AGRICULTURAL AND FORESTRY
SCIENCES ACADEMY
"GHEORGHE IONESCU - SISESTI"

ACTA AGRICOLA ROMANICA

ANIMAL SCIENCES AND VETERINARY MEDICINE

Volume 6, Year 6, No. 6.3.
August 2024

BUCHAREST

AGRICULTURAL AND FORESTRY
SCIENCES ACADEMY
"GHEORGHE IONESCU - SISESTI"

ACTA AGRICOLA ROMANICA

ANIMAL SCIENCES AND VETERINARY MEDICINE

Volume 6, Year 6, No. 6.3
August 2024

BUCHAREST

Academy of Agricultural and Forestry Sciences
"Gheorghe Ionescu-Şişeşti"
Mărăşti Blvd. 61, 011464, Bucharest, Romania
Phone: +40-21-3184450; 3184451;
E-mail: secretariat@asas.ro *Internet:* http://www.asas.ro

The magazine is edited by the Sections of " Animal Sciences and Veterinary Medicine"

The magazine appears annually, in the second semester of the year
ISSN 2784 – 0948 ISSN – L 2784 – 0948

CONTENT

Page

1. COSTACHE Mioara, ARCADE Mariana Cristina, GANCEA Marinela, DOBROTĂ Gheorghe, RADU Daniela, COSTACHE Mihail - RESEARCH ON ASSESSMENT OF SELECTIVE BREEDING POTENTIAL AND PROGRAMMING EPIGENETICS TO IMPROVE ADAPTATION TO CHANGING ENVIRONMENTAL CONDITIONS 7

2. CHIOREAN Remus, BOTA Adrian, MOLDOVAN Mădălina – STUDY OF THE METABOLIC FROFILE IN BUFFALOES WITH PROLONGED ANESTRUS 15

3. CHIOREAN Remus, BOTA Adrian, MOLDOVAN Mădălina - OVARIAN RESPONSE IN BUFFALOES WITH PROLONGED ANESTRUS DURING THE STABULATION PERIOD 19

4. DOBROTĂ Gheorghe, DOBROTĂ Nicoleta – Georgeta, RADU Daniela, COSTACHE Mihail, MARICA Nino – INNOVATIVE AQUACULTURE TECHNOLOGY REGARDING THE INFLUENCE OF THE POPULATION DENSITY ON THE DEVELOPMENT OF THE SPECIES *SANDER LUCIOPERCA* (LINNAEUS -1758) IN THE POSTEMBRYONIC PERIOD 22

5. ENCIU Ana, ZAMFIR Camelia Zoia, ILIŞIU Elena, NICOLESCU Alina - EVALUATION OF WOOL PRODUCTIVITY POTENTIAL OF CROSSBREEDS BETWEEN LOCAL SHEEP BREEDS AND IMPORTED SPECIALIZED BREEDS 30

6. ENCULESCU Marinela, NICOLAE Ioana, GAVOJDIAN Dinu - EVALUATION OF THE METABOLIC PROFILE IN LACTATING DAIRY COWS DIAGNOSED WITH MASTITIS 41

7. GAVOJDIAN Dinu, NICOLAE Ioana – BACK-CROSSING SCHEMES USED TO CREATE BEEF SYNTHETIC BREEDS 49

8. ILIŞIU Elena, NADOLU Dorina, ILIŞIU Cristian Vasile, ANGHEL Andreea Hortanse, ENCIU Ana, ILIŞIU Vasile Călin, CHIRTEŞ Ion Dumitru, ZAMFIR Camelia Zoia - COMPARATIVE STUDY REGARDING GROWTH PERFORMANCES OF LAMBS FROM **TSIGAI BREED** – RUSTY VARIETY AND TRIRACIALI CROSSBRED LAMBS OBTAINED FROM CROSSING BETWEEN **TSIGAI BREED** WITH SPECIALISED RAMS BREEDS FOR MEAT PRODUCTION ……… 54

9. MACIUC Vasile, NECHIFOR Ionică, FLOREA Alexandru Marian, NECHIFOR Bogdan Ioan, ȚURCANU Ioana, BOLDIȘOR Ana, NECHIFOR Daniel Constantin, PASCAL Constantin - INTRA-POPULATION STRUCTURE RESEARCH OF A **KARAKUL OF BOTOSANI** SHEEP FLOCK 61

10. MARICA Nino, RADU Silvia, COSTACHE Mioara, COSTACHE Mihail, DOBROTĂ Gheorghe, DOBROTĂ Nicoleta, RADU Daniela - THE INTRODUCTION OF NEW SPECIES OF FISH, A WAY TO INCREASE PRODUCTION IN AQUACULTURE 71

11. MIHALI Ciprian Valentin, NEAMȚ Radu Ionel, MIZERANSCHI Alexandru Eugeniu, ILIE Daniela Elena - ANALYSIS OF CSN2 FOR A1/A2 ALLELIC VARIANTS BY DOUBLE-SENSE DNA GENOTYPING WITH REFERENCE TO MILK QUALITY AND HUMAN HEALTH 82

12. NADOLU Dorina, ANGHEL Andreea Hortanse, ZAMFIR Camelia Zoia, ILIŞIU Elena, ANGHEL Florea, JERCAN Cătalin, ILIŞIU Vasile Călin, ILIŞIU Cristian Vasile – RESEARCH ON THE INFLUENCE OF THE a αS1-CASEIN GENOTYPE ON THE PROCESSING YIELD OF GOAT MILK INTO CHEESE 88

13. NECHIFOR Ionică, FLOREA Alexandru Marian, NECHIFOR Bogdan Ioan, ȚURCANU Ioana, BOLDIȘOR Ana, NECHIFOR Daniel Constantin, PASCAL Constantin, MACIUC Vasile - RESEARCH ON COLOR SHADES TRANSMISSION IN GRAY LINE FROM **KARAKUL OF BOTOSANI** 97

14. NEAMȚ Radu, NECIU Florin, SĂPLĂCAN Gheorghe, MIHALI Ciprian, CZISZTER Ludovic, SĂPLĂCAN Silviu, MIZERANSCHI Alexandru - RESEARCH REGARDING USING *CICHORIUM INTYBUS* IN ORDER TO IMPROVE THE PALATABILITY OF CATTLE FEEDSTUFF 104

15. NICOLESCU Alina Narcisa, RADU Răducu, CUTOVA Nicolae, ZAMFIR Camelia Zoia, ENCIU Ana, STANCIU Maria - RESEARCHES REGARDING THE OPTIMIZATION OF THE FODDERING TECHNOLOGIES FOR THE VARIOUS CATEGORIES OF SHEEP DEPENDING ON THE DIRECTION OF PRODUCTION 112

16. PALL Emoke, CENARIU Mihai, GROZA Ioan, DUCA Gheorghita, SPINU Marina, ȘANDRU Carmen Dana, BORZAN Mihai, CIUPE Simona - IMPROVING THE KINEMATIC PARAMETERS OF RAM EPIDIDYMAL SPERM USING A COCONUT WATER-BASED EXTENDER 118

17. PALL Emoke, CENARIU Mihai, DUCA Gheorghita, SPINU Marina, SANDRU Carmen Dana, CIUPE Simona, BORZAN Mihai - ASSESSMENT OF THE PROLIFERATIVE POTENTIAL OF SHEEP ENDOMETRIAL MESENCHYMAL STEM CELLS 125

18. PASCAL Constantin, CRISTIAN Costică, BULMUGĂ Dragoș – ASSESSMENT OF GROWTH INTENSITY AND PRODUCTION PERFORMANCE IN THE **ROVASI** SHEEP BREED 132

19. RADU Daniela, RADU Silvia, MARICA Nino, COSTACHE Mioara, Barbu Alin, ARCADE Mariana Cristina, GANCEA Marinela – RESEARCH ON THE OPTIMIZATION OF FISH FEEDING STRATEGY IN DIFFERENT TYPES OF PRODUCTION SYSTEMS USING ALTERNATIVE, LESS USED AND SUSTAINABLE FEED ADDITIVES AND FEEDS ……………………………….. 146
20. SAVU Vasilică, ŞAPCALIU Agripina - INFECTIOUS MYCOTIC DISEASES OF *BOMBYX MORI L.* SILKWORMS – REVIEW …… 153
21. TIȘE Nicolae, BĂIEȘ Horia-Mihai, COZMA-PETRUȚ Anamaria, MILITARU Dumitru, GROZA Ioan, GYÖRKE Adriana, SZAKÁCS Andrei, SPÎNU Marina, COZMA Vasile – CONTROL OF *BALANTIDIUM COLI* INFECTION IN SWINE WITH HYDROALCOHOLIC EXTRACTS OF WORMWOOD (*ARTEMISIA ABSINTHIUM L.*) AND TAGETES (*TAGETES PATULA*) …… 163
22. VASIU Aurel, PALL Emoke, DUCA Gheorghita, SPINU Marina, SANDRU Carmen Dana, OLAH Diana - PREDICTIVE VALUE OF CIRCULATING IMMUNE COMPLEXES' LEVELS AND LESIONAL SCORES IN CONTAGIOUS AGALACTIA OF SHEEP …… 169
23. VASIU Aurel, PALL Emoke, DUCA Gheorghita, SPINU Marina, SANDRU Carmen Dana, RUSU Mariana, OLAH Diana - STAPHYLOCOCCAL MASTITIS IN SHEEP: A SOLVED OR A PENDING PROBLEM? …………………………………………. 177
24. VICOVAN Petru Gabriel, VARTIC Alexandru Gabriel, RADU Răducu, VICOVAN Adriana Natalia, NEACȘU Corneliu Ion, NICOLESCU Alina, STANCIU Maria – CREATION BY CROSSING OF A NEW MEAT BREED OF SHEEP WITH HIGH GROWTH SPEED, HIGH YIELD AT SACRIFICE AND QUALITY CARCASSES, REQUESTED BY THE E.U. MARKET …… 185

The papers were presented at the anniversary session of the "*Gheorghe Ionescu-Sișești*" Academy of Agricultural and Forestry Sciences on May 30, 2024

ACTA AGRICOLA ROMANICA, Volume 6, Year 6, No. 6.3.

RESEARCH ON ASSESSMENT OF SELECTIVE BREEDING POTENTIAL AND EPIGENETICS PROGRAMMING TO IMPROVE ADAPTATION TO CHANGING ENVIRONMENTAL CONDITIONS

CERCETĂRI PRIVIND EVALUAREA POTENȚIALULUI DE REPRODUCERE SELECTIVĂ ȘI PROGRAMARE EPIGENETICĂ PENTRU A ÎMBUNĂTĂȚI ADAPTAREA LA CONDIȚIILE DE MEDIU ÎN SCHIMBARE

COSTACHE MIOARA[1], ARCADE MARIANA CRISTINA[1], GANCEA MARINELA[1], DOBROTĂ GHEORGHE[1], RADU DANIELA[1], COSTACHE MIHAIL[1]

[1]Fish Culture Research and Development Station of Nucet, 549 Principala Street, 137335, Nucet, Dambovita County, Romania

Correspondence address: scp_nucet@yahoo.com

Abstract

This paper is a comprehensive study of a possible strategic approach, in the context of global population growth, to create more food using current natural resources. Assessing the potential of selective breeding and epigenetic programming to improve adaptation to changing environmental conditions can provide aquaculture products in much larger quantities using the same resources involved. Harnessing the full potential of creating genetic material with superior morpho-constructive characteristics to the current specific genotype is a strategic approach to epigenetics generating multiple economic and environmental benefits. In the context of the circular economy, the principles of epigenetic programming aim at epigenetic mechanisms and their action by modifying the behavior of genes, inhibiting or activating their expression, in response to environmental stimuli, but without affecting the genome and DNA structures and restricting economic and social progress. Epigenetics highlights potentially heritable changes in gene expression that do not involve changes in DNA sequence. The results of the study can be used to improve aquaculture development strategy in the context of environmental sustainability.

Keywords: selective breeding, genetic diversity, DNA methylation, epigenetics, phenotype.

Rezumat

Această lucrare este un studiu cuprinzător al unei posibile abordării strategice, în contextul creșterii populației globale, pentru a crea mai multă hrană folosind resursele naturale actuale. Evaluarea potențialului de reproducere selective și programare epigenetică pentru a îmbunătăți adaptarea la condițiile de mediu în schimbare poate furniza produse de acvacultură în cantități mult mai mari, folosind aceleași resurse implicate. Valorificarea tuturor posibilităților de a crea material genetic, care să dețină caracteristici morfo-constructive superioare față de genofondul specific actual este o abordare strategică a epigeneticii generând multiple beneficii economice și de mediu. În contextul economiei circulare, principiile programărilor epigenetice urmăresc mecanismele epigenetice și acțiunea lor prin modificarea comportamentului genelor, inhibând sau activând expresia acestora, ca urmare a unor stimuli de mediu, fără a afecta însă, genomul și structurile ADN și a restricționa progresul economic și social. Epigenetică evidențiază modificările potențial ereditare în expresia genelor care nu implică modificări ale secvenței de ADN. Rezultatele studiului pot fi utilizate pentru a îmbunătăți strategia de dezvoltare a acvaculturii, în contextul sustenabilității mediului.

Cuvinte cheie: reproducere selectivă, diversitate genetică, metilare ADN, epigenetică, fenotip.

INTRODUCTION

In its definition, epigenetics is the branch of genetics that studies the variation of traits, phenotypic characters of organisms as a result of changing environmental conditions and thus their behavior.

Epigenetics focuses on potentially heritable changes in gene expression that do not involve changes in the DNA sequence. Epigenetic mechanisms act by modifying the behavior of genes, inhibiting or activating their expression in response to environmental stimuli, without affecting the genome and DNA structures (Gavery, M. R., et al., 2017).

The possibility that traits that are acquired during an individual's lifetime are memorized and then passed on to the next generation has led to a new theory that organisms change their behavior in response to environmental changes. The changed behavior, in turn, modifies their phenotypic characters, and their offspring inherit those 'improved' structures. Recent studies in this area suggest that an individual's early environment is a key determinant of future health and metabolism. Genetic improvement, artificial reproduction and breeding in different systems are the main preoccupations of Fish Culture Research and Development Station of Nucet. In Romania, most of the fish production is carried on in ponds, lakes and reservoirs by extensive and semi-intensive cyprinid rearing in polyculture with other fish species. These systems are very vulnerable and strongly affected by climate change, both directly, through problems related to water availability, ensuring the necessary levels in conditions of increasing water and air temperature, prolonged drought, evaporation, extreme weather phenomena, and indirectly through physiological stress induced to fish populations, due to increasing temperature, lack of oxygen, decreased metabolic rate and feeding, increased incidence of diseases. Although there are numerous studies on the influence of climate change on freshwater, marine waters, fish and other aquatic organisms at the international level, and many aspects are addressed, in Romania there are very few and they mainly concern aquatic ecosystems in general, productivity, ichthyofauna in terms of abundance and distribution, escape or avoidance of habitats that have become unsuitable, and less on fish directly; this is probably also due to their living environment, which does not offer the possibility of investigating, collecting scientific and technical data and information on a permanent and easy basis, compared to terrestrial animals. There are no studies on the influence of climate change on the essential technological processes that ensure production in fish farming: reproduction and rearing.

Adaptation of plants and animals has been essential for the expansion of the human population and the emergence of modern society as we know it (Costache, M., et al., 2023). Remarkably, the number of commercially important fish species is very limited, even though aquaculture is the world's fastest growing food production sector, accounting for almost 50% of fish for human consumption worldwide. To ensure the sustainability of the aquaculture industry, it is essential to maintain and selectively improve the main commercially valuable fish species (Gancea, M., et al., 2023).

Freshwater ecosystems are strongly threatened by climate change, and the ability of freshwater species to tolerate climate change-induced environmental fluctuations is essential for the development of adaptation and conservation strategies (Costache, M., et al., 2021).

The rapid rate of substantial phenotypic changes during adaptation to new environmental conditions to which fish species are subjected raises the hypothesis that epigenetic mechanisms are involved in this process. The overall objective of recent research and studies in the field of aquaculture biotechnology is to verify the importance of epigenetics in the adaptation of fish species, especially of valuable species of importance for sustainable aquaculture.

In the last decade, major progress has been made in genomic and genetic studies in aquaculture. However, research on epigenetic regulation of aquaculture traits is still at an early stage. It is clear that most, if not all, aquaculture traits are regulated at both genetic and epigenetic levels. To date, the molecular basis of breeding and genetic markers used in selective breeding of fish account for only a fraction of the phenotypic variation observed. The identification of epigenetic markers (DNA methylation marks and miRNA variants) will have a major impact on aquaculture biotechnology as it will allow the development and application of epigenomic selection as a new trait in future selective breeding programs.

Genetic diversity interacts with environmental variation to produce the variety of shapes, sizes, life history characteristics, behavior and colors that make aquatic species so valuable and interesting. Without genetic diversity, there would be no species diversity, no adaptation, no race, and no evolution; eventually there would be extinction as climate and habitats change as a result of natural or human actions.

Identification of parental genotypes and assessment of reproductive potential through selection is a breeding program that exploits heritable phenotypic variation; consequently, the two prerequisites for a selective breeding program are: first, the phenotype to be modified must show a degree of variation; second, the variation must be heritable. The individuals in a population fall into distinct, non-overlapping phenotypic categories.

The research results provide new answers and insights into mechanisms for the role of epigenetics in fish breeding, namely transgenerational inheritance and nutritional epigenetics. State-of-the-art applied sequencing technologies will substantially deepen our understanding of epigenome-wide genetic patterning. Although knowledge gained over the past two decades has led to a better understanding of how DNA methylation and miRNAs play a key role in fish development and physiology. Future efforts are needed to translate this into the use of miRNAs and DNA methylation as more ethical and integrative biomarkers of fish physiological status and/or innovative therapies of importance to fish farming.

DNA methylation and non-coding RNAs (ncRNAs) play a central role in the complex and tightly regulated processes that sustain life, from gene transcription to post-transcriptional regulation. A wide range of methodological approaches have been applied to identify, quantify and characterize different biological processes. Application of these methodologies, in studies with different fish species, have provided the first evidence of miRNAs and DNA methylation controlling fish development, reproduction, immunocompetence, adaptation to environmental conditions and nutrition. The development of various *in vitro* and *in vivo* biotechnological tools has allowed researchers to further develop and carry out their functional characterization.

MATERIALS AND METHODS

This study represents a synthesis of the most comprehensive articles focusing on the key concept of epigenetic programming in aquaculture. How the implementation of selective breeding potential assessment and epigenetic programming of key cultured fish species to improve adaptation to changing environmental conditions enhances fish production and boosts the entire fisheries sector. A schematic analysis of the main advantages resulting from the application of this concept highlights the strengths of developing such technology to maximize productivity and reduce the environmental footprint. In recent years, epigenetic mechanisms have been increasingly invoked in this context due to their key role in gene-environment interactions.

RESULTS AND DISCUSSIONS

Epigenetic effects affecting gene expression are triggered by environmental changes, can persist throughout life or over several generations, and can affect an individual's phenotype. In recent years, there has been increasing interest in the possible role of epigenetics in shaping complex or quantitative traits of fish species. Numerous studies have demonstrated epigenetic effects in fish, induced by environmental conditions, with benefits for the organism and evidence for heritability of adaptive phenotypic traits acquired over generations, relevant in the context of increasing aquaculture production (Figure 1).

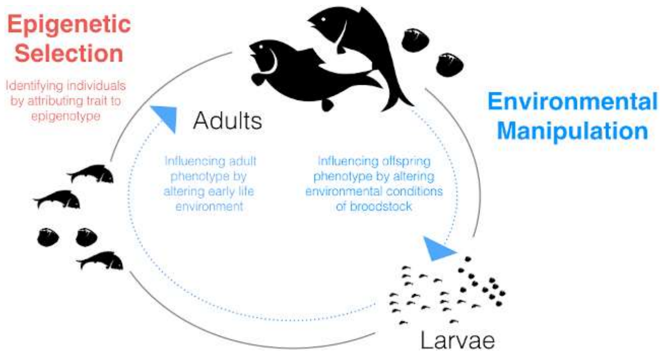


Figure 1. Epigenetic considerations in aquaculture / Considerații epigenetice în acvacultură (source: https://peerj.com/articles/4147/)

The studies have demonstrated the potential of epigenetics to positively induce stress tolerance, disease resistance and improve reproductive performance. In conclusion, understanding the epigenetic mechanisms that lead to the most favorable phenotypic traits in economically important species will contribute to the development of economically viable commercial aquaculture.

The possibility that traits that are acquired during an individual's lifetime are memorized and then passed on to the next generation has led to a new theory that organisms change their behavior in response to environmental changes. The changed behavior, in turn, changes their phenotypic traits, and their offspring inherit those 'improved' structures. Recent studies in this area suggest that an individual's early life environment is a key determinant of future health and metabolism.

Assessment of reproductive potential by identifying parental genotypes

Identification and selection of parental genotypes exploits phenotypic variation, there must be at least two phenotypes in the population and one of them must be the desired phenotype. If there is only one phenotype, there is no phenotypic variation, which means that selection cannot be used to modify the phenotype. There must be variation, but the observed variation must be heritable, otherwise selection will not be able to modify phenotypic frequencies. The distinction between heritable and non-heritable variance is important because many qualitative phenotypes that are observed are non-heritable deformities. Some deformities have a genetic basis, but most are caused by nutritional deficiencies, environmental disorders, toxins, diseases or injuries, or are developmental errors. Deformities that are produced by non-genetic factors cannot be eliminated by selection. The only way to eliminate a non-genetic malformation is to discover the environmental factor that causes it and eliminate the factor or modify the culture environment (Figure 2).

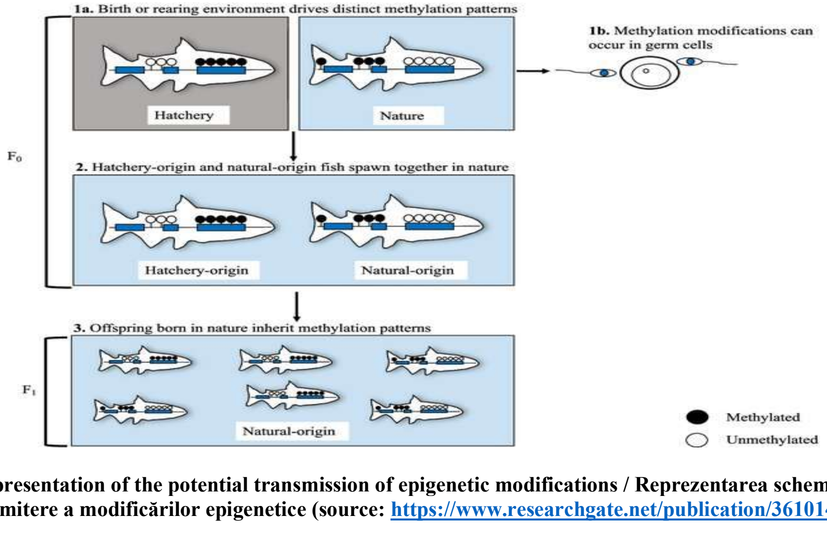


Figure 2. Schematic representation of the potential transmission of epigenetic modifications / Reprezentarea schematică a potențialului de transmitere a modificărilor epigenetice (source: https://www.researchgate.net/publication/361014176)

Methods for assessing reproductive potential

Evaluation of genetic improvement and reproductive potential are scientific parameters that provide a solution for improving production, efficiency and environmental sustainability in the aquaculture sector, and effective management of aquatic genetic resources can help address all aspects of the associated problems.

The objective of the aquaculture sector is to effectively manage genetic diversity in fish species stocks, thereby achieving benefits from breeding selection. Managing and preserving genetic diversity is the success of selective breeding.

The establishment of the basic population is a prerequisite for traditional and genetic breeding technologies and is essential for effective breeding. However, establishing a base population for breeding requires an extensive study of germplasm resources and genetic analysis of desirable traits in fish species lines (Jenko, J., et al., 2015)

Fish species have an important characteristic that is related to higher genetic diversity within species: they are highly fecund, with usually external fertilization. This feature of their reproductive biology allows flexibility in designing methods to assess reproductive potential and widely disseminate selected lines.

Assessing the reproductive potential of a genetically diverse base population is essential for adaptation and for the formation of breeding programmes, as it underpins the future genetic potential to be exploited through selective breeding (Fernández, J. et al., 2014). Adaptation of aquaculture species means that farmed species are often derived from species in the wild, and interbreeding between the two categories is possible. As species move up the adaptation ladder towards closed selective breeding populations, the genetic gap between farmed and wild populations widens. The genomes of farmed species are significantly altered by genetic breeding programs, which exert intense selection pressures (Wang, G.-D. et al., 2014). As adaptation advances, high-intensity genotyping or sequencing of multiple farmed and wild populations and genome-wide comparisons of genetic diversity can be applied to assess these effects to identify common signatures of selection (Lopez Dinamarca, M. E. et al., 2015).

The impact of breeding on the physiology of fish species has been demonstrated through gene expression and genome methylation studies, which show significant differences after several generations of hatchery breeding (Roberge, C. et al., 2006).

Conservation of the fish genofund

The use of gene banks is practiced for the conservation of aquatic species germplasm for both vulnerable wild and farmed species to avoid loss of genetic diversity. There are stocks and gene banks established for fish species, and technologies for conserving gametes, tissues and cell lines are rapidly developing, with detailed analyses available (Adams, S. L. Et al., 2015; Goswami, M. Et al., 2016).

Improper management of brood stock and hatchery practices that lead to inbreeding result in reduced population fitness, increased susceptibility to stress and disease, and ultimately boom-and-bust production cycles (You, W. & Hedgecock, D., 2019; Doyle, R. W., 2016).

An essential component of the continuous genetic improvement process, in aquaculture species, is the early understanding of sex determination, where a range of genetic and non-genetic systems have been described (Devlin, R. H. & Nagahama, Y., 2002). GBS techniques have been widely applied to evaluate the genetic basis of sex determination (Martínez, P. et al., 2014). Genetic markers identified in these studies can be applied to predict offspring sex and control sex ratio in both breeding and production stock.

For pedigree tracking (e.g. pair breeding or hormone induction stripping) (Khang, P. Van et al., 2018), genetic markers are commonly applied to track the relatedness of flocks to minimize loss of genetic diversity within a closed breeding nucleus (Vandeputte, M. & Haffray, P., 2014), and genomic tools are as a complement to phenotypic stock assessments.

Selective breeding and epigenetic programming to improve adaptability to changing environmental conditions in fish species of the *Cyprinidae* and *Polyodontidae* families, specific to South Muntenia and South-Western Romania

Selective breeding for genetic improvement of production traits has great potential to improve efficiency and reduce the environmental footprint in aquaculture.

The adoption of aquaculture breeding programs (based on selective breeding programs) for the increased demand of aquaculture production means increasing pressure, which requires the development of more efficient production systems through the adoption of technological advances, especially through improved genetic composition and selective breeding in terms of climate research and strong experimental research (Radu, D., et al., 2009).

There is potential to improve production, efficiency and environmental sustainability in the aquaculture sector, and effective management of aquatic genetic resources can help address all aspects of the problems associated with it. As well as being essential for genetic improvement programs in aquaculture, genetic resources are the necessary raw materials that allow species to adapt to short- and long-term changes in their environment; they provide species, populations and individuals with the flexibility to cope with and adapt to changes in their environment, both anthropogenic and natural; i.e. genetic diversity is necessary for the continued evolution of species.

Management of broodstock in *Cyprinidae* and *Polyodontidae* families includes optimization of critical reproductive processes such as nutrition, maturation, egg and sperm production, and reproduction; offspring from captive broodstock become the most vulnerable to disruption of reproductive activities through hormonal imbalance and unfavorable environmental parameters, leading to reproductive dysfunction (Nguyen, N. H., 2016). Such reproductive deficiencies include poor or no egg or sperm production, defective or weak eggs, lower growth and high mortality of fingerlings and sometimes mortality of chicks (Janssen, K., et al., 2017). Reproductive dysfunctions can be triggered by direct or indirect gamete and endocrine variations, captive-induced stress, inadequate breeding environment and deficiencies in the nutritional profile of the food. In order to avoid dysfunctions caused by controlled breeding, the scientific management of breeding flocks must ensure adequate physiology, immunology, activation of reproductive enzyme pathways and transcription of specific reproductive genes.

In aquaculture, a stable and predictable mating system is essential for healthy fry production, and changes in DNA methylation patterns triggered by higher temperatures can lead to transformations in physiological traits (Lacal, I., et al., 2018, Moore, L. D., Le, T., et al., 2013).

Environmental factors exert an influence on epigenetic changes to produce the phenotype, and this effect can be passed on to subsequent generations (Skinner, M. K. 2011). This creates a huge opportunity for epigenetic programming in aquaculture to select the most favorable phenotypic traits and improve production. This is often a combination not only of traits involving development, growth, reproduction, immunology and disease leading to high biomass yield, but also traits leading to healthy organisms less susceptible to disease. Recent advances in the field of epigenetics have indicated that a deeper understanding of epigenetic mechanisms and their role in physiological traits can provide not only improvements in broodstock selection, but also a measurable improvement in the link between environment and phenotype in aquaculture practices. Large-scale family-based breeding programs for genetic improvement of aquaculture species, selection programs using sibling information that have been implemented for a number of aquaculture species, relatively high heritability’s for economically important traits in fish, combined with high fecundity and short generation intervals (1-4 years) in most species, explain the high genetic gains achieved by selective breeding programs in aquaculture.

Heritability in the broad sense of a phenotype, denoted H^2, is a measure of the part of the variability of the phenotype that, in a given population, is due to genetic differences between individuals in that population. Let Vg be the phenotype variance attributed to genetic variability in the population and Vp be the total phenotype variance in the population, the heritability of the phenotype in the population is by definition equal to the ratio of the former to the latter.

For a selective breeding program to be effective, it must fulfill several basic conditions:

- There must be variation within the species for the traits under selection, because individuals may have identical phenotypes;
- part of this variation must be due to genetic differences, as only genetic variation is transferred to the next generation via eggs and sperm;
- the life cycle of the species in question must be known and controllable;
- individual specimens must be identifiable (by various marking methods) so that the genetic lineage can be kept track of.

Selective breeding plays an important role in improving key species characteristics such as growth and survival rates. Although currently breeding efforts have focused almost exclusively on genetic variation, there is growing evidence that epigenetic variation may also play an important role in selective breeding and may have the potential to contribute to significant improvements in growth rate, performance and survival (Jiang, L. et al., 2013; Houston, R.D. et al., 2020).

CONCLUSIONS

Freshwater ecosystems are under severe threat from climate change. They are one of the most important drivers of freshwater transformation, and effects include changes in their structure, composition, biodiversity and functioning. Understanding the capacity of freshwater species to tolerate climate change-induced environmental fluctuations is essential for developing conservation and adaptation strategies. In the context of climate change, phenotypic plasticity is of particular relevance as this process allows organisms to cope with the unpredictability of environmental stressors over time. In addition, the extension of phenotypic plasticity traits across generations may play an important role in the long term, allowing species to fine-tune their responses to climate change stressors. In Romania, most of the fish production is carried out in ponds, ponds and reservoirs by extensive and semi-intensive cyprinid farming in polyculture with other species. These systems are very vulnerable and strongly affected by climate change, both directly, through problems related to water availability, ensuring the necessary levels in conditions of increasing water and air temperature, prolonged drought, evaporation, extreme weather phenomena, and indirectly through physiological stress induced to fish populations, due to increasing temperature, lack of oxygen, decreased metabolic rate and feeding, increased incidence of diseases.

Considering the international developments in this field, it is necessary that fish breeding research in Romania initiate new genetic improvement programs, first of all for economically important cyprinid and sturgeon species, in order to create fish lines and hybrids that show resistance to temperature increase, low oxygen, water quality and disease resistance. Use of different techniques to identify, quantify and characterize different biological processes: identification of parental genotypes, heritable variation, determination of age of individuals, determination of heritable or non-heritable malformations, degree of genetic diversity for assessment of reproductive potential.

This review provides an overview of the status of selective breeding and reproduction of aquaculture species, highlights how the tailored application of genomic tools can accelerate sustainable genetic improvement in diverse species in different rearing environments, and explores the potential of emerging genomic and biotechnological techniques, such as genome editing or surrogate breeding technologies, to promote important improvements in aquaculture breeding and production.

BIBLIOGRAPHICAL REFERENCES

1. Adams S.L., Smith J.F., Taylor J., McGowan L.T., & Tervit H.R.; Cryopreservation of greenshell™ mussel (*Perna canaliculus*) sperm; 2015; Cryopreservation and freeze-drying protocols, 329-336.
2. Arcade M.C., Costache M., Bahaciu G.V., Dragomir N., & Nicolae C.G.; IMTA key concept for developing a strategy to increase aquaculture production and improve environmental sustainability; 2023; Scientific Papers. Series D. Animal Science, 66(1).
3. Costache M., Radu D., Marica N., Costache M., & Arcade M.C.; Growth of the North American Sturgeon (*Polyodon spathula*) during the post-embryonic development period in protected areas; 2023; International Multidisciplinary Scientific GeoConference: SGEM, 23(3.1), 61-69.
4. Devlin R.H., Nagahama Y.; Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences; 2002; Aquaculture, 208(3-4), 191-364.
5. Doyle R.W.; Inbreeding and disease in tropical shrimp aquaculture: a reappraisal and caution; 2016; Aquaculture research, 47(1), 21-35.
6. Gancea M., Arcade M.C., Costache M., Banica A.L., Dulama I.D., & Radulescu C.; Monitoring water quality of ponds and predictions related to climate change implications on fish habitats; 2023; Journal of Science and Arts, 23(4), 1035-1048.
7. Gavery M. R., & Roberts S. B.; Epigenetic considerations in aquaculture; 2017; PeerJ, 5, e4147.
8. Goswami M., Mishra A., Ninawe A., Trudeau V., & Lakra W.; Bio-banking: an emerging approach for conservation of fish germplasm; 2016; Poult. Fish. Wildl. Sci, 4, 143.
9. Houston R.D., Bean T.P., Macqueen D.J., Gundappa M.K., Jin Y.H., Jenkins T.L., & Robledo D.; Harnessing genomics to fast-track genetic improvement in aquaculture; 2020; Nature Reviews Genetics, 21(7), 389-409.
10. Janssen K., Chavanne H., Berentsen P., & Komen H.; Impact of selective breeding on European aquaculture; 2017; Aquaculture, 472, 8-16.
11. Jenko J., Gorjanc G., Cleveland M.A., Varshney R.K., Whitelaw C.B.A., Woolliams J.A., & Hickey J.M.; Potential of promotion of alleles by genome editing to improve quantitative traits in livestock breeding programs; 2015; Genetics Selection Evolution, 47, 1-14.
12. Jiang L., Zhang J., Wang J.J., Wang L., Zhang L., Li G., & Liu J.; Sperm, but not oocyte, DNA methylome is inherited by zebrafish early embryos; 2013; Cell, 153(4), 773-784.
13. Khang P.V., Phuong T.H., Dat N.K., Knibb W., & Nguyen N.H.; An 8-year breeding program for Asian seabass Lates calcarifer: Genetic evaluation, experiences, and challenges; 2018; Frontiers in genetics, 9, 191.
14. Lacal I., & Ventura R.; Epigenetic inheritance: concepts, mechanisms and perspectives; 2018; Frontiers in molecular neuroscience, 11, 292.
15. López M. E., Neira R., & Yáñez J. M.; Applications in the search for genomic selection signatures in fish; 2015; Frontiers in genetics, 5, 120295.
16. Martínez P., Viñas A.M., Sánchez L., Díaz N., Ribas L., & Piferrer F.; Genetic architecture of sex determination in fish: applications to sex ratio control in aquaculture; 2014; Frontiers in genetics, 5, 340.
17. Moore L.D., Le T., & Fan G.; DNA methylation and its basic function; 2013; Neuropsychopharmacology, 38(1), 23-38.
18. Nguyen N.H.; Genetic improvement for important farmed aquaculture species with a reference to carp, tilapia and prawns in Asia: achievements, lessons and challenges; 2016; Fish and Fisheries, 17(2), 483-506.
19. Radu D., Oprea L., Bucur C., Costache M., & Oprea D.; Characteristics of hematological parameters for carp culture and koi (*Cyprinus carpio Linneaus*, 1758) reared in an intensive system; 2009; Bulletin of the University of Agricultural Sciences & Veterinary Medicine Cluj-Napoca. Animal Science & Biotechnologies, 66.
20. Roberge C., Einum S., Guderley H., & Bernatchez L.; Rapid parallel evolutionary changes of gene transcription profiles in farmed Atlantic salmon; 2006; Molecular Ecology, 15(1), 9-20.
21. Skinner M.K.; Environmental epigenomics and disease susceptibility, 2011; EMBO reports, 12(7), 620-622.
22. Vandeputte M., & Haffray P.; Parentage assignment with genomic markers: a major advance for understanding and exploiting genetic variation of quantitative traits in farmed aquatic animals; 2014; Frontiers in genetics, 5, 118682.
23. You W., & Hedgecock D.; Boom-and-bust production cycles in animal seafood aquaculture, 2019; Reviews in Aquaculture, 11(4), 1045-1060.

ACTA AGRICOLA ROMANICA, Volume 6, Year 6, No. 6.3.

STUDY OF THE METABOLIC PROFILE IN BUFFALOES WITH PROLONGED ANESTRUS

STUDIUL PROFILULUI METABOLIC LA BIVOLIȚELE CU ANESTRU PRELUNGIT

CHIOREAN REMUS[1], BOTA ADRIAN[1], MOLDOVAN MĂDĂLINA[1]

[1] Research and Development Station for Buffalo Breeding, Sercaia, 507195,
street Campului, no 2, Brasov county, Romania

Correspondence address: scdcb.sercaia@yahoo.com

Abstract

The metabolic profile test in animals is used to assess their nutritional status, to diagnose disease and assess fertility. The aim of this paper was to investigate the metabolic profile in buffaloes. Forty buffaloes from the Research and Development Station for Buffaloes Sercaia - Romania, were divided into two groups (20 heads/group). Estrus induction protocols were applied to the first group (G1), while the second group consisted of buffaloe cows that expresses natural oestrus (G2). When the artificial insemination was performed, blood samples were taken and determined the following parameters: hemoglobin, total serum protein, phosphorus, calcium, magnesium, alkaline phosphatase, cholesterol, glucose, lipase, urea. G1 buffaloe cows had significantly higher concentrations of hemoglobin, serum protein, glucose and inorganic phosphorus, while the serum calcium levels were lower in the group. The concentration of magnesium, alkaline phosphatase, cholesterol, lipase and urea did not differ significantly between the buffalo groups (p>0.05). The Ca:P ratio for G1 buffaloes was lower (1.94:1) compared to G2 buffaloes (2.89:1). It can be concluded that the level of hemoglobin, serum protein, glucose, calcium and inorganic phosphorus plays a major role in the reproduction of buffaloes.

