of variance is a statistical procedure for testing the null hypothesis, for several univariate samples that are taken from within mite communities that have the same average. The samples are assumed to have a normal distribution and a similar variance (df = degrees of freedom, F = statistical test, p = is the probability of obtaining a result at least as extreme as the one actually observed, given that the null hypothesis is true).

The Jaccard-j (based on presence/absence data) and Bray-Curtis-bc (based on abundance data) similarity indices were used to indicate the association of species. The statistical software package PAST was used (Hammer et al., 2001)

## RESULTS AND DISCUSSIONS

Among the six measured environmental variables from the three studied transects, we observed that the soil and air temperatures, air humidity and soil acidity varied highly significantly between T1, T2 and T3 ($p<0.001$; df = 2). The highest average values for soil temperature, soil moisture content and soil resistance at penetration and pH were obtained in T3, with the lowest values being from T1. Air temperature had its highest recorded value in T2 and in air humidity in T1 (Table 1).

We identified 17 species of mite (Acari: Mesostigmata) fauna with 55 individuals and no immature stages. Transect T1 was characterised by the highest numerical abundance and number of species, as well as the Shannon-Wiener diversity index. The lowest values were found in the communities of mites from T2. The most abundant species were *Hypoaspis aculeifer* and *Rhodacarellus silesiacus*, which were also eudominant (Table 2). Transect T1 is defined by the highest number of characteristic species (9), T2 by one species and T 3 by five species.

Examining results for the dominance index, from the total number of species, in T1 22.27% are eudominant and 72.72% are subdominant ones. In transect T2, the eudominant mites represent 66.66% of the total number of mites and 33.33% dominants. In transect T3, the invertebrates were grouped as in T2, but in different percentages: 42.85% eudominant and 57.14% dominant. For the constancy index, we observed that in all transects the species were classified as accessory and accidental ones (Table 2). Analysing the equitability index for all three investigated transects, we observed that it has the same value in T1 and T2, meaning that the species were represented by a similar number of individuals.

Table 1. Average values of environmental variables from the investigated transects at Morii Lake- Bucharest, 2017 (± SE)

| Variables | T1 | T2 | T3 | p | F |
|---|---|---|---|---|---|
| Tsoil ($^0$C) | 12.90 (± 0.21) | 16.07 (± 0.48) | 17.72 (± 0.31) | <0.0001 | 46.72 |
| RP (Mpa) | 173.5 (± 6.03) | 181.2 (± 5.98) | 185 (± 9.83) | 0.545 | 0.61 |
| Hsoil (%) | 10.65 (± 1.16) | 10.68 (± 1.04) | 13.36 (± 0.60) | 0.084 | 2.58 |
| Tair ($^0$C) | 24.1 (± 0.44) | 28.66 (± 0.23) | 26.66 (± 0.20) | <0.0001 | 53.77 |
| Hair (%) | 63.8 (± 0.87) | 55.8 (± 1.73) | 56.8 (± 1.45) | 0.000237 | 9.69 |
| pH | 8.58 (± 0.04) | 8.33 (± 0.06) | 8.59 (± 0.05) | 0.001687 | 7.16 |

Table 2. The population parameters of the mites (Acari: Mesostigmata) identified in three transects (T1, T2, T3) in soil at Morii Lake urban area, Bucharest 2017

| | | T1 | | | T2 | | | T3 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Species | Code | No. ind | D% | C% | No.ind | D% | C% | No.ind | D% | C% |
| *Alliphis halleri* | *Al ha* | 4 | 15 | 20 | | | | | | |
| *Ameroseius* sp. | *Am sp* | 1 | 3.8 | 5 | | | | | | |
| *Dendrolaelaps* sp. | *De sp* | 1 | 3.8 | 5 | | | | | | |
| *Dinychus* sp. | *Di sp* | 1 | 3.8 | 5 | | | | | | |
| *Glyptholaspis americana* | *Gl am* | 1 | 3.8 | 5 | | | | | | |
| *Hypoaspis aculeifer* | *Hy ac* | 7 | 27 | 35 | 6 | 55 | 25 | 2 | 11 | 5 |
| *Hypoaspis karawaiewi* | *Hy ka* | 1 | 3.8 | 5 | | | | | | |
| *Lasioseius* sp. | *La sp* | 1 | 3.8 | 5 | | | | | | |
| *Onchodellus karawaiewi* | *On ka* | 1 | 3.8 | 5 | | | | | | |
| *Parasitus fimetorum* | *Pa fi* | 1 | 3.8 | 5 | | | | | | |
| *Rhodacarellus silesiacus* | *Rh si* | 7 | 27 | 25 | 4 | 36 | 20 | 10 | 56 | 35 |
| *Veigaia planicola* | *Ve pl* | | | | 1 | 9.1 | 5 | | | |
| *Proctolaelaps* sp. | *Pr sp* | | | | | | | 1 | 5.6 | 5 |
| *Pergamasus crassipes* | *Pe cr* | | | | | | | 1 | 5.6 | 5 |
| *Parasitus beta* | *Pa be* | | | | | | | 1 | 5.6 | 5 |
| *Pergamasus laetus* | *Pe la* | | | | | | | 1 | 5.6 | 5 |
| *Lysigamaus* sp. | *Ly sp* | | | | | | | 2 | 11 | 5 |
| Total number of individuals | | 26 | | | 11 | | | 18 | | |
| Total number species | | 11 | | | 3 | | | 7 | | |
| Dominance_D | | 0.18 | | | 0.44 | | | 0.35 | | |
| Shannon_H | | 2.00 | | | 0.92 | | | 1.46 | | |
| Equitability_J | | 0.83 | | | 0.83 | | | 0.75 | | |

Applying the similarity indices in order to highlight the affinity between mite communities from the three transects, we observed that, based on the presence/absence data, the Jaccard index of similarity recorded highest values between invertebrate communities from T2 and T3 (j=0.25) and the lowest between T1 and T 2 (j= 0.16) (Figure 3a). If we take into consideration the abundance data, the Bray-Curtis index of similarity recorded the highest value between mite communities from T1 and T 2 (bc=0.54) and the lowest between T1 and T3 (bc=0.40) (Figure 3b).

Analysing the relationship between environmental variables and numerical abundance of mite species, canonical correspondence analysis demonstrated that soil temperature influenced *Pergamasus laetus* and the soil moisture content affected *Parasitus beta*, from T3 samples. Air humidity was another factor that influenced *Alliphis halleri*, from T1 (Figure 4).

Each transect was characterised by specific microclimatic conditions: T1 had the lowest average values of soil and air temperature, soil moisture content, soil resistance at penetration and the highest average value of air humidity;

T2 had the highest average values air temperature and the lowest average values of air moisture content and soil acidity; in T3 some environmental parameters had their highest average values i.e. soil temperature and air moisture content, soil resistance at penetration and soil pH. The habitat of T1 was described as park, being managed as a green urban green area with mowing of the grass, and inputs of allochthonous soil through tree-planting, meaning external inputs of organic matter. Transect T2 had an aspect of island, close to the water of Morii lake (explaining the highest average value of soil moisture content), and the habitat of T3 was a grazed grassland (with the driest soil and more compacted) (Grădinaru et al., 2018).

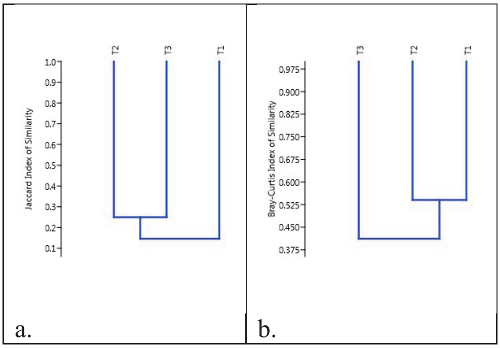


Figure 3. Indices of similarity (a- Jaccard; b- Bray-Curtis) between mite communities from the three transects on Morii Lake, Bucharest 2017

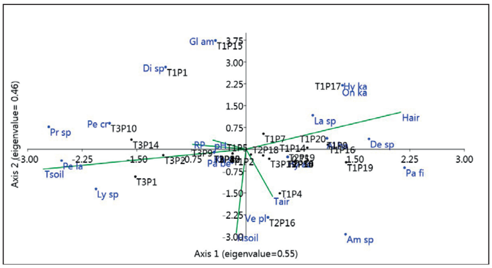


Figure 4. Canonical correspondence analysis between soil mite communities and environmental variables in Morii Lake urban area, Bucharest 2017. The short names of species are listed in Table 2

Comparing the Morii Lake results with those from elsewhere in Romania, the total number of species and individuals are closest to those obtained in some unmanaged urban green areas of Bucharest (e.g. Pantelimon, Băneasa, Griviţa with 12-22 species and 48-49 individuals) or much higher than some managed urban parks (e.g. Cişmigiu, Unirea, Izvor, Carol, Plumbuita, Floreasca, Văcăreşti with 3-10 species and with 25-43 individuals) or lower than those from Tineretului and Fundeni areas (17-18 species with 110-157 individuals) (Manu et al., 2018, 2021).

Comparing the number of Mesostigmata species from urban habitats at European level, the value at Morii Lake is comparable with that obtained in housing estates in Warsaw (18 species), higher than that in urban bamboo plantations from urban green areas in Szada, Gödöllő, Szeged and Budapest, Hungary (1-8 species) and lower than either parks and the city centre in Warsaw (22 and 39 species) or urban forest (28 species), greenery (32 species) and roadsides (33 species) from Riga, Latvia (Niedbała et al., 1982; Kontschán et al., 2015; Salmane, 2018).

Examining the number of species and the numerical abundance within the three investigated transects, we observed that T1 was characterised by the highest value of species diversity and number of individuals, whilst T2 had the lowest values of the two parameters. This result was further confirmed by the values of the Shannon index of diversity. Although the soil moisture content in T1 had the lowest average values, it is possible that it was still suitable for development of mite communities due to the reduced soil resistance at penetration, to the highest average value of air moisture content and to the reduced average value of air temperature. Lower soil resistance at penetration is correlated with larger values of organic matter, which is one of the most important abiotic factors for soil mites (Jones & Arp, 2017). Urban green areas were characterised by an impervious surface, with differing radiative, thermal, aerodynamic and moisture properties, resulting in elevated air temperatures compared to the adjacent ecosystems (Edmondson et al., 2016). Although Morii Lake is a wetland, frequent higher temperatures during summer and autumn cause high evapotranspiration and water depletion at depth in the soil (Zaharia & Găitănaru, 2018; Manu et al., 2021). Transect T2 on a peninsula had a microclimate that did not offer suitable environmental conditions for mite fauna (i.e. lowest average values of soil and air moisture content and the highest average value of air temperature). Despite T3 being grazed grassland, a more humid soil could be a favourable factor for the 7 species of mites, with 18 individuals. All transects were characterised by accidental and accessory species, demonstrating that these invertebrates are opportunistic and mobile, permanently searching for food, mainly as predators (Walter & Proctor, 2013; Klarner et al., 2013).

The most abundant species in the Morii Lake urban area were *Hypoaspis aculeifer* and *Rhodacarellus silesiacus*. In acarological studies, *Hypoaspis aculeifer* has been identified in various types of habitat from sand dunes to forest ecosystems. *Rhodacarellus silesiacus* has been found mainly in anthropogenic ecosystems, being used as a good indicator for the type of habitats, as well as for the ecological processes (as ecological succession) (Kaczmarek et al., 2012; Manu et al., 2013, 2015; Santarufo et al., 2015). Considering their preferences for urban habitats, both species were identified in managed and unmanaged green areas, as well as forests and meadows from suburban ecosystems (Manu et al., 2015, 2018, 2021; Niedbała et al., 1982, 1990). Based on the presence-absence of species (Jaccard index of similarity), we observed that there was a high similarity between the mite communities from the two habitats, island-T2 and grassland-T3 and dissimilarity between invertebrates from urban area-T1 and island T2. These differences appeared due to the difference of climatic conditions (Table 1). Comparing the abundance of mite communities in transects, through Bray-Curtis index of similarity, we observed a high similarity between the invertebrates in T1 and T2 and dissimilarity between T1 and T3. A significant factor influencing the abundance of mite communities was the soil resistance at penetration which was lowest in T1, possibly correlated with higher soil porosity and a higher quantity of organic matter (Jones & Arp, 2017).

Canonical correspondence analysis demonstrated that three environmental parameters influenced the distribution of three species: *Pergamasus laetus* (soil temperature), *Parasitus beta* (soil moisture content) and *Alliphis halleri* (air humidity). *Parasitus beta* is a predator species, widespread in soil, especially in grasslands, and found in managed and unmanaged urban areas (Manu et al., 2021). *Pergamasus laetus* is not so common in urban ecosystems, but is often recorded in forests, in habitats rich in organic matter (Manu et al., 2013, 2015, 2021). In Bucharest, species *Alliphis halleri* was found in three managed urban areas (Manu et al., 2021). In general, this species occurs in soil, leaf litter, dung and compost, where it appears to prey on nematodes (Halliday, 2019).

A more comprehensive study made in 2017, in eleven urban habitats from Bucharest, which investigated the relation between the type of management, environmental variables and structure of the soil mite communities, revealed that in unmanaged green areas the values of the community parameters (Shannon diversity, dominance and equitability) and the soil maturity index, were higher than those from managed green areas (Manu et al., 2021). If we make a comparison at the small scale, the present study, concerning the soil mite communities from the largest urban area from Bucharest, demonstrated that due to the higher anthropic impact from T3 (overgrazed grassland), due to the isolation of the transect T2 (natural area-island) and due to the better environmental conditions from T1 (park), where the area was irrigated, the results were reversed. On the other hand, acarological study from three types of managed green areas revealed that the species communities from metropolitan parks were richer than those from district parks. The study showed different values in relation to environmental factors, demonstrating important connections between mites and urban ecosystems, which are under anthropogenic pressure (Manu et al., 2021).

All this analysis constitutes a valuable argument in favour of the ecological study of the mite communities on a small scale.

## CONCLUSIONS

In order to assess and compare mite communities from the Morii Lake urban area, three transects where established, investigating the taxonomic and compositional response of mites to the type of urban habitat (park area, natural area-island and grassland) and to selected environmental variables i.e. soil and air temperature; soil pH; soil and air moisture content; soil penetration resistance. Each transect was characterised by specific environmental conditions, which varied significantly between them. In total 17 mite species were identified, with 55 individuals and no immature stages. From the acarological point of view, transect T1 offered the most suitable conditions for development of mites, the least suitable being T2. Two species were identified as most abundant: *Hypoaspis aculeifer* and *Rhodacarellus silesiacus*. Ecological characterisation of an urban green area in Bucharest, Romania, revealed that, even at the local scale, the type of habitat and particular environmental variables (i.e. soil temperature, soil moisture content, air humidity) influenced the taxonomic and structural composition of the mite populations significantly.

The urban acarological investigations, either on small scale, either at regional large scale, are very important, demonstrating the role of the soil mite communities as bioindicators.

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# MEAT BIOCHEMICAL COMPOSITION OF SOME FISHES FROM DANUBE RIVER, ROMANIA

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***Abstract***

*Fish represent a good source of animal protein which contains all the essential amino acids. Also, fish meat is an important source of valuable lipids, micronutrients, vitamins, and minerals with several benefits for human health. In this context, the biochemical composition of common carp (Cyprinus carpio), white bream (Blicca bjoerkna), barbel (Barbus barbus), asp (Aspius aspius),common bream (Abramis brama), ide (Leuciscus idus), Prussian carp (Carassius gibelio), European perch (Perca fluviatilis), and Pontic shad (Alosa immaculata) from the Danube River was studied to evaluate their nutritional value. Fish were captured in the year 2020, during the spring season (march-may), between km 169 of the river (Brăila) and km 197 of the Danube River (Gropeni). Fish samples were analyzed for water, protein, fat, moisture and ash, at the Nutrition Laboratory of Faculty of Food Science, University Dunărea de Jos, from Galați. From the obtained results we can conclude that the analyzed fish meat of some species from the Danube River represents a valuable source the consumers healthy.*

***Key words****: freshwater fish,, lipids, proteins, proximate composition.*

## INTRODUCTION

Fish is a major source of protein in humans' diet (Sarojnalini & Abdul, 2019; Wennberg et al., 2012; Oyase et al., 2016). With a higher protein content and lower fat, fish meat is an excellent source of omega-3 fatty acids. The high nutritional quality is determined by the high quality of proteins (which contain all the essential amino acids) and a wide variety of vitamins and minerals, which include vitamins A, D, and B vitamins, phosphorus, magnesium, selenium, cobalt, and iodine (Banu et al., 2010; Dhaneesh et al., 2012).

According to EUMOFA (2017), the average per capita consumption of fish in Romania is very low compared with other European countries. In 2017, Romanians consume around 7.9 kg of fish per capita. Among the most consumed fish are Cyprinids and trout (European Commission Report, 2021). Regarding the preferences of Romanian consumers about the provenience source of fish (wild or farmed) those express high percentages of preference for farmed products, (EUMOFA 2017), mainly because these products are more available on the market.

The principal constituents of fish meat are divided into Protein, Lipid, Ash, and Water and traditionally are used as indicators of the nutritional value of fish. Generally, the variation of these constituents depends on species, feeding mode, migrations, age, size, sex, environment, or season (Bud et al., 2008; Herawati et al, 2018).

Previously the composition of aquaculture fish, provided from Romanian farms, has been investigated to analyze their nutritional quality (Paltenea et al., 2007; Mocanu et al., 2019). However, there is a lack of information about the nutritional values of fish species provided from the Danube River, Romania. In this context, the present study was carried out to analyze the proximate composition of some of the most preferred species of fish by consumers from Romania. Therefore, the data of this study provides information regarding the nutrient qualities of these species for the benefit of consumers and the scientific community as a whole, since for most of them there were not published any relevant data. The list of investigated species includes common carp (*Cyprinus carpio*), white bream (*Blicca* *bjoerkna*), barbel (*Barbus barbus*), asp (*Aspius aspius*), common bream (*Abramis brama*), ide (*Leuciscus idus*), Prussian carp (*Carassius gibelio*), European perch (*Perca fluviatilis*), and Pontic shad (*Alosa immaculata*).