Key words: anestrous, buffaloes, estrus, metabolic profile.

Rezumat

Testul profilului metabolic la animale este folosit pentru a evalua starea nutrițională, pentru a diagnostica bolile și pentru a evalua fertilitatea. Scopul acestei lucrări a fost de a investiga profilul metabolic la bivolițe. Patruzeci de bivolițe de la S.C.D.C.B. Șercaia au fost împărțite în două grupe (20/grup). La primul grup s-a aplicat protocoale de inducere a estrului. Grupul al doilea a fost format din bivolițe care au manifestat normal estrul. Când s-a efectuat inseminarea artificială s-au recoltat probele de sânge. Din sânge s-a determinat: hemoglobina, proteina serică totală, fosforul, calciul, magneziul, fosfataza alcalină, colesterolul, glucoza, lipaza, ureea. Bivolițele cu ciclu normal au avut concentrații semnificativ mai mari de hemoglobină, proteine serice, glucoză și fosfor anorganic. Nivelul calciului seric a fost mai mic la bivolițele cu ciclu normal. Concentrația de magneziu, fosfataza alcalină, colesterol, lipaza și ureea nu diferă semnificativ între grupurile de bivolițe. Raportul Ca:P la bivolițele cu ciclu normal a fost mai mic (1.94:1) în comparație cu bivolițele cu anestru prelungit (2.89:1). Raportul Ca:P ar trebui să fie aproape de 2:1 pentru o mai bună reproducere. Din acest studiu se poate concluziona că nivelul hemoglobinei, proteinelor serice, glucozei, calciului și fosforului anorganic joacă un rol major în reproducerea bubalinelor.

Cuvinte cheie: anestru, bivolițe, estru, profil metabolic.

INTRODUCTION

The metabolic profile test in dairy animals is used to assess nutritional status, predict the occurrence of metabolic diseases, diagnose diseases, and assess animal fertility status [1]. Among the factors that influence the metabolic profile in buffaloes, the following were highlighted: nutrition, reproduction, age, sex and climatic factors [6, 8]. A food deficiency causes metabolic, endocrine and nervous disorders, disrupting hypothalamic-pituitary-ovarian activity, with negative effects on the reproductive process, oogenesis and folliculogenesis, extending the postpartum anestrous period and decreasing fertility indices in cows [2, 3, 4].

Data on the metabolic profile test in **Romanian Buffalo** are limited, as there are no available references on normal blood metabolite values [5]. In this metabolic profile research is necessary to continue on a larger number of animals with different sex, age, physiological state and health status [8].

The aim of this work was to investigate the metabolic profile of buffaloes with disorders of the reproductive cycle and of which estrus was induced through therapeutic protocols, compared to buffaloes that exhibited normal estrus.

MATERIAL AND METHOD

The study was carried out at the Sercaia Buffalo Breeding Research Development Station, during 2023, on buffaloes cows. Forty female buffaloes, aged between 7 and 13 years, in good maintenance condition and in different physiological states, were divided into two groups (20 buffaloes cows/group). The first group consisted of buffaloes cows with disorders of the reproductive cycle that are more than 60 days after calving (active anestrus caused by the presence of the corpus luteum and passive anestrus without the corpus luteum on the ovary) to which specific induction treatments were applied and estrus synchronization. The second group consisted of female buffaloes that showed normal estrus after calving. The buffaloes cows in both groups were maintained both in free housing and in the shelter being fed uniformly with green fodder, hay, corn silage and concentrates.

Sample collection and studied parameters

When artificial insemination was performed, blood samples were collected. Blood samples (five to ten ml) were taken from the jugular vein in the morning. Blood was transported to the laboratory on ice, where serum was extracted and stored at -20°C until further analysis. From the serum samples, the following were determined by spectrophotometric and enzymatic colorimetric methods: hemoglobin (g/dL), total serum protein (g/dL), phosphorus (mg/dL), calcium (mg/dL), alkaline phosphatase (IU /L), magnesium ((mg/dL), cholesterol (mg/dL), glucose (mg/dL), lipase (UI/L) and urea (mg/dL), using commercial kits (Span Diagnostics) according to protocol the manufacturer.

Statistical analyses

Data obtained from biochemical estimations were expressed as mean ± standard error (X±SD) and coefficients of variation (CV) for each group of buffaloes. The data were statistically analyzed by applying the Student's t-test to obtain the significance of the difference of the mean values of the two groups ($p < 0.05$ was considered statistically significant and 95% confidence). The experimental procedures were carried out in accordance with the Romanian Legislation no. 43/2014 and Council Directive 2010/63/EU on the protection of animals used for scientific purposes.

RESULTS AND DISCUSSION

The results of laboratory determinations in buffaloes from the two groups are presented in table 1.

The hemoglobin level was 9.73±1.02 g/dL in group 1 and 12.68±0.73 g/dL in group 2. The results showed a significant variation ($p < 0.05$) in the hemoglobin level in buffaloes that showed normal estrus before of buffaloes with prolonged anestrus. The higher hemoglobin concentration in buffaloes that exhibited normal estrus can also be attributed to the fact that an animal requires more oxygen in any stressful condition and consequently the hemoglobin concentration can rise [10]. Although the level of hemoglobin does not directly influence reproductive disorders, still a low value of it could indirectly affect the functioning of the reproductive organs. A low level of hemoglobin influences the oxygenation of the tissues of the reproductive organs, which in turn could affect the cycling [7].

Table 1. Results of blood biochemical parameters in buffaloes cows from the two experimental groups

Blood parameters	Group 1 N=20		Group 2 N=20		t-test
	X±SD	CV %	X±SD	CV %	
Hemoglobin (g/dL)	9.73±1.02	14.37	12.68±0,73	20.12	$p < 0.05$
Total serum protein (g/dL)	6.12±0.36	22.16	8.40±0.56	17.46	$p < 0.05$
Calcium (mg/dL)	10.52±0.35	24.35	8.28±0.37	18.27	$p < 0.05$
Phosphorus (mg/dL)	3.64±0.48	18.42	4.27±0.77	11.31	$p < 0.05$
Magnesium (mg/dL)	3.06±1.22	6.83	3.63±1.32	8.46	ns
Alkaline phosphatase (UI/L)	181.95±53.17	23.17	175.19±48.29	27.36	ns
Cholesterol (mg/dL)	98.52±12.63	14.72	93.40±10.84	18.57	ns
Glucose (mg/dL)	42.18±2.62	34.58	54.18±4.95	26.12	$p < 0.05$
Lipase (UI/L)	7.76±2.45	14.38	7.12±2.90	16.74	ns
Urea (mg/dL)	40.80±5.77	46.17	38.20±4.49	35.92	ns

X±SD = mean ± standard error; CV = coefficient of variation; t-test = Student's t-test; ns = not significant ($p > 0.05$).

Total serum protein recorded values of 6.12±0.36 g/dL in group 1 and 8.40±0.56 g/dL in group 2. A significant difference ($p < 0.05$) was recorded in buffaloes in group 2. Protein deficiency causes uterine subinvolution which leads to prolonged anestrus after calving. Excess protein increases the incidence of retained fetal adnexa, genital infections and low fecundity.

Serum calcium was 10.52±0.35 mg/dL in group 1 and 8.28±0.37 mg/dL in group 2 and was within the limits described in the specialized literature by other authors (8 – 10.8 mg/dl) [5, 6, 8]. There was a significantly greater difference in calcium concentration ($p < 0.05$) in buffaloes from group 1.

The serum phosphorus level in buffaloes with anestrus (3.64±0.48 mg/dl) was significantly lower ($P < 0.05$) compared to than that of buffaloes with a normal cycle (4.27±0.77 mg/dl). The lower calcium level in G2 buffaloes is influenced by the existence of lactation [6]. Ca:P ratio for buffaloes cows with normal cycle was lower (1.94:1) compared to buffaloes cows with anestrus (2.89:1). Ca:P ratio should be 2:1 for better reproduction [7].

Serum magnesium (G1 - 3.06±1.22 mg/dl; G2 - 3.63±1.32 mg/dl) was within the physiological limits described in the specialized literature for buffaloes cows (1.8 – 3.8 mg/dl) [5, 6, 7, 8]. Magnesium plays a vital role in the metabolism of carbohydrates, lipids, nucleic acids and proteins.

Alkaline phosphatase (ALP) (G1 - 181.95±53.17 IU/L; G2 – 175.19±48.29 IU/L), had values within the physiological limits in buffaloes [5].

The individual cholesterol values in the buffaloes from both groups did not have significant differences (G1 - 98.52±12.63 mg/dL; G2 - 93.40±10.84 mg/dL), the reference range in the specialized literature being 73-280 mg/dL [5, 6, 8]. In the present study, buffaloes cows with normal cycle had significantly higher glucose concentration - $P < 0.05$, (54.18±4.95 mg/dL) compared to buffaloes with anestrus (42.18±2.62 mg/dL). Several authors have supported the opinion that glucose concentration reflects the energy status and reproductive activity of animals [7].

Hypoglycemia influences ovarian activity in animals by reducing the release of gonadotrophins from the hypothalamus [7]. Blood glucose variations in buffaloes are related to cycling and fertility [7].

Serum lipase level was 7.76±2.45 IU/L in G1 buffaloes and 7.12±2.90 IU/L in G2 buffaloes. Reference values of serum lipase are not presented in the specialized literature for buffaloes cows. Lipase is a key enzyme in triglyceride metabolism. Urea recorded values in the physiological thresholds (21-53 mg/dl) in buffaloes from both experimental groups.

CONCLUSIONS

From the present study it can be concluded that the levels of hemoglobin, serum proteins, glucose, calcium, inorganic phosphorus play an important role in the reproduction of buffaloes. The Ca:P ratio should also be close to 2:1 for better reproduction. Subclinical nutrient deficiencies are a cause of clinical anestry in buffaloes cows.

BIBLIOGRAPHICAL REFERENCES

1. Enculescu M. et al.; The Season Influence on Blood Parameters in Lactating Buffalo Females; 2017; Scientific Papers: Animal Science and Biotechnologies, 50 (1).
2. Enculescu M. et al.; Comparative study on metabolic biomarkers in lactating dairy cows and buffaloess; 2020; Scientific Papers. Series D. Animal Science, Vol. LXIII, No. 1.
3. Fagiolo A. et. al.; Environmental factors and different managements that influence metabolic, endocrine and immuno responses in water buffalo during lactation, 2004; p. 24-26. In Proceedings of the 7th World Buffalo Congress, Manila, Philippines.
4. Fabry L.; Erreurs alimentaires et troubles de la reproduction; 1993; Les élevages belges, no. 6.
5. Ferguson J.D.; Nutrition and reproduction in dairy cows; 1991; Ref. in Animal Breeding Abstract, no. 9, vol. 60.
6. Ghergariu S. (coord.); Patologia nutriţională şi metabolică a animalelor domestice (Nutritional and metabolic pathology in domestic animals); 1990; Edit. Academiei Române, Bucureşti.
7. Ingraham R.H. and Kappus L.C.; Metabolic profile testing; 1988; Veterinary Clinics North America. Food Animal Practice, 4: 391-411.
8. Patel M.D. et. al.; Estimation of blood biochemical parameters of **Banni buffalo** (*Bubalus bubalis*) at different age, sex and physiological stages; 2016; J. Livestock Sci. 7: 250-255.
9. Serdaru M. et. al.; Seasonal variations of some hematological and biochemical parameters of the **Carpathian Romanian Buffaloes**. I. The Winter Period; 2011; Scientific Papers: Animal Science and Biotechnologies, 2011, 44 (1).
10. Sharad K. et. al.; Comparative studies on metabolic profile of anestrous and normal cyclic **Murrah** buffaloes; 2010; Buffalo Bulletin (March 2010) Vol.29, No. 1.

OVARIAN RESPONSE IN BUFFALOES COWS WITH PROLONGED ANESTRUS DURING THE STABULATION PERIOD

RĂSPUNSUL OVARIAN LA BIVOLIȚELE CU ANESTRU PRELUNGIT ÎN PERIOADA DE STABULAȚIE

CHIOREAN REMUS[1], BOTA ADRIAN[1], MOLDOVAN MĂDĂLINA[1]

[1] Research and Development Station for Buffalo Breeding, Sercaia, 507195,
street Campului, no 2, Brasov county, Romania

Correspondence address: scdcb.sercaia@yahoo.com

Abstract

Large calving interval at buffalo cow is considered guilty for the existence of periods of the year when sexual activity is missing. It is considered that if fertilization did not take place before the buffaloes cows entered the cold, stabling period, then it will take a long time until they enter again in the heat and the calving interval will be much longer. The work was conducted over 4 years and consisted of evaluation of ovarian response following administration of a prostaglandin hormone preparation dose of prostaglandin $PGF_{2\alpha}$ *during the stabling period to a number of 61 buffaloes cows aged between 5 and 14 years of age, with a good maintenance status, and the interval from the last cure to treatment was over 90 days. In 22 buffaloes cows that showed estrus within 5 days of* $PGF_{2\alpha}$ *administration, two artificial inseminations were performed at 12 hours. In 39 buffaloes cows that did not show signs of the estrus, a second dose of* $PGF_{2\alpha}$ *was given 11 days after the first administration, after which they were artificially inseminated at 48 and 78 hours, respectively. Of the 22 buffaloes cows that showed estrus, 19 heads remained pregnant, and out of the 39 buffaloes cows with prolonged treatment, only 20 heads remained. The treatment of sexual inactivity in buffaloes cows during stabling by hormonal methods with* $PGF_{2\alpha}$ *derivatives results in a shortening of the calving interval, the cost of hormonal treatments being offset by the increase in the number of calves obtained and the production of milk.*

Key words: buffaloes cows, calving interval, anestrus, prostaglandins, artificial insemination, estrus

Rezumat

Pentru intervalul mare dintre fătări la bivoliţe, se consideră că vina o poartă existenţa unor perioade din an în care activitatea sexuală lipseşte. Se consideră că dacă fecundarea nu a avut loc înainte de intrarea bivoliţelor în perioada rece, de stabulaţie, atunci va trece un timp îndelungat până ce acestea vor intra din nou în călduri şi intervalul dintre fătări se va prelungi mult. Lucrarea s-a realizat pe parcursul a 4 ani şi a constat în evaluarea răspunsului ovarian, în urma administrării unei doze de preparate hormonale pe bază de derivaţi ai prostaglandinei $PGF_{2\alpha}$*, în perioada de stabulaţie, la un număr de 61 bivoliţe, cu vârste cuprinse între 5 şi 14 ani, care prezentau o stare de întreţinere bună, iar intervalul de la ultima fătare la tratament a fost de peste 90 zile. La 22 bivoliţe care au manifestat călduri până în 5 zile de la administrarea* $PGF_{2\alpha}$*, s-au efectuat două inseminări artificiale la interval de 12 ore. La 39 bivoliţe care nu au manifestat semnele căldurilor, s-a administrat o a doua doză de* $PGF_{2\alpha}$*, la 11 zile de la prima administrare, după care s-au inseminat artificial la 48 respectiv 78 ore. Din cele 22 bivoliţe care au manifestat estru, 19 capete au rămas gestante, iar din cele 39 bivoliţe cu tratament prelungit, au rămas gestante numai 20 capete. Tratamentul anestrului la bivoliţe, în perioada de stabulaţie, prin metode hormonale, cu derivaţi ai* $PGF_{2\alpha}$*, duce la scurtarea intervalului între fătări, costul tratamentelor hormonale fiind compensat de creşterea numărului de viţei obţinuţi şi a producţiei de lapte.*

Cuvinte cheie: bivoliţe, interval între fătări, anestrus, prostaglandine, inseminare artificială, estru.

INTRODUCTION

The long interval between calvings in buffaloes cows is due to the long gestation period, dull, hardly observable heats, and the seasonality of buffalo reproduction. Cleared heats are more difficult for the breeder to observe, but in the case of natural mounts, the turmacs are able to detect all buffaloes cows in heat.

However, even in the case of natural pairing there are long intervals between calvings caused by returns at long intervals of time, a fact explained by some researchers by a high frequency of embryonic mortality.

The greatest influence on the long duration between calvings is the seasonality of reproduction, the existence of periods of the year when sexual activity is absent. It is believed that if fertilization has not occurred before the buffaloes cows enter anestrus then it will be a long time before they come into heat again and the interval between calvings will be much longer.

The size of the interval between calvings is dependent on the resumption of ovarian activity after calving, fecundity and preimplantation embryonic mortality.

During pregnancy, the lack of heat is due to the secretion of progesterone, which maintains the relaxation of the uterine muscles and inhibits the maturation of the ovarian follicles. Parturition, triggered by the mass and secretions of the fetal adrenal gland, empties the uterus of its contents and allows the secretion of prostaglandins that lyse the corpus luteum and suppress the secretion of progesterone.

As a result, the gonadotropic hormones of the pituitary (FSH and LH) cause the activation of the ovary and the initiation of the ovarian cycle. The delay in the exit of the ovary from the state of anestry is attributed, in the off-season (winter period), to the absence of pituitary hormonal stimuli that are not released by GnRH. This explanation is, however, tempered by the fact that in the cold season the fodder resources are less, lacking the necessary tonic energy to trigger follicular maturation. Deficiencies in hormonal stimuli, insufficiency of progesterone secretion, or an energy deficit in the diet leads to a high rate of ovulatory or blastocystic embryonic mortality, produced before nidation, or, to a lesser extent, even to later embryonic mortality. In buffalo bulls, unlike female buffaloes, in which the sexual activity manifests cyclically in the form of heat cycles, the sexual desire (libido) is continuous.

However, the concrete fact that in the winter period, the manifestation of libido is very low, we can blame it on the thyroid and pituitary gland, which through the decreased secretion of thyroxine and respectively ICSH (hormone to stimulate the interstitial cells of the testicle) discourages the secretion of testosterone and the expression of sexual reflexes.

MATERIAL AND METHOD

The work was carried out for four years and consisted in the administration of $PGF_{2\alpha}$ based on hormonal products under different commercial names, aiming at the resumption of the sexual cycle, with the appearance of heat followed by artificial insemination or natural heat, using the luteolytic effect of prostaglandins.

Following the transrectal examination, 61 anestrus buffaloes were identified, with a period greater than 90 days since calving, aged between 5 and 14 years and in good maintenance condition. The protocol consisted of administration of $PGF_{2\alpha}$, watching for heat onset and artificial insemination or natural mating.

In buffaloes that did not show heat after the first administration, another dose of $PGF_{2\alpha}$ was administered 11 days after the first inoculation, the onset of heat and artificial insemination or natural mating were followed. In those that did not show heat, blind artificial insemination was performed at 72 and 96 hours, respectively.

RESULTS AND DISCUSSION

Ovarian response after prostaglandin treatment in buffaloes cows is shown in Table 1.

Table 1. Results obtained as a result of prostaglandin treatments in buffaloes cows

Reference year	Treated buffaloes cows (heads)	Preparation used (trade name)	First administration of $PGF_{2\alpha}$		Second administration of $PGF_{2\alpha}$				Total pregnancies (number and %)	Birth rate %
			Artificial insemination (heads)	Gestation (number)	Artificial insemination (heads)	Gestation (number)	Artificial insemination at 72-96 hours (heads)	Pregnancies (number)		
2020	18	Flavoliz	5	5	-	-	13	8	13 (72,2 %)	92,3
2021	21	Prosolvin	7	7	4	4	10	4	15 (71,4 %)	93,3
2022	12	Enzaprost	4	4	-	-	8	3	7 (58,3 %)	100
2023	10	Prosolvin	6	3	2*	1	2	-	4 (40%)	-
Total	**61**	**-**	**22**	**19**	**6**	**5**	**33**	**15**	**39 (63,9 %)**	**-**

***- natural mount**

Of the 22 buffaloes cows that showed estrus after the first treatment, 19 cows remained pregnant, the fecundity being 86.36%, and of the 39 buffaloes cows with prolonged treatment (after the second inoculation), only 20 remained pregnant, the fecundity obtained being of 51.28%. Overall, the fecundity obtained was 63.9%, the birth rate being higher than 92%.

Although the detection of ovarian formations in buffalo cow is more difficult than in cows due to the small size of the ovaries, following a correct diagnosis by transrectal examination, it is possible to act with hormonal preparations to relaunch ovarian function and implicitly to obtain pregnancies even in the cold season of the year.

At the same time, it was found that animals with poor maintenance conditions, those with advanced age or those that presented various ailments in the post-partum period, did not respond positively to this treatment. The daily presence of the buffalo bull around the females has beneficial effects, even if he has a reduced libido in the cold season.

CONCLUSIONS

1. The use of preparations based on $PGF_{2\alpha}$ helps to increase the percentage of pregnancies and implicitly the live products obtained as well as increase the production of buffalo milk;
2. The method is especially beneficial for family farms raising buffaloes cows, where as a rule the buffaloes are sexually inactive during the winter period, the cost of the treatment being affordable;
3. The method also helps to expand artificial insemination, especially for farmers who are only used to natural breeding, bulls usually not breeding during the cold period of the year.

BIBLIOGRAPHICAL REFERENCES

1. Braselli S.P. et al; Ovarian follicular by nomeres during the estrous cycle in buffalo; 1997; Technology, 44(8).
2. Bogdan A.T. et al.; Livestock reproduction; 1981; Scrisul romanesc Publishing House, Craiova.
3. Creta V. et al.; Some indices of breeding and their influence on buffaloes milk production; 1993; Lucrări științifice USAMV, seria C, Vol XXII, Bucharest.
4. Ilinca N. et al.; Research on the introduction of the poliovulation to buffalo cows; 1993; Lucrări științifice taurine, vol. 14, Bucharest.

INNOVATIVE AQUACULTURE TECHNOLOGY REGARDING THE INFLUENCE OF THE POPULATION DENSITY ON THE DEVELOPMENT OF THE SPECIES *SANDER LUCIOPERCA* (LINNAEUS - 1758) IN THE POSTEMBRYONIC PERIOD

TEHNOLOGIE INOVATIVĂ DE ACVACULTURĂ PRIVIND INFLUENȚA DENSITĂȚII DE POPULARE ASUPRA DEZVOLTĂRII SPECIEI *SANDER LUCIOPERCA (*LINNEAUS – 1758) ÎN PERIOADA POSTEMBRIONARĂ

DOBROTĂ GHEORGHE[1], DOBROTĂ NICOLETA–GEORGETA[1], RADU DANIELA[1], COSTACHE MIHAIL[1], MARICA NINO[1]

[1]Fish Culture Research and Development Station of Nucet, 549 Principala Street, 137335, Nucet, Dambovita County, Romania, tel. 0245267003, email: scp_nucet@yahoo.com

Correspondence address: dobrota19dng@yahoo.com

Abstract

The pikeperch (Sander lucioperca, L.- 1758) is one of the freshwater species recently introduced in intensive aquaculture. In the last decade great efforts have been made 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, the most significant losses being recorded in the post-embryonic period. Therefore, the growth of the pikeperch in the post-embryonic period was experienced in "Evos" type fiberglass pools, in three experimental versions, with three different population densities as fallows: 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 Fish Culture Research and Development Station of Nucet, in triplicate, during three growing seasons (2021, 2022 and 2023). The best results were obtained in V1 version, where the survival rate was 69.5% in 2023, the average individual growth rate in 2022 was 1.555 g/ex., and the Fulton coefficient was between 0.93 - 1.13.

Key words: density, fry, pikeperch, survival weight

Rezumat

Șalăul *(Sander lucioperca, L.- 1758) este una din speciile de apă dulce recent introduse în acvacultura intensivă, în ultima decadă, depunându-se eforturi mari în direcția dezvoltării culturii intensive a acestei specii. Densitatea de populare este un parametru tehnologic deosebit de important pentru creșterea peștelui, în toate etapele de dezvoltare și este specifică speciei, vârstei și tehnologiei aplicate, cel mai greu de realizat și cu pierderile cele mai însemnate fiind în perioada postembrionară. Prin urmare, s-a experimentat creșterea șalăului în perioada postembrionară, în bazine din fibră de sticlă de tip „Evos", în trei variante experimentale, cu densități de populare diferite: V1- 1000 ex/bazin, V2- 2000 ex/bazin și V3- 3000 ex/bazin. Au fost două momente critice în perioada de dezvoltare postembrionară, primul a fost la începutul hrănirii exogene și al doilea în timpul umflării vezicii gazoase. Experimentele s-au realizat la S.C.D.P - Nucet pe parcursul a trei sezoane de creștere (2021, 2022 și 2023), în triplicat. Rezultatele cele mai bune au fost obținute în varianta V1, unde rata de supraviețuire a fost de 69,5% în anul 2023, sporul mediu de creștere individual a fost de 1,555 g/ex în anul 2022 și coeficientul Fulton cuprins între 0,93 - 1,13.*

Cuvinte cheie: alevinaj, densitate, șalău, greutate, supraviețuire

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 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 and Schmidt, 1980; Steffens, 1986); Ruuhijärvi et al., 1991; Hilge and Steffens, 1996; Ruuhijärvi and 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 one European part (*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 2021-2022-2023 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 / minute, depending on the temperature.

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 populating;
- 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.

Figure 1. Pilot installation ("Evos" type basin) for raising and the development of pikeperch larvae in the post-embryonic period / Instalație pilot (bazin tip „Evos”) pentru creșterea și dezvoltarea larvelor de șalău în perioada post-embrionară

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 variation from 1-10 exemplars.

The working methodology considered the following aspects:

- three experimental variants were established in terms of population density;
- “Evos” type pools/experimental variants: 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 no. 1 and figure 2 and 3):

- variant I: 1000 ex./basin;
- variant II: 2000 ex./basin;
- variant III: 3000 ex./basin.

Table 1. Population of the "*Evos*" baths with 7-8 days old larvae / Popularea bazinelor „Evos” cu larve de 7-8 zile

No. crt.	Experimental Variant	Basin Volume (l)	Ex. / basin	Nr. basin	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

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 the unit;
- 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;

- almost 20 days of feeding exclusively with fodder, the live food being administered sporadically, once every three days for 5 days, and 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 2021-2022-2023 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.

Figure 2. The 7-8 day old pikeperch larvae population / Populația de larve de șalău în vârstă de 7-8 zile

During the experiments, the main water physico-chemical parameters (temperature, dissolved oxygen, pH, total nitrogen and nitrites) were within normal limits for a fish water, according to OMMGA no. 161/2006 and were not significantly affected by population density (table no. 2).

Table 2. The water physical and chemical indicators average values for the period 2021-2023 / Valorile medii ale indicatorilor fizico-chimici ai apei pentru perioada 2021-2023

No. crt.	The chemical parameter		U.M.	Parameter values		
				Source	Experimental basins	Optimal according to OMMGA no. 161/2006
				Average years 2021–2022- 2023		
1	pH		Units pH	7.1	7.5	7-7.8
2	Alkalinity		mg /l	158	168	200-400
3	Calcium (Ca^{2+})		mg/l	32.0	31.2	90-120
4	Magnesium (Mg^{2+})		mg/l	15.07	16.05	10-40
5	Ca^{2+}/ Mg^{2+}		mg/l	2.12	1.32	5
6	Organic Matter		mg $KMnO_4$/l	16.18	22.76	20-60
7	Oxygen		mg/l	7.54	6.76	5-12
8	Ammonia (NH^+_3)		mg/l	missing	missing	missing
9	Nitrogen (NO^-_3)		mg/l	missing	0.106	2.5-4
10	Nitrogen (NO^-_2)		mg/l	0.0023	0.003	0.03
11	Phosphates (PO^{3-}_4)		mg/l	missing	0.04	0.05-1.5
12	Chlorides	Cl^-	mg/l	8.48	10.60	40
		Na Cl	mg/l	14.03	17.53	30
13	Ammonium (NH^+_4)		mg/l	0	0.065	0.5-1
14	Total hardness		(^{0}D)	7.95	8.06	12

Analyzing the data on the larvae growth and development in the post-embryonic period (table no. 3 and 4) by the following biotechnological indicators: average mass (W med./ex., figure 4), total length (TL), survival rate (figure 5) and the Fulton coefficient (figure 6) the following results are found:

Average mass (W) - g/ex.

- the pikeperch larvae highest average mass after 40 days was obtained in the experimental version V2 in 2022 (1.555 g/ex.), and the lowest was obtained in the V3 version (2021 of 0.821 g/ex);
- in 2021 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 2022 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 2023 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.

Table 3. The values of the biotechnological indicators for the development of the pikeperch by variants/years in the post-embryonic period in the years 2021, 2022 and 2023 / Valorile indicatorilor biotehnologici pentru dezvoltarea șalăului pe variante / ani în perioada post-embrionară în anii 2021, 2022 și 2023

Year	Variant	Basin	No. ex. populated	No. ex. achieved	Average W (g)	Average L (mm)	Sv (%)
2021	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
2022	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
2023	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

Total length (TL) - mm/ex

- the highest average total length of pike larvae after 40 days was obtained in the experimental variant V1 in 2022 (55.0 mm/ex.), and the smallest was obtained in the variant V3 (41 mm/ex.) in the year of 2022;
- in 2021, 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 2022, 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;

- in 2023, 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.

Table 4. Average results on experimental variants / Rezultate medii pe variante experimentale

Year	Variant	Basin	Average survival. (%)	Average W/variant (g)	Average Total Length / variant (mm)	Fulton Coefficient
2021	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
2022	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
2023	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

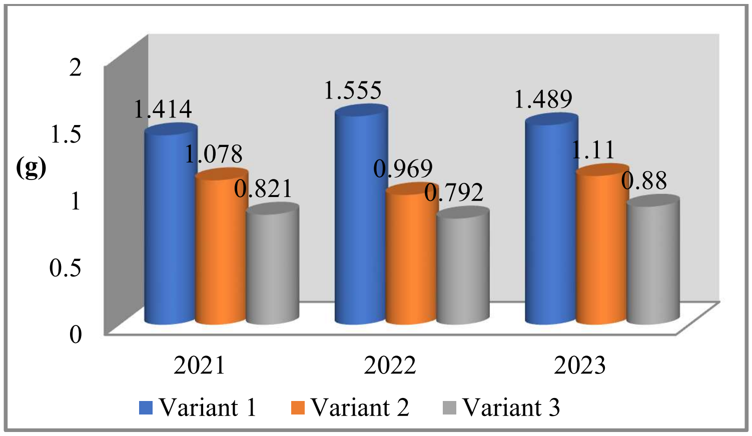


Figure 5. Average weight variation / Variația medie a greutății

Survival rate (%)

- after 40 days of post-embryonic growth, the highest survival rate was obtained in the experimental variant V1 in 2023 (69.5%), and the lowest was obtained in the variant V3 in 2021 of 44.2%;
- in 2021, 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;
- in 2022, 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 2023, 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:

- in 2021 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 2022 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 2023 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.

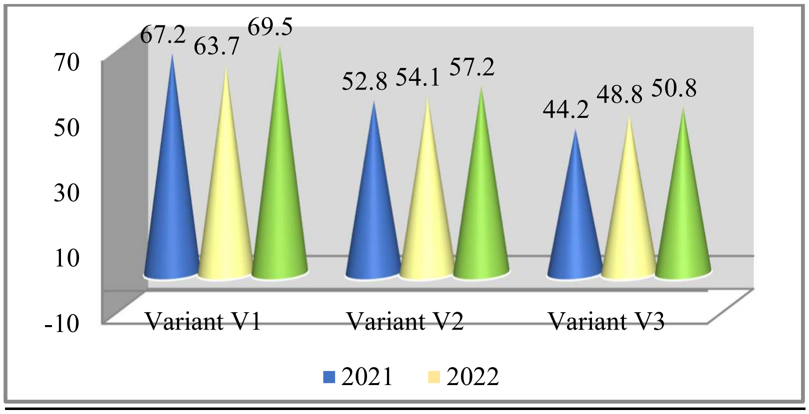


Figure 6. Survival rate variation / Variația ratei de supraviețuire

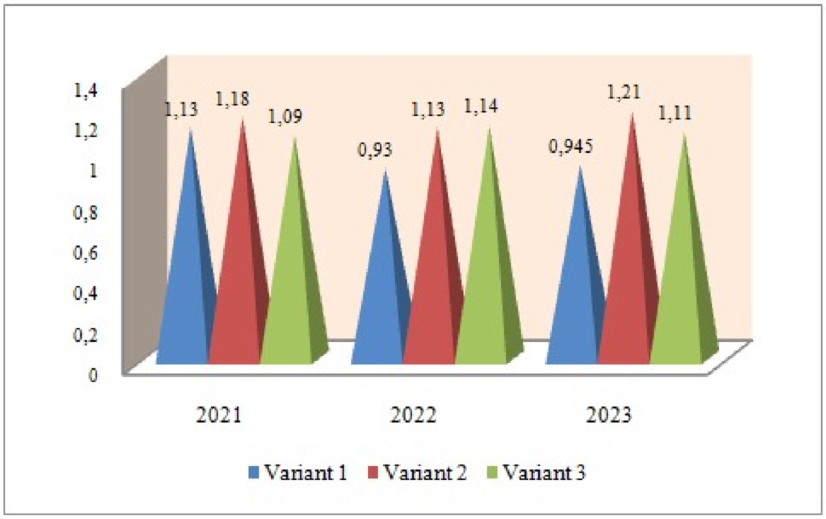


Figure 7. Fulton coefficient variation / Variația coeficientului Fulton

CONCLUSIONS

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 8):

- existing yolk sac but with small dimensions;
- gill buds without being covered by lids;
- transparent body through which the brain and the spine primordia are observed;
- the larvae swim lightly.

II. At the age of 17-18 days (figure 9):

- less transparent body with a tendency to colorate to yellowish, with pigment spots that cover almost entirely the body surface;
- 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, Cladocera's, 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 10):

- the body is no longer transparent, the pigment spots of brown color cover almost the entire body surface;
- 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 Cladocera from the water of the settling tank.

Figure 8. - 10. Post embryonic larvae of 7-10 days old, pikeperch larvae of 17-18 day sold, pikeperch larvae of 27-28 days old / Larve post-embrionare de 7-10 zile, larve de șalău de 17-18 zile vândute, larve de șalău de 27-28 de zile

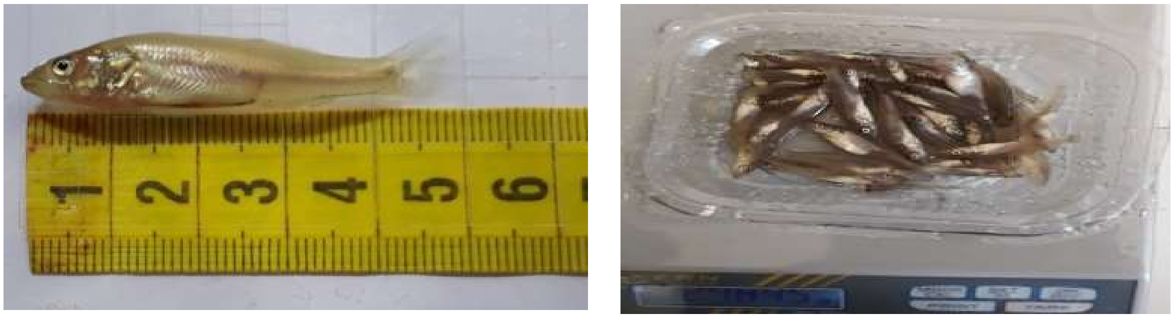


Figure 11. Pikeperch larvae at the end of the post-embryonic period / Larve de șalău la sfârșitul perioadei post-embrionare

IV. At the age of 40 days (figure 11):

- 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 the remaining 10% live food.

BIBLIOGRAPHICAL REFERENCES

1. Bnińska M., Wołos A.; Management of selected Polish commercial and recreational lake fisheries activities; 2001; Fish Manage Ecol 8:333–343.
2. Dobrotă Ghe., Oprea L., Dobrotă N G., Costache M., Marica N., Radu S.; Aspects regarding the controlled reproduction of pikeperch (*Sander lucioperca Linne, 1758*) in industrial aquaculture systems; 2021; Scientific Papers. Series D. Animal Science. Vol. LXIV, No. 2, 2021, ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285.
3. Hilge V., Steffens W.; Aquaculture of fry and fingerlings of pike-perch (*Stizostedion lucioperca L.*) – a short review; 1996; Journal of Applied Ichthyology 12:167–170.
4. Korycki A.; Pikeperch (in Polish); 1976; PWRiL, Warsaw, Poland.
5. Jankowska B, Zakęś Z., Zmijewski T, Szczepkowski M.; Fatty acid profile and meat utility of _ wild and culture dzander, Sander lucioperca (L.); 2003; Electronic Journal of Polish Agricultural Universities 6(1): http://www.ejpau.media.pl/ volume 6/issue1/ fisheries/art-02.html.
6. Mamcarz A., Kucharczyk D., Kujawa R., Skrzypczak A.; Influence of fishdensity on survival, growth and cannibalism development of pikeperch (*Stizostedion lucioperca L.)* larvae; 1997; In: Creswell L, Harache Y (eds) Island aquaculture and tropical aquaculture: Proc 2nd Int Workshop Aquaculture Percid Fish. European Aquaculture Society, Oostende, Belgium, pp 343–346.
7. Molnar T., Hancz Cs., Bodis M., Müller T., Bercsényi M., Horn P.; The effect of the initial stocking density on the growth and survival of the pikeperch finger lingreared under intensive conditions; 2004; Aquaculture Int. 12:181-189.
8. Nagiéć M.; The growth of pikeperch Lucioperca lucioperca (L.) in the lakes of Northern Poland (in Polish); 1961; Roczniki Nauk Rolniczych 77B:549–580.
9. Ruuhijärvi J., Hyvärinen P.; The status of pike-perch culture in Finland; 1996; Journal of Applied Ichthyology 12:185–188.
10. Ruuhijärvi M., Virtanen E., Salminen J., Muyunda M.; The growth and survival of pike-perch (*Stizostedion lucioperca L.*), larvae fed formulated feeds; 1991; In: Lavens P, Sorgeloos P, Jespers E, Ollevier F (eds) LARVI'91 – Fish Crustacean Larvicult Symposium, European Aquaculture Society, Gent, Belgium, pp 154–156.
11. Schlumpberger W., Schmidt K.; Vorlaufiger Stand der Technologie zur Aufzucht von vorgestreckten Zandern, (*Stizostedion lucioperca L.*); 1980; Z. Binnenfischerei, DDR 27:284–286.
12. Steffens W.; Intensive fish production; 1986; 1st edition (in Polish). PWRiL, Warsaw, Poland.
13. Szkudlarek M.; Factors influencing the rearing effectiveness of zander, Sander lucioperca (L.) larvae under recirculation system conditions (in Polish); 2004; PhD thesis, Inland Fisheries Institute, Olsztyn, Poland Klein Breteler, (1998).
14. Szkudlarek M., Zakes Z.; The effect of stocking density on the effectiveness of rearing pikeperch, *Sander lucioperca* (L.), summer fry, 2004; Arch Pol Fish 10:115-119.
15. Terlecki W.; Rearing of pikeperch stock material; 1955; 1st edition (in Polish), PWRiL, Warsaw, Poland.
16. Wołos A., Teodorowicz M., Brylski H.; Socio-economic analysis of recreational fisheries in two departments of the Polish Anglers Association, based on the results of the registration of anglers' catches; 1998; In: Hickley P, Tompkins H (eds) Recreational fisheries, social economic and management aspects. Fishing News Book, Oxford, pp 34–47.
17. Zakęś Z., Szczepkowski M., Szkudlarek M.; Production of pikeperch, Stizostedion lucioperca (L.) to market size in water recirculation systems; 2000; In: Grinzevskij MV (ed) Presnovodnaja akvakultura v Centralnoj I Vostocnoj Evropie: dostizenija I perspektivy. Institut Rybnogo Hozjaistva, Kiev, Ukraine, pp 214–216.