## MATERIALS AND METHODS

*Sample collection.* All fish were captured between km 169 of the Danube River (Brăila) and km 197 (Chiscani-Gropeni). Fish were collected weekly during the spring season (March-May). After collection, fishes were immediately placed in an icebox and transported to the Nutrition laboratory of the Romanian Center for Modelling Recirculating Aquaculture Systems (MoRAS) of "Dunărea de Jos" University of Galați, România. All fishes were eviscerated and filleted in the laboratory. Only the muscle tissue was mixed with the blender and used for further analysis.
*Proximate composition analysis.* The moisture content (%) was determined by drying flesh in a convection oven (Jeiotech, Jeio Tech Co., Inc, Korea) at 105 °C until a constant weight was obtained (Chemists, 1990). It was removed and allowed to cool in a desiccator and weighed. The difference between the wet and dry weights gave the moisture content. After the determination of moisture content, dry samples were finely ground and used for the determination of protein, fats, and ash. Crude protein content (%) was calculated by converting the nitrogen content (using the common conversion factor of N×6.25), quantified by Dumas's method, by combustion of dry samples at 1100°C (Primacs SNC 100, Skalar Analytical B.V., The Netherlands). Lipid content (%) in fish tissue was analyzed using the Soxhlet extraction method using petroleum ether as the solvent (C. Gerhardt GmbH & Co. KG, Germany), AOAC, 1997. Ash content (%) of the sample was determined using a muffle furnace (Nabertherm, Applied Scientific Instruments Co., Ltd. Thailand) at 525±25 °C for 8 hours.
*Statistical Analysis.* The proximate composition of fish was statistically analyzed using SPSS for Windows, Version 21.0 (SPSS Inc., Chicago, United States). The results obtained after the analysis of ten fish of each species are presented as means±standard deviation (S.D). For common carp and carp bream, fish samples were divided into two class sizes, and the number of analyzed fish was seven for each size class. The differences between the mean values of proximate composition were calculated using a one-factor analysis of variance (ANOVA). Tukey HSD test was used to find out which specific groups' means (compared with each other) are different. Statistically significant differences were reported at $p<0.05$.

## RESULTS AND DISCUSSIONS

The present study analyzed the muscle composition of fish. In this study, we analyze only fish that had reached the marketable size (Table 1).
Table 1 presents the summary of fish weights, while the biochemical composition of the studied fish species from the Danube River, km 169 of the Danube River (Brăila), and km 197 (Gropeni) is presented in Figures 1, 2, 3, and 4.

Table 1 Weight of investigated fish

| Fish species | Fish weight (g) Mean ± SD | N* |
|---|---|---|
| *Cyprinus carpio* | 3660±113.14 | 7 |
| | 538±22.12 | 7 |
| *Blica bjoerkna* | 112.4±12.25 | 10 |
| *Barbus barbus* | 259±21.12 | 10 |
| *Aspius aspius* | 260±18.69 | 10 |
| *Abramis brama* | 342±21.21 | 7 |
| | 1184±24.21 | 7 |
| *Leuciscus idus* | 930±28.22 | 10 |
| *Carassius gibelio* | 512.5±36.06 | 10 |
| *Perca fluviatilis* | 193.5±4.95 | 10 |
| *Alosa immaculata* | 265±21.22 | 10 |

*N= number of analyzed fish

The statistical analysis revealed significant differences ($p<0.05$) between all constituents from the analysed species.
*Protein contents*. From the analyzed fish species, the highest protein content was obtained for *Perca fluviatilis* (18.86±1.96 %), *Abramis brama* (18.3±2.16 % for fish with a mean weight of 342±21.21 g; respectively 18.31±2.23 % for fish with a mean weight of 1184±24.21 g) and *Leuciscus idus* (18.05±2.56 %), with no statistical differences ($p>0.05$) between these species. A significant ($p<0.05$) lower protein content (%) was obtained for *Cyprinus carpio* (with the mean weight of 3660±113.14 g;

protein content - 17.84±2.85 %), *Blica bjoerkna* (17.59±2.12%), *Barbus barbus* (17.04±3.10 %) and for *Aspius aspius* (17.61±1.90%). The lowest protein content was in the case of the *Pontic shad* (16.95±2.41%), *Cyprinus carpio* (with the mean weight of 538±22.12 g; protein content-16.50 %), respectively for *Carassius gibelio* (16.95±2.41 %) (Figure 1).

*Lipids content*. *Alosa immaculata* registered the highest lipid content of 19.18±1.01 %. *Cyprinus carpio,* with the mean weight of 3660±113.14 g, has a lipid content of 4.24±0.62 %, while the mean values of the lipid content of *Barbus barbus, Cyprinus carpio* (with the mean weight of 538±22.12 g), *Abramis brama* (both class sizes), and *Blica bjoerkna*, recorded no significant difference ($p>0.05$) and was 3.97±0.12%, 3.54±0.65% (for fish with a mean weight of 1184±24.21 g), 3.58±0.25% (for fish with a mean weight of 342±21.21 g), respectively 2.58±0.69%. *Carrasius gibelio, Perca fluviatilis,* and *Aspius aspius* registered similar content of lipids: 1.9±0.51%, 1.89±0.51%, 1.1±0.56% ($p>0.05$), and the lowest lipid content was obtained for *Leuciscus idus* (0.61±0.29%).

*Ash content.* According to the present results *Perca fluviatilis (1.82±0.16 %)* had the highest ash content. *Aspius aspius* (1.59±0.16%) and *Abramis brama* (1.39±0.12% and 1.45±0.11%) registered similar lipid content. *Barbus barbus*, *Cyprinus carpio* (with the mean weight of 3660±113.14 g), and *Alosa immaculata* have similar ash content: 1.32±0.04 %, 1.29±0.08% respectively 1.22±0.09 %. Mean values between *Cyprinus carpio* (with the mean weight of 538±22.12 g; 1.06±0.08%)*,* *Leuciscus idus* (1.19±0.09%), *Blica bjoerkna* (1.15±0.06%), and *Carrasius gibelio* (1.14±0.04%) recorded no significant difference ($p>0.05$) in ash content.

*Moisture content.* Water is the main constituent of fish and accounts for between 70 and 80% of the weight of the fish (Ionescu et al. 2006). Moisture content was significantly different ($p < 0.05$) among species. In our study, *Leuciscus idus* (79.79±4.12%), *Aspius aspius* (79.12±4.11%)*,* *Carrasius gibelio* (79.51±3.08%), *Cyprinus carpio* (with the mean weight of 538±22.12 g; 78.78±4.42%), and *Blica bjoerkna* (78.02±4.62 %) tissue contained a significantly higher amount of moisture in comparison with *Barbus barbus* (77.4±5.23%), *Perca fluviatilis* (76.62±4.09%)*,* *Abramis brama* (76.09±3.56%, for fish with the mean weight of 342±21.21 g, respectively 75.83±4.49% for fish with the mean weight of 1184±24.21 g), and *Cyprinus carpio* (with the mean weight of 3660±113.14 g). The lowest moisture content was obtained for *Alosa immaculata,* 61.97±16.95%.

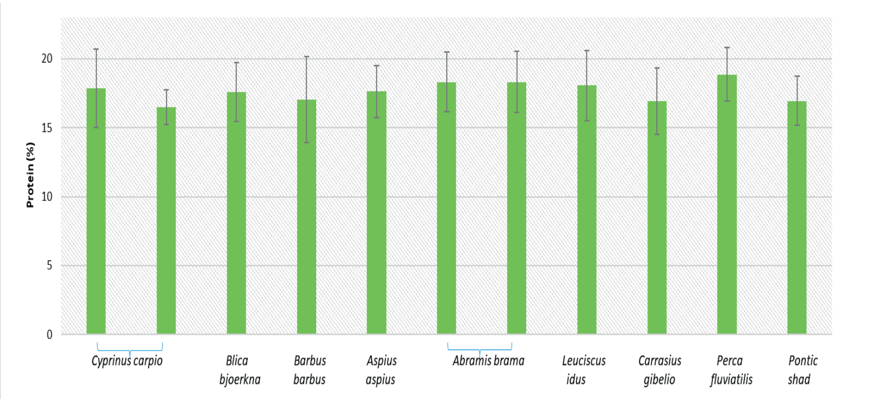


Figure 1. The protein content of fish meat

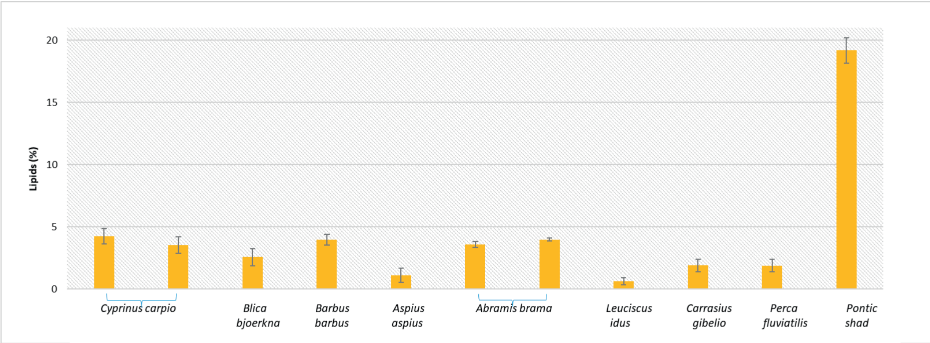


Figure 2. The lipids content of fish meat

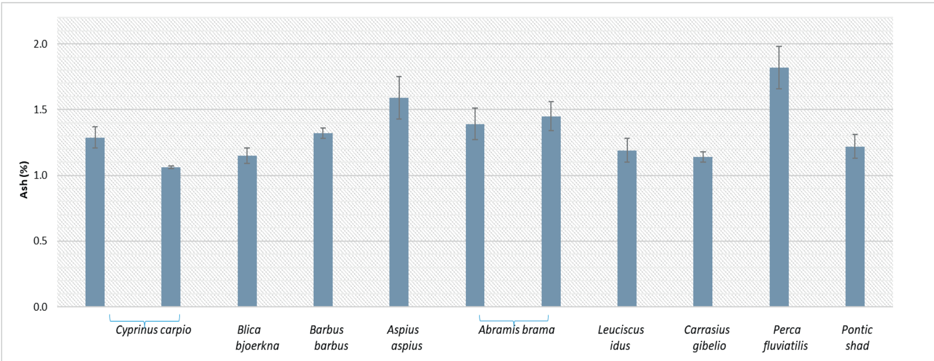


Figure 3. The ash content of fish meat

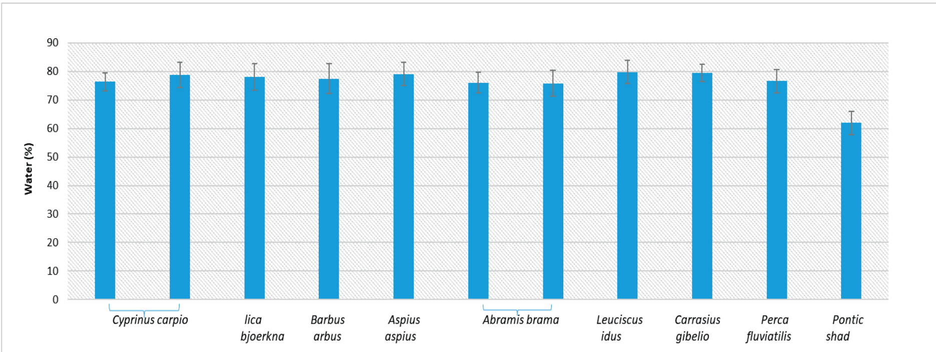


Figure 4. The water content of fish meat

The body composition of fish can be an indicator of their physiological condition and nutritional status (Zafar et al., 2004). Generally, the biochemical composition of fish varies from one species to another and within the same species (Mohamed, 2013; Nasef, 2021). The biochemical composition of fish is made up of 70-84 % water, 15-24% protein, 0.1-22% fat and 1-2 % minerals, and 0.1-1% carbohydrates (Ogunlade et al., 2005; FAO/WHO, 2011; Suganthi & Venkatraman, 2015; Kundam et al., 2018; Khawli et al., 2019). The results obtained in our study are in line with the range quoted above by these authors for fishes.

The higher content of water from all studied species supports the fact that water is the main constituent, ranging in our study from 61 to 79 %. Also, it can be observed that there is an inverse correlation between the fat and protein content of fish meat and the percentage of water.

Mainly the relationship between these indicators depends on the ambient conditions of the water ecosystem.

According to the fat content, Ackman (1989) classified fish into lean fish (fat content of less than 2%), fish with small fat content (2-4%), moderate fatty fish (4-8%), and fatty fish (more than 8% fat). In our study, *Leuciscus idus, Aspius aspius, Carrasius gibelio* and *Perca fluviatilis* are classified as lean fish, registered a lipid content between 0.6-1.9%, while *Blica bjoerkna, Cyprinus carpio* (weight of 538±22.12 g), *Abramis brama* and *Barbus barbus* are fish with small fat content. Although some authors (Aggelouis & Lazos, 1991) state that *Abramis brama* is a lean fish, with a lipid content under 1%, in our study the lipid content was 3.58 % (for fish with the mean weight of 342 g) and 3.97 % (for fish with the mean weight of 1184 g). Similar results such in our study or even higher (3.63- 5.51%) were reported in the case of *Abramis brama* by Zmijewski et al. (2006) and Zivkovic et al. (2013). Also, Mielcarek et al. (2020) reported for bream fished in the lakes of Warmia and Mazury Region, Poland, a mean fat content of 3.14±0.78%, and mean protein content of 19.33±0.6 %.

*Cyprinus carpio* with the weight of 3660±113.14 g is moderate fatty fish, while *Alosa immaculata* is classified as a fatty fish, with lipid content of 19.18 %. The lipid content of *Alosa immaculata* in the present study is in correlation with the findings of Savin et al. (2020), in the same fishing area (19.05 %). Analyzing the chemical composition of open water carp from Romania, Bud et al. (2008) obtained higher values of protein content (16.6%), fat (8.97%), while the water content (73.22%) and ash (1.20%) was lower in comparison with our study. Also, in research regarding the biochemical composition of carp from waters in Bulgaria, Hadjinikolova (2008) registered a protein content of 16.21%, fat 8.30%, water 74.55%, and 0.94% ash. Ljuboević et al. (2013) studying the chemical composition of common carp with an average weight of 1420 g from open water (Danube River) in Serbia obtained a protein content of 16.69%, fat 7.13%, ash 0.88%, and 73.73% water. Also, in the same study Ljubojevic et al. (2013) reported for *Barbus barbus* and *Aspius aspius* a higher protein, fat, and water content in comparison with our results (*Barbus barbus* 18.61±0.37%; 7.78±0.15%, 72.39±0.29%, respectively 18.07±0.09%; 2.78±0.11%; 78.51±0.2% for *Aspius aspius*). These differences provides from the location of the fish samples, the season, the nutritional condition of the fish, the conditions in the aquatic environment, the fish size and age, etc.

## CONCLUSIONS

Fish is the most preferable food for human consumption because of its relatively high value of protein content. In perspective of nutritional value of the fish meat, the results of our study revealed that all studied species are rich in protein content being an important source of animal protein. Also, the obtained results by us are valuable information to food scientist and nutritionists, since there were no data on meat quality of freshwater fish species from the Danube River in Romania region.

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# LENGTH-WEIGHT RELATIONSHIPS OF THE MONKEY GOBY (*NEOGOBIUS FLUVIATILIS*, PALLAS, 1814) FROM THE SOMEȘ RIVER CATCHMENT

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***Abstract***

*The monkey goby (Neogobius fluviatilis) is a species present in the ichthyofauna of Romania, which naturally inhabits the seaside area, the mouths of the Danube, the Danube, and the Danube tributaries lower sections. The species currently expands its range across the European continent, and it is now inhabiting new aquatic environments, including the Someș River catchment, in the Transylvania region of Romania. The purpose of this study is to analyse the biometric aspects regarding the Neogobius fluviatilis populations from the Someș River catchment. Specimens of Neogobius fluviatilis were collected from 9 locations situated on the main course of the Someș River and from a lake built on a small river from the Someș catchment, the Țaga Lake. The collected specimens were preserved, and morphometric analysis was conducted in the laboratory with the following biometric aspects being analysed: length classes distribution and the length-weight relationships (LWR), such as the Fulton condition factor (K) and the allometric growth. In the present research work, we managed to obtain important information regarding the biometric aspects of the monkey goby (Neogobius fluviatilis) population from the Someș River catchment.*

***Key words***: *Fulton, ichthyology, morphometry.*

## INTRODUCTION

The monkey goby (*Neogobius fluviatilis*) is a native species from the ichthyofauna of Romania, native to the aquatic habitats found along the Danube (Kirin et al., 2013). *Neogobius fluviatilis* has a high degree of adaptability, that tolerates both freshwater ecosystems and brackish or saltwater ecosystems and easily adapts to new environments (Bănărescu, 1964; Cocan & Mireșan, 2018).

Species from the Gobiidae family currently expand their ranges across the European continent, and their expansion must be documented by scientists (Roche et al., 2013). These species made their way across the European continent due to the anthropic intervention of connecting interior navigable paths, which helps the fish to actively migrate, and they are also transported in the water tanks of commercial vessels, thus passively migrating (Ahnelt et al., 1998).

*Neogobius fluviatilis* reached parts of the European continent (Dutch Rhine) it had never inhabited by actively migrating through channels that connect different river systems (Kessel et al., 2009).

Gobiid species also expand their range in the river catchments they naturally inhabit by populating river sections that are at a higher altitude than the sections where they were naturally present, and this is considered to be an effect of climate change and that increasing annual temperatures provide suitable environments for *Neogobius fluviatilis* in new places (Harka & Bíró, 2007).

The species presence in the Someș River (Transylvania, Romania) was first documented by Cocan et al. (2014), along with another gobiid species, the racer goby (*Babka gymnotrachelus*).

For the species to reach the Someș River, it had to populate the Danube sector above Orșova, where Bănărescu makes the upmost mention of *Neogobius fluviatilis* on the Danube in 1964. By anthropic means, the species reached the Tisa River, which receives the waters of the Someș River on the territory of Hungary and from there to make its way up the Someș River to the central region of Transylvania. This study aimed to assess the biometric aspects of the population of *Neogobius fluviatilis* across different locations in the Someș River catchment.

## MATERIALS AND METHODS

The sampling of *Neogobius fluviatilis* specimens from the Someș River catchment took place between 2014 and 2018 and all the laboratory work and data analysis was done from 2018 to 2021. Sampling stations were determined in such manner that as many possible aquatic habitats across the Someș River catchment to be explored and in as many regions as possible. 10 stations were situated on the course of the main river in the vicinity of the following settlements: Aciua, Benesat, Cărășeu, Cetan, Cuciulat, Letca, Mica, Năpradea, Oar, Valea Groșilor. One station was determined on the Țaga Lake, an artificial lake situated on a secondary tributary of the Someș River, the Fizeș River. The coordinates of the sampling stations are presented in Table 1. For each sampling location, the water depth was measured where captures occurred. Water flow was evaluated as stagnant, low, moderate, or fast and turbidity was evaluated as low, moderate, or high. The substrate was described as well.

Table 1. Someș River catchment *Neogobius fluviatilis* sampling stations coordinates

| Sampling station | Coordinates |
|---|---|
| Aciua | 47°68'42.6"N, 23°38'59.5"E |
| Benesat | 47°41'52.7"N, 23°31'19.8"E |
| Cărășeu | 47°75'71.5"N, 23°10'88.2"E |
| Cetan | 47°11'59.1"N, 23°46'15.5"E |
| Cuciulat | 47°18'34.2"N, 23°25'03.0"E |
| Letca | 47°20'08.1"N, 23°28'23.5"E |
| Mica | 47°14'81.1"N, 23°91'37.3"E |
| Năpradea | 47°30'20.4"N, 23°27'14.1"E |
| Oar | 47°81'23.1"N, 22°73'30.0"E |
| Țaga Lake | 46°55'08.0"N, 24°04'48.9"E |
| Valea Groșilor | 47°14'45.8"N, 23°43'32.2"E |

Specimens were caught using angling techniques, such as float fishing and bottom fishing. Housefly larvae (*Musca domestica*) and red wigglers (*Eisenia fetida*) were the most successful baits, but the *Neogobius fluviatilis* specimens also responded to baits such as bread flakes, sweetcorn, or grasshoppers (*Tettigonia* sp.). Baits were fished on the bottom or near it, because *Neogobius fluviatilis* is a demersal species.

A total of 237 specimens of *Neogobius fluviatilis* were captured in this research. From each location the following number of specimens was captured: 27 at Aciua station, 14 at Benesat station, 16 at Cărășeu, 5 at Cetan, 43 at Cuciulat, 47 at Letca, 7 at Mica, 10 at Năpradea, 8 at Oar, 53 at Țaga Lake and 7 at Valea Groșilor.

The samples were immediately stored in recipients with formalin solution, so they could be properly stored for the laboratory analysis. Laboratory analysis of the specimens was conducted at the Ichthyology Laboratory from the Faculty of Animal Science and Biotechnologies, at the University of Agricultural Sciences and Veterinary Medicine, in Cluj-Napoca.

For each specimen a meristic determination was condoned, to prove that it belongs to the *Neogobius fluviatilis* species. The sex of the analyzed specimens was determined according to the instructions of Oțel (2007) and the male to female ratio in each sampling location was determined.

Gravimetric determinations were done using the Dune Compact DCT 5000 weighing scale and a Petri dish was used to set the tare of the scale. The total length (TL) of the fish was measured using a digital caliper or a ruler.

Information obtained in the laboratory was tabulated and analyzed using the Microsoft Office 365 Excel software (www.microsoft.com). The distribution of sexes and the following biometric aspects were analyzed: length classes distribution and length-weight relationships (LWR) such as the Fulton condition factor (K) and allometric growth.

The Fulton condition factor is a useful tool in assessing the individual condition of fish (Ricker, 1975) and for this study, we used the formula from Cocan & Mireșan (2015), as follows:

$$K = \frac{W \cdot 100}{TL^3}$$

where:
K – Fulton condition factor
W – weight (g)
TL – total length (cm)

Regarding the allometric growth we used the formula from Cocan and Mireșan (2015), as follows:

$$\boldsymbol{W = aTL^b}$$

where:
W – weight (g)
TL – total length (cm)
a – constant
b – slope

## RESULTS AND DISCUSSIONS

*Habitat conditions*
Habitual conditions for each sampling station are presented in Table 2. Since we encounter *Neogobius fluviatilis* in such varied conditions, we can assume that the species is highly adaptable to new environmental conditions and that the Someș catchment suits the general requirements of the species.

Table 2. Habitual conditions from each sampling station

| Sampling station | Depths (m) | Water flow | Turbidity | Substrate |
|---|---|---|---|---|
| Aciua | 0.2-0.4 | stagnant | high | sand and clay |
| Benesat | 1-1.5 | moderate | low | rocks |
| Cărășeu | 0.2-0.5 | fast | moderate | sand |
| Cetan | 2 | fast | low | sand |
| Cuciulat | 0.7 | moderate | moderate | rocks |
| Letca | 0.5 | moderate | moderate | sand and clay |
| Mica | 0.5-1 | fast | high | clay |
| Năpradea | 0.3-1.2 | moderate | low | rocks |
| Oar | 0.2-0.5 | moderate | low | sand and clay |
| Țaga Lake | 0.4 | stagnant | moderate | muddy |
| Valea Groșilor | 0.4 | low | high | sand and clay |

*Distribution of sexes across sampling stations*
The work of Oțel (2007) describes that the male of *Neogobius fluviatilis* has a higher second dorsal fin than the first dorsal fin, thus we can distinguish between males and females. We identified 143 males and 94 females. The proportions between males and females varied at the sampling locations.
At the Cetan sampling station, only females were captured, at Oar station, there was an equal number of males and females, but in the rest of the sampling locations males were more numerous at 8 stations, and only at one station both sexes were present, but there were more females captured.
Table 3 displays how many specimens of each sex were captured at each sampling station and the percentage of males and females.
In general, the number of males at each station was higher than the number of females, except Letca station, where the number of females was slightly higher, and at Cetan station, where no males were captured.

Table 3. Distribution of sexes across sampling stations

| Sampling station | Males | Females | ♂% | ♀% |
|---|---|---|---|---|
| Aciua | 16 | 11 | 59.25 | 40.75 |
| Benesat | 12 | 2 | 85.72 | 14.28 |
| Cărășeu | 13 | 3 | 81.25 | 18.75 |
| Cetan | 0 | 5 | 0 | 100 |
| Cuciulat | 25 | 18 | 58.13 | 41.87 |
| Letca | 21 | 26 | 44.68 | 55.32 |
| Mica | 5 | 2 | 71.42 | 28.52 |
| Năpradea | 7 | 3 | 70 | 30 |
| Oar | 4 | 4 | 50 | 50 |
| Țaga Lake | 36 | 17 | 67.92 | 32.08 |
| Valea Groșilor | 4 | 3 | 57.14 | 42.86 |
| TOTAL | 143 | 94 | 60.33 | 39.67 |

*Length classes distribution*
Specimens with similar lengths can be classified by this aspect and the number of length classes present and how numerous they provide valuable information about the population from a particular place. For this analysis, we adapted the model presented by Jkrpo-Ćetković et al.

(2018), in which he attributes a specimen to a length class based on how many centimeters the specimen has, such as a fish of 7.1 cm belongs to the length class 7, and we classified specimens by their total length, like in the following example: if a specimen has a total length of 2.5 cm, then it belongs to the class of "2-3" because it is longer than 2 cm and shorter than 3 cm.

A total of 10 length classes were identified. The smallest number of length classes identified was at the sampling station of Oar, where only two length classes were accounted for, "8-9" and "9-10". The biggest number of length classes we identified in the Țaga Lake, a total of 9 classes, all between class "4-5" and "11-12", which shows that *Neogobius fluviatilis* dwells well in lentic habitats and this could pose a threat for lacustrine ecosystems that this species ends up inhabiting. The smallest class was class "4-5" and the biggest class was class "13-14". The results of the length classes analysis are presented in Table 4.

*Length-weight relationships*

Length-weight relationships give us insight into a fish population based on the lengths and weights of the specimens across the population. In determining the Fulton condition factor (K) and the allometric growth we used the total length and weight of specimens. The minimums, maximums, and means with standard deviations (SD) of the lengths and weights of the captured specimens from the sampling stations are presented in Table 5.

Table 4. Distribution of length classes across sampling stations

| Length class | Aciua | Benesat | Cărășeu | Cetan | Cuciulat | Letca | Mica | Năpradea | Oar | Țaga | Valea Groșilor |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 4-5 | | | | | | | | | | 1 | |
| 5-6 | 2 | | | | | 5 | 1 | | | 4 | |
| 6-7 | 2 | 2 | | 1 | 2 | 9 | | 3 | | 17 | |
| 7-8 | 7 | 3 | 4 | 2 | 6 | 8 | 3 | 3 | | 15 | |
| 8-9 | 9 | 6 | 6 | 1 | 12 | 8 | 2 | 1 | 3 | 10 | |
| 9-10 | 5 | 3 | 6 | | 14 | 7 | | | 5 | 2 | 2 |
| 10-11 | 1 | | | 1 | 9 | 8 | 1 | | | 1 | |
| 11-12 | 1 | | | | | 2 | | 3 | | 2 | 2 |
| 12-13 | | | | | | | | | | 1 | 2 |
| 13-14 | | | | | | | | | | | 1 |

Table 5. Total lengths and weights overview across sampling stations

| | Total length (cm) | | | Weight (g) | | |
|---|---|---|---|---|---|---|
| Station | Minimum | Maximum | Mean±SD | Minimum | Maximum | Mean±SD |
| Aciua | 5.63 | 11.73 | 8.19±1.17 | 2 | 16 | 6.03±3.21 |
| Benesat | 6.88 | 9.66 | 8.25±0.84 | 2 | 8 | 5.07±2.01 |
| Cărășeu | 7.32 | 9.92 | 8.64±0.76 | 4 | 12 | 7.31±2.62 |
| Cetan | 6.82 | 10.36 | 8.36±1.47 | 3.09 | 12.11 | 6.45±3.66 |
| Cuciulat | 6.05 | 10.96 | 9.10±1.11 | 1.85 | 13.35 | 8.08±3.06 |
| Letca | 5.28 | 11.2 | 8.23±1.76 | 1.43 | 18.32 | 6.75±4.71 |
| Mica | 5.84 | 10.89 | 8.26±1.53 | 2 | 17 | 6.85±4.91 |
| Năpradea | 6.14 | 11.46 | 8.41±2.12 | 2 | 18 | 6.8±6.4 |
| Oar | 8.47 | 9.79 | 9.21±0.5 | 6 | 11 | 9.25±2.18 |
| Țaga Lake | 4.88 | 12.47 | 7.49±1.51 | 1 | 21.44 | 4.66±3.67 |
| Valea Groșilor | 9.71 | 13.65 | 11.65±1.44 | 9.37 | 28.60 | 18.87±7.47 |

*Fulton condition factor (K)*

Fulton's condition factor value describes a good physical condition of the fish if the value is above 1 and a poorer condition if the value is below 1.

The smallest male (♂**)** mean value for the Fulton condition factor occurred at Benesat station with a value of 0.85±0.11 and the highest mean value for males Fulton condition factor occurred at Oar, with a value of 1.21±0.04. The smallest female (♀**)** mean value for the Fulton condition factor occurred at Benesat station with a value of 0.84±0.32 and the highest mean value for females Fulton condition factor occurred at Oar, with a value of 1.08±0.09. These results show that in the Benesat river section the population of *Neogobius fluviatilis* is not doing as well as in other river sectors. The Oar River section displays the highest values of the Fulton condition factor for both sexes, and we can underline the fact that this river section suits very well the needs of the species. The Fulton condition value for the Țaga Lake shows a mean value for males of 0.95±0.07 and a mean value for females of 0.96±0.11, values close to 1, which describe an almost good condition, but it looks more like the species is still adapting to the lacustrine environment. Regarding the other sampling sites, the Fulton condition factor value varies around the value of 1, which means that *Neogobius fluviatilis* finds the environmental conditions of the Someș catchment quite suitable. Table 6 presents the overview regarding the Fulton condition factor (K) from all the sampling stations.

Table 6. Fulton condition factor (K) and allometric growth for all sampling stations

| Sampling station | Sex | K mean±SD | LWR equation | Growth type |
|---|---|---|---|---|
| Aciua | ♂ | 0.92±0.1 | $W = 0.0063Lt^{3.2004}$ | Allometric (+) |
| | ♀ | 1.05±0.11 | $W = 0.0095Lt^{3.0462}$ | Isometric |
| Benesat | ♂ | 0.85±0.11 | $W = 0.0013Lt^{3.8728}$ | Allometric (+) |
| | ♀ | 0.84±0.32 | $W = 2E\text{-}05\ Lt^{5.8057}$ | Allometric (+) |
| Cărășeu | ♂ | 1.09±0.1 | $W = 0.0017\ Lt^{3.8582}$ | Allometric (+) |
| | ♀ | 1.05±0.02 | $W = 0.013\ Lt^{2.9007}$ | Allometric (-) |
| Cetan | ♂ | N/A | N/A | N/A |
| | ♀ | 1.01±0.07 | $W = 0.0071\ Lt^{3.1669}$ | Allometric (+) |
| Cuciulat | ♂ | 1.04±0.07 | $W = 0.0047\ Lt^{3.357}$ | Allometric (+) |
| | ♀ | 0.96±0.08 | $W = 0.0038\ Lt^{3.4354}$ | Allometric (+) |
| Letca | ♂ | 1.05±0.12 | $W = 0.0039\ Lt^{3.4591}$ | Allometric (+) |
| | ♀ | 0.98±0.07 | $W = 0.0065\ Lt^{3.2018}$ | Allometric (+) |
| Mica | ♂ | 1.1±0.16 | $W = 0.0018\ Lt^{3.8446}$ | Allometric (+) |
| | ♀ | 0.92±0.1 | $W = 2.6034\ Lt^{0.6196}$ | Allometric (-) |
| Năpradea | ♂ | 0.87±0.23 | $W = 0.5311\ Lt^{0.2655}$ | Allometric (-) |
| | ♀ | 0.86±0.15 | $W = 0.0469\ Lt^{2.1218}$ | Allometric (-) |
| Oar | ♂ | 1.21±0.04 | $W = 0.0862\ Lt^{2.1323}$ | Allometric (-) |
| | ♀ | 1.08±0.09 | $W = 0.0001\ Lt^{5.0302}$ | Allometric (+) |
| Țaga Lake | ♂ | 0.95±0.07 | $W = 0.006\ Lt^{3.2346}$ | Allometric (+) |
| | ♀ | 0.96±0.11 | $W = 0.0086\ Lt^{3.0547}$ | Isometric |
| Valea Groșilor | ♂ | 1.18±0.21 | $W = 0.0103\ Lt^{3.0528}$ | Isometric |
| | ♀ | 1.06±0.06 | $W = 0.0063\ Lt^{3.2241}$ | Allometric (+) |

*Allometric growth*

The study of allometry in fish lets us see with what intensity they grow in length and weight, which is stronger, or if they are equal. Allometric growth is exponential, and it is determined by the value of the slope (b) from the equation, which represents the allometric condition factor (Mogodan et al., 2021). $b=3$ or close to 3 means an isometric growth (weight and length grow with the same intensity), $b>3$ means a positive allometric growth (weight grows with higher intensity), $b<3$ means a negative allometric growth (length grows with higher intensity).

Table 6 displays the growth equation for each sex, from all sampling sites, and based on the generated equation and the value of the slope (b), we obtain the value of the allometric condition factor, from which we assessed the growth type for males and females throughout the research.

## CONCLUSIONS

The biometric aspects analysis of the monkey goby (*Neogobius fluviatilis*) from the Someș River catchment shows a good adaptation of the species to this new range. Our analysis shows overall a good situation for the population throughout the studied areas and regarding the allometric growth. Length-class analysis and Fulton condition factor (K) analysis point out the effectiveness of the species to adapt to lacustrine environments, where they can pose a threat to destabilize lentic environments and can also affect aquaculture activities.

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# ECOLOGICAL IMPACT OF EUROPEAN BEAVER, *CASTOR FIBER*

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***Abstract***

*Beavers are large, semiaquatic rodents in the genus Castor native to the temperate Northern Hemisphere. The European beaver (Castor fiber) has an undoubtedly positive impact on the environment: it is a key species, which means that it plays a critical role in the biodiversity of ecosystems and that many species, some endangered or threatened, rely on beavers and the landscapes they build. In this way, there are many benefits that humans and other animal species can get from beavers. Also, dam construction has the potential to alter the hydrology, geomorphology, biogeochemistry, and ecosystems of river corridors and the feedbacks between them, thus the beaver is also recognized as an 'ecosystem engineer'. However, beavers can also generate conflict situations because not all watercourses can withstand the intense construction of dams. Thus, in many contexts, the engineering activities of the beaver may come into direct conflict with other priorities: agriculture, urban land use, forestry, irrigation. Beavers occasionally damage selected trees, but the worst damage is caused by their burrows, which raise water levels in streams, ponds or lakes, flooding the ground and frequently killing large areas of valuable trees in the forest. There are proven costs to agriculture that result from the impact of beavers, and these will have to be fully taken into account in future decisions to manage the beaver population. The ecological impact of the Eurasian beaver on habitat structure has been little investigated in Europe and includes in particular the changes that take place during dam construction activities. The purpose of this study was to summarize the publications that analyse the ecological impact of beaver (Castor fiber).*

***Key words**: beaver, dam, ecological, impact.*

## INTRODUCTION

Beavers have the ability to profoundly modify ecosystems to satisfy their ecological needs, with significant associated hydrological, geomorphological, ecological and societal impacts (Brazier et al., 2021). In some areas of Romania, the complaints of the owners of agricultural lands flooded due to the dams built by beavers are multiplying from year to year and claiming hundreds of hectares transformed into swamp. Experts say the solution could only come from amending and supplementing existing legislation.
The European beaver, *Castor fiber*, is the largest rodent mammal in Europe. It is a semi-aquatic animal with multiple anatomical adaptations that allow it to successfully explore the aquatic environment.
The beaver is a nocturnal and twilight animal that is particularly prudent and suspicious of the presence of predators and humans.
The species *Castor fiber* is included in Annex II of the Habitats Directive, respectively Annex 3 of GEO no. 57/2007, which includes species of wild fauna and flora of Community interest, the conservation of which requires the declaration of Special Areas of Conservation forming the Natura 2000 Network.
The species is also included in the list of species of Annex IV of the Habitats Directive, respectively Annex 4 A of GEO no. 57/2007, as a species of community interest that requires strict protection. The obligations arising from the Habitats Directive include: maintaining the favorable conservation status of the beaver population, monitoring and regularly reporting the conservation status of the species to the European Commission.
Also, the *Castor fiber* species is included in Annex II (Strictly protected wildlife species) of the Convention on the Conservation of European Wildlife and Natural Habitats, adopted in Bern on September 19, 1979, to which Romania acceded by Law no. 13/1993 (Pașca et al., 2020). Known to us as the sheep, the beaver is nicknamed the "ecosystem engineer" for its ingenuity in building a mosaic of natural surfaces where it retains water and expands wetlands, so necessary in the current

conditions of climate change and prolonged droughts (https://www.carpathia.org/ro/castorul-revine-in-sud-estul-muntilor-fagaras/).