ACTA AGRICOLA ROMANICA, Volume 6, Year 6, No. 6.3.

EVALUATION OF WOOL PRODUCTIVITY POTENTIAL OF CROSSBREEDS BETWEEN LOCAL SHEEP BREEDS AND IMPORTED SPECIALIZED BREEDS

APRECIEREA POTENȚIALULUI PRODUCTIV PENTRU LÂNĂ A HIBRIZILOR RASELOR LOCALE DE OVINE CU RASE SPECIALIZATE IMPORTATE

ENCIU ANA[1], ZAMFIR ZOIA CAMELIA[1], ILIȘIU ELENA[2], NICOLESCU ALINA[1]

[1]Institute of Research and Development for Sheep and Goats Breeding Palas, Constanta, I.C. Bratianu, 248, Constanta, 0241.639.506, icdcocpalas@yahoo.com
[2] Institute of Research and Development for Sheep and Goats Breeding Palas, Constanta, Reghin Experimental Base, Mures, 11 Dedradului, 0241.639.506, statiuneareghinmures@yahoo.co.uk

Correspondence address: ana_enciu @yahoo.com

Summary

The present study aimed to evaluate the quantitative level of wool production in local sheep breeds and their crossbreeds with imported meat breeds and to determine the quality parameters of the fibres that can be used as criteria for selection and improvement of wool production in sheep and that can form the basis of a classification/sorting system appropriate to the current structure of sheep breeds and breeding directions in Romania. Productive potential was assessed by individual weighing of wool fleeces collected during the sheep shearing season and by laboratory analysis of randomly samples, on which the following quality parameters were determined: fibre fineness, relative length of the staples, individual fibre length, degree of crimp, degree of impurification.

The results obtained led to the conclusion that under the current conditions of sheep breeding and exploitation the quantitative and qualitative level of wool production is below the breeds-specific potential, as illustrated by both the average value of these parameters and the high percentage of individuals performing above the flock average. There is therefore a potential to create groups (sheep lines) in which, through efficient selection, quantitative and qualitative improvements in wool production can be achieved. These sheep lines can achieve increases in wool production of 8 ≈ 17%, improvements in wool fineness expressed by a reduction in fiber diameter of 5 ≈ 12% and increases in absolute wool length of 2 ≈ 5%.

Keywords: sheep crossbreed, wool production, fibre diameter

Rezumat

Prezentul studiu a avut ca scop evaluarea nivelului cantitativ al producției de lână la rasele locale de ovine și la metișii acestora cu rase de carne importate și determinarea parametrilor de calitate ai fibrelor care pot fi utilizați ca criterii de selecție și îmbunătățire a producției de lână la ovine și care pot sta la baza unui sistem de clasificare/sortare adecvat structurii actuale a raselor de ovine și direcțiilor de creștere din România. Potențialul productiv a fost evaluat prin cântărirea individuală a cojoacelor de lână recoltate în timpul sezonului de tundere a oilor și prin analiza de laborator a probelor de lână colectate randomizat, pe care s-au determinat următorii parametri de calitate: finețea fibrelor, lungimea relativă a șuvițelor, lungimea individuală a fibrelor, gradul de ondulare, gradul de impurificare.

Rezultatele obținute au evidențiat faptul că în condițiile actuale de creștere și exploatare a ovinelor nivelul cantitativ și calitativ al producției de lână este sub potențialul specific raselor, fapt ilustrat atât de valoarea medie a acestor parametri, cât și de procentul mare de indivizi cu performanțe peste media efectivului (plusvariante). Prin urmare, există oportunitatea de a crea nuclee (linii de ovine) în care, printr-o selecție eficientă, se pot obține îmbunătățiri cantitative și calitative ale producției de lână. Aceste linii de ovine pot înregistra creșteri ale producției de lână de 8 ≈ 17%, îmbunătățiri ale fineții lânii exprimate printr-o reducere a diametrului fibrelor de 5 ≈ 12% și creșteri ale lungimii absolute a lânii cu 2 ≈ 5%.

Cuvinte cheie*: *metiși ovine, producție de lână, diametrul fibrelor

INTRODUCTION

Sheep breeding is a field of animal husbandry with a long tradition in Romania and the profitability of this sector require the orientation towards productions that correspond to the biological characteristics of the breeds, the current market requirements and the ecological principles of production. The reconsideration of the exploitation directions and the orientation of sheep farming on market economy principles have stimulated the concerns of breeders to increase milk and meat production. In this context, the selection and improvement of local sheep breeds and crossbreeding with specialised breeds for these productions have led to quantitative and, above all, qualitative changes in wool production. Newly created sheep populations are characterised by a higher variability of wool parameters within the fleece, which in the context of the lack of a standard for classifying raw wool obtained in Romania implies a more difficult and elaborate classification process.

At present, when the raw wool produced in the country is no longer used by existing textile mills, the collecting activity is carried out by economic agents, who in most cases carry out the grading, sorting and baling operations. The wool sorting activity is done on the basis of criteria related to: breed, fineness, fibre length, colour, health, age, origin (from shearing sheep, from slaughtered sheep).

The operation that is carried on at the shearing site (at the farm) is more of a grading and pre-sorting operation, which consists of removing from the fleece of the extremely dirty and more degraded portions. The actual sorting, which involves breaking the wool fleeces into pieces and dividing the batches of wool according to fineness and length, corresponding to the textile processing systems (carding, combing), is usually performed at the industrial processing centre.

A better understanding of the properties of fibres and how they are classified will enable the sheep breeder to identify the major issues to be considered, namely the genetic uniformity of the flock and the management of the wool harvesting and preparation process (Emma K Doyle, et..al. .[1], C. J. Lupton et.al.[3], Muhammad Jamshed Khan et.al.[6]).

The commercial value of wool is determined by its grade, class and quality. The grade (category) to which wool belongs refers mainly to the diameter or thickness of the fibres. Grade refers to the length of the individual fibres (absolute length) or the length of the wool staple (relative length). Quality in this case refers to the absence of impurities in the fleece (equivalent to a low degree of wool dirtiness). When the wool is graded (fineness is determined) the whole fleece is placed in a fineness category corresponding to an average fibre diameter and then placed in a batch containing similar values of wool fineness. Grading and classifying are different processes from sorting, which involves fragmenting and classifying the fleeces into lots based on fineness, length, tensile strength, wash yield, colour and style. The sorting of wool is the first step in the processing route of this raw material and an important step in adding value to producers (sheep farmers) (Le Valley [2], Macpherson S. [4], Clay P. Mathis et.al. [5]),

Different classification systems have been established based on certain fibre characteristics and spinning capacity. Since the fineness (diameter) of wool fibres determines the spinnability of the fibres - their ability to spin into yarn - almost all classification systems take this characteristic into account. The most important classification systems are: the English (Bradford) system; the French system; the German system; the American system; the Australian system. The standard for grading wool will become the direct measurement system, based on the exact determination of the diameter of individual fibres (size expressed in microns). According to this system there are 16 classes of wool grading in which fineness is expressed by the average fibre diameter. An increasing percentage of wool production will be sold on the basis of objective measurements as textile traders and processors demand superior fibre quality and uniformity (Clay P. Mathis et.al. [5], [7], [8]).

The analysis of the wool classification of sheep breeds raised for meat production showed that they fall into the medium-course wool class in terms of diameter. The general recommendation for their classification takes into account the following aspects: the colour of the extremities short hair, the length of the fibres, the degree of contamination with impurities and the risk associated with the presence in the fleece of pigmented fibres, the tendency to shed, the presence of significant amounts of medullar/dead fibres.

Flocks of sheep breeds for meat can be placed along different lines in terms of wool fineness when volume and market opportunities are financially advantageous. Hybrids of these breeds are classified according to essentially the same criteria as the paternal (meat) breeds and are recommended to be collected in different lines. Also, depending on needs and fibre diameter long wool lines can be separated.

In view of the mentioned issues this study aims were:

- To evaluate the quantitative level of wool production in local sheep breeds and their crossbreeds with imported meat breeds.
- To determine the qualitative parameters of the fibres for the wool samples collected and to establish on the basis of experimental data, the current limits of the qualitative parameters that can constitute criteria for selection and improvement of wool production in sheep and that can provide the basis for a classification/sorting system appropriate to the current structure of breeds and directions of sheep farming in Romania.

MATERIAL AND METHOD

An extensive study was performed at ICDCOC Palas Constanta (Institute for Research and Development Sheep and Goat Breeding Palas - Constanta) and at the Reghin and Bilciurești Experimental Bases, where flocks of six local sheep breeds (**Palas Merino, Palas Meat Breed, Palas Milk Breed, Palas Prolific Breed, Tzigai, White Tzurcana**) and their crossbreeds with imported sheep breeds for meat production (**Rouge del`Quest, Texel, German Blackhead - Schwarzkopf, Mouton Vendeen** and **Berrichon du Cher**) had been analized in terms of wool productions.

During the period 2018-2022, a number of 450 half-breeds was studied from which 170 wool samples were collected for laboratory analysis. The activities carried out aimed to:

- individual weighing of the fleeces and determination of average wool production by age and sex categories ;
- random collecting of wool samples to determine fibre quality parameters;
- determination in the laboratory of the following characteristics of the wool quality: average wool fibre diameter, relative length, absolute length, degree of crimp, degree of wool impurity.

The data obtained were centralised and statistically processed.

RESULTS AND DISCUSSION

The wool production potential of the analysed sheep crossbreeds is shown by the quantities of wool obtained, values presented in Table 1.

In the case of the flocks studied at ICDCOC Palas the paternal breeds recorded the following levels of wool production: **Rouge de l' Ouest** 2.0 ≈ 4.3 kilos, **Texel** 1.8 ≈ 4.6 kilos and the maternal breeds **Palas Prolific Breed** 2 ≈ 5 kilos, **Palas Meat Breed** 2.5 ≈ 6.5 kilos. Compared to these the F_1 half-breeds obtained intermediate amounts of wool, respectively about 4 kilos at young female sheep and 4.8-5 kilos at male yearlings.

The data presented in Table 1 show the superiority of the male yearlings in terms of wool production, the **Palas Prolific half-breeds** had approximately 18.00% more wool than the young female sheep and the **Palas Meat** young male half-breeds produced 30.69 ≈ 32.11% more wool than the female yearlings.

Table 1. Average wool production of crossbreed sheep / Producția de lână la ovinele

Breed \ Category	Parameter	Average wool quantity (kilos)		Ranges of wool quantity (kg)	% plusvariants
		$x \pm s_x$ (kg)	CV (%)		
Rouge de l' Ouest x **Palas Prolific Breed (RPP)**	Young Male TMAP	4.771 ± 0.249	13.7811	4.0 ≈ 5.7	42.86
	Young Female TFAP	3.903 ± 0.068	15.1823	2.8 ≈ 5.5	53.33
Texel x **Palas Prolific Breed (RPP)**	Young Male TMAP	4.980 ± 0.376	16.8842	3.6 ≈ 5.7	60.0
	Young Female TFAP	4.091 ± 0.105	17.4272	3.0 ≈ 6.1	36.96
Rouge de l' Ouest x **Palas Meat Breed (RCP)**	Young Male TMAP	6.200 ± 0.2006	10.2292	5.4≈ 7.4	60.0
	Young Female TFAP	4.209 ± 0.086	16.9871	2.6 ≈ 6.2	44.12
Texel x **Palas Meat Breed (RCP)**	Young Male TMAP	5.783 ± 0.3859	16.3459	4.8 ≈ 7.0	33.33
	Young Female TFAP	4.008 ± 0.1186	18.7108	2.7 ≈ 6.2	55.50
Schwarzkopf (GCN) x **Tzigai**	Ewes	2.289 ± 0.046	23.7872	1.0 ≈ 3.9	47.55
Berrichon du Cher (BC) x **Schwarzkopf** x **Tzigai**	Young Female TFAP	3.459 ± 0.213	20.4291	2.0 ≈ 4.4	63.64
Mouton Vendeen (MV) x **Schwarzkopf** x **Tzigai**	Young Female TFAP	4.189 ± 0.152	19.1535	2.5 ≈ 6.3	42.86

The coefficient of variation of wool production for the F_1 crossbreeds of **Rouge l' Ouest** and **Texel** with **Palas Prolific Breed** was 13.8 ≈ 17.4 % and respectively 10.23 ≈ 18.71 % for the F_1 crossbreeds **Rouge l' Ouest** and **Texel** with **Palas Meat Breed**. Also, the existence of more than 40 % plus variants in the flocks of the crossbreeds analysed at ICDCOC Palas shows a potential for selection for the quantitative level of wool production. Thus, for a selection group (line) of **Palas Prolific half-breeds**, an increase in average wool production of 9.2 ≈ 15.7 % can be obtained, with an average production of up to 5.5 kilos. In the case of **Palas Meat half-breeds**, selection can increase wool production by 7.74 ≈ 16.79 %, which means that the average production can reach 6.95 kilos for young male half-breeds and 4.86 kilos for young female half-breeds with **Rouge l' Ouest**.

As regards the half-breeds of the **Tzigai**, the level of wool production was intermediate compared to that of the paternal breeds **Schwarzkopf** (4-6 kilos), **Mouton Vendeen** and **Berrichon du Cher** (2-3 kilos) and the maternal breed **Tzigai** (1 -5 kilos). Thus, the average wool quantity of the adult half-breeds was 2.29 kilos and 3.5-4.2 kilos for the young female tri-racial sheep. There was a large unevenness in the level of productivity, with a CV of 20-25% of wool quantity in female yearlings **GCN** x **Tzigai** and **BC** x **GCN** x **Tzigai** half breeds. The existence of a plus variants percentage of 42 ≈ 64 % may lead to the selection of a sheep line that achieves average wool production levels of 2.7 kilos in **GCN** x **Tzigai**, 3.9 kilos in **BC** x **GCN** x **Tzigai** tri- racial half-breeds and 4.9 kilos in **MV** x **GCN** x **Tzigai** female yearlings.

The analysis of wool quality parameters was carried out on wool samples collected during the shearing season by performing laboratory measurements of fineness, relative length (staple length), absolute length (length of individual fibres), crimp degree and wool impurification degree.

Wool fibre diameter and wool unevenness (CV %) are the main quality parameters that determine the commercial value and are criteria for grading/sorting of fleeces obtained from sheep. Table 2 shows the mean values of fibre fineness, variability, limits of mean diameter and percentage of individuals with above average fineness (plus variants).

The wool fineness of the paternal breeds was 23 ≈ 30 μm in **Rouge de l' Ouest** and 30 ≈ 35 μm in **Texel**, while the wool of the maternal breeds had diameter values of 24 ≈ 37 μm in the **Prolific Palas Breed** and 25 ≈ 35 μm in the **Palas Meat Breed**. In relation to the parental breeds the young F_1 sheep half-breeds had intermediate values, respectively 26 ≈ 36 µm in the prolific half-breeds and 30 ≈ 34 µm in the meat half-breeds. Compared to the young sheep belonging to the **Prolific Palas Breed** the F1 crossbreed had a thicker wool (larger fibre diameter) by 6-11% in male yearlings and 3-6% in female yearlings.

From the data presented, it can be seen that the wool of the young female sheep is finer than that of the young males by 2.9 % in the **Rouge de l' Ouest** x **Prolific Palas half-breeds** and by 5.4 % in the **Texel** x **Prolific Palas** half-breeds. In the case of **Palas Meat Breed** crosses, the wool of the female yearlings is 5.59% finer in the **Rouge de l' Ouest** half-breeds and 9.83% finer in the **Texel** half-breeds. Also, for the half-breed variants taken in the study it was found that in both sexes the wool obtained from the F_1 **Texel** x **Palas Meat Breed** young sheep was finer.

Table 2. Wool fiber diameter of crossbreed sheep's / Finețea lânii la ovinele metise

Breed \ Category	Parameter	Mean fiber diameter		Limits of average diameter (µm)	% plusvariants
		$x \pm s_x$ (µm)	CV (%)		
Rouge de l' Ouest x **Palas Prolific Breed (RPP)**	Young Male TMAP	31.053 ± 0.477	19.1006	28.849 ≈ 33.638	60.0
	Young Female TFAP	30.164 ± 0.509	21.2917	26.228 ≈ 36.374	44.44
Texel x **Palas Prolific Breed (RPP)**	Young Male TMAP	32.729 ± 0.493	18.9460	30.204 ≈ 33.749	40.0
	Young Female TFAP	30.982 ± 0.612	21.4919	27.163 ≈ 34.752	42.11
Rouge de l' Ouest x **Palas Meat Breed (RCP)**	Young Male TMAP	34.345 ± 0.706	21.3229	30.795 ≈ 37.313	44.44
	Young Female TFAP	32.424 ± 0.631	20.0154	30.081 ≈ 35.568	50.0
Texel x **Palas Meat Breed (RCP)**	Young Male TMAP	33.466 ± 0.692	20.6552	31.221 ≈ 39.722	60.0
	Young Female TFAP	30.176 ± 0.568	20.2918	27.218 ≈ 33.593	38.46
Schwarzkopf (GCN) x **Tzigai**	Ewes	27.287 ± 0.463	20.5494	24.099 ≈ 32.593	53.33
	Young Male TMAP	30.186 ± 0.475	18.7972	26.361 ≈ 32.718	50.0
Berrichon du Cher (BC) x **Schwarzkopf** x **Tzigai**	Young Female TFAP	29.004 ± 0.392	16.1028	24.247≈ 31.756	40.0
Mouton Vendeen (MV) x **Schwarzkopf** x **Tzigai**	Young Female TFAP	29.847 ± 0.526	21.0564	24.376 ≈ 32.862	50.0

From the data presented, it can be seen that the wool of the young female sheep is finer than that of the young males by 2.9 % in the **Rouge de l' Ouest** x **Prolific Palas half-breeds** and by 5.4 % in the **Texel** x **Prolific Palas half-breeds**. In the case of **Palas Meat Breed** crosses, the wool of the female yearlings is 5.59% finer in the **Rouge de l' Ouest half-breeds** and 9.83% finer in the **Texel half-breeds**. Also, for the half-breed variants taken in the study it was found that in both sexes the wool obtained from the F_1 **Texel** x **Palas Meat Breed** young sheep was finer.

An analysis of the data in Table 2 shows that the F_1 crossbred sheep youth of the **Rouge l' Ouest** and **Texel** breeds with the **Palas Prolific Breed** and the **Palas Meat Breed** fell into the *semi-fine crossbred wool* category with average wool diameter values of 30.164 ≈ 34.345 µm. In terms of fibre fineness variability (CV) this was over 19%, with crossbred female yearlings showing a higher wool diameter unevenness (over 21%).

The existence in the population of a percentage of individuals with above average fineness allows the constitution of selection groups (lines) with higher levels of the main wool quality parameter, reaching average fineness values of 26.69 ≈ 32.36 µm in the case of **Palas Meat Breed** crossbreeds and average fineness values of 27.4 ≈ 29.5 μm for **Palas Prolific Breed** crossbreeds, thus classifying them in the *fine crossbreed wool* category (below 30 μm).

The frequency class distribution of wool fibres in terms of diameter for young sheep crossbreeds compared to their maternal breeds are shown in Figure 1 and 4.

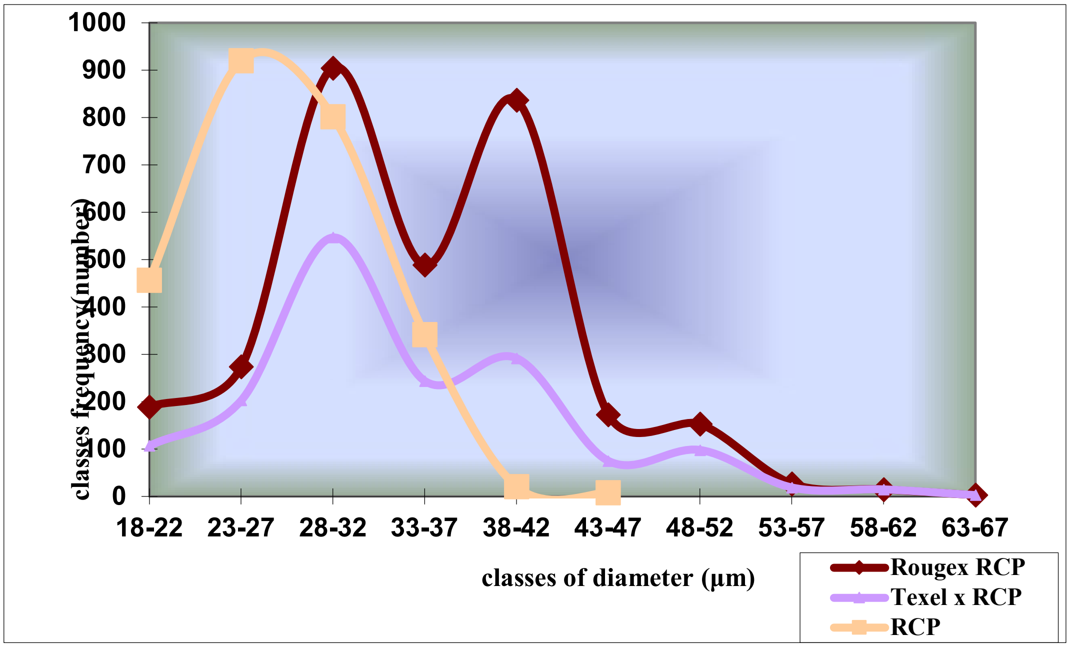


Figure 1. Frequency diagram of wool fibre diameter for young male (TMAP) / Frecvența diametrului fibrei de lână pentru masculi tineri (TMAP)

Figure 2. Rouge de Quest x Palas Meat Breed / Produși Rouge de Quest x Rasa de carne Palas

Figure 3. Texel x Palas Meat Breed / Produși Texel x Rasa de carne Palas

A higher uniformity of the wool diameter is found in the **Palas Meat Breed** where the frequency curve shows a single maximum, corresponding to the 23-27 μm class. Compared to the maternal breed, both types of half breeds have a greater variability in fineness, with frequency diagrams showing two peaks corresponding to classes 28-32 μm and 38-42 μm respectively.

Figure 4 shows a large unevenness in wool diameter, with the maternal breed sheep (**Palas Prolific Breed**) and the crossbreeds with **Rouge de l' Ouest** and **Texel** respectively showing 3 frequency curve peaks for the fineness classes 19-23 μm, 29-33 μm and 39-43 μm.

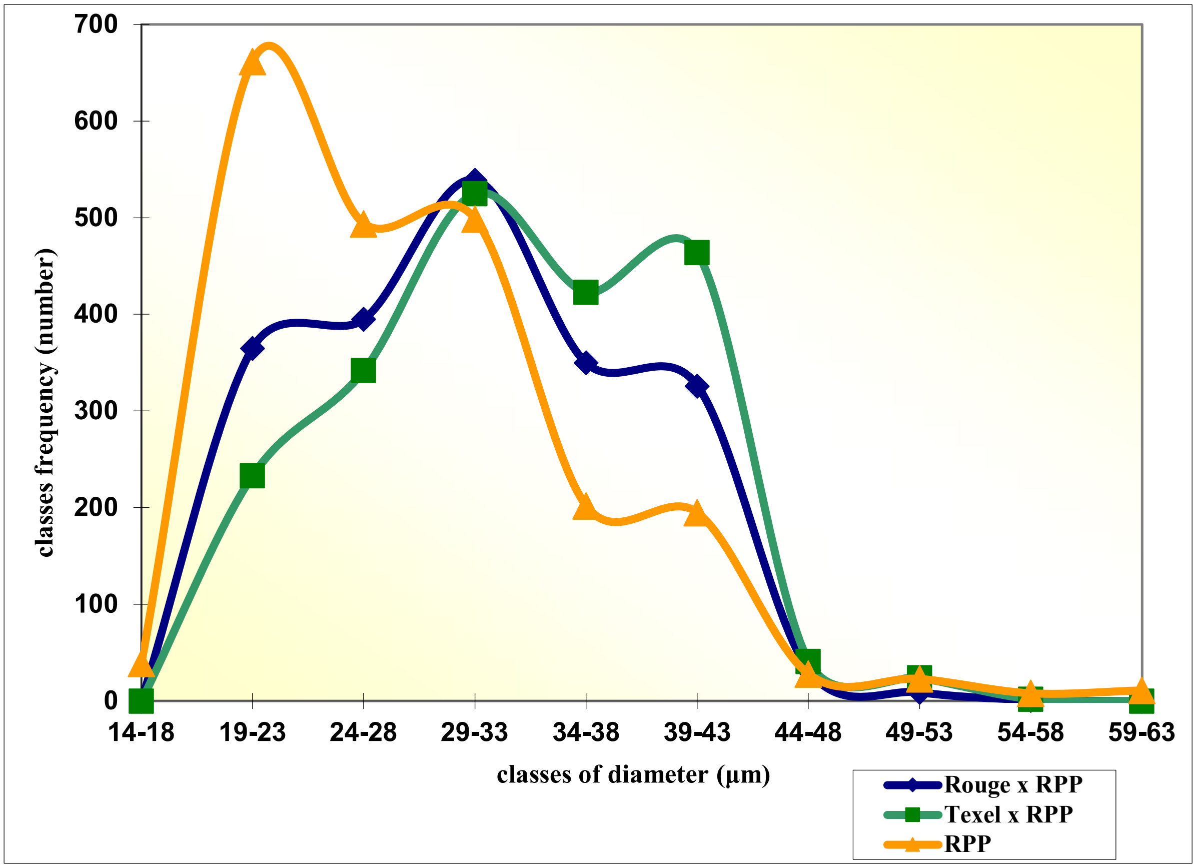


Figure 4. Frequency diagram of wool fibre diameter for young male (TMAP) / Frecvența diametrului fibrei de lână pentru masculi tineri (TMAP)

Figure 5. Rouge de Quest x Palas Prolific Breed / Produși Rouge de Quest x Rasa Prolifică Palas

Figure 6. Texel x Palas Prolific Breed / Produși Texel x Rasa Prolifică Palas

The data presented in Table 2 for the analysed samples collected from the Reghin Experimental Base show the following aspects:

- the half-breeds of the **Tzigai** breed fall into the *semifine crossbreed* category, the average wool diameter 27.29 ≈ 29.85 µm having intermediate values between the paternal breeds **Schwarzkopf** (29-33 µm), **Mouton Vendeen** (25-30 µm) and **Berrichon du Cher** (30-32 µm) respectively that of the maternal breed **Tzigai**. Wool fineness unevenness was higher for young female crossbreeds **MV** x **GCN** x **Tzigai** and **GCN** x **Tzigai** ewes (CV over 20%);
- selection groups of sheep with fineness values higher than the average of the analysed flock (more than 40 % of individuals) can record values 5.36 ≈ 11.52 % higher than the average diameter,

reaching wool fineness values of 25.381 ≈ 29.339 µm, thus classifying them in the *fine crossbreed* category (below 30 µm).

The distribution of wool diameter in young sheep by frequency class is shown in Fig.3. It is found that both the **Tzigai** sheep and the tri-racial **MV** x **GCN** x **Tzigai** half-breeds show a higher diameter unevenness, with two peaks corresponding to classes 21-25 µm and 41-45 µm respectively.

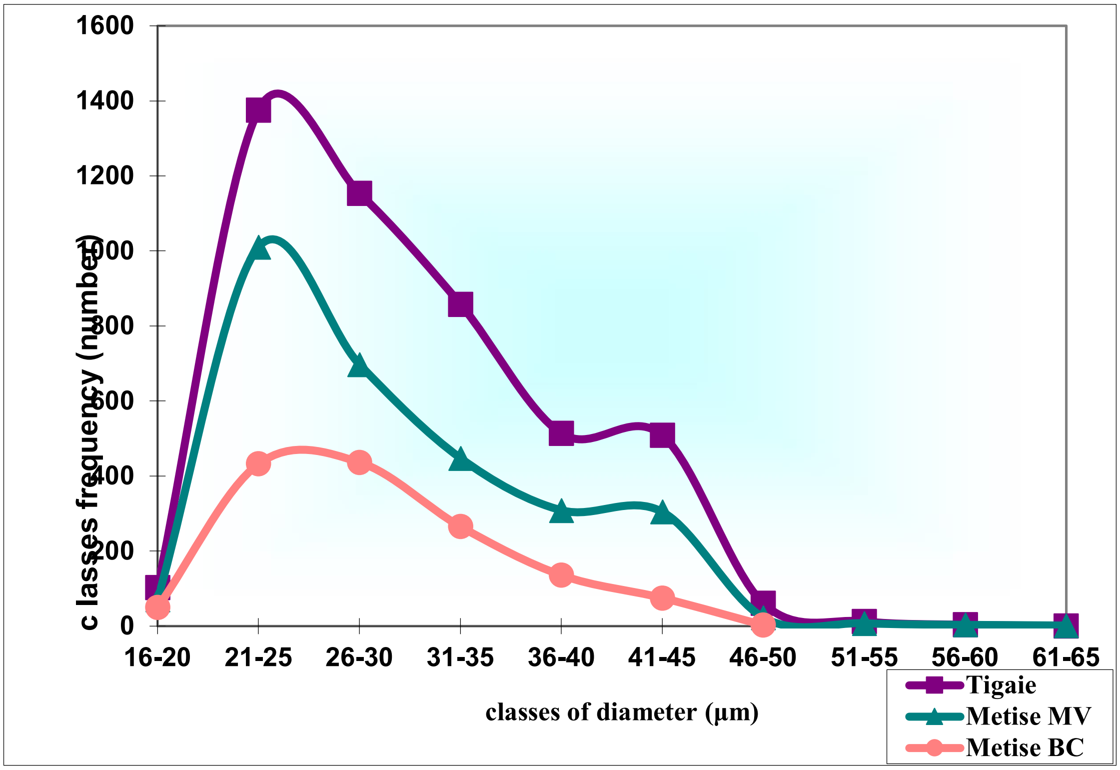


Figure 7. Frequency diagram of wool fibre diameter for young female (TFAP) / Frecvența diametrului fibrei de lână pentru femelele tinere (TFAP)

Data on wool length parameters (relative length of the staple fibres and absolute length of the individual fibres), their variability, ranges values and the percentage of the flock performing above average are presented in Table 3.

In the flocks studied at ICDCOC Palas the paternal breeds had a relative wool length (staple length) of 4 ≈ 7 cm - **Rouge de l' Ouest** respectively 7 ≈ 14 cm - **Texel**. Adult ewes of the maternal breed **Prolific Palas Breed** recorded for this parameter values of 10 ≈ 13 cm and young sheep of 11 ≈ 16 cm, in the maternal breed **Palas Meat Breed** adults had values of 6 ≈ 12 cm and young of 7 ≈ 13 cm. Compared to the parental breeds the young F_1 half-breed's sheep had intermediate values for relative wool length, 11 ≈ 14 cm.

Comparative analysis of the wool length values in the young crossbreed's sheep shows higher values in young males than females **Texel** x **Palas Prolific Breed**, which are 8.54% higher for absolute fibre length and 2.94% higher for relative length. Differences of less than 1% between the sexes were found for absolute wool length in young **Rouge de l' Ouest** x **Prolific Palas Breed** sheep. In terms of unevenness of this parameter, both variants of the half-breed had up to 16% lower values compared to the CV of absolute length in young **Prolific Palas Breed** (maternal breed).

Both young crossbreed sheep flocks show percentages of over 50 % individuals with average absolute wool length values above the population average. Thus, a selection group consisting of plus variants with higher values of this parameter would show an increase of 3.2 ≈ 6.0 % for absolute wool length of young males (reaching 16.21 cm in the case of **Texel** x **Palas Prolific half-breeds**) and 2. 6 ≈ 2.7 % in case of young females (14.28 cm in the **Texel** x **Prolific Palas half-breeds**), respectively an increase of 2.48 ≈ 3.49 % for the absolute length of young males wool and 2.12 ≈ 4.09 % for young females in the case of **Texel** and **Rouge de l' Ouest** crosses with the **Palas Meat Breed**.

Table 3. Wool fiber length of crossbreed sheep's / Lungimea lânii la ovinele metise

Breed \ Category	Parameter	Individual fibre length				Relative length of the staples	
		$x \pm s_x$ (cm)	CV (%)	Limits of average length	% plusvariants	(cm)	Limits of relative length
Rouge de l' Ouest x **Palas Prolific Breed (RPP)**	Young Male TMAP	13.565±0.121	12.508	12.75 ≈ 15.25	60.0	11.04	8.0 ≈ 14.0
	Young Female TFAP	13.654±0.189	13.556	12.93 ≈ 14.93	50.0	12.90	9.5 ≈ 16
Texel x **Palas Prolific Breed (RPP)**	Young Male TMAP	15.244±0.124	11.609	13.79 ≈16.85	60.0	14.27	12.5 ≈ 17.0
	Young Female TFAP	13.911± 0.167	13.115	12.82 ≈ 16.05	63.16	13.85	9.5 ≈ 18.0
Rouge de l' Ouest x **Palas Meat Breed (RCP)**	Young Male TMAP	15.571±0.118	9.5619	14.70 ≈ 16.63	37.5	13.42	11.67 ≈ 15.50
	Young Female TFAP	16.205±0.120	9.1977	14.00 ≈ 17.79	60.0	13.83	11.17 ≈ 15.33
Texel x **Palas Meat Breed (RCP)**	Young Male TMAP	14.641± 0.117	10.3544	14.26 ≈ 15.02	62.5	13.21	11.0 ≈ 16.0
	Young Female TFAP	15.226±0.119	10.0730	13.72 ≈ 16.04	61.54	12.85	10.0 ≈ 16.5
Schwarzkopf (GCN) x **Tzigai**	Ewes	10.320±0.071	14.851	9.25 ≈ 10.97	50	7.21	6.0 ≈ 9.0
	Young Male TMAP	11.778±0.102	12.641	11.29 ≈ 12.17	50	10.25	8.0 ≈ 11.3
Berrichon du Cher (BC) x Schwarzkopf x Tzigai	Young Female TFAP	12.772±0.119	13.029	12.05 ≈ 13.50	60	11.20	10.0 ≈ 12.5
Mouton Vendeen (MV) x Schwarzkopf x Tzigai	Young Female TFAP	12.681±0.110	13.428	11.92 ≈ 13.31	40.0	10.90	8.5 ≈ 13.0

The half-breeds of the **Tzigai** had higher values of relative wool length compared to the paternal breeds **Mouton Vendeen**, **Berrichon du Cher**, **Schwarzkopf** in which the wool locks are 4-7 cm long respectively with the maternal breed **Tzigai** which has 7 - 8 cm in adults and 11-12 cm in young. Thus, the relative wool length of young female crossbreeds was 10.25 ≈ 11.20 cm. As in the case of the other parameters analysed, there is a percentage of more than 40 % of individuals in the flock that perform above the population average and that can constitute selection groups with values 3 ≈ 5 % higher than the absolute length, for which average values of 11.6 cm can be obtained in adult sheep and 13.5 cm in young sheep.

The average values of the degree of wool crimp calculated on the basis of the ratio of relative length to absolute length were approximately 9% at young males and 15-21% at young females in the flocks of the half-breeds sheep analysed at ICDCOC Palas respectively 12.22 ≈ 31.35% in the half-breeds of the **Tzigai** breed with specialised sheep for meat production at the Reghin Experimental Base.

The limits of the mean values for wool crimp degree in the young sheep were as follows:

- 6.5 ≈ 17.0 % in young crossbreed sheeps **Rouge de l' Ouest** x **Palas Prolific Breed**;
- 5.2 ≈ 10.6 % in young crossbreed sheeps **Texel** x **Palas Prolific Breed**.
    - 4.58 ≈ 18.6% in young male crossbreed sheeps **Rouge de l' Ouest** x **Palas Meat Breed** and **Texel** x **Palas Meat Breed**;
    - 7.53 ≈ 25.32% in young female crossbreed sheeps **Rouge de l' Ouest** x **Palas Meat Breed** and **Texel** x **Palas Meat Breed**.

The average impurification degree calculated as the ratio between the depth of penetration of impurities and the relative length of the wool can be used as an indirect indicator to assess the scouring yield, respectively the quantity of clean wool that can be obtained from a given quantity of raw wool. Thus for the flocks studied this parameter had average values of 50.17 ≈ 69.62 % in the hybrids of imported sheep breeds for meat production.

CONCLUSIONS

1. The results obtained in the study led to the conclusion that under the current conditions of sheep breeding and exploitation the quantitative and qualitative level of wool production is below the breed-specific potential, as illustrated by both the average value of these parameters and the high percentage of individuals performing above the flock average.

2. There is therefore a potential within each breed to create groups (sheep lines) in which, through efficient selection, quantitative and qualitative improvements in wool production can be achieved. These sheep lines can achieve increases in wool production of 8 ≈ 17%, improvements in wool fineness expressed by a reduction in fibre diameter of 5 ≈ 12% and increases in absolute wool length of 2 ≈ 5%.

3. The variation range of the average wool diameter was large for all the crossbreds studied, thus the limits of the fineness at ICDCOC Palas were 26.2 ≈ 39.8 µm and at 24.1 ≈ 32.9 µm at the crossbreds of the Reghin Experimental Base. Selected lines from these flocks can be classified in a higher fineness category, namely *fine crossbred wool* (below 30 µm).

4. The wool productive potential of the flocks showed a high variability (CV), 10-19% in **Palas Prolific** and **Palas Meat half-breeds** and 19-24% in **Tzigai half-breeds**. The maximum amount of wool that can be obtained in selection groups is on average 7 kg in the case of **Rouge de l' Ouest** x **Palas Meat Breed** and 5 kg in the case of the tri-racial crossbreed **Mouton Vendeen (MV)** x **Schwarzkopf** x **Tzigai**.

BIBLIOGRAPHICAL REFERENCES

1. Emma K.D., James W.V.P., Bruce A. McG., Phil I.H.; The science behind the wool industry. The importance and value of wool production from sheep; 2021; Animal Frontiers, Volume 11, Issue 2, March 2021, Pages 15–23, https://doi.org/10.1093/af/vfab005.
2. Le Valley S.B.; Grades and lengths of grease wool; 2024; https://extension.colostate.edu/ docs/pubs/livestk/01401.pdf.
3. Lupton C.J., Pfeiffer F.A. and Blakeman N.E.; Optimizing the value of grease wool through preparation and marketing; 1989; Research Journal of Sheep Industry Development, vol. 5, https://sanangelo.tamu.edu/files /2011/11/R114-Optimizing-the-Value-of-Grease-Wool-Through-Preparation-and-Marketing.pdf.
4. Macpherson S.; Wool Classing; 2024; https://www.woolwise.com/wp-content/uploads/2017/07/WOOL-422-522-12-T-05.pdf.
5. Mathis C.P., Brian F.; WOOL GRADES Guide B-409; 2024; https://pubs.nmsu.edu/_b/B409.pdf.
6. Muhammad J.K., Asad A. et all.; Factors affecting wool quality and quantity in sheep; 2012; African Journal of Biotechnology Vol. 11(73), pp. 13761-13766.
7. ***Clip Preparation and Wool Marketing; 2024; https://www.woolwise.com/module-wool-422-522-12/.
8. ***Code of practice for preparation of wool clips; 2021; https://www.sheepusa.org/wp-content/uploads/2021/11/code-of-practice-2021.pdf.

EVALUATION OF THE METABOLIC PROFILE IN LACTATING DAIRY COWS DIAGNOSED WITH MASTITIS

EVALUAREA PROFILULUI METABOLIC LA VACILE DE LAPTE DIAGNOSTICATE CU MASTITĂ

ENCULESCU MARINELA[1], NICOLAE IOANA[1], GAVOJDIAN DINU[1]

[1]Research and Development Institute for Bovine, 21, Bucuresti-Ploiesti, 077015, Balotesti, Romania

Correspondence address: marinelaenculescu2006@yahoo.com

Abstract

*The aim of this study was to evaluate the haemato-biochemical profile of dairy cows diagnosed with clinical mastitis (CM) and the subclinical mastitis (SCM), compared to healthy cows. The study was conducted at the Experimental Farm of the Research and Development Institute for Bovine Balotesti, from September to October 2022, using 45 multiparous **Romanian Black and White** dairy cows, which were divided into three equal groups: E_1 (n=15) cows diagnosed with mastitis, E_2 (n=15) cows diagnosed with subclinical mastitis and C (n=15) of clinically healthy cows, as the control group. The categorization was based on clinical udder health, the California Mastitis Test (CMT), and the somatic cell count (SCC) of milk. One-way ANOVA was used to test the influence of mastitis on the blood parameters studied and the Tukey's test was applied for post hoc comparisons. The obtained results showed a significant difference in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and aspartatetransaminase (AST) levels in the E_1 group, compared with the E_2 group ($p<0.05$), and significant differences for white blood cell (WBC) in the E_1 and E_2 groups, compared to the C group ($p<0.001$). A tendency of significance for total protein (TPr), total cholesterol (TCho), and aspartatetransaminase (AST) in the E_1 group, compared with the C group ($p<0.10$) was observed. The magnesium (Mg) serum concentrations in the E_1 group were significantly higher than those of the C group ($p<0.05$). A significant positive correlation between mean corpuscular volume (MCV) vs. somatic cell count (SCC), as well as between mean corpuscular hemoglobin (MCH) vs. somatic cell count (SCC) in the E_1 group ($p<0.05$) was observed. Significant negative correlations for hematocrit (HCT) vs. somatic cell count (SCC), hemoglobin (HGB) vs. somatic cell count (SCC), and lymphocytes (LY) vs. somatic cell count (SCC) in the E_2 group ($p<0.05$) were recorded. In conclusion, the obtained results suggest that some blood indicators might be useful tools for the process of detecting, monitoring, and predicting mastitis cases in dairy cows, as well as for assessing the functional activity of a mammary gland.*

Key word: dairy cows, haematological profile, biochemical profile, mastitis.

Rezumat

*Scopul acestui studiu a fost de a evalua profilul hemato-biochimic al vacilor de lapte diagnosticate cu mastită clinică (MC) și mastită subclinică (MSC), comparativ cu vacile de lapte clinic sănătoase. Studiul a fost realizat în Laboratorul Experimental Zootehnic al Institutului de Cercetare-Dezvoltare pentru Creșterea Bovinelor Balotești, în perioada Septembrie-Octombrie 2022, pe un număr de 45 de vaci de lapte, multipare, rasa **Bălțată cu Negru Românească**, grupate în trei loturi egale (n=15/lot): lotul E_1, vaci diagnosticate cu mastită clinică, lotul E_2, vaci diagnosticate cu mastită subclinică și lotul de control C, vaci clinic sănătoase. Formarea loturilor s-a bazat pe sănătatea clinică a ugerului, California Mastitis Test (CMT) și numărul de celule somatice (NCS) din lapte. ANOVA unifactorială a fost utilizată pentru a testa influența mastitei asupra parametrilor sanguini studiați, iar pentru efectuarea comparațiilor între loturi s-a aplicat testul Tukey. Rezultatele obținute au relevat diferențe semnificative pentru volumul eritrocitar mediu (VEM), hemoglobina eritrocitară medie (HEM) și aspartataminotransferaza (GOT) la lotul E_1, comparativ cu lotul E_2 ($p<0.05$) și diferențe semnificative pentru numărul de leucocite (WBC) la loturile de vaci E_1 și E_2, comparativ cu lotul C ($p<0.001$). De asemenea, a fost observată o tendință pentru proteina totală (tPr), colesterolul total (tCho) și aspartataminotransferaza (GOT), la lotul E_1 comparativ cu lotul C ($p<0.10$). Nivelul seric al magneziului (Mg) la lotul E_2 a fost semnificativ mai ridicat, comparativ cu lotul C ($p<0.05$). O corelație pozitivă semnificativă a fost observată între volumul eritrocitar mediu (VEM) și numărul de celule somatice (NCS), precum și între hemoglobina eritrocitară medie (HEM) și numărul de celule somatice (NCS) la vacile din lotul E_1 ($p<0.05$). În schimb, o corelație negativă semnificativă a fost înregistrată pentru hematocrit (Ht) vs. numărul de celule somatice (NCS), hemoglobină (Hb) vs. număr celule somatice (NCS), limfocite (Ly)* *vs. număr celule somatice (NCS) la vacile din lotul* E_2 *(*$p<0.05$*). În concluzie, rezultatele obținute au relevat că anumiți parametrii sanguini pot fi utili în procesul de detectare, monitorizare și predicție a mamitei la vacile de lapte, precum și pentru evaluarea activității funcționale a glandei mamare.*

***Cuvinte cheie: vaci de lapte, profil hematologic, profil biochimic, mastită*.**

INTRODUCTION

Mastitis is an inflammation of the mammary gland, causing severe economic losses associated with reduced milk yields, milk quality and increased therapy costs (Geary et al., 2012; Azooz et al., 2020). Mastitis in dairy cows is induced by many factors (Thompson Crispi et al., 2014; Godden et al., 2016), such as microbial infection (bacteria, fungi, mycoplasma, virus), environmental factors (sanitary conditions, temperature and humidity, feed), human factors (mechanical injury, milking stress, improper feeding management) and cow's own factors (age, parity, body condition score, milk yield, lactation stage). Concerning the lactation stage, the occurrence of mastitis is lower in the first lactation (2.7%), compared with the fifth or more lactation (27.6%) in dairy cows (Nitz et al., 2021; Mhoscovas et al., 2023). Mastitis, exists in two primary forms: *clinical mastitis* and *subclinical mastitis* (Ruegg, *2017)*.

Clinical mastitis (CM), which is less prevalent, is characterized by systemic signs and visible abnormalities of the udder and milk (Jamali et al., 2018; Haxhiaj et al., 2022) such as swelling, redness and/or pain in the udder, fever, changes to the milk (colour, consistency, presence of clots, blood) and its component, increased conductivity, increased pH, and changes in water content of the milk (Leslie and Petersson, 2012; Roberson, 2012).

Clinical mastitis is categorized as *mild/subacute* clinical mastitis (most changes are found in the milk's colour and consistency), *moderate/acute* clinical mastitis (changes in the milk and mammary gland, including redness, swelling, heat and pain), and *severe/peracute* clinical mastitis (abnormal milk and mammary gland) along with systemic signs such as fever, loss of appetite, and inability or unwillingness to move (Suojala et al., 2013; Adkins and Middleton, 2018).

Clinical mastitis can reoccur in the same lactation and across lactations due to several pathogens, which can be classified as gram-positive bacteria (e.g. *Streptococcus spp*), gram-negative bacteria (e.g. *Escherichia coli*) or other organisms. Dairy cows with CM express illness behaviour after an infection (Fogsgaard et al., 2012) and has a negative impact on farm profitability due to reduced milk production and fertility (Hertl et al., 2014), impaired milk quality, and increased culling and mortality of cows (Hertl et al., 2011). These effects depend on the severity and pathogen type causing clinical mastitis. Besides the negative impact of clinical mastitis on farm profitability and on the welfare of cows, this could, also, affect GHG emissions of milk production (Mostert et al., 2019).

Subclinical mastitis (SCM) is more common and results in reduced milk production without visible clinical signs in the udder or milk (Zeryehun and Abera, 2017; Ndahetuye et al., 2019). For this reason, subclinical mastitis is challenging to diagnose, persists longer in the herd, and is associated with higher losses, compared to clinical mastitis (Abrahmsen et al., 2014). Subclinical mastitis is monitored through continuous measurements of somatic cell count (SCC) in milk (threshold lower than 200,000 cells/mL for infected quarters (Schukken et al., 2003) and bacterial cultures examination (Schukken, 2011). Subclinical mastitis can be highly significant (up to 50%) at dairy farm level (Busanello et al., 2016), decreasing milk yield and milk quality (Goncalves et al., 2016; Bobbo et al., 2017), increasing treatment costs, and culling rates (Bar et al., 2008; Halasa and Kirkeby, 2020). Even after complete recovery from SCM, milk output does not totally return.

The current study has focused on evaluating the blood indicators in dairy cows diagnosed with clinical mastitis and subclinical mastitis, compared with healthy dairy cows, in order to addressing novel directions for further research on the connections between blood markers and mastitis.

MATERIALS AND METHODS

All experimental procedures were performed in accordance with the practices and standards approved by *Romanian Law no. 43/2014* and the *Council Directive 2010/63/EU*.

Forty-five multiparous **Romanian Black and White** dairy cows were investigated for some haemato-biochemical parameters in relation to clinical and subclinical mastitis compared with healthy dairy cows. The study was carried out at the Experimental Farm of the Research and Development Institute for Bovine Balotesti, from September to October 2022. The cows were divided into three equal groups: the experimental group E_1 (n=15 heads) cows with clinical mastitis, the experimental group E_2 (n=15 heads) cows with subclinical mastitis, and the control group C (n=15 heads), which consisted of clinically healthy cows. The cows that were included in the study were balanced for age (between 4 to 7 years), body condition score (between 2.5-3), and lactation phase (between 90 and 140 days in milk). The cows were housed under tied stanchion barn conditions. All cows were fed twice daily, the diet fed was formulated to meet nutrient requirements at the stage of lactation (the diet/head/day consisting of 6 kg alfalfa hay, 25 kg corn silage, 5 kg concentrates, salt, and water *ad libitum)*.

The clinical mastitis was determined using clinical examinations conducted by veterinarians at the experimental farm (udder inflammation included swelling, pain, changes in the milk, colour alterations, and density variations), the positive California mastitis test (++), and the mean somatic cell count (2373.106±981.31 SCC x 1000 cell/ml). The subclinical mastitis was determined using the California Mastitis Test (+) and measures the somatic cell count in milk samples (575.93±154.60 SCC x 1000 cell/ml) without any clinical symptoms of udder inflammation and milk changes. The healthy dairy cows showed no clinical changes in their udders or milk samples, negative California Mastitis Test (-), and the mean somatic cell count was 45.53±22.90 SCC x 1000 cell/ml.

The analysis of the SCC was performed using Fossomatic apparatus by an ISO accredited laboratory. The amount of blood was collected in vacutainer tubes with EDTA/K_3 for hemoleukogram (1-2 ml/tube, and chilled to +4 °C), and vacutainer tubes (6 ml/tube, centrifuged at 3000 rpm for 15 minutes and stored at –20 °C till further testing) for biochemical examination. Red blood cell count (RBC), hemoglobin (HGB), hematocrit (HTC), red blood cell distribution width (RDWc), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets count (PLT), total white blood cell count (WBC), lymphocytes percentage (LY), monocytes percentage (MO), neutrophil percentage (NE) were performed using an automated hematology analyzer (Abacus Junior Vet 5). Total protein (TPr), total cholesterol (TCho), aspartatetransaminase (AST), alanineaminotransferase (ALT), alkaline phosphatase (PAL), calcium (Ca), inorganic phosphorus, (iP), and magnesium (Mg) were determined using a semiautomated biochemical analyser (StarDust MC 15).

Statistical analyses were carried out using Microsoft Excel Statistical Software. The data were expressed as mean ±standard deviations (SD). One-way ANOVA was used to test the influence of mastitis on the blood parameters studied and the Tukey's test was applied for post hoc comparisons. Differences were considered statistically significant at $p<0.05$, trends $p\leq0.10$. The correlations between blood parameters and somatic cell count (SCC) were estimated using Pearson's correlation coefficient at a statistical significance of $p<0.05$.

RESULTS AND DISCUSSIONS

The means and standard deviations of RBC, HGB, HTC, RDW, MCV, MCH, and MCHC are presented in Table 1. A significant effect between the studied groups for MCV ($F_{(2;42)}=5.83$; p=0.0058), and MCH ($F_{(2;42)}=4.26$; p=0.0205) was found. The mean value for mean corpuscular volume (MCV) was

44.06±3.80 fl in the clinical mastitis cows group (E_1), 39.4±4.7 fl in the subclinical mastitis cows group (E_2), and 41.46±2.32 fl in the healthy cows group (C).

For RBC ($F_{(2;42)}$=0.66; p=0.5218), HGB ($F_{(2;42)}$=1.50; p=0.2346), HTC ($F_{(2;42)}$=1.03; p=0.3641), MCHC ($F_{(2;42)}$=1.09; p=0.3430), and RDW ($F_{(2;42)}$=1.95; p=0.1549) no significant effect was found. The mean value for red blood cell count (RBC) was 6.13±0.40 10^6/µl in the clinical mastitis group (E_1), 6.36±0.77 10^6/µl in the subclinical mastitis group (E_2) and 6.34±0.50 10^6/µl in the healthy group (C).

The HGB concentration ranged from 9.17±0.83 g/dl in the clinical mastitis group (E_1), to 8.68±0.91 g/dl in the subclinical mastitis group (E_2) and 9.04±0.67 g/dl in the healthy group (C). The mean values for HCT were lower than physiological limits, ranges from 27.03±2.75 % (E_1), to 25.80±2.41 % (E_2) and 26.19±1.93 % at (C), respectively. RDW concentration ranges from 20.63±0.93 % to 21.25±0.81 % in the mastitis and the healthy cows. The means values recorded for PLT were situated in physiological limits, without significant differences between studied groups ($F_{(2;42)}$=0.04, p=0.9551).

Current values have been found to be similar with values reported by Saharan et al. (2022) in the clinical mastitis group, compared to the healthy group (RBC: 7.39±0.36, HGB: 10.55±0.42 g/dl, HTC: 30.39±0.84 %), without significant differences between the groups. A similar observation was, also, reported by Sarvesha et al. (2017) and Singh et al. (2014), however, Zaki et al. (2010) and Das et al. (2018) reported decreased values of HGB, RBC, and HTC in the mastitis group.

Table 1. Results of the haematological indicators in dairy cows diagnosticated with mastitis and healthy animals / Rezultatele examenului hematologic la vacile de lapte diagnosticate cu mastită și la animalele sănătoase

Blood indicators	**Groups**			**Reference values**
	E_1 (clinical mastitis)	**E_2 (subclinical mastitis)**	**C (healthy)**	
	$\bar{X}$ ±SD	$\bar{X}$ ±SD	$\bar{X}$ ±SD	
RBC, 10^6/µl	6.13±0.40	6.36±0.77	6.34±0.50	5-8
Pairwise comparisons, *p*	E_1 vs. E_2: p=0.5616; *E_1 vs. C: p=0.6084; E_2 vs. C: p=0.9968*			
HGB, g/dl	9.17±0.83	8.68±0.91	9.04±0.67	9-11
Pairwise comparisons, *p*	*E_1 vs. E_2: p=0.2293*; E_1 vs. C: p=0.9041; *E_2 vs. C: p=0.4367*			
HTC, %	27.03±2.75	25.80±2.41	26.19±1.93	32-38
Pairwise comparisons, *p*	E_1 vs. E_2: p=0.3472; *E_1 vs. C: p=0.6009*; E_2 vs. C: p=0.9000			
RDW, %	20.63±0.93	21.25±0.81	20.74±1.00	16-20
Pairwise comparisons, *p*	E_1 vs. E_2: p=0.1629; *E_1 vs. C: p=0.9325; E_2 vs. C: p=0.2994*			
MCV, fl	44.06±3.80	39.4±4.71	41.46±2.32	40-60
Pairwise comparisons, *p*	***E_1 vs. E_2: p=0.0040;*** *E_1 vs. C: p =0.1514; E_2 vs. C: p=0.2967*			
MCH, pg	14.95±1.30	13.75±1.14	14.35±0.92	11-17
Pairwise comparisons, *p*	***E_1 vs. E_2: p=0.0150;*** *E_1 vs. C: p=0.3236; E_2 vs. C: p=0.3159*			
MCHC, g/dl	33.98±2.00	33.82±1.08	34.56±0.92	30-36
Pairwise comparisons, *p*	E_1 vs. E_2: p=0.9458; *E_1 vs. C: p=0.5230; E_2 vs. C: p=0.3439*			
PLT, 10^3/µl	337.53±96.24	347.66±93.53	341.86±85.43	100-800
Pairwise comparisons, *p*	*E_1 vs. E_2: p=0.9509; E_1 vs. C: p=0.9908*; E_2 vs. C: p=0.9836			

RBC=red blood cells count, HGB=hemoglobin concentration, HTC=hematocrit percentage, RDW=red blood cells distribution width, MCV=mean corpuscular volume, MCH=mean corpuscular hemoglobin, MCHC=mean corpuscular hemoglobin concentration, PLT=platelet count.

The WBC count ($F_{(2;42)}$=20.30, p=0.0000) showed statistical differences between studied groups (Table 2), whereas, the LY percentage ($F_{(2;42)}$=2.35, p=0.1068), MO percentage ($F_{(2;42)}$=0.15, p=0.8596) and NE percentage ($F_{(2;42)}$=1.94, p=0.1550), showed no statistical differences between groups. The count of WBC was highest, even than the reference limits, in the clinical mastitis group (10.42±1.56 10^3/µl) and the subclinical mastitis group (10.13±1.22 10^3/µl).

The LY mean values were situated in physiological limits for E_1 and C groups, whereas, the NE and the MO for all studied groups were higher than reference limits. Saharan et al. (2022) reported values of 30.38±1.9 % for LY, 1.75±0.3 % for MO, and 66.25±2.11 % for NE in the mastitis group compared with the healthy group (WBC: 6.82±0.33$10^3$/µl, LY: 65.88±1.26 %, MO: 2.13±0.39 %, NE: 30.38±1.06 %), which are in contrast with our results.

Table 2. Results of the withe blood cell and leucocytes formula in dairy cows diagnosticated with mastitis and in healthy animals / Rezultatele leucogramei la vacile de lapte luate în studio, diagnosticate cu mastită și la animalele sănătoase

Blood indicators	**Groups**			**Reference values**
	E_1 (clinical mastitis)	**E_2 (subclinical mastitis)**	**C (healthy)**	
	$\bar{X}$ ±SD	$\bar{X}$ ±SD	$\bar{X}$ ±SD	
WBC, 10^3/µl	10.42±1.56	10.13±1.22	7.78±0.83	6.5-9.5
Pairwise comparisons, *p*	E_1 vs. E_2: p=0.7993; ***E_1 vs. C: p=0.0000***; **E_2 vs. C: p=0.0002**			
LY, %	50.9±17.69	42.60±12.32	52.75±9.59	45-61
Pairwise comparisons, *p*	E_1 vs. E_2: p=0.2296; *E_1 vs. C: p=0.9264; E_2 vs. C: p=0.1152*			
MO, %	4.53±3.94	5.30±4.15	5.11±4.02	0-4
Pairwise comparisons, *p*	*E_1 vs. E_2: p=0.8575*; E_1 vs. C: p=0.9166; *E_2 vs. C: p=0.9905*			
NE, %	44.22±19.26	52.14±13.36	42.47±7.96	15-41
Pairwise comparisons, *p*	E_1 vs. E_2: p=0.2930; *E_1 vs. C: p=0.9402; E_2 vs. C: p=0.1653*			

WBC=total white blood cells count, LY=lymphocytes percentage, MO=monocytes percentage, NE=neutrophil percentage.

The obtained mean values for serum biochemical indicators are presented in Table 3. The concentration of TCho ($F_{(2;42)}$=3.36, p=0.0442), AST ($F_{(2;42)}$=3.84, p=0.0294), and Mg ($F_{(2;42)}$=3.39, p=0.0429) showed statistical differences between the studied groups, with a tendency for TPr ($F_{(2;42)}$=2.49, p=0.0944), unlike concentrations of ALT ($F_{(2;42)}$=0.80, p=0.4547), ALP ($F_{(2;42)}$=1.89, p=0.1633), Ca ($F_{(2;42)}$=0.11, p=0.8898) and inorganic P ($F_{(2;42)}$=0.83, p=0.4419). The mean values for AST and ALP levels were higher than the reference limits, while the ALT level was into reference limits, higher values of this enzymes reflecting liver damage (Kew, 2000).

In a previous study, Moyes et al. (2016) showed that mammary inflammation was associated with the impairment of liver metabolism and liver function.

Table 3. Results of the biochemical serum indicators in dairy cows diagnosticated with mastitis and in healthy animals / Rezultatele examenului biochimic seric la vacile de lapte diagnosticate cu mastită și la animalele sănătoase

Blood indicators	**Groups**			**Reference values**
	E_1 (clinical mastitis)	**E_2 (subclinical mastitis)**	**C (healthy)**	
	$\bar{X}$ ±SD	$\bar{X}$ ±SD	$\bar{X}$ ±SD	
TPr, mg/dl	8.26±0.90	8.02±0.70	7.62±0.0.77	6.8-8.4
Pairwise comparisons, *p*	*E_1 vs. E_2: p=0.6869*; **E_1 vs. C: p=0.0807**; *E_2 vs. C: p=0.3590*			
TCho, mg/dl	226±50.38	191.47±43.50	186.4±43.21	124-224
Pairwise comparisons, *p*	E_1 vs. E_2: p=0.1097; **E_1 vs. C: p=0.0545**; E_2 vs. C: p=0.9429			
AST, U/L	54.4±7.52	46.53±9.25	47±9.26	21-24
Pairwise comparisons, *p*	***E_1 vs. E_2: p=0.0455; E_1 vs. C: p=0.0634***; *E_2 vs. C: p=0.9882*			
ALT, U/L	23.6±7.59	22.6±4.96	20.8±5.52	<60
Pairwise comparisons, *p*	*E_1 vs. E_2: p=0.8961; E_1 vs. C: p=0.4308; E_2 vs. C: p=0.7027*			
ALP, U/L	77.88±13.36	75.26±14.57	67.8±15.87	10-36
Pairwise comparisons, *p*	*E_1 vs. E_2: p=0.8839; E_1 vs. C: p=0.1597; E_2 vs. C: p=0.3516*			
Ca, mg/dl	8.99±0.89	8.87±0.84	8.83±0.94	8-11
Pairwise comparisons, *p*	E_1 vs. E_2: p=0.9366; *E_1 vs. C: p=0.8872*; E_2 vs. C: p=0.9918			
iP, mg/dl	4.35±0.73	4.72±0.95	4.6±0.67	4.62-7
Pairwise comparisons, *p*	E_1 vs. E_2: p=0.4221; E_1 vs. C: p=0.6734; E_2 vs. C: p=0.9099			
Mg, mg/dl	2.71±0.57	2.38±0.32	2.30±0.41	2.1-2.8
Pairwise comparisons, *p*	E_1 vs. E_2: p=0.1350; ***E_1 vs. C: p=0.0457***; *E_2 vs. C: p=0.8674*			

AST=aspartatetransaminase, ALT=alanineaminotransferase, ALP=alkaline phosphatase, Ca=calcium, iP= inorganic phosphorous, Mg=magnesium.

In contrast with our results, Stanojevic et al. (2023) reported significant difference (p=0.001) between the clinical and the subclinical groups compared to the healthy group for the TPr, ALT, and ALP.

Ali et al. (2017) reported significant increase in TPr level in the subclinical mastitis cases, compared to the healthy cows (7.90±0.10 g/dl vs. 7.15±0.18 g/dl), without significant differences observed in the clinical mastitis cows (7.30±0.13 g/dl vs. 7.15±0.18 g/dl), and no significant change was observed in values of TCho in the clinical and subclinical mastitis than the healthy animals (103.90±2.14 mg/dl in the healthy group, 103.80±2.30 mg/dl in the subclinical mastitis group, 103.66±2.14 mg/dl in the clinical mastitis group). Opposite, Saleh et al. (2022) recorded significantly lower serum levels of TPr, in cows affected by subclinical mastitis, compared to healthy cows.

In accordance with our results, Stanojevic et al., (2023) found that the concentration of Mg was significantly higher in cows with the subclinical and clinical forms of mastitis, compared to healthy cows, and no statistical differences in the concentrations of Ca and concentrations of the P between the clinical, subclinical groups, compared to the healthy group. Conversely, Saharan et al. (2022) reported no significant difference, between the mastitis cows and the healthy cows, in the Mg concentration. On the other hand, Das et al., (2018) reported no significant differences in the concentrations of Mg and ALT between the mastitis and the healthy cows groups.

The Pearson correlation coefficient between blood parameters and somatic cell count (SCC) of milk is presented in Table 4. Moderate positive correlations were established between MCV and SCC (*r*=0.5409, *p*=0.0373), as well as between MCH and SCC (*r*=0.5262, *p*=0.0439) in the clinical mastitis group. Moderate positive correlations were, also, established between MCV and SCC (*r*=0.6533, *p*=0.0082) in the healthy group.

Therefore, an increased SCC tended to be moderate negative associated with decreased of HTC (*r* =-0.5632, *p*=0.0288), HGB (*r*=-0.5659, *p*=0.0278), and LY (*r*=-0.5189, *p*=0.0474) level in the subclinical mastitis group. Furthermore, TCho in the clinical mastitis group (r=0.4647, p=0789), NE (r=0.4537, p=0.0893), and PLT (r=0.4612, p=0.0835) in the subclinical mastitis group, were positively correlated with SCC showing a tendency of week association.

A tendency weaker negative correlation with SCC was observed for MCH (*r*=-0.4555, *p*=0.0879), WBC (*r*=-0.4469, *p*=0.0949), and Ca (*r* =-0.4546, *p*=0.0886).

Table 4. Pearson correlation coefficient between blood indicators and SCC in dairy cows diagnosticated with mastitis and in healthy animals / Coeficientul de corelație Pearson între parametrii sanguini și NCS la vacile de lapte diagnosticate cu mastită și la animalele sănătoase

Blood indicators	*SCC*		
	E_1 (clinical mastitis)	E_2 (subclinical mastitis)	C (healthy)
	r	r	r
RBC	-0.3084^{ns}	-0.2562^{ns}	-0.3089^{ns}
HGB	0.2999^{ns}	**-0.5659***	0.0921^{ns}
HCT	0.2441^{ns}	**-0.5632***	0.2226^{ns}
MCV	**0.5409***	0.0644^{ns}	**0.6533****
MCH	**0.5262***	-0.2876^{ns}	*0.4555*^{ns}
MCHC	0.0170^{ns}	-0.2700^{ns}	-0.1027^{ns}
RDW	-0.3477^{ns}	-0.3164^{ns}	-0.3994^{ns}
WBC	0.3871^{ns}	0.0462^{ns}	*-0.4469*^{ns}
LY	0.1187^{ns}	**-0.5189***	0.2715^{ns}
MO	-0.2177^{ns}	0.0818^{ns}	-0.1507^{ns}
NE	-0.0401^{ns}	*0.4537*^{ns}	-0.2174^{ns}
PLT	0.1113^{ns}	*0.4612*^{ns}	0.1348^{ns}
TPr	-0.1166^{ns}	-0.0839^{ns}	-0.0165^{ns}
TCho	*0.4674*^{ns}	-0.1368^{ns}	0.0345^{ns}
AST	0.1364^{ns}	0.1075^{ns}	0.0471^{ns}
ALT	*-0.4353*^{ns}	0.1124^{ns}	-0.0821^{ns}
PAL	0.0881^{ns}	0.2363^{ns}	-0.0472^{ns}
Ca	0.3028^{ns}	0.0988^{ns}	*-0.4546*^{ns}
P	0.0399^{ns}	-0.0826^{ns}	0.1056^{ns}
Mg	0.1183^{ns}	0.0203^{ns}	-0.0677^{ns}

SCC = somatic cell count, p=significance levels (p<0.05), ns=not significant.

CONCLUSIONS

The obtained results revealed significant differences for MCV, MCH, and AST in dairy cows diagnosticated with clinical mastitis, compared to the subclinical mastitis group, while the concentrations of WBC in the clinical and the subclinical mastitis affected groups differed significantly from those of healthy cows. For the biochemical profile, differences for TCho and AST, and a tendency for TPr, in the clinical mastitis group, compared with the healthy group were observed. Serum Ca and inorganic P concentrations in the mastitis studied groups were not significantly different to those from the control group, while serum Mg levels were significantly higher in the clinical mastitis group, compared with the control group, and without statistical significance for the subclinical mastitis group, compared to the healthy group.

The results of this study show the importance of haematological and serum biochemical indicators in the process of diagnosing clinical and subclinical mastitis in dairy cows. Further research on larger numbers of animals is needed to establish connections between blood markers and mastitis. Early detection of cows changes in metabolic parameters through the identified udder health issues could improve management treatment at herd level, while improving animal welfare and milk production.

ACKNOWLEDGEMENTS

This study was supported by Research Thematic Plan of the Research and Development Institute for Bovine Balotesti, Project No. 4466/2018 "*Research on the metabolic profile in cattle and buffaloes*".

BIBLIOGRAPHICAL REFERENCES

1. Ali A., Ahmad Mir B., Bhat R.R., Baba O.K., Ishraq H., Mudasir Rashid S., Showkeen Muzamil, Ahmad S.B., Mir M.R.; Metabolic profiling of dairy cows affected with subclinical and clinical mastitis; 2017; J. Entomol. Zool. Studies, 5(6): 1026-1028.
2. Abrahmsen M., Persson Y., Kanyima B.M., Bage R.; Prevalence of subclinical mastitis in dairy farms in urban and peri-urban areas of Kampala, Uganda; 2014; Trop. AniM. Health Prod., 46, (1), 99–105.
3. Adkins P.R.F., Middleton J.R.; Methods for Diagnosing Mastitis; 2018; Vet. Clin. N. Am.Food Ani Pract., *34*, 479–491.
4. Azooz M., El-Wakeel S.A., Yousef H.; Financial and economic analyses of the impact of cattle mastitis on the profitability of Egyptian dairy farms; 2020; Vet. World, 13,1750.
5. Bar D., Gröhn Y.T., Bennett G., González R.N., Hertl J.A., Schulte H.F., Tauer L.W., Welcome F.L., Schukken, Y.H.; Effects of Repeated Episodes of Generic Clinical Mastitis on Mortality and Culling in Dairy Cows; 2008; J. Dairy Sci., 91, 2196–2204.
6. Bobbo T., Ruegg P.L., Stocco G., Fiore E., Gianesella M., Morgante M., Pasotto D., Bittante G., Cecchinato A.; Associations between pathogen-specific cases of subclinical mastitis and milk yield, quality, protein composition, and cheese-making traits in dairy cows; 2017; J. Dairy Sci., 100 (6), 4868–4883.
7. Busanello M., Rossi R.S., Cassoli L.D., Pantoja J.C.F., Machado P.F.; Estimation of Prevalence and Incidence of Subclinical Mastitis in a Large Population of Brazilian Dairy Herds; 2017; J. Dairy Sci, 100, 6545–6553.
8. Das D., Panda S.K., Kundu A.K., Jena B., Das B.C., Sahu R.K.; Haematological and metabolic profile test of mastitis affected bovines in Odisha; 2018; J. Entomol. Zool. Stud, 6, 3022–3024.
9. Directive 2010/63/EU regarding handling and protection of animals used for scientific purposes, OJEU L 276/33 IA.
10. Fogsgaard K.K., Rontved C.M., Sorensen P., Herskin M.S.; Sickness behavior in dairy cows during *Escherichia coli* mastitis; 2012; J. Dairy Sci., 95, 630–638.
11. Geary U., Lopez-Villalobos N., Begley N., McCoy F., O'Brien B., O'Grady L., Shalloo L.; Estimating the effect of mastitis on the profitability of Irish dairy farms; 2012; J. Dairy Sci. 95 (7), 3662–3673.
12. Godden S.M., Royster E., Knauer W., Sorg J., Lopez-Benavides M., Schukken Y., Leibowitz S., French E.A.; Randomized noninferiority study evaluating the efficacy of postmilking teat disinfectant for the prevention of naturally occurring intramammary infections; 2016; J. Dairy Sci. 99:3675–87, doi: 10.3168/jds.2015-10379.
13. Goncalves J.L., Tomazi T., Barreiro J.R., Beuron D.C., Arcari M.A., Lee S.H.I., de Magalhães Rodrigues Martins C.M., Araújo Junior J.P., dos Santos M.V.; Effects of bovine subclinical mastitis caused by *Corynebacterium spp.* on somatic cell count, milk yield and composition by comparing contralateral quarters; 2016; Vet. J. 209, 87–92.
14. Haxhiaj K., Wishart D.S., Ametaj B.N.; Mastitis: What It Is, Current Diagnostics, and the Potential of Metabolomics to Identify New Predictive Biomarkers; 2022; Dairy, 3(4):722-746, https://doi.org/10.3390/dairy3040050.
15. Halasa T., Kirkeby C.; Differential Somatic Cell Count: Value for Udder Health Management; 2020; Front. Vet. Sci., 7, 609055.
16. Hertl J.A., Schukken Y.H., Bar D., Bennett G.J., González R.N., Rauch B.J., Welcome F.L., Tauer L.W., Gröhn Y.T.; The effect of recurrent episodes of clinical mastitis caused by gram-positive and gram-negative bacteria and other organisms on mortality and culling in Holstein dairy cows; 2011; Journal of Dairy Science 94, 4863–4877.
17. Hertl J.A., Schukken Y.H., Welcome F.L., Tauer L.W., Gröhn Y.T.; Effects of pathogen-specific clinical mastitis on probability of conception in Holstein dairy cows; 2014; Journal of Dairy Science 97, 6942–6954.

18. Law no. 43/2014 regarding handling and protection of animals used for scientific purposes, OM no.326/Part I.

19. Jamali H., Barkema H.W., Jacques M., Lavallée-Bourget E.M., Malouin F., Saini V., Stryhn H., Dufour S.; Invited review: incidence, risk factors, and effects of clinical mastitis recurrence in dairy cows; 2018; J. Dairy Sci.,101(6):4729–4746, doi: 10.3168/jds.2017-13730.

20. Kew M.C.; Serum aminotransferase concentration as evidence of hepatocellular damage; 2000; Lancet, 355:591-592.

21. Leslie K.E., Petersson-Wolfe C.S.; Assessment and Management of Pain in Dairy Cows with Clinical Mastitis; 2012; Vet. Clin. N. Am.-Food Anim. Pract, 28, 289–305.

22. Moschovas M., Pavlatos G., Basdagianni Z., Manessis G., Bossis I. A.; Cross-Sectional Study of Risk Factors Affecting Milk Quality in Dairy Cows; 2023; Animals (Basel). Nov 10;13(22):3470, doi: 10.3390/ani13223470.

23. Mostert P.F., Bokkers E.A.M., de Boer I.J.M., van Middelaar C.E.; Estimating the impact of clinical mastitis in dairy cows on greenhouse gas emissions using a dynamic stochastic simulation model: a case study; 2019; Animal, 13(12), 2913-2921, doi.org/10.1017/S1751731119001393.

24. Moyes K., Sorensen P., Bionaz M.; The impact of intramammary *Escherichia coli* challenge on liver and mammary transcriptome and cross-talk in dairy cows during early lactation using RNAseq; 2016; PLoS ONE, 11.