The beaver was declared extinct in Romania in 1824. After more than a century and a half, in 1998, a study on the repopulation of the beaver in Romania materialized with the bringing of some specimens from Germany (https://blog.cosmeanu.ro/presa-a-semnalat-recent-prezenta-castorilor-in-delta-aflati-cum-si-prin-cine-au-ajuns-insa-simpaticele-animale-in-romania/).

The size of the beaver population in our country has undergone great variations in the last 200 years, mainly due to anthropogenic pressure.

After the reintroduction, the beaver population at national level had a slightly upward trend, in the first years, after which it increased exponentially reaching in 2017 to 2145-2250 specimens (Pașca et al., 2020).

The growing trend of the population at national level and the continuous expansion of the area of *Castor fiber* brings to the fore the need to move to another level in terms of management measures applied to the species.

If in the previous period the main concern was the monitoring of the species in the context of reintroduction, at present the success of the reintroduction project is as obvious as possible and it is necessary to establish other monitoring criteria that are essential in the long-term sustainable management of the species.

One of these directions is to determine the reasonable maximum limit of population development that allows maintaining an acceptable level of conflict.

Significant areas within protected natural areas of the NATURA 2000 network overlap with the network of flood dams in the custody of the Water Management System and the National Agency for Land Improvement.

Through their activity, beavers often contradict the interests and activities of the two institutions listed above, by creating dams, plugging riverbeds.

Although previous projects have addressed these issues in an attempt to find solutions, and at the institutional level SGA and ANIF agree with the protection and conservation of the species, maintenance work on the flood protection system must be carried out periodic.

Thus, a high anthropogenic pressure is exerted on the species, the impact being special if we refer to the works of regularization, clearing, recalibration of the riverbed or cutting of the woody vegetation on the banks.

All these have direct and indirect effects on beavers, some incompatible with the presence of the species in the area affected by works for periods between 1 and 5 years (necessary for the natural restoration of the affected habitat).

Under these conditions, the designation of new protected areas in species-friendly areas where conflict levels would be lower would be a solution to ensure a favourable conservation status of the species in areas where the system of dams and drainage channels is particularly extensive.

Also, analysing the trend of beaver populations reintroduced at European level, it is observed that most of them have reached a particularly high number.

In this context, to which is added the low degree of acceptance of the human population as a whole, it is necessary to take more drastic management measures, including questioning the introduction of a hunting intervention quota. This action will be based on studies to assess the conservation status of the species (population size, quality and habitat size), but also simulations that will show the impact on the species. It is intended that the introduction of the harvest quota be introduced as a management tool in the event that the support capacity is reached, the damage caused by the beaver is significant and relocations are no longer possible.

## MATERIALS AND METHODS

In this review, the scientific literature consists on a series of specific articles which were found to have useful information about the ecological impact of beaver, from different databases. The journals were selected through the analysis of previous data studies that classified and ranked the most significant key publications.

All the scientific research papers used within present review are indexed in GS data based, 76.47% of them are also indexed Web of Science (WOS) database and a percentage of the 23.52% are indexed in Scopus. Considering WOS scientific articles used in present study, a percentage of 17.64% are placed in red zone, 35.3% in yellow zone and 47.06 % in white zone.

The journals were selected through the analysis of previous studies that classified and ranked the most significant key journals. According to Simionov et al., the searching was considered to perform better if using a variety of search methods (electronic and manual) and by searching multiple possibly overlapping resources. In order to offer complete view of the analysed subject, papers published within a wide time period were considered (between years 1998 and 2021). The researches which have the highest visibility are priority. As a limited factor in the process of scientific papers selection, the extent of searching is determined by the research keywords and resources available to the research team.

## RESULTS AND DISCUSSIONS

**The impact of the beaver on the invertebrates**

Bush & Wissinger (2016) suggest that beaver wetland complexes support a wealth of invertebrate taxa, mainly due to high habitat heterogeneity. Beavers create wetlands with a variety of small habitats, and the barring of streams or the construction of canals by the beaver creates a mosaic of slow and lotic hydrology that provides habitat for semi-aquatic invertebrates.

The beaver also creates and maintains new wetlands and improves existing ones, helping to maintain invertebrate wetland habitat in the face of climate change and habitat destruction. Beaver ponds turn lotus habitats into slow habitats. In ponds, the aquatic invertebrate community is changing to reflect the newly created lentic habitat. Under such circumstances, shredders and scrapers become less abundant, while collectors and predators become more abundant (McDowell & Naiman, 1986). Beavers can also create unique aquatic habitats, such as canals, that support taxa not found in other wetland habitats (Hood & Larson, 2015).

Beaver dams can support a wide variety of invertebrates (Rolauffs et al., 2001).

Hering et al. (2001) reviewed in detail the literature on the aquatic invertebrate community in beaver-captured streams and uncaptured streams. They reported that on a landscape scale, beaver basins have a positive impact on the abundance and diversity of aquatic invertebrates.

Beaver activities can lead to a considerable change in the morphology and composition of the community in floodplains, in particular by creating additional habitat types, two of which can be very abundant:

1. beaver ponds, which are characterized by stagnation and a different composition of the substrate compared to the unconstituted sections;
2. beaver dams, which can have an average density of 10 dams / km section of stream in habitats suitable for beavers. They are frequently destroyed by floods, but their remains can remain for decades (Rolauffs et al., 2001).

When building dams, beavers change the ways of streams and rivers, allowing the creation of extensive wetland habitats. In one study, beavers were associated with large increases in open water areas. When beavers returned to an area, 160% more open water was available during the drought than in previous years, when they were absent. Beaver dams tend to lift the groundwater, both in mineral soils and in wet areas such as peat bogs. Especially in peatlands, their dams can stabilize the often fluctuating groundwater table, which controls both carbon and water levels. Ponds colonized by beavers offer a supportive environment even for the smallest aquatic organisms - the plankton.

Plankton play an essential function in the food chain of every aquatic habitat (Janiszewski et al., 2014).

Beaver activity has an impact on aquatic invertebrate communities.

Damage usually leads to an increase in lentil-dependent species (slow or still water), such as dragonflies, oligochaetes, snails and mussels, to the detriment of lotic species (fast water) such as blackflies, stoneflies and flies that they spin in the net.

Beaver floods create a growth of dead trees that benefit terrestrial invertebrates such as *Drosophila* flies and bark beetles, which live on dead wood (https://wikipredia.net/ro/Beaver).

Dams create places for insects to lay eggs, such as dragonflies (https://wikicro.icu/wiki/Reintroduction_of_beavers_to_Europe).

Invertebrate communities in aquatic habitats associated with beaver activities can be divided into two general groups:

(1) those with a distinct "running water perspective", which focuses on how beaver dams change invertebrate communities in the river on multiple scales, and

(2) those with a distinct wetland / pond perspective on plant and animal life living in the many types of shallow slow habitats outside river channels (Bush & Wissinger, 2016).

The influence of the beaver on the invertebrate communities derives from the modification of the physico-chemical aspects of the water: chemistry, carbon reserves, nutrient spiral, flow regimes, physical substrate, rotation of organic matter.

Beaver pond hydrology is dominated by flow inputs and outputs, and dams can reduce the peak flow of the canal by temporarily storing water and maneuvering it to the adjacent riparian zone / floodplain.

Invertebrate biomass is much higher (1.3–11.1 g $m^{-2}$) in the basins behind beaver dams than in adjacent ones (0.01–0.6 g $m^{-2}$), but taxonomic diversity between habitats is similar (McDowelland & Naiman, 1986). Large patches of wood debris associated with dams can house unique sets of invertebrate species. Rolauffs et al. (2001) found a higher diversity of invertebrates and a higher secondary productivity on the coarse woody substrates of dams than in rivers or dam-created basins.

The macroinvertebrate communities in beaver ponds are reported to be considerably different from those in unimpounded sections (Pliūraitė & Kesminas, 2012).

**The impact of the beaver on the vertebrates**

Studies show many positive effects of beavers on frog populations. Beaver activity can also increase the connectivity between ponds, due to the increased density of the lentic habitat, but also due to the creation of channels by beavers.

Beaver huts and dams can provide valuable habitat for amphibians that can be used to avoid predators, to provide and develop larval food, or as hibernation sites. It has been suggested that a higher abundance of predatory fish in beaver ponds may reduce the abundance of amphibians. However, Dalbeck et al. (2007) reported that the increase in habitat heterogeneity caused by beaver activity means that *Salmo trutta*, a key predator, does not eradicate amphibians from upstream streams.

Beaver activity has been shown to have a positive impact on abundance or biodiversity in four studies of salamanders and newts.

The impact of beavers on newt and salamander species is variable.

Many salamander species prefer running water and cannot use beaver ponds. A number of researchers have observed reptiles using the habitat created by beavers. The older a beaver pond is, the greater the diversity and abundance of reptiles. In two studies, the usefulness of beaver ponds as a habitat for reptiles was investigated. One showed that beaver ponds had a greater abundance of reptiles and greater biodiversity than streams. In particular, the creation of slow habitat and open habitats around ponds due to the roaring of beavers has been considered important for reptiles.

The effects on snakes have been shown to be mixed (Stringer & Gaywood, 2016). Most papers have shown that bird species use beaver ponds or beaver-created habitats, but this use has not been compared to the use of unaffected areas by beavers. Numerous mechanisms have been cited as reasons for increasing the abundance or diversity of birds.

The increase in wetland area caused by beaver catches is a key factor in avian biodiversity. Beaver dams often flood and kill trees in the waterfront. It attracts woodpeckers (Picinae), as standing wood is an important habitat for nesting and feeding. Woodpeckers are often classified as ecosystem engineers themselves, due to the use of woodpecker holes by a number of secondary species that nest in the cavity.

Beaver habitats provide a more abundant supply of bird food. Beaver ponds contain an abundant aquatic ensemble, including a diverse range of macronevertebrates, which are an excellent source of food for ducks. In addition, an increased abundance and diversity of fish and amphibians in beaver basins provide food for species such as herons (Ardeidae) and seagulls.

Studies investigating the impact of beavers on mammalian diversity and abundance have been reviewed. Thus, beaver-created ponds supported a greater abundance of bats than beaver-free ponds. Bats can use, for example, beaver habitat to shelter under the exfoliating bark of beaver-killed beaver trees. Also, following the activity of the beaver, bats benefit from the increase in

the abundance and availability of prey (Ciechanowski et al., 2011).
Otter, *Lutra lutra* is likely to benefit from beaver activity because it increases the habitat suitable for otter by capture. The formed ponds are rich in prey species for otters such as fish, amphibians and invertebrates. Beaver activity does not appear to affect small terrestrial mammals (Suzuki & McComb, 2004). Beavers instead influence large mammals by creating habitats, sources of prey, and because beaver-cut trees can provide food for many ungulates (Rosell et al., 2005).

**Environmental impact**
One bevear's ecology characteristic is its capacity to build shelters, dams and channels in this way it changes the landscape and increases the capacity/ability to occupy the territory. Their capacity of changing their habitation offers a special significance to beaver as a geomorphic agent and this called them in ecosystem's engineer.
As a result, a direct and significant control on the ecosystem structure, the beavers are considered a key specie/the most important specie. It's important to accept these effects before to reintroduction in order to take an documented decides regarding the feasibility of reintroduction and also the opportunity to restoration of this specie in the actual habitat.
During the vegetation period, the beaver uses dug burrowing on the river bank with the entrance above the water's level. during the cold season, the entrance inside the burrowing is always situated under the water's level. The beavers built their shelters according to environmental – local topography and environmental condition.
Natural wholes who situated /dug in the river's bank can serve/be a place of a burrow. when these natural wholes don't exist, the beavers (especially *Castor fiber*) dug burrows where the bank is tall enough and the soil is stronge enough for the construction of this kind of shelter. At least 50% of their shelters are dug burrows.
When the banks aren't tall enough for digging burrows, the beavers can build shelters under water or on the river's bank, consisting of a bilt (dug) or a masked burrow by pices of wood with an extended channel till the water.
The built shelters usually consist of a vestibular room and a living room situated above the river's level. These are, generally, made of a mixture of wood and ground. Branches and twigs can be used for covering any wholes when / in case the surface is destroyed. The new constructions start to be used as shelters when there is approximately 1 meter of construction material stored above the living room.
The shelters can serve to a family of beavers as a place for sleeping, as a refuge and for growing their babies up. The shelters are of these types:
1. Dug burrows in the tall bank of water where is possible, except the rocky banks- with entrances above the water's level- more or less temporary- in the hot period of the year, and with under water entrances during the winter period. This kind of shelter is usually specific to rivers with hight whole and tall bank.
In the aquatic habitats where the bank of the water is tall enough for digging, the acces is situated under the level of the water. Sometimes, the access in the shelter may be covered (masked) by/with pieces of wood branches, twigs linked between them with ground or mud. The dig burrow in water's bank.
2. If the configuration of the banks doesn't allow the digging of the burrows (one tall bank missing)- narrow rivers, streams, ponds- the beavers build themselves make shelters like huts, situated of linked branches between then with mud or ground, but still with under water access; generally speaking, the beavers build banks for rising the water's level, where the water is not deep enough for allowing an the underwater access in the shelter and , sometimes, the shelter's type is usually specifically for hilly areas.
3. The third type is a combination between tge first two types. There, where the banks allows excavation, but the high of the bank is not enough (<1.5-2 m) and the composition of the soil permits the fitting of a dug burrow, but with the enough distance between ceiling of the living room and the surface of the soil from above, the beavers build the burrow but the ceiling of the shelter isn't realized the bank soil, anymore, but from a similar material with a the ane of the built shelters.
Digged burrow: The structure of the shelter is complex. It can have several levels and consists of several rooms. The burrows dug in the high

shore usually have two or more entrances which continue with a tunnel which may have, in its course, from place to place, feeding chambers or refuges situated at water level, in which the sheep feed in in winter or where he retreats if he feels threatened outside the shelter.
The tunnels end with a larger room where the sheep family spends more time - sleeping, raising chicks, etc. Generally, the rooms have a ventilation chimney at the top.
This type of den can also be of two types: simple - with the entrance uncovered or with the entrance covered with branches, twigs and other woody materials, but without being glued together with mud. The shelters built (on the waterfront) can reach 3.5 m in height. The entrance to this type of den is always located below the water level, dug into the water's edge. The shelter is usually made up of two levels: the vestibular floor is located at the height of the water surface and is the place where the sheep can dry after leaving the water; the second level (round main room - approx. 60/70 cm) is lined with dry wood chips, plants and twigs, the animals using it as a place to sleep, raising chicks, etc. The roof is made of tree bark or branches welded together with mud. The part above the water consists only of branches not welded together, acting as a vent.
Shelters of this type have the shape of a half-sphere, reaching a diameter of 6 m at the base and a height of 1.70 m. It can often be seen that they are anchored by shrubs or even trees.
The shelter can reach up to 50 cm above the ground around it; it has the same shape and dimensions as a dug burrow and its organization is identical (sealed / earthed roof, ventilation chimney).
In this type of shelter, the gloss of the water is close to the feeding chamber, which in turn is very close to the living room. The shelter is usually built from the back, the animal climbing the waterfront through ramps that surround the shelter and meet at the top. Sometimes the tunnels branch in all directions up to a distance of 100 meters. The construction is made of wood materials 5 - 7 cm in diameter and can measure up to 3 m long, notched from the top, the spaces between them being covered with mud or plant debris from the bottom of the watercourse. Maintenance is done by adding a new layer of wood, which will be covered with clay and / or mud afterwards. This is done several times a year, more or less frequently, depending on the weather conditions. At first glance, it looks more like a mass of dead wood from the flood than any other type of construction. Their shape is quite irregular. However, several factors ensure the presence of sheep: their location, much of the wood and bark that make up this structure, show clear traces of bites - including defoliation of bark (bitten by sheep), traces of alleys used by sheep. (at the bottom of the stream). The sheep keep the shelter clean and repair it regularly, with alternating layers of branches, twigs, fresh wood chips, bark and mud. The shelter is constantly being expanded and rebuilt, especially in late autumn, when the sheep are preparing for winter. Both types of shelters consist of one or more tunnels leading up to one or more bedrooms.
Usually the shelter has at least two tunnels from the river to the feeding chambers, sometimes there can be a complex system of tunnels. When the ground is rocky the shelter is built above the ground, the tunnel system being built in the added material.