25. Ndahetuye J.B., Persson Y., Nyman A.-K., Tukei M., Ongol M.P., Bage R.; Aetiology and prevalence of subclinical mastitis in dairy herds in peri-urban areas of Kigali in Rwanda; 2019; Tropical Animal Health and Production, 51 (7), 2037–2044.

26. Nitz J., Wente N., Zhang Y., Klocke D., Seeth M.T., Krömker V.; Dry period or early lactation-Time of onset and associated risk factors for intramammary infections in dairy cows; 2021; Pathogens, 10:224, doi: 10.3390/pathogens10020224.

27. Roberson J.R.; Treatment of Clinical Mastitis; 2012; Vet. Clin. N. Am.-Food Anim. Pract. 2, *28*, 271–288.

28. Ruegg P.L.; A 100-year review: mastitis detection, management, and prevention; 2017; J. Dairy Sci., 100 (12), 10381–10397.

29. Saharan D.K., Jaidiya K., Meena Y., Bhalothia S.K., Choudhary S.; Mineral profile changes in cattle affected with clinical mastitis; 2022; Pharma Innov. J, 11, 2596–2599.

30. Saleh N., Allam T.S., Omran A., Abdelfattah A.M.; Evaluation of Changes in Hemato-Biochemical, Inflammatory, and Oxidative Stress Indices as Reliable Diagnostic Biomarkers for Subclinical Mastitis in Cows; 2022; Alex. J. Vet. Sci, 72, 23.

31. Sarvesha K., Satyanarayana M.L., Narayanaswamy H.D., Rao S., Yathiraj S., Isloor S. Mukartal S.Y., Singh S.V., Anuradha M.E.; Haemato-Biochemical Profile and Milk Leukocyte Count In Subclinical And Clinical Mastitis Affected Crossbred Cattle; 2017; Journal of Experimental Biology and Agricultural Sciences. 5(1):1-6.

32. Schukken Y.H., Wilson D.J., Welcome F., Garrison-Tikofsky L., Gonzalez R.N.; Monitoring Udder Health and Milk Quality Using Somatic Cell Counts; 2003; Vet. Res., *34*, 579–596.

33. Schukken Y.H., Günther J., Fitzpatrick J., Fontaine M.C., Goetze L., Holst O., Leigh J., Petzl W., Schuberth H.J., Sipka A., D.G.E., Smith R., Quesnell J., Watts R., Yancey H., Zerbe A., Gurjar R., Zadoks N., Seyfert H.M.; Host-Response Patterns of Intramammary Infections in Dairy Cows; 2011; Vet. Immunol. Immunopathol, 144, 270–289.

34. Singh R., Bhardwaj R.K., Azad M.S., Beigh S.; Effect of mastitis on haemato-biochemical and plasma mineral profile in crossbred cattle. Indian Journal of Animal Science; 2014; 48(1):63-66.

35. Stanojevic J., Kreszinger M., Radinovic M., Kladar N., Tomanic D., Ruzic Z., Kovacevic Z.; Assessment of Mastitis Patterns in Serbian Dairy Cows: Blood Serum Metabolic Profile and Milk Composition Parameters; 2023; Pathogens, 12(11):1349.

36. Suojala L., Kaartinen L., Pyörälä S.; Treatment for Bovine *Escherichia Coli* Mastitis—An Evidence-Based Approach; 2013; J. Vet. Pharmacol. Ther, *36*, 521–531.

37. Thompson Crispi K., Atalla H., Miglior F., Mallard B.A.; Bovine mastitis: frontiers in immunogenetics; 2014; Front Immunol., 5:493.

38. Zaki M.S., El-Battrawy N., Mostafa S.O.; Some biochemical Studies on Friesian suffering from subclinical mastitis; 2010; Nature and Science, 8(4):143-146.

39. Zeryehun T. Abera G.; Prevalence and bacterial isolates of mastitis in dairy farms in selected districts of Eastern Harrarghe zone, Eastern Ethiopia; 2017; Journal of Veterinary Medicine, 7.

BACK-CROSSING SCHEMES USED TO CREATE BEEF SYNTHETIC BREEDS

CREAREA UNUI NUCLEU DE TAURINE DE CARNE PRIN UTILIZAREA SCHEMEI DE ÎNCRUCIȘARE BACK-CROSS

GAVOJDIAN DINU[1], NICOLAE IOANA[1]

[1]Research and Development Institute for Bovine, Sos. Bucuresti-Ploiesti km 21, Balotesti, 077015, Romania,
Phone (+40) 213501026, E-mail icdcb.balotesti@asas.ro

Correspondence address: gavojdian_dinu@animalsci-tm.ro

Abstract

The objectives of the present study were the following: i) to develop synthetic beef breeds, adapted to the Romanian climate, while taking advantage of the heterosis effects, throughout the use of back-crossing schemes; ii) to test breed complementarity between ***Romanian Black and White (RBW)*** *dairy cows and* ***Belgian Blue***, ***Aberdeen Angus***, ***Limousin*** *and* ***Charolais*** *beef specialized breeds; iii) to increase dairy farms returns throughout the production of calves with better fattening attributes and higher marketing value. The experiments were carried out at the Experimental Farm of the Research and Development Institute for Bovine in Balotesti, within the framework of two research projects funded by the Ministry of Agriculture and Rural Development, between 2015 and 2022. The live body weights of the back-cross calves at the age of 210 days were on average of 339.8±5.1 kg in R_1* ***Belgian Blue*** *x (F_1* ***Belgian Blue*** *x* ***RBW****), 312.4±3.3 kg in R_1* ***Charolais*** *x (F_1* ***Charolais*** *x* ***RBW****), 296.2±2.9 kg in R_1* ***Limousin*** *x (F_1* ***Limousin*** *x* ***RBW****) and of 244.0±3.5 kg in R_1* ***Aberdeen Angus*** *x (F_1* ***Aberdeen Angus*** *x* ***RBW****). Growth rates were significantly influenced by the sire genetics, back-cross crossbreeding schemes being recommended when sexed semen is used at dairy farms level, or on culled/low productive cows. By applying well designed crossbreeding schemes, it is possible to increase meat production and implicitly the economic returns.*

Key words: cattle; crossbreeding schemes; beef production; growth rates

Rezumat

Obiectivele prezentului studiu au fost următoarele: i) producerea și testarea unor hibrizi specializați pentru producția de carne, care să fie bine adaptați la condițiile de creștere din România, prin utilizarea unor scheme de tipul back-cross; ii) testarea complementarității rasiale între vacile din rasa ***Bălțată cu Negru Românească (BNR)*** *și rase specializate de carne precum* ***Blanc-Bleu Belge (BBB), Aberdeen Angus (AA), Limousine (LI)*** *și* ***Charolaise (CH)****; iii) creșterea rentabilității fermelor prin producerea de viței cu aptitudini bune pentru îngrășare și cu valoare comercială ridicată. Experimentele au fost realizate în Laboratorul Experimental Zootehnic al I.C.D.C.B, în cadrul a două proiecte de cercetare finanțate de M.A.D.R, în perioada 2015-2022. Greutățile corporale ale vițeilor hibrizi la vârsta de 210 zile au fost în medie de 339,8±5,1 kg la hibrizii R_1* ***BBB*** *x (F_1* ***BBB*** *x* ***BNR****), 312,4±3,3 kg la hibrizii R_1* ***CH*** *x (F_1* ***CH*** *x* ***BNR****), 296,2±2,9 kg la hibrizii R_1* ***LI*** *x (F_1* ***LI*** *x* ***BNR****) și de 244,0±3,5 kg la hibrizii R_1* ***AA*** *x (F_1* ***AA*** *x* ***BNR****). Ratele de creștere au fost influențate în mod semnificativ de structura genetică a hibrizilor, aplicarea schemelor de hibridare de tip back-cross fiind recomandate atunci când la nivelul fermei se utilizează material seminal sexat sau la vacile de slab productive. Prin aplicarea schemelor de încrucișare de tipul back-cross, este posibilă creșterea producției de carne la nivelul fermelor și implicit, a rentabilității economice.*

Cuvinte cheie: taurine; scheme de hibridare; producția de carne; rate de creștere

INTRODUCTION

Globally, meat and dairy consumption is expected to increase by 70% until 2050 (FAO, 2009). Livestock is currently an important sector in the European Union, with revenues of 130 billion EUR, representing 48% of total agricultural income, and employing around 30 million people (EuroStat, 2022).

The current situation, as well as existing estimates, creates the conditions for EU Member States to become important meat exporters, especially to Asian and African countries.

According to a report published by the European Commission (DG-AGRI, 2017), on the EU agricultural sector production forecasts for 2030, the cattle sector will undergo a period of major challenges and changes. The dairy cattle sector will see a decrease in herds, from 24 million heads for the reference year 2017, to 21.8 million heads by 2030, with estimated decreases of 1.3% per year. In Europe, the number of cows kept for milk production is expected to decrease mainly due to the overproduction of milk, low milk prices and reduced milk consumption, from 58 kg milk/person to 52 kg milk/person forecasted for 2030. Moreover, average milk production for 2030 in the European countries is forecast at 8300 kg per dairy cow, an increase of 1400 kg compared to the reference year 2017.

The level of milk production in Romania is currently well below the European average, which makes the dairy farming sector significantly vulnerable. Furthermore, the increasing use of sexed semen on large dairy farms leads to a surplus of replacement heifers, and therefore the application of crossbreeding schemes with beef bulls can increase income at farm level, especially if low-producing and culled cows are used to produce crossbreeds for fattening.

The beef cattle sector is highly diverged in the EU member countries. The European Commission's report (DG-AGRI, 2017) estimates a decrease in the number of herds raised for beef production with 10% by 2030, compared to the number of cattle raised for meat production in 2017. The decrease in the number of cattle kept for meat production at European level is based on the forecasted reduction in beef consumption at European level, from 10.8 kg/person/year in 2017, to 10.1 kg/person/year by 2030. However, in the same DG-AGRI report, the number of cattle kept for meat production is expected to increase with 7-10% by 2030 in the Eastern European countries, mainly in Poland, Hungary, Bulgaria and Romania. This can be a market advantage for Romanian farmers, especially those who want to switch from dairy to beef production.

The objectives of the present study were as follows: *i)* to develop composite beef breeds, adapted to the Romanian climate, taking advantage of the heterosis effects, using back-crossing schemes; *ii)* to test breed complementarity between **Romanian Black and White (RBW)** dairy cows and **Belgian Blue**, **Aberdeen Angus**, **Limousin** and **Charolais** beef breeds; *iii)* to increase farm returns throughout the production of calves with better fattening attributes and higher marketing value.

MATERIAL AND METHODS

The experiments were carried out in the Experimental Farm of the Research and Development Institute for Bovine Balotesti, within the framework of two research projects funded by the Ministry of Agriculture and Rural Development, through the ADER Sectorial Plans, as follows:

- *ADER project 5.1.10*, with implementation period 2015-2018, project in which a number of four F_1 crossbreeds were produced and tested (crossbreeding scheme shown in Figure 1), using the **Romanian Black and White** breed as the maternal breed, crossed with beef bulls from the **Belgian Blue**, **Aberdeen Angus**, **Charolais** and **Limousin** breeds. The F_1 crossbred heifers obtained were kept for breeding, and the male calves were marketed after testing their growth performances at the age of 7-8 months.
- *ADER project 8.1.12*, with implementation period 2019-2022, in this project back-cross (return, R_1) calves were produced and tested (crossing scheme shown in Figure 2), using the F_1 crossbreed females, which were further mated with bulls from their respective paternal breeds. The obtained R_1 heifers were kept for further breeding, and the bull calves were marketed at the age of 7-8 months, after testing their growth performances.

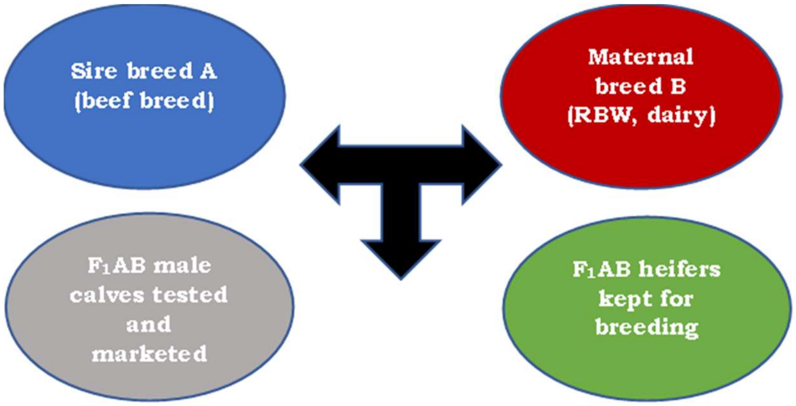


Figure 1. **Crossbreeding scheme applied to obtain the F_1 crossbred heifers, using Belgian Blue, Aberdeen Angus, Charolais and Limousin beef sire breeds (A) and the Romanian Black and White dairy cows as the maternal breed (B) / Schema de încrucișare aplicată pentru obținerea junincilor F1, utilizând ca rasă paternă rasele Blanc-Bleu Belge, Aberdeen Angus, Charolais și Limousin (A) și Bălțată cu Negru Românească ca rasă maternă (B)**

For each animal in the experimental groups (n=5-7 calves/genotype), an individual record was set-up, containing data such as: identification number, type of crossbreed, sire (ID and breed), dam (ID and type of F_1 crossbreed), date of birth, birth weight, live weights at 100±7 and 210±7 days, average daily gains (g/day), type of birth and health records.

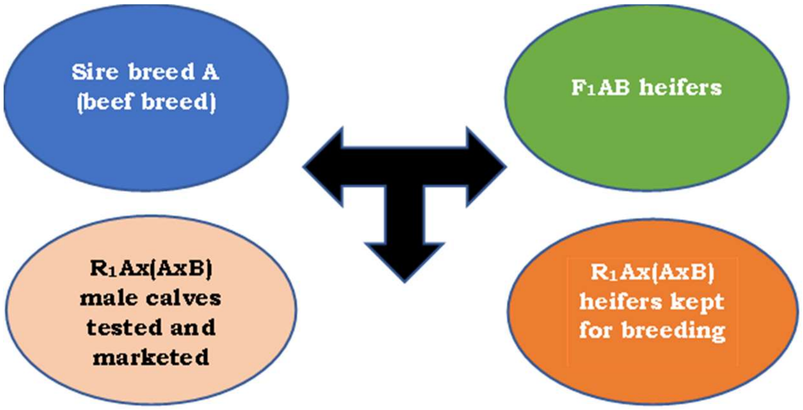


Figure 2. **Crossbreeding scheme applied to obtain the back-cross crossbred heifers ('return crossing'), using Belgian Blue, Aberdeen Angus, Charolais and Limousin beef sire breeds / Schema de încrucișare aplicată pentru obținerea junincilor („încrucișare de întoarcere"), utilizând rasele de carne Blanc-Bleu Belge, Aberdeen Angus, Charolais și Limousin**

All R_1 calves were separated from their dams at birth, after their first colostrum intake. Cows were than managed under semi-intensive dairy production system, with calves being kept in individual hutches until weaning at 95-105 days of age, and receiving a daily ration of 6-8 kg of milk/head/day, alfalfa hay and starter concentrates *ad libitum*. Male calves were kept intact and were marketed at 7-8 months of age.

Table 1. Estimated costs for producing the R_1 crossbred calves and raising them until 7 months / Costuri estimative pentru producerea viților încrucișați R1 și creșterea acestora până la 7 luni

Item	Price estimates (EUR)
AI semen (x2 doses/gestation)	6.1
Milk costs (880 kg)	363.5
Concentrates costs (445 kg)	187.0
Hay costs (335 kg)	27.4
Corn silage (1340 kg)	41.9
Other costs: electricity, veterinary care, water, etc.	52.3
Total costs	**678.5**

RESULTS AND DISCUSSION

The evaluation of live body weights and growth rates was carried out comparatively, based on the genetic structure of the back-crosses (R_1), in order to assess the growth efficiency up to the age of 7 months, using a Patura500 weighing platform, equipped with an electronic MP 800 True-Test scale, results being presented in Table 2.

The average daily gains and marketing live weights at 7 months of age for the R_1 Belgian Blue 50% x (F_1 **Belgian Blue** 25% x **Romanian Black and White** 25%) crossbreed calves were of 1373.3 g/day and 339.8 kg, respectively. The high growth performances have led to obtaining an overall selling price per calf of 992.2 EUR/calf, and after subtracting the production costs of 678.5 EUR/head, to an estimated profit of 313.7 EUR/calf produced and sold.

In R_1 **Charolais** 50% x (F_1 **Charolais** 50% x **RBW** 50%) crossbreed calves, the average daily gains were of 1307.7 g/day and the live weights at 7 months of age of 312.4 kg. The overall growth performance of the R_1 **Charolais** sired calves has led to obtaining an overall selling price per calf of 912.2 EUR, and after subtracting the production costs, to an estimate profit of 233.7 EUR/calf.

The R_1 **Limousin** 50% x (F_1 **Limousin** 50% x **RBW** 50%) crossbreed calves registered average daily gains of 1220.1 g/day, which has led to a marketing average live weight of 296.2 kg at the age of 7 months and to a selling price of 864.9 EUR, which in turn has led to obtaining an estimated profit of 186.4 EUR per calf sold.

More modest average daily gains were registered for the R_1 **Aberdeen Angus** 50% x (F_1 **Aberdeen Angus** 50% x **RBW** 50%), of 960.3 g/day, which lowered the marketing live weights at the age of 7 months, leading to average live weights of 244.0 kg, and subsequently lower selling prices of 712.4 EUR, which in return has led to a decrease of the estimated profits to 33.9 EUR per calf.

Table 2. Daily gains, marketing weights and estimated economic returns in R_1 cross-breeds / Câștigurile zilnice, greutatea comercială și randamentele economice estimate la încrucișările R1

Item/ Genotype	R_1 Belgian Blue x (F_1 Belgian Blue x RBW)	R_1 Charolais x (F_1 Charolais x RBW)	R_1 Limousin x (F_1 Limousin x RBW)	R_1 Angus x (F_1 Angus x RBW)
Average daily gains (g/day)	1373.3±4.9	1307.7±3.6	1220.1±3.2	960.3±2.8
Marketing live weights (kg)	339.8±5.1	312.4±3.3	296.2±2.9	244.0±3.5
Price per calf sold at 7 mo. (EUR)	992.2	912.2	864.9	712.4
Estimated profit (EUR)/ head	***313.7***	***233.7***	***186.4***	***33.9***

Note: Marketing price used in the estimates was of 2.92 EUR/kg live weight (VAT included)

The highest profits were obtained from marketing the R_1 **Belgian Blue** x (F_1 **Belgian Blue** x **RBW**) and the R_1 **Charolais** x (F_1 **Charolais** x **RBW**) crossbreed calves. While the lowest profits were registered in producing and selling of the R_1 **Angus** x (F_1 **Angus** x **RBW**) crossbreed calves. As a consequence, Angus genetics is not recommended for crossbreeding schemes under semi-intensive beef to dairy systems, the breed having good aptitudes under extensive grass-based systems, where the production costs are significantly lower. It is worth pointing out that in this study, only the male calves were marketed, with the R_1 female crossbreed calves being kept for further breeding purposes.

CONCLUSIONS

The growth rates of the back-cross calves obtained and tested in this study were comparable to those recorded in beef specialized breeds raised under semi-intensive production systems. As a result, the application of the back-crossbreeding schemes can increase meat production at farm level and improve incomes, given the higher selling price of crossbreed calves compared to dairy-veal calves. Crossbreeding offers a number of biological advantages, such as hybrid vigour (heterosis effects) and the high degree of complementarity between dairy and beef breeds.

The results showed that there are a number of advantages and disadvantages for each type of R_1 crossbreed, and certain crossbreeding schemes are recommended, depending on the farm specificity, such as the type of feed, the existence of pasture areas, beef market value, the degree of mechanisation and the degree of specialisation of the farm-workers.

Beef production in crossbreeding schemes was significantly influenced by the sire genetics, with this approach being advised when sexed semen is used at farm level, or on culled/low productive dairy cows. One of the main disadvantages of practicing crossbreeding is that subsidies are reduced, given that no performance recordings occur, which than negatively influences the baseline for state and EU payments.

ACKNOWLEDGEMENTS:

This research was supported by the Sectorial Plan projects ADER 5.1.10/2015 and ADER 8.1.2/2019, funded by the Romanian Ministry of Agriculture and Rural Development.

BIBLIOGRAPHICAL REFERENCES

1. DG-AGRI, EU agricultural outlook for the agricultural markets and income 2017-2030; 2017; Source: https://agriculture.ec.europa.eu/system/files/2018-07/agricultural-outlook-2017-30_en_0.pdf.
2. Eurostat, Agricultural production - livestock and meat; 2022; Source: https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Agricultural_production_-_livestock_and_meat.
3. Food and Agriculture Organization of the United Nations (FAO), HOW to feed the world, High Level Expert Forum - Global agriculture towards 2050; 2009; Rome – Italy, Source: https://www.fao.org/fileadmin/templates/wsfs/docs/Issues_papers/HLEF2050.
4. Gavojdian D., Nicolae I., Inventarierea resurselor genetice la bovine în România; 2018; Ed. TotalPublishing, București.
5. Gavojdian D., Elemente de proiectare tehnologică la taurine pentru producția de carne; 2022; Ed. TotalPublishing, București.
6. Georgescu G., Mărginean G., Vintilă I., Grosu H., Drăgănescu C.I., Tratat de creștere a bovinelor – Ameliorare; 1998; Ed. Ceres, București.
7. Grosu H., Oltenacu P.A.; Programe de ameliorare genetică în zootehnie; 2005; Ed. Ceres, București.
8. Stanciu G.; Tehnologia creșterii bovinelor; 1999; Ed. Brumar, Timişoara.

COMPARATIVE STUDY REGARDING GROWTH PERFORMANCES OF LAMBS FROM TSIGAI BREED – RUSTY VARIETY AND TRIRACIALI CROSSBRED LAMBS OBTAINED FROM CROSSING BETWEEN TSIGAI BREED WITH SPECIALISED RAMS BREEDS FOR MEAT PRODUCTION

STUDIU COMPARATIV PRIVIND PERFORMANȚELE DE CREȘTERE ALE MIEILOR DIN RASA ȚIGAIE - VARIETATEA RUGINIE ȘI A METIȘILOR TRIRASIALI OBȚINUȚI PRIN ÎNCRUCIȘAREA RASEI ȚIGAIE CU BERBECI DIN RASE SPECIALIZATE PENTRU PRODUCȚIA DE CARNE

ELENA ILIȘIU[1,2], DORINA NADOLU[1,3], CRISTIAN VASILE-ILIȘIU[1,2],
ANDREEA - HORTENSA ANGHEL[1,3], ANA ENCIU[1], VASILE - CĂLIN ILIȘIU[1,2],
ION - DUMITRU CHIRTEȘ[1,2], CAMELIA ZOIA ZAMFIR[1]

[1]Research and Development Institute for Sheep and Goat Palas - Constanta, I. C. Brătianu, 248, Romania
Phone: 0241639506, E-mail: icdcocpalas@yahoo.com
[2]Caprirom Nord Association, 545300 Reghin, Dedradului 11, Romania
Phone: 0743331553, E-mail: capriromreghin@yahoo.com
[3]Romanian National Association of Goats Breeders „Caprirom„ 248, Constanta, Romania
Phone: 0727869984, E-mail: caprirom@yahoo.com

Correspondence address: nuti.ilisiu2@yahoo.com

Abstract

The current study aimed to assess the growth performance and eye muscle (M. Longissimus dorsi) properties in 70 female lambs from two distinct genotypes: 40 individuals from the ***Tsigai*** *– rusty variety and 30 females from a crossbreed of* ***Suffolk*** *(50%) x* ***German Blackface*** *(37.5%) x* ***Tsigai*** *(12.5%). Measurements including birth weight (BW), weaning weight (WW), and weight at 5 months (W5M) were recorded for analysis. Ultrasonic measurements were utilized to assess the depth, eye muscle area, and eye muscle perimeter of the Longissimus dorsi, as well as the thickness of fat covering this muscle. The animals were fed the same diets throughout the study. The results obtained highlighted the fact that, breed structure influenced significantly ($p<0.05$) birth weight, with similar significant effects observed for weaning weight and weight at 5 months, indicating the impact of breed structure on these growth parameters. Significant differences ($p<0.001$) in favour of crossbred female were also recorded regarding the total weight gain in the birth-weaning and birth-5 months period, differences that were also reflected in the average daily gains. Means for ultrasound measurements of eye muscle properties were highly significant ($p<0.001$) only for backfat thickness, at 3rd – 4th lumbar vertebrae. Regarding phenotypic correlations between ultrasound measurements and weight at 5 months, among a total of 45 trait pairs, 46.66% exhibited small correlations (0.00-0.30), 26.67% displayed medium to high correlations (0.31-0.60), and 26.67% showed high correlations (0.61-1.00).*

Key words: lamb, genotype, Tsigai, crossbred, ultrasound

Rezumat

Scopul studiului actual îl reprezintă evaluarea performanțelor de creștere și caracteristicile ochiului de mușchi (mușchiul Longissimus dorsi) la 70 de femele din două genotipuri distincte, respectiv: 40 de capete din rasa ***Țigaie*** *– varietatea ruginie și 30 capete femele metise* ***Suffolk*** *(50%) x* ***Germană de Carne cu Capul Negru*** *(37,5%) x* ***Țigaie ruginie*** *(12,5%). În scopul determinării performanțelor de creștere au fost înregistrate măsurători, respectiv greutatea la fătare, greutatea la înțărcare și greutatea la 5 luni. Măsurătorile cu ultrasunete au fost utilizate pentru aprecierea adâncimii, suprafeței și perimetrul mușchiului Longissimus dorsi, precum și grosimea stratului de grăsime care acoperă acest mușchi. Animalele au*

fost hrănite cu aceeași dietă pe tot parcursul studiului. Rezultatele obținute au evidențiat faptul că, structura de rasă a influențat semnificativ (p<0,05) greutatea la naștere, cu efecte semnificative similare observate pentru greutatea la înțărcare și greutatea la 5 luni, indicând impactul structurii rasei asupra acestor parametri de creștere.
S-au înregistrat diferențe semnificative (p<0,001) în favoarea femelelor metise și în ceea ce privește creșterea totală în greutate în perioada naștere-înțărcare și naștere-5 luni, diferențe care s-au reflectat și în sporurile medii zilnice. Mediile măsurătorilor cu ultrasunete pentru caracteristicile ochiului de mușchi au fost foarte semnificative (p<0,001) doar pentru grosimea stratului de grăsime care acoperă mușchiul între vertebrele 3 – 4 lombare. În ceea ce privește corelațiile fenotipice dintre măsurătorile cu ultrasunete și greutatea la 5 luni, dintr-un total de 45 de perechi de trăsături, 46,66% au prezentat corelații mici (0,00-0,30), 26,67% au prezentat corelații medii spre mari (0,31-0,60), iar 26,67% au prezentat corelații mari (0,61-1,00).

Cuvinte cheie: miel, genotip, Țigaie, metis, ultrasunete

INTRODUCTION

Real-time ultrasonography is a non-invasive technique enabling the evaluation of carcass quality without causing harm to the product, as demonstrated by studies (Delfa et al., 1995; Fernández et al., 1998; Mendizabal et al., 2003). The cost-effectiveness and portability of ultrasound equipment have facilitated its integration into national genetic programs aimed at enhancing lamb carcass quality across numerous regions globally (Stanford et al., 1998).

Ultrasound measurement represents a novel, non-invasive, and efficient grading method for classifying and quantifying animal carcasses early in their lifecycle, facilitating their subsequent use for reproduction purposes. The inclusion of measured parameters such as subcutaneous fat layer thickness, muscle eye area, and muscle depth introduces new selection criteria, namely muscle depth and subcutaneous fat layer thickness, alongside traditional ones like body weight and carcass meat. The ultrasound technique is predominantly employed for sheep carcass grading in Western European Union countries with established traditions in sheep rearing and breeding for meat production. This modern technology is currently utilized in the UK, New Zealand, and Ireland for breeding programs, as evidenced by studies (Fogarty et al., 1992, 1995; Wilson, 1992; Russel, 1995; Larsgard & Kolstad, 2003), demonstrating strong correlations with conventional methods. Fernández et al. (1997) found significant correlations between ultrasound measurements and carcass measurements of LD muscle in Manchego lambs, with coefficients of 0.88 for muscle eye area, 0.74 for muscle depth, and 0.56 for subcutaneous fat layer thickness. Fernández et al., (1998) and Ibrahim et al., (2007) identified moderate correlations between ultrasound measurements and carcass assessment for muscle eye area and weaning body weight, as well as between the thickness of the subcutaneous fat layer and weaning body weight. Emenheiser et al., (2010) underlined the importance of validating the use of ultrasound in determining lamb carcass composition for meat production and highlighted its advantageous features. Thus, ultrasound offers breeders, producers, and researchers the possibility to assess carcass composition traits in live animals, thereby enhancing the precision of breeding, management, and marketing decisions (Leeds et al., 2008).

The research aimed to assess backfat thickness and *Longissimus dorsi* muscle characteristics in lambs of **Tsigai breed** and crossbred **Suffolk** (50%) x **German Blackface** (37.5%) x **Tsigai** (12.5%) using ultrasound measurements. Additionally, it aimed to examine the influence of genotype on these traits. By offering the initial benchmark for these phenotypic parameters through ultrasonic measurements, this study could facilitate the launch of a genetic breeding program. This program could incorporate ultrasonic measurements to enhance meat yield and, consequently, the quality of lambs, particularly those of the **Tsigai** – rusty variety.

MATERIALS AND METHODS

This study took place at the Experimental Base Reghin of the Research Institute for Sheep and Goat Palas Constanta, situated in Mures County at coordinates 46°46' N and 22°42' E. The location, at an altitude of 395 meters, recording an annual rainfall ranging from 650 to 700 mm, with average temperatures of 19°C during summer and -3°C during winter.

Animal management

In this study, a total number of 70 female lambs, were divided into two groups. Lot 1 (L1) comprised 40 females of the **Tsigai** - rusty variety, while Lot 2 (L2) consisted of 30 crossbred female **Suffolk** (50%) x **German Blackface** x **Tsigai** (**S** x **BF** x **Ti**). The mother ewes, were maintained on pastures from half of May until early December, and in shelter during December – May period. Concentrates were provided as supplements during certain critical periods, such as late gestation and the two months following lambing. The lambs, born between January and March, were tagged and weighed (±0.1 kg) shortly after birth. Their sex, date of birth, type of birth, as well as the dam and ram group, were recorded. Throughout the first five months of their lives, the lambs were weighed monthly (±0.1 kg). Ewes and their lambs remained together under the same management conditions for two months post-lambing, after which the lambs were weaned at approximately 60 days old. During the suckling period, the diet was tailored to achieve a growth potential of 300 g/head/day, aligning with the requirements outlined by NRC (1985) (135 g DP and 10.89 MJ NE).

The composition of the concentrated feed consisted of: 30% corn flour, 30% barley flour, 25% corn grain, 11.25% sunflower groats, 2.25% calcium, and 1.5% salt. Following weaning until reaching five months of age, both groups of lambs were fed the same diet, housed in shelters, and provided with *ad libitum* access to feed.

The structure of concentrate fodder is presented in Table 1. Additional, in the ration was added hill hay.

Table 1. Structure of concentrate fodder used in the experiment with lambs from different genotype (after weaning) / Structura furajului concentrate utilizat în experiment cu miei din genotipuri diferite (după înțărcare)

Characteristics	Composition
Corn flour (%)	4.00
Barley flour (%)	4.00
Corn grain (%)	90.00
Sunflower groats (%)	1.50
Calcium (%)	0.30
Salt (%)	0.2
Dry matter/kg concentrated fodder	830
Digestible protein g/kg dry matter	100
NE MJ/kg dry matter	11.29

The lambs underwent measurements for birth weight (BW), weaning weight (WW), and weight at five months (W5M), as well as assessments for *Longissimus dorsi* muscle (LD) depth (LMD), area (LMA), and perimeter (LMP), along with backfat thickness (BF) overlying this muscle, between the 12th and 13th rib and between the 3rd and 4th lumbar vertebrae, utilizing ultrasonic scanning. Prior to ultrasonic scanning, wool was removed from the measurement areas through shearing.

The ultrasonic measurements were conducted *in vivo* by a skilled operator using an HS-1600 (HONDA ELECTRONICS CO.), ultrasonic machine equipped with an HLV 7218 linear probe, operating at frequencies of 1.5/2.0/3.0 MHz. All captured images were analysed using the ImageJ program.

Following the capture of the scan image, measurements of muscle depth, *Longissimus dorsi* area, *Longissimus dorsi perimeter, and backfat thickness at the specified* points were conducted using the electronic calliper of the scanner, with a resolution of 0.01 cm. The *Longissimus dorsi* area and perimeter were assessed on the same image after delineating the muscle borders.

Statistical analysis was carried out using the JASP procedure, employing Student tests for data processing.

RESULTS AND DISCUSSION

The Table 2 provides data on female body development, total weight gain, and average daily gain (ADG) from birth to five months. The average birth weight of lambs ranged from 4.28 kg in L1 to 4.62 kg in L2, differences being statistically significant ($p<0.05$). The analysis revealed significant effects ($p<0.001$) of breed structures on weaning weight (WW) and weight at five months (W5M). Female lambs in L2 exhibited higher weights, with 4.79 kg at WW and 7.12 kg at W5M, compared to female lambs in L1. Significant differences ($p<0.001$) in favour of crossbred females were also noted in total weight gain during the birth-weaning and birth-five-months periods, differences that were also reflected in the average daily gains ($p<0.001$). No statistical differences ($p>0.05$) were found between the two lots with regard at total gain and ADG in weaning-five months period.

Table 3 presents statistical data on ultrasonic measurements of eye muscle properties and lamb weights at five months. Significant differences assigned to breed structure were observed in backfat thickness ($p<0.001$) at the 3rd and 4th lumbar vertebrae. Additionally, the eye muscle area was greater in L2 with 0.51 cm^2 at the 12th rib and 0.35 cm^2 at the 3rd-4th lumbar vertebrae, compared to L1. It can be mentioned that, despite having the highest body weight, the lots of crossbred females exhibited lower backfat thickness, as is resulting from Table 3.

Table 2. Mean (±SE) for body evolution, total gain and ADG of female from birth up to 5 months / Media (±SE) pentru evoluția greutății corporale, spor total și spor mediu zilnic de la fătare până la vârsta de 5 luni

Specification	Genotype	
	L1 Tsigai (N = 40)	L2 SxBFxTi (N = 30)
BW, kg	4.28 ± 0.10^{a}	4.62 ± 0.12^{b}
WW, kg	18.22 ± 0.48^{A}	23.01 ± 0.56^{B}
W5M, kg	34.71 ± 0.94^{A}	41.83 ± 1.08^{B}
Total gain birth-weaning, kg	13.95 ± 0.49^{A}	18.38 ± 0.54^{B}
Total gain weaning – 5 months, kg	16.49 ± 0.69	18.82 ± 0.78
Total gain birth - 5 months, kg	30.44 ± 0.93^{A}	37.21 ± 1.08^{B}
ADG birth-weaning, kg	235.85 ± 7.89^{A}	306.11 ± 9.11^{B}
ADG weaning - 5 months, kg	169.36 ± 6.63	182.74 ± 7.53
ADG birth- 5 months, kg	193.72 ± 5.28^{A}	227.71 ± 6.09^{B}
Age at weaning, days	59.63 ± 1.29	60.60 ± 1.49

Means with different superscripts (^{a}, ^{b}) in each trait differ (P< 0.05).
Means with different superscripts (^{A}, ^{B}) in each trait differ (P< 0.001).

Table 3. Mean (±SE) for ultrasound measurements of eye muscle properties and weights of female at 5 months / Media (±SE) pentru măsurătorile cu ultrasunete ale caracteristicilor ochiului de mușchi și greutatea femelelor la 5 luni

Specification	Genotype	
	L1 Tsigai (N = 40)	L2 SxBFxTi (N = 30)
Fat depth on 12 rib (mm)	6.46 ± 0.18	6.15 ± 0.21
Fat depth between 3 – 4 lumbar vertebra (mm)	6.01 ± 0.17^{A}	5.35 ± 0.19^{B}
LD Muscle depth on 12 rib (mm)	23.27 ± 0.48	24.05 ± 0.56
LD Muscle depth between 3 – 4 lumbar vertebra(mm)	22.52 ± 0.44	22.67 ± 0.51
Eye muscle area on 12 rib (cm^2)	15.33 ± 0.32	15.84 ± 0.37
Eye muscle area between 3 – 4 lumbar vertebra (cm^2)	13.69 ± 0.22	14.04 ± 0.43
Eye muscle perimeter on 12 rib (mm)	171.73 ± 2.23	174.90 ± 2.58
Eye muscle perimeter between 3 – 4 lumbar vertebra (mm)	164.43 ± 2.20	167.43 ± 2.54
Live weight (kg)	34.71 ± 0.94^{A}	41.83 ± 1.08^{B}

Means with different superscripts (^{A}, ^{B}) in each trait differ (P< 0.001).

Concerning the phenotypic correlations between ultrasound measurements and weight at five months, among a total of 45 trait pairs, 46.66% displayed small correlations (0.00-0.30), 26.67% exhibited medium to high correlations (0.31-0.60), and 26.67% showed high correlations (0.61-1.00). Notably, a negative correlation was observed between backfat thickness and *Longissimus dorsi* depth, area, perimeter, and weight at five months, while all other correlations were positive. The correlation between *Longissimus dorsi* area and perimeter was highest (0.77 at the 3rd-4th lumbar vertebrae and 0.72 at the 12th rib, respectively) (Table 4). Additionally, moderate correlations were found between the weight of lambs at five months and the measurements of *Longissimus dorsi* depth and area at the 12th rib, while the correlation of weights at five months with backfat thickness and perimeter was low.

Significant differences ($p<0.001$) were observed between backfat thickness at the 3rd- 4th lumbar vertebrae, as well as between *Longissimus dorsi* area with depth, perimeter, and body weight at five months.

Table 4. Correlation coefficients between ultrasound measurements and weight at 5 months (n = 70) / Coeficienții de corelație între măsurătorile cu ultrasunete și greutatea la 5 luni (n = 70)

Traits	BFT12	BFT34	LMD12	LMD34	LMA12	LMA34	LMP12	LMP34	W5M
BFT12	1.00								
BFT34	0.43***	1.00							
LMD12	-0.03	0.12	1.00						
LMD34	0.32	0.16	0.30	1.00					
LMA12	-0.04	0.01	0.56***	0.37	1.00				
LMA34	-0.07	0.08	0.43***	0.51***	0.61***	1.00			
LMP12	0.08	0.06	0.12	0.28	0.72***	0.44***	1.00		
LMP34	-0.13	0.07	0.33	0.19	0.53***	0.77***	0.42***	1.00	
W5M	0.08	-0.15	0.38	0.27	0.42***	0.16	0.24	0.06	1.00

BFT12, BFT34: BFT: Backfat Thickness; LMD12, LMD34: *Longissimus* Muscle Depth; LMA12, LMA34 – *Longissimus* Muscle Area; LMP12, LMP34 – *Longissimus* Muscle Perimeter; W5M - Weight at 5 Months; ***$p<0.001$

In this study, the average lamb weight ranged from 34.71 kg in L1 (the lot of female **Tsigai** sheep) to 41.83 kg in L1 (female crossbreed), with average ages ranging from 156 to 163 days, depending on the lot. The age of the animals at the time of measurement is significant, as differences may arise between genetic evaluation programs based on ultrasonic measurements if these scans are conducted at different time points. Australia's genetic evaluation and performance testing program, LAMBPLAN, permits ultrasonic measurements for lambs to be conducted across a broad age range, from 5 to 18 months (Gilmour et al., 1994). Meanwhile, other schemes, such as the Suffolk sire-reference programs in Canada (Gallivan & Hosford, 1997) and Britannia (MLC, 1987), focus on measurements at 100 and 147 days of age, respectively.

Regarding lamb weight, environmental influences, and genetic factors, the findings of this study indicate variations between lots, contingent upon genotype. Crossbred lambs exhibited superior values in terms of body weight at birth, weaning, and the end of the five-month period, compared to lots consisting of Tsigai lambs, with daily gains being higher in crossbred females.

Both lots of lambs were evaluated for average backfat thickness, *Longissimus dorsi* (LD) depth, LD eye muscle area, and perimeter using ultrasound measurements. Interestingly, the average backfat thickness at both points was lower in the lots consisting of crossbred females, despite the crossbred group registering the highest live weight. Additionally, the eye muscle area and perimeter were found to have the highest values in L2.

The eye muscle area was measured at 8.95 cm^2 for **Manchego** lambs, 9.67 cm^2 for **Merino**, and 10.85 cm^2 for **Ile de France** x **Merino** lambs, with live weights ranging from 22 to 28 kg (Fernandez et al., 1997). The corresponding fat thickness for these genotypes was recorded as 3.28 mm, 3.83 mm, and 4.10 mm, respectively. Similarly, Stanford et al. (2001) examined male and female lambs at 90 days old, with live weights of 27.3 kg and 25.3 kg. They reported a loin eye area of 7.15 cm² for males and 7.42 cm² for females, with fat thickness measurements of 2.74 mm and 2.96 mm, respectively.

The correlation coefficients obtained in this study fall within the range previously documented in the literature (Fernandez et al., 1997; 2001; Safari et al., 2005). Fernandez et al. (1997) reported a correlation coefficient of 0.56 between muscle area and muscle depth, a finding that aligns with the results of the present study. Lazar et al. (2016) identified very close correlations at **Tsigai Blackhead of Teleorman** between weight at 2.5 months and subcutaneous fat layer thickness, muscle depth, and muscle eye area (0.72, 0.71, and 0.82, respectively). Similarly, Ibrahim et al. (2007) reported strong correlations in **Kivircik** lambs between body weight at birth and muscle depth (0.609) as well as muscle eye area (0.649). They also found a strong correlation between muscle eye area and muscle depth (0.845). Ultrasonic measurement technology has been implemented in selection programs aimed at enhancing growth and carcass traits in sheep (Simm et al., 1987; Larsgard & Kolstad, 2003). One of its primary advantages is its ability to assess live animals at relatively low costs (Conington et al., 1995; Larsgard & Kolstad, 2003). Furthermore, moderate to high heritability estimates have been reported for ultrasonic fat and muscle measurements (Fogarty, 1995; Jones et al., 2004; Safari et al., 2005). The findings presented in this study, focusing on **Tsigai** and crossbreed lambs, will contribute to future research endeavors aimed at genetic improvement of lamb meat quality. Notably, in various regions worldwide including the UK, Australia, New Zealand, Denmark, Finland, and Norway, ultrasound measurements are integrated into national genetic evaluation programs or selection indices to enhance the quality of lamb carcasses (Stanford et al., 1998).

CONCLUSIONS

Lambs are usually sent to market at weaning, which occurs around 70 to 90 days of age, or lambs may continue grazing alongside their mothers, marketed at the age of 4-5 months. Integrating ultrasonic measurements into breeding programs for lambs of the **Tsigai breed** – rusty variety, in conjunction with weaning or market weight data, holds promise for enhancing meat yield and quality. By incorporating ultrasonic measurements alongside other records such as live weight or weight gains at various intervals, a breeding programs can potentially accelerate genetic improvements in growth and carcass characteristics of lambs.

BIBLIOGRAPHICAL REFERENCES

1. Conington J., Bishop S.C., Waterhouse A., Simm G.; A genetic analysis of early growth and ultrasonic measurements in hill sheep; 1995; Animal Science, 61, 85-93.

2. Delfa R., Teixeira A., Gonzalez C., Blasco I.; Ultrasonic estimates of fat thickness and longissimus dorsi muscle depth for predicting carcass composition of live **Aragon** lambs; 1995; Small Ruminant Research, 16,159–164.

3. Emenheiser J.C., Greiner S.P., Lewis R.M., Notter D.R.; Longitudinal changes in ultrasonic measurements of body composition during growth in Suffolk ram lambs and evaluation of alternative adjustment strategies for ultrasonic scan data; 2010; Journal of Animal Science, 88, 1341–1348.

4. European Communities, Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes; 1986; Official Journal, L, 358,1–29.

5. Fernández C., Gallego L., Quintanilla A.; Lamb fat thickness and longissimus muscle area measured by a computerized ultrasonic system; 1997; Small Ruminant Research, 26, 277–282.

6. Fernández C., García A., Vergara H., Gallego L.; Using ultrasound to determine fat thickness and longissimus dorsi area on **Manchego** lambs of different live weight; 1998; Small Ruminant Research, 27, 159–165.

7. Fogarty N.M., Banks R.G., Gilmour A.R., Brash L.D.; Enhancement of LAMBPLAN to incorporate maternal traits and the eye muscle measurements; 1992; Proceeding of Australian Association of Animal Breeding and Genetic, 10, 63–66.

8. Fogarty N.M.; Genetic parameters for live weight, fat and muscle measurements, wool production and reproduction in sheep: A review; 1995; Animal Breeding Abstracts, 63, 101–143.

9. Gallivan C., & Hosford S.; Environmental effect on weight, ultrasonic muscle depth and ultrasonic fat depth in Alberta **Suffolk** lambs; 1997; In: Proceedings of the Annual Meeting of Canadian Society of Animal Science, Montreal, Quebec, Canada, July 24-26.

10. Gilmour A.R., Luff A.F., Fogarty N.M., Bariks R.; Genetic parameters for ultrasound fat depth and eye muscle measurements in live **Poll Dorset** sheep; 1994; Australian Journal of Agricultural Research, 45, 1281-1 291.

11. Ibrahim C., Orhan K., Tufan A., Ozdal G., Murat Y., Onur Y.; Ultrasounds measurements of eye muscle properties and backfat thickness in **Kivircik** lambs; 2007; Journal of Biology Science, 7, 89–94.

12. Jones H.E., Lewi R.M., Young M.J., Simm G.; Genetic parameters for carcass composition and muscularity in sheep measured by X-ray computer tomography, ultrasound and dissection; 2004; Livestock Production Science, 90, 167-1 79.

13. Larsgard A.G., Kolstad K.; Selection for ultrasonic muscle depth; direct and correlated response in a Norwegian experimental sheep flock; 2003; Small Ruminant Research,48, 23–29.

14. Lazar C., Gras M. Al., Pelmus R.S., Rotar C.M., Ghita E., Burlacu R.; Estimation of meat amount by non-linear multiple regression equations using in vivo and carcass measurements on **Teleorman Black Head** lambs; 2016; Journal of Animal and Feed Sciences, 25, 292–301.

15. Leeds T.D., Mousel M.R., Notter D.R., Zerby H.N., Moffet C.A., Lewis G.S.; B-mode, real-time ultrasound for estimating carcass measures in live sheep: Accuracy of ultrasound measures and their relationships with carcass yield and value; 2008; Journal of Animal Science, 86, 3203-3214.

16. Mendizabal J.A., Delfa R., Arana A., Eguinoa P., Gonzalez C., Reacher T., Purroy A.; Estimating fat reserves in **Breed Aragonesa** ewes: A comparison of different methods; 2003; Canadian Journal of Animal Science, 83, 695–701.

17. MLC, Sheep Yearbook, Meat and Livestock Commission; 1987; Milton Keynes, UK., 49.

18. National Research Council, Nutrient requirement of sheep; 1985; Washington, National Academy Press, 99.

19. Russel A.J.F.; Ultrasonography and body composition in sheep; 1995; In: P.J. Goddard (Editor). Veterinary Ultrasonography. CAB International. Wallingford (UK), 315–324.

20. Safari E., Fogarty N.M., Gilmour A.R.; A review of genetic parameter estimates for wool, growth, meat and reproduction traits in sheep; 2005; Livestock Production Sciences, 92, 271-289

21. Simm G., Young M.J., Beatsori P.R.; An economic selection index for lean meat production in New Zealand sheep; 1987; Animal Production, 45, 465-475.

22. Stanford K., Jones S.D.M., Price M.A.; Methods of predicting lamb carcass composition: A review; 1998; Small Ruminant Research, 29, 241–254.

23. Wilson D.E.; Application of ultrasound for genetic improvement; 1992; Journal of Animal Science, 70, 973–983.

INTRA-POPULATION STRUCTURE RESEARCH OF A KARAKUL OF BOTOSANI SHEEP FLOCK

CERCETĂRI PRIVIND STRUCTURA INTRA POPULAȚIONALĂ A UNUI EFECTIV DE OVINE DIN RASA KARAKUL DE BOTOȘANI

MACIUC VASILE[1], NECHIFOR IONICĂ[1], FLOREA AALEXANDRU MARIAN[1], NECHIFOR BOGDAN IOAN[1] , ȚURCANU IOANA[1] , BOLDIȘOR ANA[1], NECHIFOR DANIEL CONSTANTIN[1] , PASCAL CONSTANTIN[1]

[1] Research and Development Station for Sheep and Goat Breeding "Popăuţi" – Botosani

Correspondence address: floreamarianalex@yahoo.com

Abstract

The research was conducted on a flock of 212 ***Karakul of Botoșani*** *sheep, belonging to the research station, predominantly from the White and Pink lines. This flock includes the descendants of 52 breeding rams and 164 ewes, which were included in the Official Production Control over the period from 2017 to 2023. The intrapopulation structure of the studied flock was analyzed. The obtained data, once systematized, were processed and interpreted using specific methods for such research. The intrapopulation structure of the* ***Karakul of Botoșani*** *breed taken in the study revealed 52 genetic groups with one to 18 offspring (males and females), respectively. Rams (T) with more than five offspring formed 20 genetic groups, representing 38.46%. It was found that there are very valuable genetic families with an average total score of over 630 points for Ram (T) code 651794 (Pink line), with the offspring scoring 530 points. Ram (T) code 467435 (Gray line) scored 625 points, with the offspring scoring 502 points. Ram (T) code 651786 (Greyish line) scored 640 points, with the offspring scoring 525 points. Ram (T) code 174050 (Black line) scored 650 points, with the offspring scoring 504 points. Rams (T) code 870226 (Brown line) and 422517 (Pink line) scored 615 points, with the offspring scoring 486 and 453 points, respectively. The genetic structure highlights that selection and improvement are continuous processes, especially for the White and Pink lines, this is understandable since these lines are in the process of being standardized, and we are currently in the phase of increasing the flock size by retaining and multiplying valuable genotypes from high-performing genetic structures while eliminating individuals from low-performing structures.*

Key words: breed, sheep, Karakul, intrapopulation structure, zootechnical diversity

Rezumat

Cercetările au fost efectuate pe un efectiv de 212 capete ovine din rasa ***Karakul de Botoșani****, aparținând stațiunii de cercetare, preponderent din liniile Alb și Roz, descendența a 52 de berbeci reproducători și 164 de femele, oi mame, cuprinse în Controlul Oficial al Producțiilor pe perioada 2017-2023. S-a urmărit structura intrapopulaționala a efectivului de ovine studiat. Datele obținute, odată sistematizate, au fost prelucrate și interpretate prin metode specifice unor astfel de cercetări. Structura intrapopulațională a rasei de ovine* ***Karakul de Botoșani*** *luată în studiu a evidențiat 52 grupe genetice cu unu și până la 18 produși (fii și fiice) respectiv, Tați (T) cu mai mult de 5 produși au înregistrat 20 de grupe genetice ceea ce reprezintă 38,46 %. S-a constatat existența unor familii genetice foarte valoroase cu valoarea medie la punctajul total de peste 630 puncte la Tata (T) cod 651794 (linia Roz), iar produsul de 530 puncte, Tata (T) cod 467435 (linia Sur) cu punctaj de 625, iar produsul 502 puncte, Tata (T) cod 651786 (linia Brumăriu) cu punctaj de 640, iar produsul 525 puncte, Tata (T) cod 174050 (linia Negru) cu punctaj de 650, iar produsul 504 puncte și Tata (T) cod 870226 (linia Maro) respectiv 422517 (linia Roz) cu 615 puncte, iar produșii care au avut un punctaj de 486 și 453. Structura genetică evidențiază faptul că selecția și ameliorarea sunt un proces continuu, mai ales în cazul liniilor Alb și Roz, explicabil întrucât aceste linii sunt în curs de omologare iar acum suntem în faza de înmulțire a efectivului, cu reținerea și multiplicarea genotipurilor valoroase din structurile genetice performante, concomitent cu eliminarea de la reproducție a indivizilor din structuri slab performante.*

Cuvinte cheie**: **rasă, ovine, Karakul, structură intrapopalatională, diversitate zootehnică

INTRODUCTION

The **Karakul** breed is one of the oldest domesticated sheep breeds. Evidence of its existence dates back to 1400 BCE. The birthplace of the **Karakul** breed is Central Asia. This breed is also known by the names Astrakhan, Bukhara, or Persian lamb.

Regarding species improvement activities, it should be noted that researchers at the Popăuți Research and Development Station for Sheep and Goat Breeding have developed the **Karakul of Botoșani** breed after three generations of crossbreeding and well-directed selection.

The **Karakul of Botoșani** breed is a Romanian breed specialized for pelt production, created by combining the **Karakul** breed (males originating from the Bukhara basin - Turkmenistan, Kazakhstan, Germany, Austria, Bessarabia) with females from the indigenous **Țurcană** breed, which is black and greyish, a coarse-wool breed well adapted to the specific conditions of northeastern Moldova. Of the seven color varieties within the breed, the first four have been officially recognized to date: black, greyish, brown, and gray (Alexandru M.F. et al., 2020, Nechifor I. 2019, Pascal C. et al. 1994-2001, Albertyn L.R. et al. 1993, Alemneh T., et al., 2019).

In the case of local breeds, the animals resemble each other, although diversity can be observed among individuals for many phenotypic traits. In standardized breeds, the animals resemble each other more than in the case of ancestral local breeds, making them more uniform. However, differences can still be observed among individuals of the standardized breed. Therefore, within animal populations (species, primitive breeds, standardized breeds, or livestock lines), there are varying degrees of diversity stemming from genetic origin or zootechnical diversity (Huțu I. et al., 2020, Tittensor D., et al 2011, Vinke C.M, 2001, Wilson, E.O., 1988, Woolliams J., et al. 2007, Johansson A.M., et al. 2010).

Genetic diversity arises because animals possess different DNA; DNA differs more between species than between primitive breeds, more between primitive breeds than within specialized breeds, and more within specialized breeds than within livestock lines.

Zootechnical diversity expresses differences within a breed (intra-breed diversity) or differences between different breeds (inter-breed diversity). Zootechnical diversity has emerged due to the combined influence of four types of factors: genetic drift, migration, selection, and mutation.

Considering the above aspects, we aimed to conduct the present research to examine the intrapopulation structure of a flock of sheep belonging to the **Karakul of Botoșani** breed.

MATERIAL AND METHOD

The research was conducted on a flock of 212 individuals from the active population of the **Karakul of Botoșani** breed, representing families that were genotyped for resistance to sheep spongiform encephalopathy. This group consisted of the offspring of 52 breeding rams and 164 ewes, all included in the Official Production Control from 2017 to 2023. The majority of individuals in the flock are from the White and Pink lines. Using data from the official control, supplemented with direct personal observations, we analyzed the total score and birth weight for rams (T), ewes (M), and offspring (F), both for the entire flock and by genetic groups.

Once systematized, the data were processed and interpreted using specific methods for such research-arithmetic mean, standard error of the mean, standard deviation, coefficient of variation, range, and significance tests (Fisher and Tukey, etc.) using the statistical program for variance and covariance analysis (S.A.V.C.) developed by V. Maciuc in 2002-2003 at U.S.V. Iasi (Cucu Gr. I., et al., 2004). Statistical measures are noted with Latin letters: arithmetic mean ($\overline{x}$), variance (s^2), and standard deviation (s).

The coefficient of variation (V%) directly indicates the relative variability of the population compared to the mean, namely the degree of homogeneity of the population. It is calculated using the formula:

$$V\,\% = \frac{s}{\overline{X}} \times 100$$

Depending on the value of the coefficient of variation, it is determined whether a population is homogeneous (when V% is less than 10%), moderately homogeneous (when V% is between 10% and 20%), or very heterogeneous (when V% is greater than 20%).

The range of variation is used to evaluate a quantitative characteristic in terms of its average value and degree of variability. For this purpose, statistical methods are used, and observations must be presented as figures to be processed. These figures exhibit variability or variation. The property or characteristic under study that shows variability or variation represents the variable.

The Fisher (F) test is used to verify the equality of variances between two independently normally distributed variables. The null hypothesis is $H_0:\sigma 12=\sigma 22$.

The Tukey test, also known as the Tukey Honest Significant Difference test, is the most commonly used multiple comparison procedure, typically used in conjunction with ANOVA statistical models. When the null hypothesis of the F test in the analysis of variance is rejected, it becomes important to determine which means are not equal. This gives rise to multiple comparison techniques since sequential pairwise comparisons cannot be controlled regarding the significance threshold. The Tukey method tests all pairwise differences simultaneously to determine if at least one is significantly different from zero.

The Tukey-Kramer test is very similar to the Tukey method for equal groups, but the denominator differs slightly. The formula for calculating Q using Tukey-Kramer is:

$$Q = \frac{M_i - M_j}{\sqrt{MS_{intra} * \left(\frac{\frac{1}{n_i} + \frac{1}{n_j}}{2}\right)}}$$

Where: ni and nj are the means of the ith and jth groups, Mi and Mj is the mean square within groups.

The number of degrees of freedom is determined similarly to the Tukey method. The first degree of freedom is k (the number of groups in the experiment), and the second is $N-k$ (degrees of freedom for within-group variance).

The complexity of the aspects under study necessitated the use of a diversified working methodology depending on the aspects being investigated, while also utilizing and adhering to the investigation methodology recommended by the specialized literature (Drucker, A.G., et al., 2001).

RESULTS AND DISCUSSION

The study regarding the total score and birth weight for rams (T), ewes (M), and lambs (P), in the population of **Karakul of Botoșani** sheep (Table 1) under investigation, revealed an average score of 576.21 for rams (T), with limits ranging from 465 to 668 points, an average value of 519.47 points for ewes (M), with limits ranging from 395 to 650 points, and an average value of 495.38 points for offspring (P) with minimum and maximum values between 395 and 663 points. We observe that the lambs have a lower score than the parents, and the differences are highly significant, with $p<0.001p<0.001$, C.I. = 95%, as indicated by the Fisher and Tukey tests (Tables 1A and 1B).

Table 1 Statistical Analysis for the characters studied in the Karakul Sheep Breed / Statisticii pentru caracterele studiate la rasa de ovine Karakul

Characters	n	$\overline{X}$	$\pm s_{\overline{x}}$	s	V%	Minimum	Maximum
Ram total score (T)	52	576.21	7.083	51.075	8.864	465	668
Ram birth weight (T)	52	4.89	0.158	1.141	23.337	3	9.2
Ewe score (M)	164	519.47	4.148	53.124	10.227	395	650
Ewe birth weight (M)	164	4.1	0.078	0.997	24.333	2.3	6.7
Lamb total score (P)	212	495.38	3.129	45.563	9.198	395	663
Lamb brith weight (P)	212	4.44	0.069	1.001	22.566	2.5	7.9

Total outer score: The Fisher test : 118,338 (F) > F0,001 (2 ; 633) 6,91 *** f.s. very significant

The Tukey test:

Character 1	Character 2	Average diff.	Q1	Q2	W1	W2	The meaning	Treshold
Lambs points	Ram points	74.12	0	0	0	0	significantly	0.01
Lambs points	Ewe points	21.86	0	0	0	0	significantly	0.01
Ewe points	Ram points	52.26	3.31	4.12	11.58	14.42	significantly	0.01

Birth weight Kg: The Fisher test : 32,9469 (F) > F0,001 (2 ; 633) 6,91 *** f.s. very significant

The Tukey test:

Character 1	Character 2	Average diff.	Q1	Q2	W1	W2	The meaning	Treshold
Lambs birth weight	Ram birth weight	0.41	0	0	0	0	significantly	0.01
Lambs birth weight	Ewe birth weight	0.44	0	0	0	0	significantly	0.01
Ewe birth weight	Ram birth weight	0.85	3.31	4.12	0.24	0.3	significantly	0.01

Accordingly, the best average value for birth weight (Table 1) was recorded for Rams (T) at 4.89 kg, followed by 4.44 kg for Lambs (P), and 4.1 kg for Ewes (M). The differences among the three experimental groups for birth weight are highly significant, as indicated by the Fisher and Tukey tests (Table 1B).

The explanation for the total score lies in the fact that when pairing for the White and Pink lines, the total score of the parents should not have statistically significant differences, as shown in Table 1A. Additionally, we believe that birth weight influences the total score. For birth weight, the coefficient of variation was over 20%, with values ranging between 22% and 24%, indicating a highly heterogeneous population.

For the **Karakul** sheep population in the Moldova region, the analysis of the studied traits was conducted on genetic groups of half-siblings (Table 2).

Table 2. Statistics by genetic groups for the characters studied in the Karakul sheep breed (>5 records) / Statistici pe grupe genetice pentru caracterele studiate la rasa de ovine Karakul (>5 înregistrări)

No crt.	No. Matriculation ram	Characters	n	$\overline{X}$	$\pm s_{\overline{x}}$	s	V%	Min.	Max.
1.	870226 the Brown line	Ram points	5	615.00	0.000	0.000	0.000	615.00	615.00
		Ram birth weight	5	5.00	0.000	0.000	0.000	5.00	5.00
		Ewe points	5	501.00	31.121	69.588	13.890	405.00	590.00
		Ewe birth weight	5	3.72	0.379	0.847	22.762	3.00	5.00
		Lambs points	5	486.00	26.571	59.414	12.225	420.00	570.00
		Lambs birth weight	5	5.74	0.303	0.677	11.790	5.00	6.60
2.	229680 the White line	Ram points	14	485.00	0.000	0.000	0.000	485.00	485.00
		Ram birth weight	14	3.40	0.000	0.000	0.000	3.40	3.40
		Ewe points	14	515.07	16.545	61.906	12.019	435.00	620.00
		Ewe birth weight	14	3.31	0.154	0.576	17.409	2.50	4.50
		Lambs points	14	513.57	13.772	51.531	10.034	460.00	655.00
		Lambs birth weight	14	4.42	0.199	0.743	16.813	3.00	5.30
3.	467435 the Grey line	Ram points	8	625.00	0.000	0.000	0.000	625.00	625.00
		Ram birth weight	8	3.40	0.000	0.000	0.000	3.40	3.40
		Ewe points	8	547.38	15.353	43.425	7.933	465.00	600.00
		Ewe birth weight	8	4.43	0.242	0.684	15.458	3.00	5.10
		Lambs points	8	502.75	20.037	56.674	11.273	412.00	565.00
		Lambs birth weight	8	4.28	0.417	1.180	27.614	3.10	6.70
4.	651786 the Greyish line	Ram points	7	640.00	0.000	0.000	0.000	640.00	640.00
		Ram birth weight	7	4.30	0.000	0.000	0.000	4.30	4.30
		Ewe points	7	534.43	35.839	94.821	17.742	395.00	633.00
		Ewe birth weight	7	3.44	0.227	0.600	17.416	2.80	4.40
		Lambs points	7	525.00	19.086	50.498	9.619	485.00	630.00

		Lambs birth weight	7	3.79	0.388	1.025	27.086	2.50	5.20
5.	**651794 the Pink line**	Ram points	9	**630.00**	**0.000**	**0.000**	**0.000**	**630.00**	**630.00**
		Ram birth weight	9	**5.70**	**0.000**	**0.000**	**0.000**	**5.70**	**5.70**
		Ewe points	9	**495.22**	**21.716**	**65.147**	**13.155**	**400.00**	**600.00**
		Ewe birth weight	9	**3.64**	**0.226**	**0.677**	**18.565**	**2.80**	**4.40**
		Lambs points	9	**530.00**	**11.932**	**35.795**	**6.754**	**470.00**	**605.00**
		Lambs birth weight	9	**4.49**	**0.204**	**0.611**	**13.617**	**3.50**	**5.20**
6.	651799 the Grey line	Ram points	5	608.00	0.000	0.000	0.000	608.00	608.00
		Ram birth weight	5	4.40	0.000	0.000	0.000	4.40	4.40
		Ewe points	5	540.00	27.249	60.930	11.283	495.00	645.00
		Ewe birth weight	5	3.50	0.255	0.570	16.288	3.00	4.30
		Lambs points	5	550.20	30.523	68.251	12.405	490.00	663.00
		Lambs birth weight	5	4.28	0.381	0.853	19.922	3.30	5.60
7.	231809 the Black line	Ram points	8	595.00	0.000	0.000	0.000	595.00	595.00
		Ram birth weight	8	4.60	0.000	0.000	0.000	4.60	4.60
		Ewe points	8	511.50	23.007	65.074	12.722	440.00	638.00
		Ewe birth weight	8	3.90	0.306	0.865	22.185	3.40	5.80
		Lambs points	8	523.75	15.945	45.099	8.611	455.00	605.00
		Lambs birth weight	8	5.54	0.443	1.252	22.616	3.80	7.70
8.	**422517 the Pink Line**	Ram points	5	**615.00**	**0.000**	**0.000**	**0.000**	**615.00**	**615.00**
		Ram birth weight	5	**4.40**	**0.000**	**0.000**	**0.000**	**4.40**	**4.40**
		Ewe points	5	**498.20**	**21.639**	**48.386**	**9.712**	**430.00**	**545.00**
		Ewe birth weight	5	**3.06**	**0.060**	**0.134**	**4.384**	**3.00**	**3.30**
		Lambs points	5	**453.00**	**21.943**	**49.066**	**10.831**	**405.00**	**530.00**
		Lambs birth weight	5	**4.04**	**0.075**	**0.167**	**4.142**	**3.80**	**4.20**
9.	173905 the Black line	Ram points	10	505.00	0.000	0.000	0.000	505.00	505.00
		Ram birth weight	10	4.00	0.000	0.000	0.000	4.00	4.00
		Ewe points	10	514.10	13.294	42.038	8.177	460.00	568.00
		Ewe birth weight	10	4.36	0.366	1.157	26.532	3.00	6.10
		Lambs points	10	499.10	12.122	38.333	7.680	455.00	558.00
		Lambs birth weight	10	4.81	0.240	0.758	15.756	3.60	5.60
10.	174050 the Black line	Ram points	7	650.00	0.000	0.000	0.000	650.00	650.00
		Ram birth weight	7	6.30	0.000	0.000	0.000	6.30	6.30
		Ewe points	7	501.57	12.897	34.122	6.803	440.00	543.00
		Ewe birth weight	7	5.30	0.395	1.046	19.729	3.60	6.70
		Lambs points	7	504.00	13.777	36.451	7.232	445.00	535.00
		Lambs birth weight	7	5.43	0.284	0.752	13.855	4.00	6.10
11.	186804 the Pink line	Ram points	18	535.00	0.000	0.000	0.000	535.00	535.00
		Ram birth weight	18	4.00	0.000	0.000	0.000	4.00	4.00
		Ewe points	18	517.67	13.840	58.720	11.343	395.00	650.00
		Ewe birth weight	18	3.84	0.175	0.742	19.306	2.80	5.20
		Lambs points	18	498.33	10.298	43.690	8.767	430.00	570.00
		Lambs birth weight	18	4.16	0.136	0.576	13.846	3.30	5.20
12.	186913 the Pink line	Ram points	5	535.00	0.000	0.000	0.000	535.00	535.00
		Ram birth weight	5	3.70	0.000	0.000	0.000	3.70	3.70
		Ewe points	5	509.00	22.271	49.800	9.784	445.00	545.00
		Ewe birth weight	5	3.82	0.514	1.150	30.099	3.00	5.40
		Lambs points	5	478.00	16.325	36.503	7.637	420.00	510.00
		Lambs birth weight	5	4.42	0.422	0.944	21.368	3.50	5.70
13.	**486585 the Pink line**	Ram points	6	**583.00**	**0.000**	**0.000**	**0.000**	**583.00**	**583.00**
		Ram birth weight	6	**5.20**	**0.000**	**0.000**	**0.000**	**5.20**	**5.20**
		Ewe points	6	**488.33**	**16.159**	**39.581**	**8.105**	**430.00**	**530.00**
		Ewe birth weight	6	**4.62**	**0.398**	**0.975**	**21.108**	**3.20**	**6.10**
		Lambs points	6	**445.83**	**7.236**	**17.725**	**3.976**	**425.00**	**465.00**
		Lambs birth weight	6	**4.75**	**0.281**	**0.689**	**14.510**	**3.50**	**5.60**
14.	405048 the Black line	Ram points	6	545.00	0.000	0.000	0.000	545.00	545.00
		Ram birth weight	6	9.20	0.000	0.000	0.000	9.20	9.20
		Ewe points	6	526.83	24.761	60.651	11.512	425.00	578.00
		Ewe birth weight	6	4.28	0.361	0.884	20.641	3.20	5.40
		Lambs points	6	497.00	18.430	45.144	9.083	462.00	555.00
		Lambs birth weight	6	6.23	0.420	1.029	16.507	5.00	7.90
15.	405270 the Grey line	Ram points	5	585.00	0.000	0.000	0.000	585.00	585.00
		Ram birth weight	5	5.20	0.000	0.000	0.000	5.20	5.20
		Ewe points	5	524.00	33.963	75.944	14.493	425.00	620.00
		Ewe birth weight	5	4.22	0.317	0.709	16.790	3.40	4.80
		Lambs points	5	455.00	9.618	21.506	4.727	425.00	475.00
		Lambs birth weight	5	3.76	0.380	0.850	22.614	2.80	4.80
16.	405298 the Grey line	Ram points	7	580.00	0.000	0.000	0.000	580.00	580.00
		Ram birth weight	7	5.70	0.000	0.000	0.000	5.70	5.70

		Ewe points	7	526.14	11.927	31.556	5.998	485.00	578.00
		Ewe birth weight	7	4.03	0.300	0.793	19.688	3.00	5.10
		Lambs points	7	507.86	16.252	42.998	8.467	485.00	605.00
		Lambs birth weight	7	3.93	0.345	0.912	23.223	2.80	5.10
17.	405400 the Black line	Ram points	5	605.00	0.000	0.000	0.000	605.00	605.00
		Ram birth weight	5	5.70	0.000	0.000	0.000	5.70	5.70
		Ewe points	5	545.20	26.194	58.572	10.743	468.00	608.00
		Ewe birth weight	5	4.92	0.503	1.126	22.878	4.00	6.50
		Lambs points	5	554.00	15.665	35.029	6.323	522.00	605.00
		Lambs birth weight	5	5.24	0.250	0.559	10.677	4.60	5.90
18.	485073 the Grey line	Ram points	5	590.00	0.000	0.000	0.000	590.00	590.00
		Ram birth weight	5	5.40	0.000	0.000	0.000	5.40	5.40
		Ewe points	5	529.00	18.262	40.835	7.719	495.00	600.00
		Ewe birth weight	5	5.00	0.261	0.583	11.662	4.60	6.00
		Lambs points	5	470.00	8.367	18.708	3.980	445.00	495.00
		Lambs birth weight	5	4.08	0.524	1.171	28.709	2.50	5.30
19.	485106 the Halili line	Ram points	5	475.00	0.000	0.000	0.000	475.00	475.00
		Ram birth weight	5	5.00	0.000	0.000	0.000	5.00	5.00
		Ewe points	5	533.60	23.462	52.462	9.832	470.00	610.00
		Ewe birth weight	5	4.56	0.447	0.999	21.908	3.50	6.10
		Lambs points	5	488.60	20.444	45.714	9.356	410.00	520.00
		Lambs birth weight	5	4.60	0.339	0.758	16.485	3.40	5.30
20.	485705 the Brown line	Ram points	6	490.00	0.000	0.000	0.000	490.00	490.00
		Ram birth weight	6	6.20	0.000	0.000	0.000	6.20	6.20
		Ewe points	6	483.83	17.758	43.499	8.991	440.00	550.00
		Ewe birth weight	6	4.75	0.423	1.037	21.828	3.00	6.00
		Lambs points	6	471.67	17.638	43.205	9.160	425.00	550.00
		Lambs birth weight	6	5.32	0.256	0.627	11.801	4.20	6.00

In the intrapopulational structure of the **Karakul of Botoșani** sheep breed, 52 genetic groups were identified, each with one to 18 offspring (half-siblings through the father). For the analysis, we considered Rams (T) with more than 5 offspring, and 20 genetic groups were recorded, whose average indices are presented in Table 2.

Of the 20 genetic groups with more than 5 offspring, representing 38.46% of the total 52 genetic groups, the Rams (T) with the most offspring were: code 186804 (Pink line) with 18 offspring, code 229680 (White line) with 14 offspring, code 173905 (Black line) with 10 offspring, code 651794 (Pink line) with 9 offspring, and codes 467435 (Grey line) and 231809 (Black line) each with 8 offspring.

Analyzing the average values of the studied indicators in these genetic structures, it was found that there are very valuable genetic families with an average total score of over 630 points for Rams (T) code 651794 (Pink line), and the offspring scored 530 points. Rams (T) code 467435 (Gray line) scored 625 points, with offspring scoring 502 points. Rams (T) code 651786 (Greyish line) scored 640 points, with offspring scoring 525 points. Sire (S) code 174050 (Black line) scored 650 points, with offspring scoring 504 points. Sires (S) codes 870226 (Brown line) and 422517 (Pink line) each scored 615 points, with offspring scoring 486 and 453 points, respectively. The amplitude of total score variability ranged from a minimum of 615 points to a maximum of 650 points, highlighting the value and genetic potential of the **Karakul of Botoșani** sheep breed.

From the table of 20 genetic families identified with more than 5 offspring, 7 Rams (T) had scores below the population average studied (576.21 points). These include codes 229680 (White line), 173905 (Black line), 186804 (Pink line), 186804 (Pink line), 186913 (Pink line), 405048 (Black line), and 485106 (Halili line), representing 35%, a very significant proportion that must be considered in both selection and genetic improvement of the studied sheep population. Of the total active population at the station, individuals from the White and Pink lines represent approximately 15%, and as observed in the study, the limits, especially the lower values, are mainly maintained by individuals from these two lines.

All these genetic structures highlight the genetic value of the **Karakul of Botoșani** sheep studied, as well as the careful selection process, with the retention and multiplication of valuable genotypes from performant genetic structures, while eliminating low-performing individuals from reproduction. The studied population includes offspring regardless of their destination, and only individuals with higher genetic value were kept for reproduction, even in the White and Pink lines.

The analysis of the variation series for the total score and birth weight for Rams (T), Ewes (M), and Lambs (P) in the entire studied population is presented in Figures 1 to 6.