**Human-beaver conflicts**

A major challenge for conservation biology is to facilitate coexistence between humans and wildlife. On the other hand, growing beaver populations cause increasing conflicts with man, and population and/or damage control may therefore be required (Nolet & Rosell, 1998).
Human-animal conflicts occur when the activities of wild animals or their presence have a negative influence on humans (Treves et al., 2006). Beaver-man conflicts arise when beavers get close to populated areas and through specific activities affect the interests of the local population.
Thus, conflicts arise in the following cases:

- causing damage to agricultural crops in the immediate vicinity of watercourses;
- felling of valuable trees on the banks of watercourses populated by beavers;
- flooding of certain areas by raising the water level due to the construction of dams;
- damage to the defensive dams by digging burrows.

Beavers' engineering activity has a significant impact on the ecosystem and the economy. The most common alterations are land flooding due to the construction of dams. Beavers also clog culverts, girdle fruit and garden trees, and also forage on crops (mainly corn, carrots and beets) on a field nearby (Swiecicka, 2014).

Eurasian beavers can be destructive when they cut down trees and flood areas. They may be removed for nuisance behavior. The most numerous nuisance complaints are flooding farm lands and crop destruction from eating and flooding. Eurasian beavers also flood roadways and culverts and can cause extensive timber damage (Nolet, 2000).

## CONCLUSIONS

The Eurasian beaver experienced a spectacular comeback in the early 20th century.

The reduction in hunting pressure has made it possible to restore the population and increase the area occupied by the species.

The natural recovery of beaver populations has also been supported by reintroduction projects in many European countries.

The biggest challenge of the future is to adopt certain management measures to help man and beaver coexist. For the repopulation of new territories with beavers, it is necessary to elaborate impact / feasibility studies, which are requested by the environmental authority as a basis for repopulation.

Given that in some areas the density of beavers has increased beyond the optimum and conflicts occur, it is very important to identify areas where repopulation can take place and preparatory steps have been taken so that relocation is as rapid as possible.

As a last resort solution, it is recommended to relocate the "problem" beaver specimens to new territories, without beavers or with very low densities.

The relocation will take place in autumn and spring, avoiding the period of calving and raising the chicks.

This will be done immediately after the installation of beavers in the area at risk.

The important thing is to capture the whole family. During the capture period, the specimens will be kept in the rehabilitation center, until the capture of all its members.

For all conflicting cases in which it is necessary to relocate / extract specimens or the habitat of the species is affected, it is mandatory to obtain a ministerial order regarding the derogation from the profile legislation.

The elaboration of a guide for living with beavers is increasingly important in the conditions in which there is an increase in the number of human-beaver conflicts and the diversification of the issue.

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# MACRONUTRIENTS MODIFICATION IN THE MUSCLE OF COMMON CARP (*CYPRINUS CARPIO*) DURING WINTER

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***Abstract***

*Climate change can affect the life cycle of fish reared in earthen ponds, especially in winter, when the metabolism is reduced and the fish no longer feeds due to low temperatures. In Romania, in recent years, winter temperatures have been higher than normal, justifying the need to assess biochemical changes in fish meat. The biochemical characterization of macronutrients in carp meat was performed by monitoring during the winter, the following parameters: moisture, proteins, lipids, ash, fatty acids in fish meat, temperature and oxygen of the water. The experiment was conducted between November 2020 - March 2021, the biological material being represented by common carp (Cyprinus carpio), aged one summer. At the beginning of winter, saturated fatty acids (25.80%) and monounsaturated fatty acids (49.03%) were found in a higher proportion compared to the end of winter, when polyunsaturated fatty acids had the highest percentage (55.38%) of the entire amount of lipids. The amount of protein, fat and ash during the winter period decreased but insignificantly, while the water content of the meat increased. The biological material recorded a physiological loss of 27.28% of the initial average mass.*

***Key words***: *biochemistry, Cyprinus carpio, fatty acids, macronutrients.*

## INTRODUCTION

Fisheries and aquaculture, worldwide, are threatened by climate change through rising water temperatures and levels, melting glaciers, significant variations in rainfall, changes in abundant ichthyological stocks and changes in water salinity and acidity. Fish meat plays an important role in human nutrition. The effects of consuming fishery products are to reduce the incidence of cardiovascular diseases, to lower the total cholesterol level, by reducing the level of triglycerides, to moderate the inflammatory response and to improve the metabolism of carbohydrates.

Among the potential sources of winter mortality, heat stress and starvation have received the most scientific attention. Other sources, such as trophic chains and pathogens, have a significant impact but there are not enough studies to date. Recent experimental designs highlight the effects of the interaction between these stressors on end-of-winter mortality (Hurst, 2007).

Taking into account the demands on water temperature, carp is a eurythermic species, preferring higher temperatures and wide seasonal variations, this category includes representatives of the cyprinid family. Knowing the optimal thermal range for each species and age category is of particular importance in rearing and production technologies.

Jobling (1993) investigated the growth rate of carp in relation to the increase in water temperature. The results are directly proportional to the increase in water temperature.

In the case of low temperatures and those exceeding the upper limit of thermal comfort, the metabolic activity of fish is greatly diminished. The tolerated variation limits of the aquatic environment temperature for the species *Cyprinus carpio* are between 3 - 35˚C, according to Froese & Pauly (2022). The optimal development temperature being in the thermal range 20 - 25˚C.

This paper evaluated winter losses and changes in the biochemical composition of carp meat between November 2020 and March 2021. During the years 2020-2021, our studies were carried out within the fish farm Brateș Experimental Base belonging to the Institute of Research and Development for Aquatic Ecology, Fishing and Aquaculture, Galați.

## MATERIALS AND METHODS

The experiment took place in 3 earth ponds, belonging to the Brateș Experimental Base, Galați County. In these ponds, carp in monoculture was monitored from the beginning of November 2020 until the end of March 2021. Water temperatures were measured daily at 8 o'clock in all 3 ponds, in the same point, at a depth of 1 meter, using a multiparameter model HQ40D - Hach.

The fish were kept in a concentrated solution of clove oil to be anesthetized before slaughter. This procedure complies with law no. 43/2014 on the protection of animals used for scientific purposes and with the Directive 2010/63/Eu of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

Once a month, 100 specimens were fished from each pond for measurement and weighing, followed by biochemical analysis of the meat for 5 specimens from each weight category. The biochemical analysis of the meat was performed by weight categories, as follows: under 20 g, between 21 and 100 g, between 101 and 200 g.

The analysis of fish meat samples was performed using the procedures indicated by the standard methods of analysis for fish meat.

The moisture was determined by Standard Official Methods of the AOAC (1990).

The total ash was determined by Furnace Incineration described by AOAC (1990).

The crude proteins content of the samples was determined using the Kjeldahl method of AOAC 17th edition, 2000, Official Method 928.08 Nitrogen in Meat (Alternative II), which involved protein digestion and distillation, where F (conversion factor), is equivalent to 6.25.

The total fats were determined using the Soxhlet method, equipped with Gerhardt Brand Multistate Controller, with modified ether extraction methods AOAC 960.39.

Fatty acids in meat were determined at the beginning and at the end of the experiment. A homogeneous sample was achieved from the meat of 10 specimens of *Cyprinus carpio* fished from the 3 ponds.

The determination of fatty acids in fish meat was determined by gas chromatography (GC). For lipid extraction, the homogenized samples were dried for 1 h at 105°C. The fatty acid methyl esters were analysed with a Clarus-500 gas chromatograph with a Perkin-Elmer mass spectrometry detector, equipped with a system of injection into the capillary column (ratio of 1:100). The change of fatty acids from the sample to methyl ester was followed by separation of the components on the capillary column and the identification by comparison with a chromatography standard.

All analyses were performed in triplicate. Data are presented as mean ± standard deviation (SD). Comparison of several samples was done using the ANOVA test - Single factor followed by T test. Significance was defined as $p<0.05$.

## RESULTS AND DISCUSSIONS

The water temperature values, monitored daily are presented as weekly averages and are found in Table 1.

Table 1. Evolution of water temperature starting with November 1st 2020 until March 31st 2021, in the wintering ponds of the common carp

| | U.M. | Pond 1 | Pond 2 | Pond 3 | Average ± SD |
|---|---|---|---|---|---|
| | ˚C | Average±SD | Average±SD | Average±SD | |
| Week 1 (1-7.11.2020) | ˚C | 12.53±0.63 | 13.64±0.65 | 13.99±0.77 | 13.39±0.76 |
| Week 2 | ˚C | 10.96±0.32 | 11.60±0.35 | 12.83±0.14 | 11.80±0.95 |
| Week 3 | ˚C | 10.30±0.02 | 11.01±0.03 | 12.26±0.04 | 11.19±0.99 |
| Week 4 | ˚C | 9.61±0.10 | 10.10±0.09 | 11.39±0.09 | 10.37±0.92 |
| Week 5 | ˚C | 7.90±1.02 | 8.30±0.97 | 9.11±0.81 | 8.44±0.62 |
| Week 6 | ˚C | 6.60±0.38 | 6.86±0.44 | 7.87±0.52 | 7.11±0.67 |
| Week 7 | ˚C | 5.86±0.11 | 6.04±0.08 | 7.01±0.12 | 6.30±0.62 |
| Week 8 | ˚C | 5.41±0.45 | 5.19±0.68 | 6.93±0.25 | 5.84±0.95 |
| Week 9 | ˚C | 6.44±0.55 | 6.57±0.62 | 7.51±0.46 | 6.84±0.59 |
| Week 10 | ˚C | 7.17±0.45 | 7.16±0.35 | 8.04±0.16 | 7.46±0.51 |
| Week 11 | ˚C | 4.64±0.58 | 4.61±0.53 | 7.29±0.41 | 5.51±1.53 |
| Week 12 | ˚C | 3.89±0.35 | 3.80±0.37 | 4.11±0.97 | 3.93±0.16 |
| Week 13 | ˚C | 4.31±0.17 | 4.30±0.19 | 5.44±0.63 | 4.69±0.66 |
| Week 14 | ˚C | 3.69±0.12 | 3.41±0.27 | 5.10±0.24 | 4.07±0.91 |
| Week 15 | ˚C | 4.49±0.33 | 3.87±0.38 | 5.40±0.26 | 4.59±0.77 |
| Week 16 | ˚C | 3.99±0.13 | 3.50±0.45 | 5.47±0.10 | 4.32±1.03 |
| Week 17 | ˚C | 4.46±0.16 | 3.84±0.32 | 5.61±0.30 | 4.64±0.90 |
| Week 18 | ˚C | 5.43±0.49 | 4.60±0.18 | 5.81±0.28 | 5.28±0.62 |
| Week 19 | ˚C | 5.64±0.17 | 5.16±0.19 | 5.51±0.29 | 5.44±0.25 |
| Week 20 | ˚C | 5.93±0.26 | 5.20±0.13 | 5.06±0.22 | 5.40±0.47 |
| Week 21 | ˚C | 6.40±0.28 | 5.69±0.25 | 6.17±0.33 | 6.09±0.36 |
| Week 22 (28-31.03.2021) | ˚C | 7.28±0.17 | 5.85±0.13 | 7.08±0.35 | 6.73±0.77 |

*SD = standard deviation

The temperature in the 3 ponds varied both due to their location on the farm and the dynamics of the fish population associated with each pond. However, the range of values was close, the average difference between the data collected for each pond was 0.92˚C. These values were compared with the water temperature measured at the Brateș Experimental Base over the past 10 years. Table 2 shows the differences between the average monthly temperature measured between

01.11.2020 - 31.03.2021 and the average monthly water temperature calculated from the data collected in the Brateș Experimental Base. The average monthly temperatures in the experimental period were higher than the average values recorded in previous years, the largest temperature difference being in December. Statistically speaking, the differences are significant, p>0.05.

This increase in temperature, amid global warming, influences the metabolic processes of reared fish species.

Table 2. Average monthly water temperature for the 5 experimental months compared to the averages from the same months from the past 10 years

| | Average water temperature in the experiment | Average water temperature in the past 10 years |
|---|---|---|
| November | 11.04±1.84 | 10.22±1.65 |
| December | 6.91±1.09 | 6.13±1.11 |
| January | 5.69±1.52 | 2.95±1.86 |
| February | 4.46±0.77 | 3.18±1.14 |
| March | 5.79±0.72 | 5.07±1.27 |

*Values presented as average ± standard deviation

The beginning of the winter period is considered when the water temperature drops below 10˚C, any feeding being superfluous. At this water temperature, the carp no longer feeds, entering the hibernation phase.

The physiological losses were assessed at the end of the winter period, when the control fishing was carried out. In Table 3, the losses recorded, both in number of specimens and in mass, are presented in comparison with the losses allowed in the technological norms.

At the end of March, the biomass of the biological material was 27.28% lower compared to the biomass parked in the winter basins at the beginning of November. This value is higher than the technological losses and physiological declines allowed for the fish material during its winter hibernation, for each weight category, determined within the Institute of Research and Development for Aquatic Ecology, Fishing and Aquaculture, Galați. Technological norms in the field of fish farming for the eastern and south-eastern areas of Romania were carried out in the Brateș Experimental Base. The set of rules was established in 2009 based on measurements made in previous years.

Mortality due to energy consumption from pre-winter reserves is considered the main cause of declining stocks of young fish at the end of the winter period, according to Pratt & Fox (2002). Resistance to starvation, which depends on the size of the fish, is the main mechanism considered for mortalities at the end of hibernation.

Table 3. Technological losses and physiological declines recorded at the end of winter in the experiment, as well as the values allowed in the technological norms

| Average Weight Category g/fish | Technological Norms | | Experimental Values | |
|---|---|---|---|---|
| | No. Specimens | Physiological Declines | No. Specimens | Physiological Declines |
| 0 – 20 g | Up to – 35% | Up to – 30% | 36.12% | 33.27% |
| 21 – 100 g | Up to – 25% | Up to – 25% | 23.33% | 26.44% |
| 101 – 250 g | Up to – 20% | Up to – 20% | 18.51% | 22.13% |
| Average | 26.6% | 25% | 25.99% | 27.28% |

In periods of prolonged drought and excessive temperatures compared to the normal of that period, losses of 3-5% are allowed, above the norm of technological losses for that period, for fish specimens up to 1 kg inclusive.

Technological losses during the winter period 01.11.2020 - 31.03.2021 were 1.12% higher than the allowed value for carp under 20 g, but lower by 1.67% for carp weighing between 21 - 100 g and 1.49% for carp weighing between 101 and 250 g. Fish biomass recorded weight losses higher than the allowed values, for all weight categories.

The values of macronutrients in the meat of common carp, *Cyprinus carpio*, were influenced by the degree of activity of the biological material, triggered by winters with higher temperatures than in previous years. Table 4 shows the biochemical parameters measured in the experiment, at the beginning, at the end and monthly during the experiment, from the beginning of November to the end of March. The water content showed a tendency to increase in the muscular tissue of carp, during the winter, in all the weight categories. At the end of March, the moisture content of common carp meat was declining, coinciding with the end of the hibernation period.

The amount of lipids and proteins decreased during the winter period, but the range of variation was not large. This change in biochemical composition is due to the energy consumption necessary for fish to survive the winter. The differences are not statistically significant between the values of macronutrients in fish meat obtained between December 1st, 2020 and March 1st, 2021 (p<0.05), but are

statistically significant compared to the values of macronutrients in fish meat obtained on November 2nd, 2020 and March 31st, 2021 ($p>0.05$).
The heavier fish also showed higher amounts of lipids at the beginning of the winter period. The energy value of carp meat highlights the loss of fat and protein in muscle tissue during the winter. Fish use lipid reserves both to survive hibernation and for activity triggered by high winter temperatures, considering that the fish does not have a food source to supplement energy consumption.
Takeuchi et al. (1986), in the experiment of feeding carp before winter with diets low in protein but rich in energy, obtained values of moisture and lipids lower but the amount of protein higher, compared to the carp used in the present experiment, both at the beginning and at the end of the winter period.

Table 4. Variation of the biochemical profile of the common carp meat, *Cyprinus carpio*, during the winter period November 2020 - March 2021

| Weight | Standard length | | **November 2nd 2020** | **December 1st 2020** | **January 1st 2021** | **February 2nd 2021** | **March 1st 2021** | **March 31st 2021** |
|---|---|---|---|---|---|---|---|---|
| Under 20 g | Between 8 - 11cm | Moisture | 79.01±0.73 | 80.38±0.04 | 81.14±0.01 | 80.69±0.34 | 80.83±0.01 | 79.73±0.03 |
| | | Proteins | 17.67±0.08 | 16.89±0.01 | 16.77±0.10 | 16.58±0.29 | 16.98±0.21 | 17.63±0.32 |
| | | Lipids | 1.36±0.04 | 1.22±0.03 | 0.76±0.18 | 0.86±0.01 | 0.95±0.02 | 1.35±0.06 |
| | | Ash | 1.19±0.04 | 1.17±0.02 | 1.11±0.07 | 1.08±0.08 | 1.03±0.06 | 1.05±0.06 |
| | | Energy value* kcal/100g | 85.095 | 80.595 | 75.825 | 75.976 | 78.453 | 84.838 |
| Between 21-100g | Between 11 - 15cm | Moisture | 78.42±0.40 | 80.19±0.49 | 80.53±0.90 | 80.48±1.12 | 80.51±0.37 | 79.84±0.43 |
| | | Proteins | 17.51±0.35 | 16.78±0.19 | 16.69±0.12 | 16.37±0.64 | 16.66±0.45 | 17.11±0.43 |
| | | Lipids | 1.90±0.41 | 1.55±0.41 | 1.41±0.95 | 1.16±1.26 | 1.49±0.44 | 1.69±0.32 |
| | | Ash | 1.17±0.04 | 1.16±0.02 | 1.05±0.03 | 1.05±0.06 | 1.02±0.08 | 1.05±0.08 |
| | | Energy value* kcal/100g | 89.461 | 83.213 | 81.542 | 77.905 | 82.163 | 85.868 |
| Between 101-200g | Over 15 cm | Moisture | 78.71±0.01 | 80.35±0.25 | 80.58±0.45 | 80.14±0.81 | 80.82±0.67 | 80.31±0.81 |
| | | Proteins | 17.17±0.13 | 16.65±0.03 | 16.96±0.34 | 16.97±0.28 | 17.08±0.04 | 17.52±0.22 |
| | | Lipids | 1.99±0.23 | 1.46±0.49 | 0.98±0.09 | 0.93±0.71 | 0.86±0.66 | 0.82±0.52 |
| | | Ash | 1.19±0.04 | 1.21±0.03 | 1.08±0.01 | 1.03±0.02 | 1.03±0.08 | 1.03±0.16 |
| | | Energy value* kcal/100g | 88.904 | 81.843 | 78.65 | 78.226 | 78.026 | 79.458 |

*Calories conversion factors used: for proteins 4.1 kcal/g, for lipids 9.3 kcal/g
**Values presented as average ± standard deviation

Larger fish in the *Cyprinus carpio* species, considered in the experiment, had higher lipid reserves than small fish in early winter. Post & Parkinson (2001) demonstrated the dependence between fish size and specific metabolic rate. The larger the fish size is, the lower the specific metabolic rate will be. This phenomenon, coupled with higher lipid reserves at the beginning of winter, leads to a higher degree of survival of larger specimens. Therefore, smaller specimens of fish will have a higher mortality rate during the winter due to reduced energy reserves and higher metabolism, in conditions of starvation.
High mortality depending on lipid content and biological mass at the beginning of the winter period was also recorded by Biro et al. (2004), in a study conducted to determine lipid consumption and survival rate of the species *Oncorhynchus mykiss*. The initial weight and starvation during the winter represent important selective pressures for the one-year-old *Oncorhynchus mykiss* specimens, leading to the depletion of lipid reserves up to a minimum critical amount depending on the initial state of maintenance of the biological material.
Cho (2005), for specimens of *Puvulichthys olivuceus* starving for 4 weeks, obtained protein and lipid content values from meat, similar to those in the present experiment.
Table 5 shows the percentage distribution of fatty acids, measured before and after winter, in the meat of *Cyprinus carpio*.
Of the saturated fatty acids, the largest amount is palmitic acid (C16:0), both at the beginning of the winter period and at the end of it. All saturated fatty acids had lower values at the end of winter.
The main share of monounsaturated fatty acids is oleic acid (C18:1), before and after winter. Myristoleic (C14:1), 11-eicosenoic (C20:1), erucic (C22:1), and nervonic (C24:1) acids are found in higher percentages at the end of winter than at the beginning of this period.