Class nr.	Class	Absolute frequency	Relative frequency
1	465 - 485.3	3	5.77
2	485.31 - 505.6	3	5.77
3	505.61 - 525.9	3	5.77
4	525.91 - 546.2	9	17.31
5	546.21 - 566.5	4	7.69
6	566.51 - 586.8	5	9.62
7	586.81 - 607.1	10	19.23
8	607.11 - 627.4	7	13.46
9	627.41 - 647.7	4	7.69
10	647.71 - 668	4	7.69

Figure 1. Histogram of rams total score (T) / Histograma scorului total de berbeci (T)

Class nr.	Class	Absolute frequency	Relative frequency
1	3 - 3.62	5	9.62
2	3.63 - 4.24	14	26.92
3	4.25 - 4.86	7	13.46
4	4.87 - 5.48	9	17.31
5	5.49 - 6.1	12	23.08
6	6.11 - 6.72	3	5.77
7	6.73 - 7.34	1	1.92
8	7.35 - 7.96	0	0
9	7.97 - 8.58	0	0
10	8.59 - 9.2	1	1.92

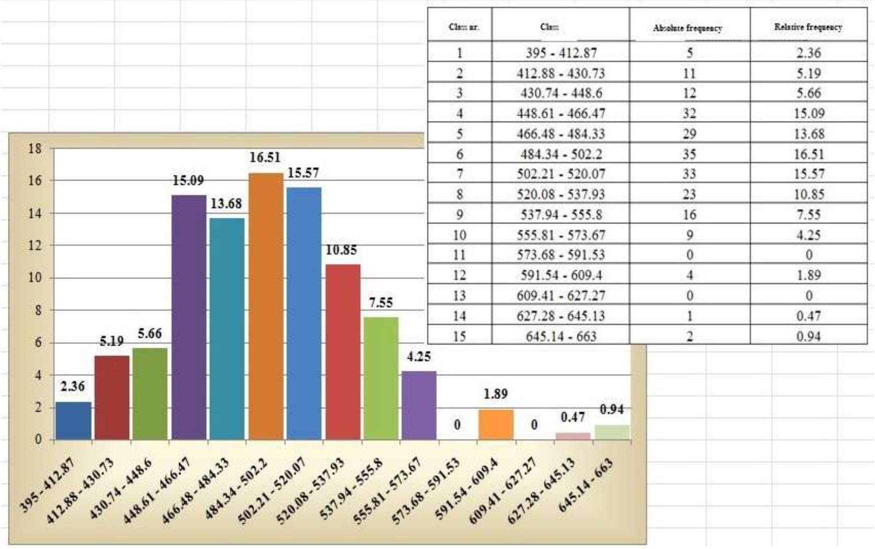
Figure 2. Frequency polygon of rams birth weight (T) / Poligonul de frecvență a greutății berbecilor la naștere (T)

Class nr.	Class	Absolute frequency	Relative frequency
1	395 - 420.5	4	2.44
2	420.51 - 446	14	8.54
3	446.01 - 471.5	17	10.37
4	471.51 - 497	19	11.59
5	497.01 - 522.5	33	20.12
6	522.51 - 548	33	20.12
7	548.01 - 573.5	19	11.59
8	573.51 - 599	11	6.71
9	599.01 - 624.5	9	5.49
10	624.51 - 650	5	3.05

Figure 3. Histogram of ewes total score (M) / Histograma scorului total al oilor (M)

Class nr.	Class	Absolute frequency	Relative frequency
1	2.3 - 2.74	4	2.44
2	2.75 - 3.18	34	20.73
3	3.19 - 3.62	31	18.9
4	3.63 - 4.06	20	12.2
5	4.07 - 4.5	21	12.8
6	4.51 - 4.94	16	9.76
7	4.95 - 5.38	17	10.37
8	5.39 - 5.82	11	6.71
9	5.83 - 6.26	8	4.88
10	6.27 - 6.7	2	1.22

Figure 4. Frequency polygon of ewes birth weight (M) / Frecvența greutății la naștere a oilor (M)



Class nr.	Class	Absolute frequency	Relative frequency
1	395 - 412.87	5	2.36
2	412.88 - 430.73	11	5.19
3	430.74 - 448.6	12	5.66
4	448.61 - 466.47	32	15.09
5	466.48 - 484.33	29	13.68
6	484.34 - 502.2	35	16.51
7	502.21 - 520.07	33	15.57
8	520.08 - 537.93	23	10.85
9	537.94 - 555.8	16	7.55
10	555.81 - 573.67	9	4.25
11	573.68 - 591.53	0	0
12	591.54 - 609.4	4	1.89
13	609.41 - 627.27	0	0
14	627.28 - 645.13	1	0.47
15	645.14 - 663	2	0.94

Figure 5. Histogram of lambs total score (P) / Scorul total al histogramei mieilor (P)

Class nr.	Class	Absolute frequency	Relative frequency
1	2.5 - 2.86	9	4.25
2	2.87 - 3.22	18	8.49
3	3.23 - 3.58	23	10.85
4	3.59 - 3.94	17	8.02
5	3.95 - 4.3	32	15.09
6	4.31 - 4.66	23	10.85
7	4.67 - 5.02	30	14.15
8	5.03 - 5.38	26	12.26
9	5.39 - 5.74	18	8.49
10	5.75 - 6.1	7	3.3
11	6.11 - 6.46	3	1.42
12	6.47 - 6.82	4	1.89
13	6.83 - 7.18	0	0
14	7.19 - 7.54	0	0
15	7.55 - 7.9	2	0.94

Figure 6. Poligon of frequencies for lambs birth weight (P) / Poligon de frecvențe pentru greutatea la naștere a mieilor (P)

For rams total score (T), the class with the highest relative frequency is between 484.34 - 502.2 points, representing 16.53%. This will be the basis for starting the selection of plus variants in the genetic improvement of the studied population. The plus variants represent a proportion of 58.03% and form a foundation for the initiation of the genetic improvement program.

The variation series for rams birth weight (T) has a class with values between 3.63 - 4.24, with a proportion of 26.92%, the highest. The class with the highest frequency represents the module with which the selection of plus variants in the genetic improvement of the **Karakul of Botoșani** sheep population will begin.

The variation series for the total score and birth weight in Ewes (M) has a module with values between 497.01 - 522.5 points, representing 33%. The module for birth weight had values between 2.75 - 3.18, representing 34%.

For Lambs (P), the class with the highest frequency is between 484.34 - 502.2 points, representing 34%. For birth weight, the class with the highest frequency is between 3.95 - 4.3, representing 32%. Both classes constitute the module for males and females with which the selection of plus variants in the genetic improvement of the **Karakul of Botoșani** sheep population will begin.

CONCLUSIONS

Based on the study conducted, the following conclusions can be drawn:

1. Lambs Score: The lambs (males and females) had a lower score than their ancestors, with very significant differences ($p < 0.001$, C.I. = 95%), as indicated by the Fisher and Tukey tests. The explanation for the total score is that the studied population is not the entire active population of **Karakul of Botoșani** sheep at the station, but a significant population for the White and Pink lines, lines that are in the process of being homologated. Thus, not all individuals will be kept for breeding, only those that fall within the frequency limits calculated for the selection of plus variants.

2. Genetic Structure: The intrapopulational structure of the **Karakul of Botoșani** sheep breed studied revealed 52 genetic groups with one to 18 offspring (half-siblings through the sire), with Sires (S) having more than 5 offspring recorded in 20 genetic groups, representing 38.46%.

3. Valuable Genetic Families: Even though the studied population predominantly comprises individuals from the White and Pink lines, there are still very valuable genetic families with an average total score of over 630 points for Ram (T) code 651794 (Pink line), and an offspring score of 530 points. Sire (S) code 467435 (Grey line) had a score of 625, with an offspring score of 502 points. Rams (T) code 651786 (Greyish line) scored 640, with an offspring score of 525 points. Rams (T) code 174050 (Black line) scored 650, with an offspring score of 504 points. Rams (T) codes 870226 (Brown line) and 422517 (Pink line) each scored 615 points, with offspring scores of 486 and 453 points, respectively.

4. Value and Selection: The identified genetic structures highlight the genetic value of the **Karakul of Botoșani** sheep studied and the necessity for careful selection, retaining and multiplying valuable genotypes from high-performing genetic structures, while eliminating low-performing individuals from reproduction. The studied population includes offspring regardless of their destination, and only individuals with higher genetic value were kept for reproduction, even in the White and Pink lines.

ACKNOWLEDGMENTS:

The White and Pink lines are an important category of **Karakul of Botoșani** sheep, and this study helps us to perfect the documentation for their homologation.

BIBLIOGRAPHICAL REFERENCES

1. Albertyn L.R., Schoeman S., Groeneveld H.T.; Factors influencing the quality of **Karakul** pelts, with emphasis on discrete characteristics; 1993; S. Afr. 1. Anim. Sci. 23, p.183.
2. Alemneh T., Getabalew M.; Factors Influencing the Growth and Development of Meat Animals; 2019; Int J Anim Sci; 3(2): 1048.
3. Cucu Gr. I., Maciuc V., Maciuc Domnica; Cercetarea ştiintifică şi elemente de tehnică experimentală în Zootehnie; 2004; Edit. Alfa, Iaşi.
4. Drucker A.G., Gomez V., Anderson S.; The Economic Valuation of Farm Animal Genetic Resources: a Survey of Available Methods; 2001; Ecological Economics, 36 (1) p. 1-18.
5. Florea A. M., Nechifor I., Crîşmaru A., Pascal C.; Researches regarding weight evolution considering the young female mating's age; 2020; Scientific Papers-Series D-Animal Science, Volume: 6 , Issue: 2 Pages: p. 281-286.
6. Huţu I., Oldenbroek K, van der Waaij L.; Animal breeding and husbandry; 2020; Ed. Agroprind, Timişoara, p.427.
7. Johansson A.M., Pettersson M.E., Siegel P.B., Carlborg O.; Genome-Wide Effects of Long-Term Divergent Selection; 2010; PLoS Genet 6(11): e1001188. https://doi.org/10.1371/journal.pgen.1001188.
8. Nechifor I.; Evaluarea gradului de ameliorare a caracterelor de producție la rasa **Karakul of Botoșani**; 2019; Edit, PIM, Iași, p.49-57.
9. Pascal C., Gilca I., Creanga St., Vintila V.; Cercetări comparative privind unele însușiri ce influențează calitatea pielicelelor la mieii de rasă **Karakul** și metiși; 1994; Lucrări Ştiinţifice, vol. 37/38. Seria Zootehnie, USAMV Iasi, p. 216-221.
10. Pascal C.; Calitatea pielicelelor obţinute de la ovinele de rasa **Karakul** crescute şi exploatate în ferma Andrieșeni, jud. Iași; 2001; Lucrări Ştiinţifice, Seria Zootehnie, USAMV Iași, vol. 43/44, p. 327-330.
11. Tittensor D**.** et al.; Global patterns and predictors of marine biodiversity across taxa; 2011; Nature. 466 (7310): 1098-1101.
12. Vinke C.M.; Some comments on the review of Nimon and Broom on the welfare of farmed mink; 2001; Animal welfare, 10:315-323.
13. Wilson E.O**.;** Biodiversity; 1988; National Academy Press.
14. Woolliams J., Toroin M.; Chapter 3. What is genetic diversity? In: Utilization and conservation of farm animal genetic resources; 2007; Editor. Kor Oldenbroek. Wageningen Academic Publishers.

THE INTRODUCTION OF NEW SPECIES OF FISH, A WAY TO INCREASE PRODUCTION IN AQUACULTURE

INTRODUCEREA SPECIILOR NOI DE PEŞTI, MIJLOC DE CREŞTERE A PRODUCŢIEI ÎN ACVACULTURĂ

MARICA NINO, RADU SILVIA, COSTACHE MIOARA, COSTACHE MIHAIL, DOBROTA GHEORGHE, DOBROTA NICOLETA, RADU DANIELA

Research and Development Station for Fisheries Nucet
549 Principala, 137335, Nucet, Dâmboviţa County
Phone: 040245267003, Fax: 040245267003; Email: scp_nucet@yahoo.com;

Correspondence address: nino.marica@yahoo.com

Summary

The voluntary, deliberately introduction (transfer) of plant and animal species from one area to another has been, since ancient times, one of the important dynamizing factors of agricultural development and animal husbandry in different areas of the world. Among vertebrates, fish represent the largest group that has been the subject of transfer and acclimatization actions. The vast majority belong to freshwater fish species - 2904, of which 1205 cases were aimed at introduction into aquaculture. (Froese and Pauly 2012, Casal 2006, Savini et al. 2010). This paper presents the results of studies and researches on new species of fish introduced / entered in Romania, respectively: ways of introduction / enter, their evolution, current situation and impact on local ichthyofauna, on aquatic ecosystem functionality and on fish farms production. Over time, a number of 28 new species have been introduced in Romania. A number of 24 species were introduced voluntarily or accidentally and another 4 species entered naturally. The vast majority were introduced in order to increase fish production, to capitalize on some trophic levels underused by local ichthyofauna or to expand the range of cultivated species. At the moment, 7 species are subject to aquaculture.

Keywords: new species of fish, aquaculture, production, fish farms.

Rezumat

Introducerea voluntară, conştientă (transferul) de specii vegetale şi animale dintr-un areal în altul a constituit, din cele mai vechi timpuri, unul din factorii dinamizatori importanţi ai dezvoltării agriculturii şi ai creşterii animalelor, în diferite zone la nivel planetar. Dintre vertebrate peştii reprezintă grupul cel mai numeros care a făcut obiectul transferului şi acțiunilor de aclimatizare. Majoritatea aparţine speciilor de peşti de apă dulce - 2904, din care 1205 cazuri au avut ca obiectiv introducerea în acvacultură. (Froese şi Pauly 2012, Casal 2006, Savini și colab. 2010). În lucrare sunt prezentate rezultatele studiilor și cercetărilor referitoare la speciile noi de peşti introduse / pătrunse în România, respectiv: modalităţile introducerii / pătrunderii, evolutia acestora, situatia actuală și impactul asupra ihtiofaunei autohtone, a functionalităţii ecosistemelor acvatice şi a producției din fermele piscicole. De-a lungul timpului, în România, au fost introduse un număr de 28 de specii noi. Un număr de 24 specii au fost introduse voluntar sau accidental, iar alte 4 specii au pătruns pe cale naturală. Marea majoritate au fost introduse în scopul creşterii producției piscicole, al valorificării unor verigi trofice insuficient valorificate de ihtiofauna autohtonă sau pentru lărgirea paletei de specii cultivate. În acest moment, 7 specii de pești fac obiectul creşterii dirijate.

Cuvinte cheie: specii noi de pești, acvacultură, producție, ferme piscicole.

INTRODUCTION

The voluntary, conscious introduction (transfer) of plant and animal species from one area to another has been, since ancient times, one of the important dynamizing factors of the development of agriculture and animal husbandry, in different areas on a planetary level.

According to FAO data, in the case of aquatic organisms, the fish group is the most often used among vertebrates in the case of intentional acclimatizations and introductions, and of these, freshwater fish species have the largest share in (intentional or accidental) introductions of foreign species. Historically speaking, one of the first actions in this field was the introduction of carp (*Cyprinius carpio*, Linnaeus 1758) first from Asia to Europe, and then from the Danube to the lakes of Italy and Greece.

It is currently assumed that in Europe alone there are around 40 species of introduced fish and many more have been translocated from various European countries to others.

In the case of Romania, up to now, more than 20 species of fish have been introduced, of which more than half have acclimatized and naturalized. The problems related to invasive species have become so serious that the regulations regarding the introduction of new species already appear in the legislation of developed countries and especially in the case of the European Union. The concept of "*increasing biodiversity*", under which the introduction of new fish species was promoted, turned out to be, in reality, an impoverishment of biodiversity. Invasive or potentially invasive species are responsible for this phenomenon and these situations. The term "invasive" implies that they succeed in reproducing in the new environment and taking advantage of the lack of parasites and specific predators, they reproduce excessively, invading the new habitat. Invasive fish species have proven to be serious competitors for native species in the niche, and in many cases, the foreign species have emerged victorious from this competition, affecting the new environment both biotically and abiotically (composition, structure, relationships, population density, biodiversity, behavior, etc.) (Bud I. et al. 2006).

As for the effects of the invasion, they are mostly detrimental and very rarely beneficial to the respective environment. The negative effects can be: competition for food, competition for space, predation, the introduction of new animal parasites, fungi and microbial pathogens, habitat alteration, hybridization etc.

MATERIALS AND METHODS

This study was performed based on works and specialized studies from the country and abroad, by consulting several databases: Google Scholar, JSTOR, Science Direct, ISI Web of Science, Springer Link, Academic Search Elite and AGRICOLA (AGRICultural Online Access).

In order to establish the inventory of new fish species in Romanian aquaculture, a series of practical, theoretical and bibliographic approaches were carried out and the main characteristics were highlighted.

RESULTS AND DISCUSSION

Among vertebrates, freshwater fish species have the highest share in the (intentional or accidental) introductions of foreign species. There are more than 40 introduced fish species in Europe, and many more have been translocated from various European countries to others. Regarding the introduction of foreign species of fish into Romania, naturally or with the help of man, three major periods can be mentioned: the first dates from ancient times until the year 1956, the second period begins in 1956 until 1989, when the first batch of embryonated whitefish (*Coregonus sp.*) eggs was brought for the acclimatization and the third period begins in 1989 and continues to this day. According to all observations, determinations made and existing data on the territory of our country, a total of 31 non-native species of fish (with different varieties and subspecies) were inventoried, of which 4 entered naturally through the hydrographic network, 26 species were introduced deliberately or accidentally by man, and about a species of African catfish (*Clarias ngamensis*) it is not yet known how it entered the waters. Of the 4 alien species introduced naturally, 2 reproduce in natural waters (*Ameiurus nebulosus* and *Lepomis gibbosus*), and about the other two (*Ictalurus melas* and *Odontobutis glenii*) there is still not enough information, given the fact that they were recently reported in the ichthyofauna of our country.

As for the introduced foreign fish species, 12 of them already reproduce naturally, without human help, locally or in larger areas (*Cyprinus carpio*, *Oncorhynchus mykiss*, *Carassius auratus gibelio*, *Gambusia holbrooki*, *Pseudorasbora parva*, etc.), 8 species reproduce artificially only in aquaculture units, through the intervention of specialists (*Ctenopharyngodon idella, Hypophthalmichthys molitrix*, *Hypophthalmichthys nobilis, Mylopharyngodon piceus, Ictiobus niger*, *Polyodon spathula, Acipenser baerii,* etc.), and about the other 8 introduced fish species there is not enough data regarding their status (*Coregonus lavaretus maraenoides*, *Coregonus albula ladogensis*, *Ictalurus punctatus*, *Ictiobus cyprinellus*, *Ictiobus bubalus* etc.) (Iacob M. et al. 2008).

Foreign species of fish entered Romania

Foreign species of fish introduced into Romania naturally or through human intervention take into account the three major stages mentioned (Table 1):

- stage I : from the earliest times – 1956;
- stage II: 1956 - 1989;
- stage III: 1989 - present.

Table 1. The area of origin of fish species introduced in Romania / Zona de provenienţă a specilor de peşti introduse în România

AREA OF ORIGIN	THE NUMBER OF SPECIES
North America	14
Asia (including Siberia)	10
Africa	2
Other European countries	2
TOTAL	28

Stage I

The fish species that entered Romania naturally or through human intervention, in stage I, come from Asia and North America (Table 2). The most important species for aquaculture are: carp (*Cyprinus carpio* , L), rainbow trout *(Oncorhynchus mykiss, Walbaum 1792),* brook trout *(Salvelinus fontinalis).*

Table 2. Fish species introduced in stage I / Specii de peşti introduse în etapa I

NO.	SPECIES	COMMON NAME	YEAR	STATUTE
1	*Cyprinus carpio*	Common carp	≈ 1300	Autochthonous
2	*Oncorhynchus mykiss*	Rainbow trout	1 885	Naturalized
3	*Salvelinus fontinalis*	Brook trout	1900	Naturalized
4	*Ameiurus nebulosus*	Brown bullhead	1908	Naturalized
5	*Carassius auratus gibelio*	Prussian carp	1 912	Autochthonous
6	*Lepomis gibbosus*	Pumpkinseed	1929	Naturalized
7	*Gambusia holbrooki*	Eastern mosquitofish	1927	Naturalized

Stage II

During this period, most of the introduction actions of new freshwater fish species were carried out. Thus, a total of 16 fish species were introduced, in order to develop aquaculture in Romania (Table 3).

In this second period of the introduction of foreign fish species in Romania, species from the Coregonidae family, Asian cyprinid species, buffalo fish etc. were brought.

Among the fish species introduced in the II stage, only some Asian cyprinids has the status of being naturalized today. They were acclimatized perfectly for growing in the environmental and climatic conditions in Romania, being from the acclimatization until now basic species with economic value in aquaculture:

- silver carp (*Hypophthalmichthys molitrix*, Val., 1844), phytoplankton consumer;
- grass carp (*Ctenopharyngodon idella*, Val., 1844), consumer of aquatic macrovegetation;
- bighead carp (*Hypophthalmichthys nobilis*, Rich., 1845), zooplankton consumer.

Table 3. Fish species introduced in stage II / Specii de peşti introduse în etapa a-II-a

NO.	SPECIES	COMMON NAME	YEAR	STATUTE
1	*Coregonus lavaretus maraenoides*	Peipsi whitefish	1956	Locally naturalized
2	*Coregonus albula ladogensis*	Vendace	1956	Locally naturalized?
3	*Ctenopharyngodon idella*	Grass carp	1960-1962	Naturalized
4	*Hypophthalmichthys molitrix*	Silver carp	1960-1962	Naturalized
5	*Hypophthalmichthys nobilis*	Bighead carp	1960-1962	Naturalized
6	*Mylopharyngodon piceus*	Black carp	1960-1962	-
7	*Parabramis pekinensis*	White amur bream	1960-1962	-
8	*Megalobrama terminalis*	Black Amur bream	1960-1962	-
9	*Pseudorasbora parva*	Moroccan stone	1960-1962	Naturalized / Invasive
10	*Ichthyobus cyprinellus*	Bigmouth buffalo	1978-1980	-
11	*Ichthyobus niger*	Black buffalo	1978-1980	Acclimatized?
12	*Ichthyobus bubalus*	Smallmouth buffalo	1978-1980	-
13	*Ictalurus punctatus*	Channel catfish	1978	Acclimatized?
14	*Coregonus pelid*	Peled	1980	?
15	*Poecilia reticulate*	Guppy	?	Locally naturalized
16	*Cyprinus rubrofuscus*	Punakas karpkala	1990	-

Stage III

The third period of the introduction of foreign fish species into Romania (from 1989 to the present) for the purpose of aquaculture development, is dominated by the acclimatization and introduction into aquaculture of the North American sturgeon (*Polyodon spathula*, Walbaum 1792). Also, in this third stage, during this period the following species were introduced: black bullhead (*Ameiurus melas* Rafinesque 1820), North African catfish (*Clarias gariepinus,* Burchell 1842), Siberian sturgeon (*Acipenser baerii* Brandt, 1869) and Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758) (Table 4).

Table 4. Fish species introduced in stage III / Specii de peşti introduse în etapa a-III-a

NO.	SPECIES	COMMON NAME	YEAR	STATUTE
1	*Polyodon spathula*	Mississippi paddlefish	1992	Acclimatized
2	*Ameiurus melas*	Black bullhead	*1997*	-
3	*Acipenser baerii*	Siberian sturgeon	*2003*	Acclimatized
4	*Clarias gariepinus*	North African catfish	*2004*	-
5	*Oreochromis niloticus*	Nile tilapia	*2007*	-

Objectives of acclimatization

The experimental works carried out with a view to acclimatization, in various water categories of Romania, had to clarify, for the new living conditions, the following problems:

- the preferential food regime of each species, depending on the natural resources specific to the fish ponds and the seasons. The biological changes occurring in the flora and fauna of the water basins, as an effect of population with new species;
- the behavior of the new species towards the fodder used for carp, in the situation of the stocking of these species mixed with the carp, in the fodder ponds. Fodder specific to the new species, to supplement their feeding and the convenient way of administration;
- the period of active feeding and growth capacity depending on the microclimate of the region. The annual increase in production - achievable fish - due to the new introduced species and the native ones, with which they are stocked in polyculture;
- indices of stocking at different ages, in populations mixed with carp, depending on the natural food of the different categories of fish ponds, on the fertilizers and feeds administered;
- the minimum requirements for each new species, for their life, growth and wintering. The annual average losses - numerical percentage - by age, by growing season and wintering season and for various categories of living conditions that can be offered;
- the conditions for ensuring the development process of the gonads, in conditions of growth in stagnant water, as well as the process of reproduction, fertilization, incubation, pre-larval, larval growth and fry growth in the first summer, the method of efficient transportation in the larval or fry phase in various stages of development.
- specific diseases and parasites, their detection and combat. Diseases and parasites due to the new living conditions and their combat. The influence of specific diseases and parasites on native fish and vice versa.

Fish species are subject to directed growth

Currently, 7 species are the object of directed growth: silver carp, grass carp, bighead carp, paddlefish, rainbow trout, African catfish and Siberian sturgeon. The first three are also present in a significant proportion in natural ecosystems, as there is information on their natural reproduction in the Danube river.

The North American paddlefish (*Polyodon spathula* Walbaum, 1792)
Order*: Acipenseriformes*
Family*: Polyodontidae*

Polyodon spathula is native from hydrographic basin of Mississippi River (Figure 1). It's a fresh water sturgeon with a big size (as 2 m length and 70 kg weight), fast growing, and the most important for technological and economic part, it is not a feed consumer.

After quite difficult steps, started in 1988, the action to introduce the species in Romania was triggered by the import in 1992 of a batch of 2000 embryonated eggs from the company Osage Catfisheries Inc., Osage Beach, Missouri, USA. The batch arrived at SCDP Nucet, Dâmboviţa county on May 8, 1992. Since then, new batches of embryonated eggs have been imported every spring, all being brought and grown in Nucet Station.

In 2002 have been achieved the first artificial reproduction experiments with positive results, which represent a big achievement for fish culture research from Romania, the biological material obtained was stocked in almost 20 fish farms from Romania (Alba, Arad, Calarasi, Arges, Iasi, Vaslui, Constanta, Dolj and Tulcea counties). By results of the research and by number of specimens from this species that station owns, SCDP Nucet comes in third place in the world after USA and China and is in first place from Europe (Costache M. 2010).

Figure 1. North American paddlefish / *Polyodonul Nord American* (*Polyodon spathula* Walbaum, 1792)

The scythe (*Ctenopharyngodon idella* Valenciennes, 1844*)*
Order*: Cypriniformes*
Family*: Cyprinidae*

The grass carp is part of the cyprinid family, widespread in the Amur river basin and the Sungari and Ussuri tributaries and in the lakes fed by the rivers (Figure 2) .

Until the age of one month, the juvenile feeds on plankton and later, like the adult fish, on vegetable food, avoiding places that are too sandy or clogged. It feeds actively at 20°C.

Grass carp develops very quickly depending on the climate and food; under natural conditions it reaches 30-100 g in the first year, the maximum weight being on average 20 kg.

It is a very greedy fish, because at an optimal temperature of 20-28°C in 24 hours it ends up consuming an amount of vegetation equal to its weight and more, up to 120%. The female reaches sexual maturity at 3-5 years.

Figure 2. Grass carp / *Cosaş* (*Ctenopharyngodon idella* Valenciennes, 1844)

Bighead carp (*Hypophthalmichthys nobilis* Richardson, 1845)
Order: Cipriniformes
Family: Cyprinidae

The bighead carp (*Hypophthalmychtys nobilis*) called "marbled carp", morphologically resembles with silver carp (*H. molitrix).*

The head is wider, the ventral part more rounded. The coloring is darker, sometimes yellowish, with darker marbled spots. The gill filter is wider, compared to the silver carp and adapted to filter zooplankton, which forms the main food, and phytoplankton secondarily. When it has sufficient amounts of food, this species grows quickly.

Bighead carp has a faster growth, under optimal environmental and food conditions, reaching a weight of 200-500 g/ex in one year. Its food consists mainly of zooplankton and secondarily of detritus, phytoplankton, occasionally insects and larvae (Figure 3) (Nichifor F. 2004).

Figure 3. Bighead carp / ***Novac*** **(*Hypophthalmichthys nobilis* Richardson, 1845)**

Silver carp (*Hypophthalmychtys molitrix* Valenciennes, 1844)
Order: ***Cipriniformes***
Family: ***Cyprinidae***

The silver carp is spread in the Amur basin, in the tributaries of the rivers: Sungari, Usurii, in the Iantzî river (P. R. China). It was brought to our country in 1960 (Nucet Station, Dâmboviţa County). They have quite tasty meat, with a fat content of aprox. 23,5%. The basic food of this species is phytoplankton, with which it feeds throughout its life, apart from the first period of development, when, together with phytoplankton, it also consumes zooplankton. It develops very quickly in ponds. In our country, this species grows faster in ponds: 25-50 g/ex in the first year (Figure 4) (Nichifor F. 2004).

Figure 4. Silver carp / ***Sânger*** **(*Hypophthalmichthys molitrix* Valenciennes, 1844)**

Rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792*)*
Order: ***Salmoniformes***
Family: ***Salmonidae***

The rainbow trout is a salmonid, native to the cold-water tributaries of the Pacific Ocean in Asia and North America.

Adult freshwater rainbow trout average between 0.5 - 2.5 kg, while lacustrine and anadromous forms can reach 9 kg. Coloration varies greatly among subspecies and habitat. Adult fish are distinguished by a broad reddish stripe along the lateral line, from the operculum to the tail, which is more intense in males ready to reproduce .

This fish prefers bodies of water such as lakes, rivers and streams that do not reach temperatures above 25°C or in lakes with very high oxygen concentrations (Figure 5).

Figure 5. Rainbow trout / ***Păstrăv curcubeu*** **(*****Oncorhynchus mykiss*** **Walbaum, 1792)**

African catfish (*Clarias gariepinus* Burchell, 1822)
Order: ***Siluriformes***
Family: ***Clariidae***

The African catfish is a species of fish that has the ability to breathe from atmospheric air. It is widespread throughout Africa and the Middle East. It lives in freshwater lakes, rivers and swamps, as well as in man-made habitats such as ponds or even in urban channels systems.

The African catfish was introduced worldwide in the early 1980s for aquaculture purposes, so it is found in countries outside its natural habitat, such as Romania, Hungary, Brazil, Vietnam, Indonesia, India etc. It reaches a maximum length of 1.7 m and can weigh up to 60 kg (Figure 6).

Figure 6. African catfish / ***Somn African*** **(*****Clarias gariepinus*** **Burchell, 1822)**

Siberian sturgeon (*Acipenser baerii Brandt, 1869)*
Order: *Acipenseriformes*
Family: *Acipenseridae*

The natural area of the Siberian sturgeon is represented in Russia by all the big rivers in Siberia, being a semi-anadromous or freshwater species, meeting most frequently in the middle and lower sections of the rivers.

Acipenser baerii can reach a maximum length of 2 m and a weight of 210 kg. As a rule, it does not exceed 65 kg with a maximum age of 60 years.

The breeding season is in the spring, at a water temperature varying between 9 – 18^0 C. For females, the interval between two reproductions is at least three to five years, while males can reproduce again after two, three years. It feeds mainly on benthic organisms, including chironomid larvae and amphipods, isopods and river polychaetes (Figure 7).

Figure 7. Siberian sturgeon / *Nisetru Siberian* (*Acipenser baerii* Brandt, 1869)

Currently, four species: Brown bullhead *(Ameiurus nebulosus*), Stone moroko (*Pseudorasbora parva*), Prussian carp (*Carassius auratus gibelio*) and Pumpkinseed (*Lepomis gibbosus*) are naturalized and integrated into the aquatic ecosystems in Romania, showing a strongly invasive character, being considered the species with the most negative impact on aquaculture (Figure 8).

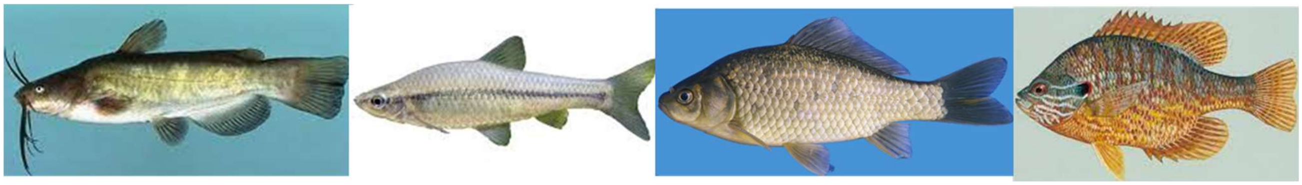


Figure 8. Brown bullhead *(Ameiurus nebulosus*), Stone moroko (*Pseudorasbora parva*), Prussian carp (*Carassius auratus gibelio*) and Pumpkinseed (*Lepomis gibbosus*) / Somnul pitic *(Ameiurus nebulosus*), murgoiul bălțat (*Pseudorasbora parva*), carasul argintiu (*Carassius auratus gibelio*) și bibanul soare (*Lepomis gibbosus*)

At present, the contribution of the 7 species (silver carp, grass carp, bighead carp, North American paddlefish, rainbow trout, African catfish and Siberian sturgeon) to the national aquaculture production, represents a significant percentage quantitatively, qualitatively and from the point of view of the economic value obtained.

The quantity of fish for consumption obtained from aquaculture, at the level of 2020, was 12,150 tons. From the point of view of the diversity of cultivated species, the aquaculture of 2020 is still dominated by farms that practice the cultivation of cyprinid species, some in combination with predatory species (catfish, pikeperch etc.) followed by trouts dominated by rainbow trout (Table 5).

Table 5. Aquaculture production 2015-2020 / ***Producţia din acvacultură*** **- 2015-2020**

Species	2015	2016	2017	2018	2019	2020
	t	t	t	t	t	t
Common Carp	4,349	4,841	4,539	4,357	4,191	3,647
Bighead Carp	1,840	2,121	2,771	2,548	2,870	2,237
Silver Carp	1,843	2,364	1,854	1,692	1,465	1,743
Grass Carp	128	96	89	164	143	178
Crucian Carp	873	883	862	730	975	1,120
Sturgeons	14	35	252	53	94	84
European Catfish	86	48	46	28	53	62
Pikeperch	84	167	124	62	78	70
Pike	42	18	20	17	21	16
Other species	182	279	161	175	340	357
Trouts	1,542	1,597	2,078	2,476	2,618	2,636
Mussels	35	25	-	-	-	-
TOTAL	**11,018**	**12,472**	**12,796**	**12,300**	**12,848**	**12,150**

In 2020, the share of crop species in the reported production was common carp as the main crop species (3,647 tons).

For Asian cyprinids the reported production was:

- 2,237 tons of *A. nobilis* ;
- 1,743 tons of *H. molitrix;*
- 178 tons of *Ct. idella*.

These species grown mostly in polyculture, represent over 64% of the total production, obtained in 2020 (Figure 9).

The production of *Oncorhynchus mykiss* and *Salvelinus fontinalis* was 2,636 tons; crucian carp production of 1,120 tons.

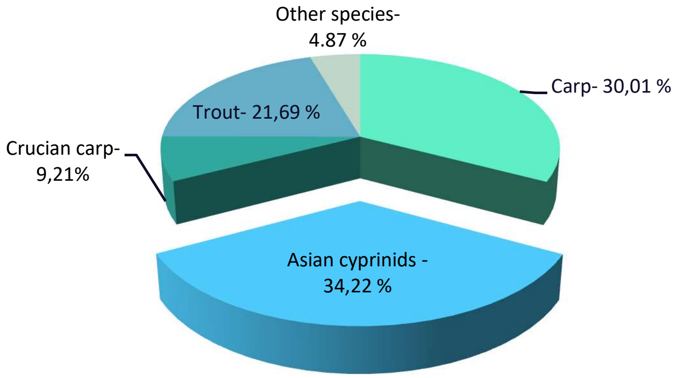


Figure 9. The share of crop species in consumer production marketed in 2020 / Ponderea speciilor de acvacultură în producția de consum comercializată în 2020

CONCLUSIONS

At present, 7 species are the object of directed growth: silver carp, grass carp, bighead carp, North American paddlefish, rainbow trout, African catfish and Siberian sturgeon.

When Asian cyprinids are grown in polyculture with other species, trophic niches are exploited more efficiently, and the quality of the technological water is maintained in the optimal growth parameters.

The contribution of the 7 species to the national aquaculture production represents a significant percentage both quantitatively, qualitatively and from the point of view of the economic value obtained.

Voluntary or accidental introductions of non-native species do not always have the expected results, causing, in some cases, serious ecological disturbances, with sometimes disastrous economic consequences.

Through the phenomena of competition between species, of genetic introgression, through the introduction of new pathogenic agents, these introductions can cause the disappearance of some populations, native species (Tendron G., 1997).

Considering the biological characteristics of fish and the characteristics of the aquatic environment, in the case of the introduction of new fish species, there may be a danger of producing some unwanted phenomena. Thus, the small size of the larvae and the "continuity" of the aquatic environment favor the "escape" from culture and the rapid spread in the natural environment of the newly introduced fish species. The same "continuity" of the aquatic environment favors the spread of new pathogens.

External fertilization, characteristic of most fish species and the lack of effective cytological barriers make possible the hybridization of related species, thus introgression, resulting in the disappearance of some native species.

BIBLIOGRAPHICAL REFERENCES

1. Bud I., Mag I.V., Petrescu R.M.; Speciile invadatoare de peşti din apele dulci ale României şi impactul lor asupra mediului acvatic; 2006; Environment & Progress.
2. Casal C.M.V.; Global documentation of fish introductions: the growing crisis and recommendations for action; 2006; Biol. Inv. 8:3-11.
3. Costache M.; Studii şi cercetări privind tehnologia de reproducere artifcială şi dezvoltare posetmbrionară a speciei de sturion Nord American *Polyodon spathula* (1792) în condiţiile din România; 2010; PhD Thesis.
4. Froese R. , Pauly D.; Comments on FAO's State of Fisheries and Aquaculture; 2012; Marine Policy, 36(3).
5. Iacob M., Petrescu-Mag I.V.; Inventarul speciilor nonnative de peşti din apele dulci ale României; 2008; Bioflux, Cluj-Napoca;
6. Nichifor F.; Cercetări privind perfecţionarea tehnologiilor de reproducere şi dezvoltare postembrionară la speciile *Hypophthalmichthys molitrix* (Val., 1844) şi *Aristichthys nobilis* (Rich., 1845); 2004; PhD Thesis.
7. Savini D., Occhipinti–Ambrogi A., Marchini A., Tricarico E., Gherardi F., Olenin S., Gollasch S.; The top 27 animal alien species introduced into Europe for aquaculture and related activities; 2010; Journal of Applied Ichthyology 26(s2):1–7.
8. Tendron G.; Les introductions des especes dans les milieux aquatiques continentaux en metropole Avant-propos; 1997; Bulletin Francais de la Peche et de la Pisciculture 344-345:1-2.

ANALYSIS OF CSN2 FOR A1/A2 ALLELIC VARIANTS BY DOUBLE-SENSE DNA GENOTYPING WITH REFERENCE TO MILK QUALITY AND HUMAN HEALTH

ANALIZA CSN2 PENTRU VARIANTELE ALELICE A1/A2 PRIN GENOTIPARE ADN DUBLU-SENS CU REFERIRE LA CALITATEA LAPTELUI ȘI A SĂNĂTĂȚII LA OM

MIHALI CIPRIAN VALENTIN[1], NEAMȚ RADU IONEL[1], MIZERANSCHI ALEXANDRU EUGENIU[1], ILIE DANIELA ELENA[1]

[1] Research and Development Station for Bovine – Arad, Calea Bodrogului No. 32, 310059, Arad, Romania,
Phone: +40-257-339130, Fax: +40-257-339133, secretariat@scdcbarad.ro

Correspondence address: ciprian.mihali@scdcbarad.ro

Abstract

The method of CSN2 gene cow selection for animals which are included in the production of milk for human consumption includes the following steps: genomic DNA extraction, checking the presence of DNA molecules by electrophoretic migration, examining the gel with UV light, obtaining PCR products and sequencing the products obtained by PCR using the Sanger method characterized by the fact that for each DNA sample extracted and amplified by PCR, 2 samples are sent for sequencing, one containing the forward primer + PCR products and the second one containing the reverse primer + PCR products, so that at position 8101 for the CSN2 gene the 3 possible allelic variants will be identified. Then the animals homozygous for the A2 allele, the only one with interest for the production of a non-allergenic milk and which does not induce pathological conditions in humans through its consumption, will be selected and lotted.