Eicosapentaenoic (C20:5) and linoleic (C18:2) acids represent the largest share of polyunsaturated fatty acids at the beginning of the winter period. This percentage distribution changes considerably at the end of the winter period, when linoleic acid (C18:2) and α-linoleic acid (C18:3) are the majority of polyunsaturated fatty acids.

Overall, during the winter, the fatty acid profile changes, the carp preferentially consuming the reserves of monounsaturated and saturated fatty acids. Thus, at the end of March, polyunsaturated fatty acids, which have been used less in basal metabolism, are found in the largest amount. This evolution of fatty acid changes is similar to that from the experiment conducted by Kminkova et al. (2001), in which the fatty acid content of carp tissues over a year was evaluated.

In a study conducted by Guler et al. (2008), related to fatty acid changes in common carp meat, from a lake in Turkey, over the course of a year, palmitic acid (saturated fatty acid) had a lower value in early winter but a higher value in early spring than in the present experiment. The same phenomenon is found for the percentage of oleic acid (monounsaturated fatty acid) and linoleic acid (polyunsaturated fatty acid), the main representatives of each category of fatty acids.

Guler et al. (2011), repeated the experiment, using common carp *Cyprinus carpio*, from another natural environment, where the fatty acid values were lower for palmitic and oleic acid but higher for docosahexaenoic acid (C22:6).

The ratio of saturated to unsaturated fatty acids is an indicator of the importance of nutritional fat for humans. Ratio values above 0.35 indicate fats with a beneficial quality for consumption. In Table 5, this ratio (n-3)/(n-6) has values greater than 0.35, both before and after wintering, which means that this species, *Cyprinus carpio*, can be introduced into diets for humans, at any time of the year.

In a 2004 study by Bauer & Schlott, common carp activity level was monitored during the winter. The carp was active during the winter, and it began to feed when the water temperature exceeded 3.1˚C, justifying the need for in-depth studies on the feeding behaviour and population densities of the carp in wintering ponds.

Table 5. Percentage distribution of fatty acids in common carp meat, at the beginning and end of the winter period

| | Start of the experiment 01.11.2020 | End of the experiment 31.03.2021 |
|---|---|---|
| C 14:0 | 2.03 | 1.92 |
| C 15:0 | 0.69 | 0.50 |
| C 16:0 | 16.85 | 12.10 |
| C 17:0 | 1.07 | 1.00 |
| C 18:0 | 5.16 | 4.89 |
| **∑SFA** | **25.80** | **20.41** |
| C 14:1 (n-5) | 0.57 | 0.62 |
| C 15:1 (n-5) | 0.96 | 0.44 |
| C 16:1 (n-7) | 12.01 | 6.63 |
| C 17:1 (n-8) | 2.40 | 0.95 |
| C 18:1 (n-9) | 30.70 | 9.46 |
| C 20:1 (n-9) | 1.63 | 2.29 |
| C 22:1 (n-9) | 0.70 | 1.11 |
| C 24:1 (n-9) | 0.06 | 0.24 |
| **∑MUFA** | **49.03** | **21.74** |
| C 18:2 (n-6) | 5.06 | 26.86 |
| C 18:3 (n-6) | 0.63 | 0.51 |
| C 18:3 (n-3) | 3.42 | 10.54 |
| C 20:2 (n-6) | 0.58 | 0.40 |
| C 20:3 (n-6) | 0.07 | 2.96 |
| C 20:3 (n-3) | 0.31 | 0.77 |
| C 20:4 (n-6) | 2.64 | 2.32 |
| C 20:5 (n-3) | 5.52 | 6.21 |
| C 22:4 (n-6) | 0.48 | 0.39 |
| C 22:5 (n-3) | 1.34 | 0.93 |
| C 22:6 (n-3) | 2.81 | 3.49 |
| **∑PUFA** | **22.86** | **55.38** |
| Other fatty acids | 2.19 | 2.47 |
| n-3 | 13.40 | 21.94 |
| n-6 | 9.46 | 33.44 |
| **(n-3)/(n-6)** | **1.42** | **0.66** |

Steffens (1996), determined the efficiency of diets enriched with lipids and a high energy intake, for the efficient growth and food conversion of common carp. These lipid-supplemented diets lead to an increase in the amount of fat in the fish tissues, carp juveniles with higher fat deposits showing greater resistance to winter conditions.

This correlation between the state of maintenance of the biological material and the survival rate during the winter was also analysed by Sogard & Olla (2000), in the species *Theragra chalcogramma*, aged one summer in the absence of food. Fish survival followed the same bioenergetic pattern, high survival rates after 200 days at average temperatures below 3°C were observed in fish that gained sufficient body weight and a good state of maintenance during the summer growing season. Biochemical analyses performed on the meat of *Theragra chalcogramma* showed a substantial increase of the water amount in the tissues to the detriment of lipids during the winter, a phenomenon also observed in the present experiment.

For a higher survival rate of the fish during the winter period, methods are required to ensure the best possible maintenance of the biological material at the beginning of winter. In the context of climate change, the reassessment of the norms of technological losses and physiological declines allowable during the winter, must be taken into account in order to ensure the efficiency of fish production.

## CONCLUSIONS

The temperature of the aquatic environment between November 2020 and March 2021, recorded higher values than the monthly average measured in previous years, which characterizes the influence of climate change on aquatic ecosystems.

Winter mortality is inversely proportional to the energy value of the weight groups considered in the experiment. The risk of starvation due to a winter with higher temperatures in which the fish are active is much higher.

The physiological losses during the winter period are 2.28% higher than the normative ones, motivated by the increased activity of the carp during the hibernation period, a value that is placed at the upper allowed limit.

The percentage of moisture has increased to the detriment of proteins and lipids in common carp meat over the winter.

Saturated and monounsaturated fatty acids decreased during the winter in the meat of *Cyprinus carpio*. Polyunsaturated fatty acids accounted for a larger share of carp meat at the end of winter, both compared to the beginning of fish hibernation and compared to other categories of fatty acids at the end of the experiment.

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# HEALTH PROFILE OF SOME FRESHWATER FISHES COLLECTED FROM DANUBE RIVER SECTOR (KM 169-197) IN RELATION TO WATER QUALITY INDICATORS

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***Abstract***

*Evaluating the fish parasitic fauna should constitute a major concern, especially in the climate change context because the parasites have a significant impact both on the natural fish population and the farm yield, economic viability, or sustainability. In this context, this study aimed to present the influence of water quality parameters upon the distribution and variety of parasites of 14 freshwater species, belonging to 5 systematic families: Cyprinidae, Percidae, Siluridae, Clupeidae, Esocidae. Parasitofauna analyses were performed through classic methods and the results were expressed as the prevalence of parasitic fauna and their intensity grades. Although the experimental groups have a similar environment, they present a distinct parasitofauna which shows a strong influence of the environmental factors upon its development. Adequate knowledge and periodic monitoring of the prevalence of parasites on the fish populations can have multiple implications and can be used as an indicator of anthropogenic impacts on other aquatic environments.*

***Key words***: *ectoparasites, endoparasites, water's physical-chemical factors, wild fish.*

## INTRODUCTION

The Danube River covers a large hydrographical basin, being the second biggest river in Europe which passes over seven European countries.

A significant part of the lower sector flows either close by or into Romanian territory where it forms a large delta before getting to the Black Sea. Even though the Danube present both ecological and economical importance, providing a valuable habitat for numerous species, various aspects need a profound insight.

The functional integrity of riverine ecosystems and the sustainable management of their natural resources are frequently threatened, especially in heavily polluted areas that are subjected to numerous anthropogenic impacts (Zaharieva et al., 2021).

Diminishing the effect of pollution on organisms and ecosystems constitute nowadays one of the key topics to improve the quality of life in developed countries. Induced changes in aquatic ecosystems as a consequence of human activity and its influence on organisms can have important effects on the abundance and quality of natural resources and therefore on the economic development since they can alter both biodiversity and the functioning of ecosystems and their carrying capacity (Chunchukova et al., 2020; Mocanu et al., 2021).

Knowledge of species composition changes at different time scales is crucial for understanding the dynamics of riverine communities (Sinclair & Byron, 2006). Due to the lack of a previous historic data baseline on ecosystems functioning, try-outs to evaluate human impact on the ecosystems are often extremely difficult to be successfully applied.

An environmental factor in the riverine ecosystems, which can bring a large amount of important information, is represented by the study of diseases. Illnesses/infections play an important role in the evolution of fish populations, especially commercial ones (Miller et al., 2014).

A disease state is defined as a complex of manifestations found in different relationships with one or more pathogens, from the moment of contact with the host until the disappearance of the consequences (Casadevall et al., 2000).

The manifestation of the disease in a population is generally established after the state of stress, which reduces the resistance of organisms and promotes the onset of infectious, parasitic, or other diseases (Bagge et al., 2004).

Environmental disturbance can have a positive, negative, or neutral effect on the fish and also on their parasites, depending on the type of ecological factor fluctuation and parasite taxa (Sures, 2006, 2008).
A regular parasite phase may incorporate the fish definitive host and several intermediate invertebrate hosts and for the parasite to survive all hosts must co-occur in a stable population structure. Changes in environmental conditions that affect any of the hosts, directly and indirectly, will have a significant effect on the prevalence and intensity of the infection, and on the diversity of parasites that infect the fish (Hudson et al., 2006; Lafferty et al., 2008). In this sense, parasite communities of fish have been used as comprehensive marks of ecosystem health, parasites being also used as a source of information that can provide data about fish stock separation, fish recruitment migrations, fish diet, or fish behavior, in relation to environmental pollution (D'Amelio & Gerasi, 1997; Overstreet, 1997; Marcogliese, 2003). That is why, for a complete fish health profile a report of the hydrochemical parameters (e.g. temperature, salinity, nutrient and oxygen concentrations should be given (Araújo et al., 1999; Marshall and Elliott, 1998; Snigirov et al., 2012; Thiel et al., 1995).
Therefore, in our study, we aimed to analyze the structure of fish's parasitofauna, from the Danube - Brăila region as a health profile tool, in relation to the environmental factors affecting their habitat.

## MATERIALS AND METHODS

Our research was conducted during the three seasons along the year: spring (April-May), summer (June-August), autumn (September-November) in 2021, on the Danube River sector (Brăila station) (Figure 1). The sampling sites were randomly taken within the 169-197 km of the river and the fishing activity was carried out with the help of a fishing net wall. Seasonally there were randomly selected between 15-30 samples from each species for ichtyopatological analysis. During the entire activity, there were collected water samples and diverse specimens belonging to 14 fish species: *Abramis brama* (Linnaeus, 1758), *Alosa immaculata* (Bennett,1835), *Aspius aspius* (Linnaeus, 1758), *Barbus barbus* (Linnaeus, 1758), *Carassius auratus gibelio* (Bloch, 1782), *Cyprinus carpio* (Linnaeus, 1758), *Esox lucius* (Linnaeus, 1758), *Hypophthalmichthys molitrix* (Valenciennes, 1844), *Perca fluviatilis* (Linnaeus, 1758), *Rutilus rutilus* (Linnaeus, 1758), *Sander lucioperca* (Linnaeus, 1758), *Scardinius erythrophtamus* (Linnaeus, 1758), *Silurus glanis* (Linnaeus, 1758), *Vimba vimba* (Linnaeus, 1758) (Table 1). After catching, the fish were immediately frozen at -20°C and transported to the Institute of Research and Development for Aquatic Ecology, Fishing and Aquaculture Galați where examinations were completed. After the standard measurements (total length – TL, body weight – BW) each fish was analyzed for endo- and ectoparasites. According to the parasite category, each fish was consequently dissected and analyzed for parasites using standard parasitological techniques (Docan et al., 2021). The gills, skin, eyes, intestine, muscle and liver were examined using a Zeiss microscope. Further, parasites were taxonomically classified using identification keys (Bauer, 1987; Dykova, 1989; Lom & Moravec, 1994; Munteanu & Bogatu 2003). Morphological parameters of all collected fishes are shown in Table 1. The parasitic infection was described in terms of prevalence (number of infected hosts/number of examined hosts, as a percentage) and mean intensity (total number of parasites/number of infected hosts). The water quality parameters were analyzed in the laboratory except for the temperature (which was measured on the field with a portable multiparameter). The obtained values were reported at the Order of the Ministry of Environment and Waters Management 161/2006 about surface water quality classification to establish water body ecological status.
*Statistical analysis*. Data were analyzed using SPSS program version 21. One-way ANOVA and Duncan's multiple range tests were used to compare the differences between the experimental groups (p=0.05). If differences among seasons were recorded, a Duncan test was performed. A p-value lower than 0.05 was considered statistically significant.


Figure.1. Fishing area – Brăila region (169 and 197 km)

Table 1. Morphological parameters of the examined fish

| Fish species | Body mass (g) | | Total length (cm) | |
|---|---|---|---|---|
| | Average±S.D. | Body range | Average±S.D. | Body range |
| *Abramis brama* | 358.16±85.53 | 201-515 | 30.43±3.05 | 23.2-34.6 |
| *Alosa immaculata* | 259.63±49.31 | 170-350 | 31.11±2.09 | 26-35 |
| *Aspius aspius* | 2040±850.61 | 1100-2770 | 59±7.39 | 50-68 |
| *Barbus barbus* | 937.37±308.84 | 475-1685 | 44.38±4.15 | 38-52.5 |
| *Carasus auratus gibelio* | 809.3±613.86 | 200-3200 | 34.11±9.33 | 21.6-60 |
| *Cyprinus carpio* | 1962.6±923.02 | 215-5250 | 50.57±7.92 | 28.2-72 |
| *Esox lucius* | 870±346.41 | 320-1430 | 48.6±6.69 | 37-61 |
| *H. molitrix* | 4382.5±2233.8 | 2400-6430 | 66.75±10.88 | 57-78.5 |
| *Perca fluviatilis* | 333.3±158.41 | 115-630 | 26.3±3.94 | 20-33 |
| *Rutilus rutilus* | 3567.5±3739.97 | 175-8300 | 25.1±1.39 | 22.6-27.5 |
| *Sander lucioperca* | 1241.67±452.79 | 235-2373 | 49.37±5.88 | 32.2-60.5 |
| *S. erythrophthamus* | 225.56±58.48 | 142-360 | 24.78±9.8 | 21.3-28.5 |
| *Silurus glanis* | 1935.63±1056.6 | 835-5780 | 64.5±10.43 | 35-90 |
| *Vimba vimba* | 342.68±67.64 | 155-490 | 30.98±1.68 | 29.5-37 |

## RESULTS AND DISCUSSIONS

*Water analysis*

The Danube River is a fluctuating aquatic ecosystem, that is why for high accuracy of physicochemical characterization, the water samples were collected every month being analyzed using the same laboratory methods.

The mean values (per season) of the analyzed parameters are summarized in Table 2.

With few exceptions, the majority of the parameters place the water quality into II - III classes of the Order no. 161/2006 emitted by the Ministry of Environment and Water Management regarding the classification of surface water quality. Generally, the reaction of the water was favorable for the living aquatic organisms and primarily fish. The values recorded for pH were constantly high during all seasons, slightly exceeding the recommended range for fish life (i.e. 6,4-8,5 upH).

The values recorded for pH did not show major oscillations which proves a relatively constant composition of water over time. The organic substances detected during the autumn season exceeded the maximum allowed limits, according to the literature (i.e. 30-55 mg $KMnO_4$/l) (Roccaro et al., 2007). Being an active aquatic ecosystem, these variations of organic substances are normal, being subjected to a complex of biotic and abiotic factors. Regarding the phosphates, the obtained values from all the samples surpass the detection limit. The statistical analysis applied to all parameters revealed different trends.

Table 2. Synthetic table with the average values of the main physicochemical parameters of water

| Water parameters | U.M. | Spring | | Summer | | Autumn | |
|---|---|---|---|---|---|---|---|
| | | Mean | ±S.D. | Mean | ±S.D. | Mean | ±S.D. |
| Temperature | °C | 13.81 | 4.49 | 24.79 | 3.79 | 17.83 | 4.71 |
| pH | upH | 8.49 | 0.22 | 8.65 | 0.38 | 8.85 | 0.15 |
| Organic matter | mg $KMnO_4$/l | 26.23 | 1.87 | 34.74 | 10.81 | 59.45 | 9.32 |
| Chemical oxygen consumption | mg $O_2$/l | 6.63 | 0.48 | 8.79 | 2.74 | 13.51 | 4.52 |
| Calcium ($Ca^{2+}$) | mg/l | 56.11 | 17.01 | 58.78 | 8.34 | 57.62 | 3.54 |
| Magnesium ($Mg^{2+}$) | mg/l | 26.73 | 13.75 | 12.96 | 1.40 | 20.38 | 11.63 |
| Water hardness | °D | 14.02 | 0.79 | 10.84 | 0.87 | 15.43 | 5.95 |
| Nitrites ($NO_2^-$) | mg/l | 0.015 | 0.01 | 0.030 | 0.013 | 0.02 | 0.01 |
| Nitrates ($NO^-_3$) | mg/l | 1.83 | 0.18 | 1.64 | 0.18 | 1.25 | 0.29 |
| Chlorides ($Cl^-$) | mg/l | 20.74 | 6.77 | 10.99 | 1.62 | 11.17 | 2.26 |
| Ammonium ($NH_4^+$) | mg/l | 0.09 | 0.11 | 2.19 | 0.21 | 0.15 | 0.10 |
| Ammonia ($NH_3^-$) | mg/l | 0.11 | 0.03 | 0.16 | 0.01 | 0.03 | 0.01 |
| Phosphates (P-$PO_4$) | mg/l | | | > detection limit | | | |

To measure specific differences between pairs of values, Duncan post-hoc test was applied (p=0.05). The results varied, as follows: temperatures were separated into three distinct groups, organic matter was separated into two groups - spring-summer and autumn, chemical oxygen consumption varied significantly among all seasons (three groups), magnesium ions were divided into 2 groups – spring and summer-autumn, water hardness and nitrites displayed the same pattern (spring-autumn group and summer group), nitrates were lower in the autumn than in the spring and summer, chlorides were higher in the spring than the other two seasons, the ammonium was lower in the spring and autumn versus summer and the ammonia was higher in the autumn than in the rest of the year.

*Fish*

The aim of this study was the investigation the parasitofauna of the fish community as a reflection of the health profile. The fish are part of the commercial species, being caught in 2021 from the Danube river, in the Brăila sector. The majority of species are Cyprinids, followed by Percids, Clupeids, Esocids and Silurids with important economic values.

Parasitological examination of the fish species from the Danube River (Brăila), showed the presence of twelve species of parasites: one specie from the Ciliates group - *Trichodina* spp., four parasite species of class Monogenea - *Dactylogyrus vastator* (Nybelin, 1924), *Diplozoon paradoxus* (Nordmann, 1832), *Gyrodactylus* sp., *Mazocraes alosae* (Hermann, 1782), two parasite species of class Trematoda: *Diplostomum spathaceum* (Rudolphi, 1819) and *Neascus cuticola* (von Nordmann, 1832) Hughes, 1927, three species of class Nematoda - *Contracaecum aduncum* (Rudolphi, 1802), *Eustrongylides excisus* (Jägerskiöld, 1909) and *Hepaticola* sp., one parasite species of class Acanthocephala: *Pomphorhynchus laevis* (Zoega in Müller, 1776) and one parasite species of class Cestoda: *Ligula intestinalis* (Linnaeus, 1758) (Tables 3, 4). Regarding the infection with Trichodina spp. there was found a seasonal variability at the prevalence and mean intensity parameters (Duncan post-hoc test, p<0.05), with higher values during the colder seasons (spring and autumn). Our results align with our studies which states that trichodiniasis infection is more frequent at low temperatures, being favored by the accumulation of organic matter and mal-nutrition or poorly developed fish (Rumokoy et al., 2018). The freshwater ecosystem of the Danube River displays a significantly larger number of taxa in the parasite communities, especially worms (Kirin et al., 2013, Munteanu & Bogatu, 2008). Fluctuation of the monogeneans had different patterns between the seasons (Table 3). The most frequent are monogeneans which are either oviparous or viviparous with a single host, spreading being uninterrupted (Munteanu & Bogatu, 2008).

Table 3. Prevalence and intensity of fish's parasites from the Danube River, Brăila station

| Systematic group | Parasite | Host | Site | Seasons | N | P (%) | MI |
|---|---|---|---|---|---|---|---|
| Protozoa/Ciliata | *Trichodina* sp. | *Carasus auratus gibelio* | G | Spring | 30 | 53.57 | 13.67 |
| | | | | Summer | 28 | 33.33 | 10.77 |
| | | | | Autumn | 27 | 36.67 | 11.13 |
| | | *Sander lucioperca* | G | Spring | 17 | 47.06 | 6.35 |
| | | | | Summer | 24 | 38.18 | 12 |
| | | | | Autumn | 21 | 54.17 | 10.33 |
| | | *Rutilus rutilus* | G | Spring | 26 | 50 | 8.76 |
| | | | | Summer | 23 | 43.43 | 8.63 |
| | | | | Autumn | 28 | 47.83 | 7.15 |
| Monogenea | *Dactylogirus vastator* | *Carasus auratus gibelio* | G | Spring | 30 | 33.33 | 9.12 |
| | | | | Summer | 28 | 53.57 | 9.25 |
| | | | | Autumn | 27 | 51.85 | 10 |
| | | *Cyprinus carpio* | G | Spring | 29 | 37.93 | 8 |
| | | | | Summer | 30 | 56.67 | 6.5 |
| | | | | Autumn | 30 | 46.67 | 5.25 |
| | | *Abramis brama* | G | Spring | 30 | 43.33 | 5.66 |
| | | | | Summer | 28 | 57.14 | 6.75 |
| | | | | Autumn | 30 | 50 | 5.5 |
| | *Diplozoon paradoxus* | *Abramis brama* | G | Spring | 30 | 50 | 7.6 |
| | | | | Summer | 28 | 50 | 5.9 |
| | | | | Autumn | 30 | 36.67 | 4.53 |
| | | *Rutilus rutilus* | G | Spring | 26 | 53.85 | 3.99 |
| | | | | Summer | 23 | 56.52 | 4.87 |
| | | | | Autumn | 28 | 57.14 | 9.25 |
| | | *Scardinius erythrophthamus* | G | Spring | 25 | 32 | 14.3 |
| | | | | Summer | 22 | 45.45 | 8.8 |
| | | | | Autumn | 27 | 33.33 | 6.5 |
| | *Gyrodactylus* sp. | *Vimba vimba* | G | Spring | 23 | 47,62 | 4.75 |
| | | | | Summer | 27 | 65.22 | 5.25 |
| | | | | Autumn | 21 | 48.15 | 4.66 |
| | | *Cyprinus carpio* | G | Spring | 29 | 40 | 4.8 |
| | | | | Summer | 30 | 55.17 | 8,25 |
| | | | | Autumn | 30 | 46.67 | 6,12 |
| | *Mazocraes alosae* | *Alosa immaculata* | G | Spring | 15 | 53.33 | 10.3 |
| | | | | Summer | 23 | 65.22 | 13.1 |
| | | | | Autumn | 15 | 46.67 | 11.2 |
| Acantocephala | *Pomphorhynchus laevis* | *Silurus glanis* | I | Spring | 18 | 44.44 | 5,3 |
| | | | | Summer | 24 | 46,3 | 6 |
| | | | | Autumn | 15 | 46.67 | 4,7 |
| | | *Barbus barbus* | I | Spring | 19 | 42.11 | 6.21 |
| | | | | Summer | 23 | 47.83 | 7.72 |
| | | | | Autumn | 27 | 33.33 | 5.25 |

*G = gills, I = intestine, N = total number of examined fish specimens, P% = prevalence, MI = mean intensity*

Investigates on the helminth community revealed 11 species during all the seasons.

*Dactylogirus vastator* was found on gills of common carp (*Cyprinus carpio*), Prussian carp (*Carasus auratus gibelio*) and common bream (*Abramis brama*). The number of parasitic specimens was situated between 4-15 per examined fish. Statistical analysis showed significant differences between the prevalence and mean intensity found in the spring season and summer-autumn seasons (ANOVA, $p<0.05$), the incidence of the virus being temperature-dependant.

Infection with *Diplozoon paradoxus* was found throughout the year in the Cyprinids group, and the prevalence of infection varied, ranging from 32% (found in spring) to around 57%, calculated during the autumn seasons. *Gyrodactylus* monogeneans are viviparous, possessing no specific transmission stage or swimming ability (Chunchukova et al., 2020; Soleng et al., 1999).

Table 4. Prevalence and intensity of fish's parasites from the Danube River, Brăila station

| Systematic group | Parasite | Host | Site | Seasons | N | P (%) | MI |
|---|---|---|---|---|---|---|---|
| Trematoda | *Diplostomum spathaceum* | *Rutilus rutilus* | E | Spring | 26 | 46.15 | 7.3 |
| | | | | Summer | 23 | 52.17 | 4.67 |
| | | | | Autumn | 28 | 42.86 | 5.5 |
| | | *Hypophthalmychtis molitrix* | E | Spring | 29 | 48.28 | 6.33 |
| | | | | Summer | 30 | 53.33 | 6.4 |
| | | | | Autumn | 30 | 56.67 | 6.75 |
| | *Neascus cuticola* | *Rutilus rutilus* | S | Spring | 26 | 61.54 | 7 |
| | | | | Summer | 23 | 63.91 | 5.5 |
| | | | | Autumn | 28 | 64.29 | 5.13 |
| Nematoda | *Hepaticola* sp. | *Cyprinus carpio* | L | Spring | 29 | 51.72 | 5.45 |
| | | | | Summer | 30 | 50 | 4.25 |
| | | | | Autumn | 30 | 53.33 | 4.67 |
| | | *Abramis brama* | L | Spring | 30 | 52.33 | 5.8 |
| | | | | Summer | 28 | 50 | 7.14 |
| | | | | Autumn | 30 | 43.33 | 4.67 |
| | | *Vimba vimba* | L | Spring | 23 | 65.22 | 5.52 |
| | | | | Summer | 27 | 51.85 | 6.65 |
| | | | | Autumn | 21 | 61.9 | 7.44 |
| | *Contracaecum aduncum* | *Alosa immaculata* | I | Spring | 15 | 33.33 | 7.44 |
| | | | | Summer | 23 | 39.13 | 6.98 |
| | | | | Autumn | 15 | 46.67 | 7.67 |
| | *Eustrongylides excisus* | *Perca fluviatilis* | M | Spring | 16 | 37.5 | 6.98 |
| | | | | Summer | 17 | 41.18 | 8.14 |
| | | | | Autumn | 23 | 47.83 | 7.21 |
| | | *Sander lucioperca* | M | Spring | 17 | 52.94 | 6.98 |
| | | | | Summer | 24 | 41.67 | 7.67 |
| | | | | Autumn | 21 | 42.86 | 7.91 |
| | | *Silurus glanis* | M | Spring | 18 | 33.33 | 8.14 |
| | | | | Summer | 24 | 45.83 | 7.21 |
| | | | | Autumn | 15 | 46.67 | 6.74 |
| | | *Esox lucius* | M | Spring | 15 | 40.2 | 6.98 |
| | | | | Summer | 16 | 35.71 | 7.44 |
| | | | | Autumn | 20 | 55.42 | 7.44 |
| | | *Aspius aspius* | M | Spring | 28 | 39.29 | 7.44 |
| | | | | Summer | 26 | 46.15 | 6.98 |
| | | | | Autumn | 29 | 44.83 | 7.67 |
| Cestoda | *Ligula intestinalis* | *Cyprinus carpio* | I | Spring | 29 | 40.27 | 7.21 |
| | | | | Summer | 30 | 43.33 | 6.65 |
| | | | | Autumn | 30 | 41.27 | 7.12 |
| | | *Rutilus rutilus* | I | Spring | 26 | 41.69 | 7.69 |
| | | | | Summer | 23 | 43.52 | 7.46 |
| | | | | Autumn | 28 | 42.1 | 7.39 |

*E= eyes, S= skin, L= liver, I= intestine, M = muscle, N = total number of examined fish specimens, P% = prevalence, MI = mean intensity*

There was observed a clear variance in the transmission proportion between the three seasons, positively correlated with temperature. At an average temperature of 15°C (registered in the spring and autumn), the prevalence and mean intensity of parasites varied between 25-50%, respectively 2-9 parasites/fish, statistically different than the calculated parameters for the summertime, mean temperature 24,79±3,79°C (50-65% prevalence, 3-12 parasites/fish). (Duncan, post-hoc analysis, $p<0.05$). The findings correlate with other studies which state that the intensifying water temperature increases the rate of transmission of *Gyrodactylus* species (Bakke et al., 1991; Bakke et al., 1992; Soleng et al., 1999).

In the case of the last detected monogenean, *Mazocraes alosae* it can be observed the same pattern among the examined fish samples. During the summer, the infection increases substantially compared with the other two seasons. Nevertheless, *Mazocraes alosae* can also be attained earlier, in spring, when about 30–60% of them have eggs, and later through autumn (September–November).

Identified *Pomphorhynchus laevis* parasite didn't have a similar trend in the two examined hosts, *Silurus glanis* and *Barbus barbus*. The mean intensity obtained on both species in autumn was found to be significantly lower compared to summer and spring indices, the calculated data suggesting that the parasite populations in autumn consist mainly of young preadult individuals. On the other hand, the prevalence was similar for the wells catfish during all three seasons (Duncan post-hoc test, $p>0.05$), while the common barbel had a lower prevalence during the autumn, compared to spring-summer seasons (Duncan post-hoc test, $p<0.05$).

The Cestoda parasite, *Ligula intestinalis*, was found only on the Cyprinids species, *Cyprinus carpio* and *Rutilus rutilus*. *Ligula intestinalis* is a widely distributed cestode species with a complex life cycle, which involves a copepod as the first intermediate host, fish as a second intermediate host and an avian definitive host (Chunchukova et al., 2019; Dubinina, 1980).

Prevalence was lowest in spring, slightly increasing in summer, whereas mean intensity was marked by spring peaks (7.21 and 7.69 respectively), in both species. However, there were no substantial seasonal changes for any of the parameters.

Trematoda worms were observed with prevalence indices (P%) situated between 40-60% and mean intensity, MI=49.91±0.94 for *Diplostomum spathaceum* and MI=63.5±1.5 for *Neascus cuticola*. Both detected trematodes have birds as intermediate hosts, which, together with the excrements, discard huge quantities of eggs in the water (Chunchukova et al., 2020).

In the current study, the nematodes were represented by *Hepaticola* species, *Contracaecum aduncum* and *Eustrongylides excisus*. Fish's nematodes development cycle is closely related to aquatic invertebrates groups, therefore their developmental cycles are very different.

Invertebrates play-acting as intermediate hosts, and fish as intermediate, accumulating, or definitive hosts (Kuzmanova et al., 2019). Nematodes are found in freshwater fish under scales, in the digestive tract, or other organs and tissues (Zaharieva et al., 2021).

*Eustrongylides excisus* was a core parasite, being found on five fish species. Its pattern was different among species, statistical analysis highlighting a lower prevalence during the spring, among the following species: *Perca fluviatilis*, *Silurus glanis* and *Aspius aspius* (Duncan post hoc test, $p<0.05$), and during the summer, on the *Sander lucioperca* and *Esox lucius* (Duncan post hoc test, $p<0.05$). *Hepaticola* sp., which were also a core species in the nematode community registered a lower prevalence during the summer (on *Cyprinus carpio* and *Vimba vimba* species) and autumn (*Abramis brama*) with mean intensities situated between 2 and 14 parasites/fish.

The *Contracaecum aduncum* was only found on *Alosa immaculata* with a significantly different prevalence (Duncan post hoc test, $p<0.05$), calculated between seasons. Also, the mean intensity varied between studied exemplars, with parasites ranging from 4 to 12 per fish.

Following the study, the best-represented class is that of Monogeans, with four species of parasites identified during all seasons.

Our results suggest that there is a significant fluctuation of the parasite's fauna among the seasons (Figure 2 a-j), demonstrating the importance of water temperature, as the main factor that draws major changes in the chemical composition of the water, as previously observed by Özer et al. (2004) and Kennedy (2006).

For example, researches conducted on the seasonal incidence of *Gyrodactylus* species show that there is a strict influence of temperature on the prevalence and intensity of infestations which generates a definite seasonal cycle of the parasites (Bakke et al., 1992; Jansen & Bakke, 1991; Mo, 1992; Özer et al., 2004). A significant number of studies have suggested that the prevalence and intensity of monogeneans are seriously influenced by the developmental stage of the fishes, as hosts (Khidr et al., 2012; Özer et al., 2004, 2015; Sailaja et al., 2017). Bakke et al. (1992) state that the role of drifting of detached larval stages of the parasites in the water column depends on a sum of factors. Some parasites separate from their hosts by accident, by active migration, or as a result of a host response (Özer et al., 2004; Scott & Nokes, 1984) and higher temperatures are forcing out accidental dislodgement during transmission (Harris, 1994).


Figures 2 a-j. Parasites found in the Danube River fishes, Brăila region

Seasonal transmission rates were reported for numerous aquatic parasites, with climate conditions playing a major role (Moravec & Scholz, 1994; Nachev et al., 2016). Moravec & Scholz (1994) and Nachev et al. (2016) observed seasonality in the occurrence and maturation of some acanthocephalans detected in barbel, the main conclusions being in accordance with our results.
Some parasites species display a maximum prevalence in spring (Benovics et al., 2018), summer (Wrona et al., 2006), or during the cold seasons (Sultana et al., 1994), being related to fish's activity and thermal's limits. Some fish's activity differs strongly in terms of water temperature, as it decreases progressively with the decrease of water temperature until reaching their thermal limit for activity at a certain temperature (dormancy phase) (Baras, 1995). Consequently, the diminished fish activity and adjusted feeding behavior probably conduct to a diminished infection during colder months, as the final host ceases feeding on intermediate hosts (Kennedy, 2006; Molloy et al., 1995). With increasing temperatures, the fishes start feeding again, but an increase in new parasite infection occurs in late summer when more infected intermediate hosts are available (Finlay et al., 2021).
The presence of parasites can provide information about the state of the environment: the ciliates and nematodes should be sensitive indicators of eutrophication and thermal effluent, while digeneans and acanthocephalans should make good indicators of heavy metals and human disturbances (Docan et al., 2021; Lafferty et al., 2008).

## CONCLUSIONS

Our results provide information about a particular parasite's population upon the fish, as hosts, adding important information regarding their incidence and prevalence. Depending on the parasite's systematic group, the calculated indices displayed a different pattern, showing an affinity for a specific host or organ, signifying that both ecto- and endoparasites are linked to their hosts along with their ecosystem. In this study, the parasites do not seem to affect the health status of their hosts. However, further research is needed, studies that cover larger areas from the species habitats, in order to obtain a parasites full scale seasonal variations table. Also, there is a need for data regarding updated parasites reproductive cycles, their spreading and development and relationship with their hosts.

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# DIFFERENT TYPES OF NEST BOXES USED BY LESSER KESTREL (*FALCO NAUMANNI*) AFTER BEING RECOVERED AS A BREEDER IN BULGARIA

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***Abstract***

*Lesser Kestrel often nests in urban areas surrounded by agricultural areas. This makes the species largely dependent on human activity to availability of nesting places. The loss of natural nesting sites was one of the main reasons the species to become extinct in the late 20th century in Bulgaria. After it was recovered as a breeding species in country, one of the main goals of the expert is to make the colony stable. For implementation of these goals, it is necessary to provide a suitable nesting place for the species. Field studies show that the provision of artificial nest boxes for Lesser Kestrel resulted in increasing of numbers and strengthening existing colonies. There are different types of artificial nest boxes like: Classic wall, cavity wall, under-roof and etc. Conducting daily observations of the birds, the colony's nesting territory was determined - concentrated around the Lesser Kestrel Release and Adaptation Module. In the past years large proportion of the Lesser Kestrel population in Bulgaria nested in artificial nest boxes thus proving that species easily occupies artificial nests designed for it.*

***Key words***: *endangered species, Falcon, raptors, Sakar SPA.*

## INTRODUCTION

Lesser Kestrel (*Falco naumanni,* Fleischer, 1818) is a small species of falcon, one of the smallest falcons found in Europe. The species nests in Southern Europe, North Africa, Asia Minor and the Middle East, Southern Russia, the Caucasus, Central Asia east to China. Most birds breeding in western Europe winter in Africa south of the Sahara, although some individuals remain in Spain (Negro, 1991), southern Turkey (Cade, 1982). Some of these birds may be early migrants, depending on climatic conditions and food availability (Global Raptor Information Network, 2022). Lesser Kestrel often nests in urban areas, as they provide nesting sites and the level of threat of predation in the nest is lowland are usually surrounded by agricultural areas or open uncultivated areas providing food resources. That is why the species largely depends on human activity not only in terms of feeding places, but also because of the presence of nesting places. Despite being widespread in Bulgaria in the mid-XIX (Radakoff, 1879), but has suffered dramatic declines in many parts of its breeding area, as well as its disappearance from some countries where it had previously multiplied. In Europe, declines equivalent to 46% in each decade since 1950 have occurred and on the wintering grounds in South Africa, there have been declines equivalent to 25% in each decade since 1971 (BirdLife International, 2004).

In the breeding range, problems include demolition of older buildings where the birds nested, loss of habitat through afforestation, intensification of agriculture, and urbanization, pesticide poisoning, human persecution, and interspecific competition (Biber, 1996). The principal threats in South Africa are the loss of grassland habitat to overgrazing and pesticide effects, particularly when the birds are attracted to outbreaks of locusts or crickets, which are sprayed by farmers (Pepler, 2000).

The reduction of the nutritional base of the Lesser Kestrel as a result of the intensive use of pesticides and poisons, together with the overgrowth and abandonment of pastures and agriculture lands, are considered to be one of the main reasons for the extinction of the species in Bulgaria.

In Bulgaria, at the close of the 19th century the Lesser Kestrel was reported as "nesting everywhere" (Radakoff, 1879), and in the mid-

20th century as "fairly common" and widely distributed (Patev, 1950; Arabadzhiev, 1962).
In 2000-2010, there were no breeding birds reported (Barov, 2002) i.e., no confirmed breeding of the species (Iñigo & Barov, 2010). In according to the updated edition of the Red Data Book of Bulgaria, the species was announced critically endangered (CR) without being reported for nesting population (Barov et al., 2015). The species has been reported with negative trends for all population parameters (Marin et al., 2020).The most serious problem today is the critically low number of populations and isolation, which do not allow the species to recover naturally. With the help of a specialized project "Lesser Kestrel Recovery" LIFE11 NAT/BG/360 implemented by "Green Balkans – Stara Zagora" NGO the Lesser Kestrel has been successfully recovered as a breeder in Bulgaria. To preserve and ensure the sustainable existence of the recovered colony are necessary additional conservation efforts. Due to the drastic reduction of natural habitats, the placement of artificial nest boxes provides reliable nesting sites with a low risk of predation.

## MATERIALS AND METHODS

After being recovered as a breeding species in Bulgaria, one of the main objects of conservationists is to make the colony stable. To achieve these objectives, it is necessary to provide suitable nesting places for the species. Field studies indicate that the provision of artificial nesting structures for Lesser Kestrel is an old and traditional practice in the Sakar region, and probably other parts of the country (Marin et al., 2020). The field research was implemented on the territory of Lesser Kestrel Release and Adaptation Module in village Levka SPA "Sakar" (BG0002021) part of European Ecological Network NATURA 2000, where 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.
SPA "Sakar" (BG0002021) classified as SPA by Council of Ministers Decision No. 802/04.12.2007 (promulgated SG 107/2007).
Issued designation order by the Minister of Environment and Water of Bulgaria with prohibitions and restrictions on activities contradicting the conservation objectives of the site – Order No. RD – 758/19.08.2010 (promulgated SG 72/2010), amended by Order No. RD – 70/28.01.2013 (promulgated SG 10/2013).
A low-mountain region with rounded hilltops and comparatively open river valleys of the Maritsa and Tundzha tributaries, close to the state border with Turkey. The terrain altitude is between 50 and 856 m. On its territory there about 30 small settlements, the town of Topolovgrad and a poorly developed road network. Lower parts of the Sakar territory is occupied by farmland, which has replaced forests of *Quercus pubescens* and *Quercus virgiliana*. On about 15% of the area there are dispersed xerothermal grass associations, dominated by *Dichantium ischaemum*, *Poa bulbosa*, *Chrisopogon grillus*, etc., and, more rarely, meso-xerothermal vegetation. The shrubs of *Paliurus spina-christi*, mixed with *Jasminum fruticans* in combination and the xerothermal grass formations determine the comparatively high numbers of the Hare *(Lepus europeus)* and the Souslik *(Spermophilus citellus)* respectively. The area of Sakar currently supports 220 bird species, 59 of which are listed in the Red Data Book for Bulgaria. From the birds occurring there 96 species are of European conservation concern (SPEC), 11 of them being listed in category SPEC 1 as globally threatened, 23 in SPEC 2 and 62 in SPEC 3 as species threatened in Europe. The area provides suitable habitats for 76 species, included in Annex 2 of the Biodiversity Act, which need special conservation measures. Sixty-eight of them are listed also in Annex I of the Birds Directive and more than half of them breed in the region in significant populations. Sakar holds the biggest populations in the country of Imperial Eagle (*Aquila heliacal*), Lesser Spotted Eagle (*Aquila pomarine*), Booted Eagle (*Hieraaetus pennatus*), Black Kite (*Milvus migrans*) and Long-legged Buzzard (*Buteo rufinus*). It is one of the most important areas in the country on European Union scale for the species mentioned above, as well as for the Levant Sparrowhawk (*Accipiter brevipes*), the Tawny Pipit (*Anthus campestris*), Calandra Lark (*Melanocorypha calandra*), Greater Short-toed Lark (*Calandrella brachydactyla*), Masked Shrike (*Lanius nubicus*), Stone Curlew (*Burchinus

*oedicnemus*), Montagu`s Harrier (*Circus pygargus*), Syrian Woodpecker (*Dendrocopos syriacus*) and the Olive-tree Warbler (*Hippolais olivetorum*) (Natura 2000, standard data form, 2015).

During the communist regime in Bulgaria, for several decades, the border areas with Greece and Turkey remained closed and inaccessible to tourists, visitors and guests of the region.

The strict control and restricted access to the border areas was part of the division of Eastern and Western Europe into two warring camps during the Cold War. Sakar mountain fell into the trap of the so-called "Iron Curtain" and were unfamiliar to the public.

Due to the limited access to these areas, the strict controls and not infrequently the geographical isolation of the border areas, they have preserved key habitats, rare and protected species and unique landscapes.

These areas are united together in the European Green Belt initiative. At present, when we have United Europe, these regions, which were unknown until recently, are gradually opening up and showing their treasures.

For nature and history lovers, the heritage reveals emblematic etalons of conservate nature, preserved historical sites and distinctive culture and traditions. Gradually, not only for the general public, but also for the scientific community, monuments and landmarks of Sakar mountain region are getting popular and accessible used to be unfamiliar until recently.

The region also reveals its unique nature at the gorge of the Tundzha River, the floodplain forests along the islands of the Maritza River, the habitats of the Eastern Imperial Eagle and Lesser Kestrel, for which Sakar Mountain is one of the most important nesting areas in the whole country.

In the Transboundary region of Bulgaria, Greece and Turkey there are some emblematic species that have been known to people on both sides of the border for centuries and are now conservated by NATURA 2000.

Particularly popular in this respect are various rare and protected birds. They fly over large areas, with state borders and natural barriers being no obstacle for them. Many of these feathered creatures live close to people and can be identified by them. At the same time, these emblematic species are quite sensitive to the environmental changes, direct violations on them or large-scale investment actions (Gradev, 2021).

For these purposes was chosen the area of the village Levka SPA "Sakar" (BG0002021) for recovered Lesser Kestrel as a breeding.

That the Sakar Mountain are part of European ecological network Natura 2000 and European Green Belt, which areas are with preserved nature and biodiversity (Yaneva et al., 2021).

The monitoring of the occupied nesting places was carried out in the period March-September 2021, when is the breeding season of Lesser Kestrel. For its implementation standard internationally established methods are used, including observation and follow-up with:

- Binoculars; (Figure 1)
- Field scope tube; (Figure 2)
- Camera;
- Video surveillance. (Figure 3)

Figure 1 Observation of Lesser Kestrel with binoculars / Photo credit: "Green Balkans – Stara Zagora" NGO/


Figure 2. Observation of Lesser Kestrel with field scope tube / Photo credit: "Green Balkans – Stara Zagora" NGO/


Figure 3. Observation of Lesser Kestrel with video surveillance /Photo credit: "Green Balkans – Stara Zagora" NGO/

In addition to following the nesting sites occupied and breeding success, except daily monitoring through field observation methods direct inspections of artificial nest boxes have been carried out, which are performed during a certain period in order to determine the exact number of hatched chicks (Figure 4).

Figure 4. Check the artificial nest box /Photo credit: "Green Balkans – Stara Zagora" NGO/

All methods of observation Lesser Kestrel during the breeding period are selected for the purpose birds should be monitored without direct disturbance. Direct inspections of nesting sites are carried out in a period in which the probability of abandoning eggs or small hatched chicks is minimal.

## RESULTS AND DISCUSSIONS

From the surveys conducted in 2021 three types of artificial nest boxes have been identified:
1. Classical wall nest box; (Figure 5)
2. Cavity wall nest box; (Figure 6)
3. Under-roof nest box. (Figure 7)


Figure 5. Classical wall nest box /Photo credit: "Green Balkans – Stara Zagora" NGO/


Figure 6. Cavity wall nest box /Photo credit: "Green Balkans – Stara Zagora" NGO/

Figure 7. Under-roof nest box /Photo credit: "Green Balkans – Stara Zagora" NGO/

All artificial nest boxes are specially designed for the Lesser Kestrel in accordance with the methodology of the Spanish organization DEMA.
The nest boxes are made from materials to be strong, reliable and to protect birds, eggs and young chicks. The material and the structure have to be impermeable to water to avoid flooding of the nest box, which would lead to drowning of eggs or chicks. Several small holes

should be opened in the floor of the nest box to allow drainage of water when necessary. Such openings should be provided in the higher parts of all walls to allow ventilation inside the nest. It is recommended to cover the floor of the nest box with fine sand, to enable the birds form egg-laying cavities and secure mechanical protection of the eggs, since Lesser Kestrels do not build typical nests and there is a risk for the eggs to roll over the smooth flat surface of the nest bottom. The measurements of the entrance hole should be about 6-6.5 cm, to avoid penetration of other bird species, competing with Lesser Kestrels for breeding sites. It is good to put a door on one of the walls, in order to allow the implementation of manipulations or examinations when needed. In the bottom of the nest box have to be with an extra hole and a shutter, allowing easy cleaning of the nest box after the end of every breeding season. The door for inspection and the shutter of the opening for cleaning the nest box was securely fixed and tightened, so that they cannot be opened by martens, domestic cats, rats or other predators.

During the breeding season 2021 in the colony of Lesser Kestrel in the village Levka, part of SPA "Sakar" (BG0002021) is established 25 formed pairs in artificial nest boxes and и between 3-4 pairs in natural nesting sites. After the end of the breeding season and data processing it was found that 85% of breeding pairs of Lesser Kestrel prefer to nest in artificial nest boxes. Artificial nest boxes are of great importance in providing safe nesting sites, more than 60 % of the population in Bulgaria use those (Gradev et al., 2021).

In 2020 Society "Nature Park Sakar" and "Green Balkans – Stara Zagora" NGO made and installed 40 artificial nest boxes for Lesser Kestrel near to breeding colony, according to an innovative methodology for the country. The model is borrowed from the Spanish organization DEMA, part of specialized project under the Program LIFE of EU - LIFE-ZEPAURBAN, which is aimed at improving the nesting areas for Lesser Kestrel in some villages in Spain. The artificial nest boxes are not made of ordinary wood and are made of modern building materials with very good insulating properties. This provides additional comfort for the Lesser Kestrel, protecting them from rain and high temperatures in the summer in the Sakar Mountains.

In 2021 the first successful occupied of the new artificial nests by a pair of Lesser Kestrel was proven (Figure 8).

In 2021, a new method for releasing Lesser Kestrel in Bulgaria was experimented for the first time. To this moment in the framework of the recovery activities of the Lesser Kestrel, the Green Balkans team released only zero-year-old chickens - birds aged between 25 and 30 days, in down plumage.


Figure 8. First occupied artificial nest box from the new methodology /Photo credit: "Green Balkans – Stara Zagora" NGO/

New in the approach was the adaptive aviary - a cage on wheels, which is used as a temporary home for the birds, from where they were later released. This time the released birds were hatched in 2020, i.e., at the age of almost one year. After the aviary was opened, more than half of the released birds were noticed around the release site – Lesser Kestrel Release and Adaptation Module in village Levka, not just in flight, but also actively eating at the feeding place. At least three of the birds have formed pairs in artificial nest boxes placed specifically for the species.

All of these cases confirmation that the artificial nest boxes, constructions especially for the Lesser Kestrel, are recognizable for the species and them easily occupied it. It is an interesting fact that in addition to Lesser Kestrel, artificial nest boxes are also occupied by other birds in the area such as Little owl, Eurasian scops owl, Common starling and sparrow.

## CONCLUSIONS

From the surveys have been identified three different types of artificial nest boxes: Classical wall nest box, cavity wall nest box, under-roof nest box.

All studies conducted in 2021 show that Lesser Kestrel adapt extremely successfully to artificial nest boxes and this is a major way to conserve the species as well as increase its numbers. In recent years, a large part of the population of the Lesser Kestrel in Bulgaria nests in artificial nest boxes, which proves that the species easily occupied it.

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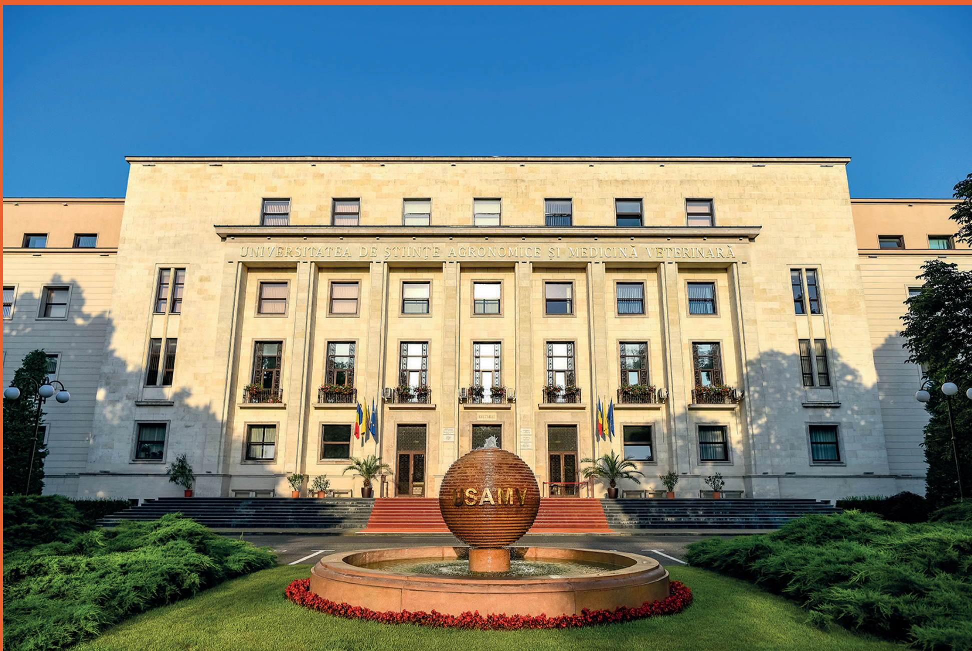


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