Keywords: milk proteins, beta casein, CSN2 gene, milk human consumption

Rezumat

Metoda de selecție a bovinelor pentru gena CSN2 pentru animale care sunt incluse în producția de lapte pentru consumul uman cuprinde următoarele etape: etapele de extracție a ADN-ului genomic, verificarea prezenței moleculelor de ADN prin migrare electroforetică, examinarea gelului cu lumina UV, obținerea produșilor PCR și secvențierea produșilor obținuți prin PCR prin metoda Sanger, caracterizată prin aceea că pentru fiecare probă de ADN extrasă și amplificată prin PCR se trimit spre secvențiere 2 probe, una care conține amorsă forward + produși PCR și o a doua care conține amorsă reverse + produși PCR astfel încât la nivelul poziției 8101 pentru gena CSN2 se vor identifica cele 3 variante alelice posibile, apoi se selectează și se lotizează animalele homozigote pentru alela A2, singura de interes pentru producția unui lapte non-alergenic și care nu induce condiții patologice la oameni prin consumul acestuia.

Cuvinte cheie: proteine lapte, beta cazeina, gena CSN2, consumul de lapte la om

INTRODUCTION

Milk is a highly nutritious liquid that contains almost every single nutrient that our body needs (www. healthline.com, 2024). It's a rich source of protein, providing approximately 1 gram of this nutrient in each fluid ounce (30 mL), or 8.14 grams in each cup (249 grams). The proteins in milk can be divided into two groups based on their solubility in water: insoluble milk proteins, known as casein, and soluble milk proteins, known as whey proteins.

Casein makes up more than 70% of the total protein in milk. It is digested slowly, making it a great choice for late evening protein diet. Whey protein accounts for approximately 20% of the protein found in milk. It's particularly rich in branched-chain amino acids (BCAAs) — such as leucine, isoleucine, and valine. Whey protein is excellent for growing and maintaining muscles. The human milk consumption, it varies around the world (www.ourworldindata.org). While milk has long been seen as an important source of calcium and other nutrients, particularly for children, some researchers suggest that it is not a necessary part of a healthy diet for most adults (www.hsph.harvard.edu). In fact, humans do not need to consume dairy milk to be healthy (www.milk.procon.org). The U.S. Department of Agriculture (USDA) recommends three cups per day of dairy milk, but this might be too high for most people. However, milk consumption has been associated with a lower risk of several health conditions, including cardiovascular disease, stroke, hypertension, colorectal cancer, metabolic syndrome, obesity, and osteoporosis (Zhang, X. et al., 2021). Conversely, milk intake might be associated with a higher risk of certain conditions, such as prostate cancer, Parkinson's disease, acne, and iron-deficiency anemia in infancy. Potential allergy or lactose intolerance also needs to be considered.

Proteins from cow's milk are responsible for producing allergies through consumption in the human population. Allergy means the situation in which an individual's immune system reacts to protein (from milk), considering the protein a harmful structure, physically defending itself by triggering one or more hypersensitivity reactions (Monaci L. et al., 2007). The frequency of manifestation of these allergies through the consumption of cow's milk in the human population is estimated to be 10% globally (Miluchová M et al., 2014). The allergy-causing milk proteins are especially β-lactoglobulin and β-casein. The consumption of cow's milk in the human population is due to the fact that this food is a source of essential amino acids (Massella E. et al., 2017), respectively it modulates the intestinal flora (if we refer to whey proteins), (Kanwar J.R. et al., 2009). Based on the latest studies, bovine milk proteins are classified into 2 large categories: caseins (as1, as2, beta, kappa) constituting 80% of the total milk protein and the 2nd category represented by whey proteins which represents the difference of 20% of the total milk protein. With reference to the first category of protein, here beta-casein constitutes approximately 35% of the total casein in cow's milk, the weight is 23,983 kDa being given by a polypeptide chain comprising 209 amino acids. 12 types of beta caseins (A1, A2, A3, B, C, D, E, F, G, H1, H2 and I) were characterized in cava milk, depending on the genotype of each cow (Sodhi M., Kataria R.S. et al., 2018). The most important of these are the allelic forms A1 and A2. Based on the latest studies, between the 2 allelic forms there is only one point mutation in the CSN2 gene, resulting in the case of A1 β-casein and A2 β-casein respectively (Miluchová M. et al. 2009). As a consequence, between the 2 allelic forms, this point mutation produces the modification of an amino acid in the polypeptide chain. As a result, β-casein A2 contains proline (CCT) at position 67, while β-casein A1 contains histidine (CAT), (Kumar A. et al., 2019). Following this change in the structure of the polypeptide chain, the proteolysis at the level of the primary structure of the protein changes as a consequence, resulting in structurally different peptides. Therefore, and by way of consequence, by splitting β-casein A1, the opioid peptide called β-casomorphin-7 (BCM-7) is formed, and from the splitting of A2, β-casomorphin-9 (BCM-9) results (Massella E. et al., 2017). The first secondary metabolite (BCM-7) has a lower molecular weight, it can enter the blood circuit more easily inducing opioid-type symptoms throughout the body. These are presented in the form of gastrointestinal disorders (bloating/tympanism), high flatulence (Woodford K.B., 2021).

Currently in Romania, 3 breeds of cows are bred with high frequency, namely Baltata Romaneasca, Bruna de Maramures and Holstein respectively. These cows are mainly raised in semi-montane and lowland areas in free range systems.

The objective of the present study was to identify the frequency and type of genetic polymorphisms in exon 7 of the CSN2 β-casein gene and to apply a protocol for identifying existing allelic polymorphisms in cows from the Bruna de Maramures and Baltata Romaneasca breeds for this gene. The results from this will be able to help in the selection of cattle, having as a criterion the quality of milk protein with importance in the health of man and his diet.

MATERIALS AND METHODS

The methodology of genetic analysis of double-stranded DNA (ie on both DNA strands, 5' - 3' and 3' - 5') in dairy cows using sequencing and analysis of the region of the 8101 locus for the CSN2 gene (having as a reference the public sequence code X14711.1/ https://www.ncbi.nlm.nih.gov/nuccore/120, the sequence contains 10338 nucleotides) with high precision and the establishment of the allelic form/variant in the analysed individuals involves the following steps: the extraction of genomic DNA, i.e. all existing DNA, in a volume of 300 µl of blood taken in collection tubes containing an anticoagulant (heparin). DNA extraction is done using a classic genomic DNA extraction protocol (www.cdc.gov/dpdx/diagnosticprocedures/blood/dnaextraction), the protocol being one with free public access. After the extraction of genomic DNA from the collected blood samples, the presence of DNA molecules in the solution resulting from the extraction protocol is checked.

This verification is carried out by electrophoretic migration in a 2.5% agarose gel (which also contains ethidium bromide) of a quantity of 5 µl DNA + 1 µl loading buffer solution in the presence of a molecular weight marker for 30 minutes at a voltage of 100V. The electrophoretic migration protocol can be accessed for free using various bibliographic resources (www.addgene.org/protocols/gel-electrophoresis/), both printed and electronic. In a next step, an examination of the gel is carried out in a device with UV light transmitted through the gel where, in the case of the existence of DNA molecules, their presence will be visually highlighted in the form of bands in the analysed gel. The next stage consists in obtaining the PCR (Polymerization Chain Reaction) products which will later be sequenced by the Sanger method in order to be able to study the sequence order of the nucleotides in the sequences obtained by the amplification reaction of the DNA molecules corresponding to the previously taken blood samples. The PCR products are obtained using a public protocol existing in the specialized literature (Massella E. *et al.,* 2017). PCR products are Sanger sequenced using an outsourced public sequencing protocol. From each DNA sample extracted and amplified by PCR, 2 samples are sent for sequencing, one containing forward primer + PCR products and a second containing reverse primer + PCR products. Following the sequencing of each extracted DNA sample (which represents an individual), 2 sequences are obtained, one corresponding to the forward direction and one corresponding to the reverse sequence for the 2 strands of the DNA molecule.

From this stage, the methodology by which the computerized analysis of the 2 sequences (one forward and one reverse) at the level of position 8101 for the CSN2 gene is carried out where the variant / allelic form of the CSN2 gene in the sample can be precisely determined analyzed. The three variants that can exist at this locus are A1A1, A1A2 (which induce pathological conditions in humans through consumption of cow's milk) and A2A2 which do not induce pathological conditions by consumption. To achieve this evaluation, computer analysis is used using programs with free public licenses, text editors, both those encapsulated in operating environments on different Linux distributions, or free programs that can be used for free in Linux distributions. With the help of these programs the sequences are aligned at position 8101. The reverse sequence is processed to obtain its reverse complement also with the help of a free computer program available in Linux distributions. In the next step, the analysis is done by aligning the forward sequence at position 8101 with the reverse sequence of the complement for the reverse sequence for the CAT codon (A1 allelic variant) and the CCT codon (A2 allelic variant). The previously marked red nucleotide in the 2 codons represents position 8101. Additional explanatory details are shown in Figure 1.

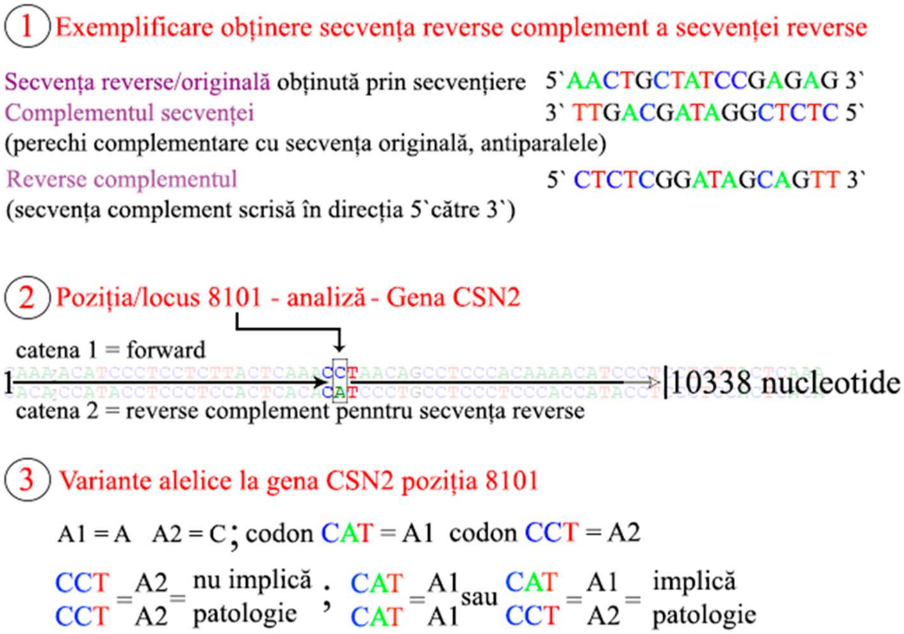


Figure 1. Position/locus 8101 in the CSN2 gene (public reference code X14711) where accurate discrimination can be made between A1 and A2 allelic forms/variants. The A1 allelic variant has an adenine nitrogenous base at position 8101 while the A2 allelic variant has a cytosine nitrogenous base / Poziția/locusul 8101 din gena CSN2 (referință publică cod X14711) unde se poate face discriminarea precisă între formele/variantele alelice A1 și A2. Varianta alelică A1 prezintă în poziția 8101 o bază azotată adenină în timp ce varianta alelică A2 prezintă o bază azotată citozină

For the safety of the analysis, a number of 100 nucleotides both upstream and downstream of position 8101 are evaluated to confirm the similarity between the 2 analyzed sequences. Sequence alignment is performed on a computer using free stand-alone programs Bioedit v7.7 (Hall, T.A. 1999) or included in various free operating environments such as Linux in various distributions as previously mentioned. The sequencing was done by outsourcing the service to the company Macrogen Europe.

RESULTS AND DISCUSSION

Following Sanger sequencing on the DNA animal samples (36 individuals belonging to SCDCB Arad), the sequences that entered the *''in silico''* analysis were generated. The forward-reverse tandem sequences were followed for each animal, respectively, a total of 72 DNA sequences were obtained for the entire herd of animals taken in the present study. Furthermore, using the software analysis (Bioedit v.7.7), the individual analysis of the forward and reverse complement sequences was carried out for each individual animal, with the identification of the A1 and A2 allelic forms for each animal, as shown in table 1.

Following the precise determination of the allelic forms (A1, A2) for the CSN2 gene through the double-sense analysis of the DNA in each animal, it was subsequently possible to identify the frequency with which these allelic forms are manifested in the studied animals herd. As can be seen in figure 2, the highest percentage is represented by the homozygous A2A2 genotype followed by A1A2 and the least represented homozygous form A1A1.

Tabel 1. Animal CSN2 gene genotyping, dsDNA analysis, A1 / A2 – allelic forms / Animale luate în studiu în vederea genotipării genei CSN2, analiză ADNds, A1 / A2 – forme alelice

Nr. crt.	ADER No.	Matricula No.	Genotype	
			F	R - reverse complement
1	623	RO505005656415	A2	A2
2	312	RO020000144078	A2	A1
3	324	RO023000061030	A2	A2
4	369	RO026000133748	A2	A2
5	408	RO025000104374	A1	A1
6	117	RO501004505314	A2	A2
7	149	RO508004505469	A2	A1
8	161	RO509004505350	A2	A2
9	178	RO500004505283	A2	A2
10	182	RO501004505369	A2	A1
11	183	RO503004505349	A2	A2
12	189	RO508004505357	A2	A1
13	201	RO500001104827	A2	A2
14	203	RO029001025526	A2	A2
15	207	RO021000060307	A2	A2
16	209	RO500002214307	A2	A2
17	210	RO503001104785	A2	A2
18	211	RO506000104206	A2	A1
19	212	RO022001018611	A2	A2
20	222	RO505001757811	A2	A2
21	225	RO505000131061	A2	A1
22	226	RO504002214354	A2	A2
23	230	RO507001104787	A2	A2
24	234	RO509000131089	A2	A2
25	248	RO503003324039	A2	A1
26	261	RO508003323987	A2	A2
27	272	RO506003323994	A2	A2
28	276	RO506003324070	A2	A2
29	277	RO503002214463	A1	A1
30	284	RO504003324115	A2	A1
31	285	RO508003324076	A2	A2
32	296	RO028000117920	A1	A1
33	299	RO501000131001	A2	A2
34	375	RO021001009810	A2	A1
35	1016	RO502002214486	A1	A2
36	1020	RO503009113949	A1	A1

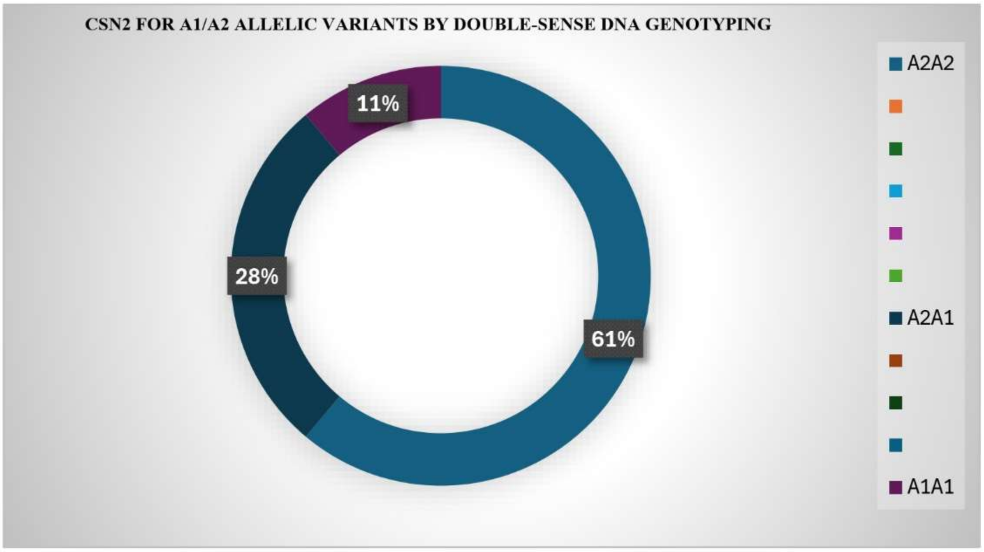


Figure 2. A1 and A2 allelic forms distribution of the CSN2 gene in the experimental animal group / Distribuția formelor alelice A1 și A2 pentru gena CSN2 în lotul de animale luate în experiment

A2A2 allelic form is found in 61% from the studied animals. This percent could be explained due to the fact that within our farm we use directed herds in which the bulls' sperm is genotyped for the CSN2 gene with the favourable allelic variant, i.e. A2A2. Over time this led to the elimination of a significant percentage of A1A2 heterozygosity and A1A1 homozygosity respectively, both undesirable forms from the point of view of the milk quality.

CONCLUSIONS

The presented new protocol relates to a method by which precise analysis (ie on both DNA strands, 5' - 3' and 3' - 5') of a region of the CSN2 gene locus in dairy cows can be done using sequencing and analysis of the region of the 8101 locus for the CSN2 gene that expresses the β-casein protein in milk (having as a reference the public sequence code X14711, the sequence contains 10338 nucleotides). Following the application of this method, the allelic forms/variants in the analysed individuals are established with great precision. Based on the analysis, the screening of individuals who produce milk for human consumption can be carried out and that in individuals possessing the A2A2 allelic form, the resulting milk does not induce allergic/pathological gastrointestinal conditions. By implementing this method of DNA analysis followed by the screening of individuals, it is possible to obtain a milk that presents food safety conditions. Through the certification of this analysis method, the diversification of the methodologies/technologies used in the dairy industry can be achieved by increasing and differentiating the assortments of milk intended for human consumption under food safety conditions.

BIBLIOGRAPHICAL REFERENCES

1. Hall T.A.; BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT; 1999; Nucl. Acids. Symp. Ser. 41:95-98.
2. Kanwar J.R., Kanwar R.K., Sun X., Punj V., Matta H., Morley S.M., Parrat A., Puri M., Sehgal R.; Molecular and Biotechnological Advances in Milk Proteins in Relation to Human Health; 2009; Curr. Protein Pept. Sci. 2009; 10:308–338. doi: 10.2174/138920309788922234.
3. Kumar A., Singh R.V., Chauhan A., Ilayakumar K., Kumar S., Kumar A., Sonwane A., Kumar S., Panigrahi M., Bhushan B.; Genetic association analysis reveals significant effect of β-casein A1/A2 loci on production & reproduction traits in Frieswal crossbred cows; 2019; Biol. Rhythm Res. 2019; 51:1259–1272. doi: 10.1080/09291016.2019.1571705.
4. Massella E., Piva S., Giacometti F., Liuzzo G., Zambrini A.V., Serraino A.; 2017; Evaluation of bovine beta casein polymorphism in two dairy farms located in northern Italy, Ital. J. Food Saf. 2017; 6:131–133. doi: 10.4081/ijfs.2017.6904.
5. Miluchová M., Trakovická A., Gábor M.; Analysis Polymorphism of Beta Casein of Slovak Pinzgau Cattle by Pcr-Rflp for Allels A1 and A2; 2009; Sci. Pap. Anim. Sci. Biotechnol. 2009; 42:288–292.
6. Miluchová M., Gábor M., Trakovická A.; Analysis of Beta-Casein Gene (CSN2) Polymorphism in Different Breeds of Cattle; 2014; Sci. Pap. Anim. Sci. Biotechnol. 2014; 47:56–59.
7. Monaci L., Anklam A., Tregoat V.; Milk allergens, their characteristics and their detection in food: A review; 2007; Aust. J. Dairy Technol. 2007; 62:62–71. doi: 10.1007/s00217-005-0178-8.
8. Sodhi M., Kataria R.S., Niranjan S.K., Verma P., Swami S.K., Mukesh M.; Sequence 38 Characterisation and Genotyping of Allelic Variants of Beta Casein Gene Establishes Native Cattle of Ladakh to be a Natural Resource for A2 Milk; 2018; Def. Lif. Sci. J. 2018; 3:177. doi: 10.14429/dlsj.3.12574.
9. Woodford K.B.; Proceedings of the 16th International Farm Management Congress: Peer Reviewed Papers. International Farm Management Association; Bristol, UK; 2007; [(accessed on 25 June 2021)]. A2 milk, farmer decisions, and risk management; pp. 641–648. Available online: http://researcharchive.lincoln.ac.nz/dspace/handle/10182/417.
10. Zhang X., Chen X., Xu Y. et al.; Milk consumption and multiple health outcomes: umbrella review of systematic reviews and meta-analyses in humans; 2021; Nutr Metab (Lond) 18, 7 (2021). https://doi.org/10.1186/s12986-020-00527-y.
11. https://www.healthline.com/nutrition/milk, accessed at 28.03.2024.
12. https://ourworldindata.org/grapher/per-capita-milk-consumption, accessed at 20.03.2024.
13. https://www.hsph.harvard.edu/news/hsph-in-the-news/milk-healthy-diet/, accessed at 08.03.2024.
14. https://milk.procon.org/, accessed at 09.03.2024.
15. https://www.cdc.gov/dpdx/diagnosticprocedures/blood/dnaextraction.html, accessed at 01.02.2024.
16. https://www.addgene.org/protocols/gel-electrophoresis/ accessed at 01.12.2023.

RESEARCH ON THE INFLUENCE OF THE a αS1-CASEIN GENOTYPE ON THE PROCESSING YIELD OF GOAT MILK INTO CHEESE

CERCETĂRI PRIVIND INFLUENȚA GENOTIPULUI αS1-CAZEINA ASUPRA RANDAMENTULUI DE TRANSFORMARE ÎN BRÂNZETURI A LAPTELUI DE CAPRĂ

NADOLU DORINA[1], ANGHEL ANDREEA HORTANSE[1], ZAMFIR ZOIA CAMELIA, ILIȘIU ELENA[2], ANGHEL FLOREA[3], JERCAN CĂTĂLIN[4], ILIȘIU VASILE CALIN[2], ILIȘIU CRISTIAN-VASILE[5]

[1]Institute of Research and Development for Sheep and Goat Breeding Palas, Constanta, I.C. Bratianu, 248, Constanta, 0241.639.506, icdcocpalas@yahoo.com
[2] Institute of Research and Development for Sheep and Goat Breeding Palas, Constanta, Reghin experimental baze, Mures, Dedradului, 11, 0241.639.506, statiuneareghinmures@yahoo.co.uk
[3]CMV Anghel Florea, Corbu, Constanta, 0721388213, afanghel@yahoo.com
[4]ANCC CAPRIROM, I.C. Bratianu, 248, Constanta, 0726880793, caprirom@yahoo.com
[5]Reghin CAPRIROM Association, Dedradului, 11, Mures, 0265512041, capriromreghin@yahoo.com

Correspondence address: icdcocpalas@yahoo.com

Summary

The genome of sheep and goats has been deciphered since 2008. Each quantitative trait, such as milk production, growth rate, body weight etc. is determined by several genes, distributed throughout the genome, but especially in some of the chromosomes that the species possesses. Through the use of genetic markers in breeding programs, it is possible to detect genes that determine quantitative or qualitative characteristics, which is why most of the proteins synthesized in milk or other tissues have become the subject of research that attempts to improve the efficiency of meat and milk production through selection assisted at the molecular level. Casein is a protein specific to milk. In the last decades, several studies have been carried out on the αs1-casein locus to individualize allelic variants and their relationship with goat milk composition and technological properties. The research of the present study aimed to evaluate in farm conditions the effect of polymorphism of the αS1 casein gene (CSN1S1) on the percentage of coagulable casein in milk. Thus, the efficiency of raw milk transformation into cheese is higher in the case of milk collected from females carrying the CSN1S1 AA genotype (19,206%), compared to milk collected from goats carrying the CSN1S1 FF genotype (16,197%).

Key words: casein, goat milk, cheeses

Rezumat

Genomul ovinelor și al caprinelor a fost descifrat încă din anul 2008. Fiecare caracter cantitativ, cum ar fi producția de lapte, viteza de creștere, greutatea corporală etc. este determinat de mai multe gene, distribuite în tot genomul, dar mai ales în unii dintre cromozomii pe care-i posedă specia. Prin utilizarea markerilor genetici în programele de ameliorare se oferă posibilitatea depistării unor gene care determină caracterele cantitative sau calitative, fapt pentru care, majoritatea proteinelor sintetizate în lapte sau în alte țesuturi au devenit subiectul cercetărilor prin care se încearcă eficientizarea producției de carne și lapte, prin selecția asistată la nivel molecular. Cazeina este o proteină specifică laptelui. În ultimele decenii au fost efectuate mai multe studii asupra locusului αs1-cazeinei care să individualizeze variantele alelice și relația lor cu compoziția laptelui de capră și proprietățile tehnologice. Cercetările prezentului studiu au avut drept scop evaluarea în condiții de fermă a efectului polimorfismului genei αS1 cazeinei (CSN1S1) asupra procentului de cazeină coagulabilă din lapte. Astfel, randamentul de transformare a laptelui crud în brânză este mai mare în cazul laptelui colectat de la femelele purtătoare ale genotipului CSN1S1 AA (19,206%) față de laptele colectat de la caprele purtătoare ale genotipului CSN1S1 FF (16,197%).

Cuvinte cheie: cazeină, lapte capră, branzeturi

INTRODUCTION

The genome of sheep and goats has been deciphered since 2008 by a consortium of american, australian, spanish, french, english, norwegian, new zealand, chinese, german researchers, which made possible to apply modern methods of genetic improvement breeds and herds of animals, respectively genomic selection (Vintila et al., 2005).

Until the genome reading it was considered that sheep and goats are related and that the differences between them are only due to the different number of chromosomes they possess in the nucleus of the body's cells, in sheep 27 pairs of chromosomes, and in goats 30 pairs of chromosomes. Research has shown that the sheep genome consists of a DNA chain of 2.53 billion nucleotides, and that of goats of 2.66 billion nucleotides.

Each quantitative trait, such as milk yield, growth rate, body weight etc. it is determined by several genes, distributed throughout the genome, but especially in some of the chromosomes that the species possesses. It is possible to identify and select, within the populations, individuals with the desired genotypes at the level of the gene loci that encode the desired characters, thus achieving a safe, fast and efficient selection within the animal populations. Of the total number of genes coding for proteins necessary for the functioning and development of cells and the whole organism of almost 21,000 in sheep and 22,000 in goats, approximately 15,967 are similar in structure and function to those of humans, and 16,604 of them are homologous to those of taurines **(**Vintila et al., 2005).

By using genetic markers in breeding programs, it is possible to detect genes that determine quantitative or qualitative traits, which is why most of the proteins synthesized in milk (k-casein, β lactoglobulin) or in other tissues (Pit-1, leptin), have become the subject of research that attempts to increase the quantitative and qualitative production of meat and milk in bulls, sheep and goats, through assisted selection at the molecular level.

Advanced biotechnologies based on the methods of biology and molecular genetics aim at the direct improvement of the body's genetic mechanisms. All of them contribute either to the enrichment of the animal genome, or to obtaining more offspring beyond the natural reproduction limits of the species (Vintila et al., 2005).

The composition of goat's milk is no different from cow's milk in terms of total fat, protein and lactose content. The main difference lies in the ''quality'' of fats and proteins. Compared to human milk, goat milk differs in both quantity and quality of constituents. Goat's milk is richer in protein, while the lactose content is lower; in addition, the protein composition differs greatly because human milk contains more proteins from the whey protein category than goat milk. All these differences give cow, goat and human milk different physical, chemical and nutritional characteristics (Anghel et al, 2022).

The protein content of cow's milk is on average 3,3%, in buffalo's milk 4,1%, in sheep's milk 5,3 %, and in goat's 3,7%. Of the total proteins, the casein fraction represents 80%, and serum proteins 20%.

Milk proteins are divided into two large groups depending on their behavior at an acidic pH of 4,6, respectively the soluble fraction at this pH, called „whey proteins'' that are not included in the composition of cheeses (β-lactoglobulin, α-lactalbumin, other minor proteins) and the insoluble fraction at this pH, called „total casein'', consisting of four types of casein: αS1-casein (αS1-CN), αS2-casein (αS2-CN), β-casein (β-CN) and K-casein (K-CN). Casein is a phosphoprotein with a molecular mass of 75-100.000 kD. Caseins are found in fresh milk dispersed in the form of a very large number of solid particles in suspension, being joined together by calcium phosphate molecules. These particles are called micelles (Balteanu et al., 2015).

Casein is a protein specific to milk. In cow, sheep, goat milk, casein represents about 4/5 of the total protein, the milk being also called casein milk. In the milk of odd-headed animals, herbivores, carnivores and omnivores, the ratio between casein and serum proteins is approximately 1/1, and this milk is called albuminous milk. In milk, casein is found in the form of calcium phosphocaseinate micelles in colloidal solution (Anghel et al, 2022).

Also present in milk is a non-protein nitrogen (NPN) component that includes ammonia, urea, creatinine, creatine and uric acid. The main proteins in goat milk are α-lactalbumin (α-Lac), β-lactoglobulin (β-Lg), immunoglobulins (Ig), lactoferrin (Lf), αs1-casein (αs1-CN), αs2-casein (αs2-CN), k-casein (κ-CN) and β-casein (β-CN) and other minor proteins and enzymes. Although the protein components of goat's milk are the same as those of cow's and sheep's milk, goat's milk has inferior clotting properties.

The identification of genes responsible for milk quality is now being exploited in sheep and goats, with the aim of identifying the molecular mechanisms that lead to obtaining milk of a different quality. The most intensively analysed genes are milk protein genes, where, to date, major effects have been assessed for αs1-casein, both in sheep and goats. The genetic polymorphism of αs1-casein causes a wide range of variation (0-30%) in the presence of this protein in goat milk; in addition, several studies have found that strong genotypes are associated with high total milk protein and casein content. If the direct effect on protein content and αs1-casein is evident, less evidence is available to clarify whether they also have some direct effects on the biosynthesis of other caseins (Anghel et al, 2021).

In the last decades, several studies have been carried out on the αs1-casein locus to individualize allelic variants and their relationship with goat milk composition and technological properties. All variants can be divided into four categories (strong, intermediate, weak and null) as a function of the amount of αs1-CN in the milk.

The research of this study was carried out within the ADER 818 project and aimed to evaluate in farm conditions the effect of the polymorphism of the αS1 casein gene (CSN1S1) on the percentage of coagulable casein in milk, respectively, the correlation between the presence of αS1-CASEIN and the efficiency of converting milk into cheeses.

MATERIAL AND METHOD

The research was carried out on 133 goats of the **Carpathian breed**, belonging to a farmer from the town of Moiad, Salaj County. In the first stage of the study, blood samples were collected in order to carry out DNA tests to identify the genotype for casein, and the genotypes AA (64 goats) and FF (69 goats) of the CSN1S1 gene were identified. Also, milk samples with a specific preservative were collected and sent for processing to the Milk Quality Control Laboratory owned by the Cluj-Napoca Milk Quality Control Foundation, establishing the biochemical profile, respectively total protein, casein, fat and dry substance.

Based on the biochemical analyses of the milk and the genotypes of the CSN1S 1 gene, 2 groups of females were formed, corresponding to genotypes AA and FF, respectively group 1 and group 2.

The two groups of goats were kept together, benefiting from the same feeding and maintenance conditions, and monthly quantitative measurements of milk production were made, 3 consecutive months, with an interval of 28-34 days between checks.

The collection of milk in order to establish the yield of conversion into cheeses was carried out after the end of the 3 official controls, namely the 5th month of lactation, of which 1-1,5 months the females stayed with the kids, breast-feeding them. The average length of lactation in goats of the **Carpathian breed** is 180-210 days, after which the milk secretion decreases so that, after approximately 240 days of lactation, the goats are weaned. Therefore, at the 4th milk quantity measurement and collection for processing, the milk production is placed on the downward side of the lactation curve, and the assessment of the total milk quantity per lactation was made according to the Fleischmann formula.

The differentiation of goats into batches (batch 1 and batch 2), on the control day, was carried out at the time of milking by reading the registration number, identifying the batch to which the goat belongs (according to genotyping) and collecting the milk in different containers.

The technological process of obtaining coagulum at farm level

Immediately after milking, the containers with the milked milk were transported to the cheese factory and coagulation valves, separately for each lot in the farm, depending on the established genotype, proceeding to its second filtering through filters of silk, the first filtering being carried out on the farm immediately after manual milking (figure 1, 2). It should be noted that the milk processing activity was carried out at the same time, in the same room, using the same type of curd with the quantities corresponding to the amount of milk to be coagulated, but in separate vessels.

Figure 1. 2nd filtration through silk filter of the milk in the two coagulation valves / Filtrarea a 2-a prin filtru de mătase a laptelui în cele două vane de închegare

Figure 2. Milk residues retained by the silk filter / Reziduuri din lapte reținute de filtrul de mătase

The total quantity of milk corresponding to each lot was weighed and after filtering, the temperature of the milk was checked and if necessary heated to 33-34^{0}C, using indirect heat and then the *Fromase granule* rennet was added from the leaflet (1 gram/ 50 kg of milk, dissolved for 15-20 min in 100 ml of cold chlorine-free water) (figure 3).

Figure 3. Milk thermometry and curd addition / Termometria laptelui și adăugarea cheagului

The milk was thoroughly homogenized with the curd and then left to coagulate for about 40 minutes. Checking the formation of the clot is done by the detachment test from the edge of the valve, which must be done in a block without leaving traces of milk or clot on the walls. Another test is the breaking of the clot, an action in which we observed differences on the section between the two samples of clot from the milk from batches 1 (genotype AA) and 2 (genotype FF). When removing the curd by hand, the curd section of the milk from the goats in batch 1 is smooth, firm, glassy, with a gelatinous appearance. The clot from the milk of the goats in batch 2 is slightly crumbly, of a slightly softer consistency compared to the first situation, and on section it has a granular, pea-like appearance. The next step in obtaining the cheeses is cutting the curd, or fractioning the curd with a stainless-steel spatula by making 8-10 cm squares, then leaving another 10–15-minutes to express the whey. (figure 4 and figure 5).

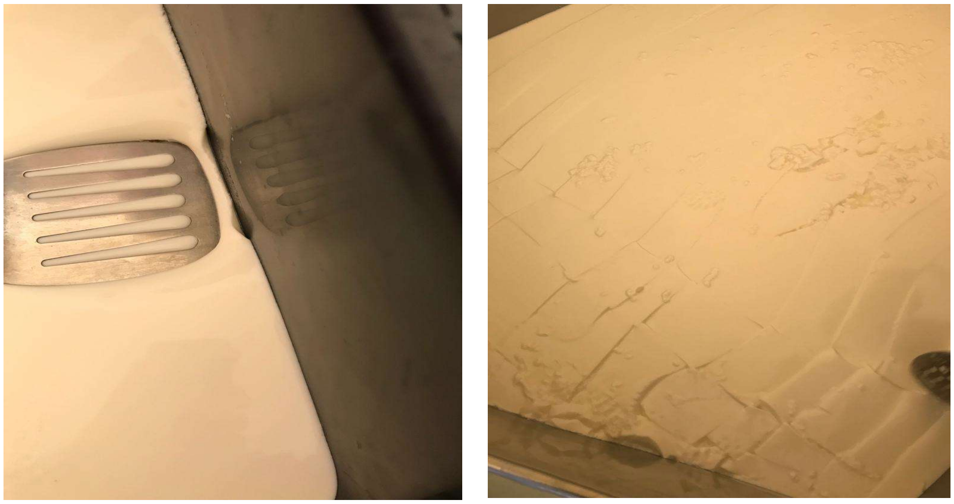
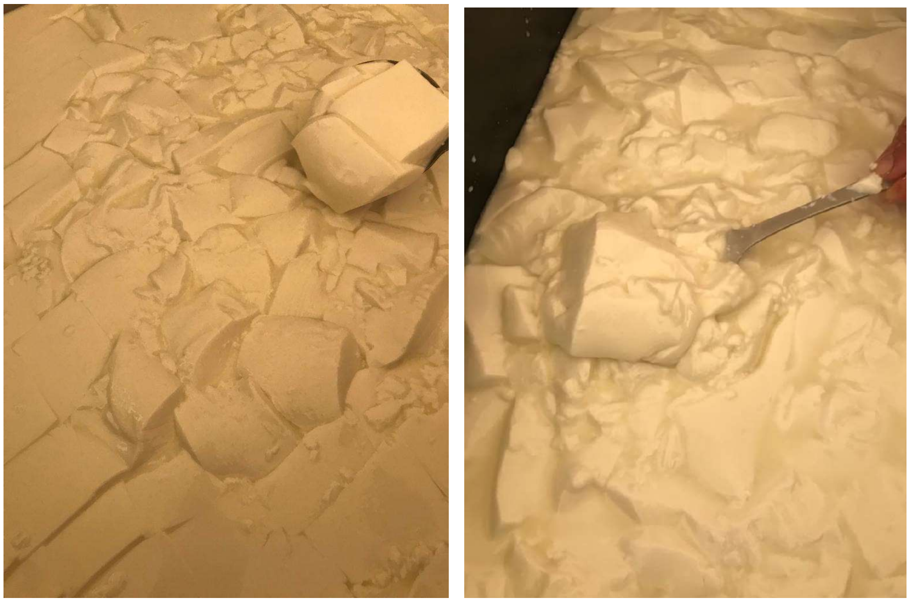


Figure 4. Checking milk coagulation and cutting the curd / Verificarea coagulării laptelui și tăierea coagulului



a b

Figure 5. Fractionation of the clot: a- coagulum from milk from goats carrying the AA allele; b- coagulum from milk from goats carrying the FF allele / Fracționarea coagulului: a- coagul din lapte de la capre purtătoare ale alelei AA; b- coagul din lapte de la capre purtătoare ale alelei FF

After coagulation, the curd was placed in special moulds (figure 6) so that the expressed whey flowed, separated from those two processing valves. The obtained whey was collected separately and weighed to be used to obtain the wort. The processing of milk into cheeses was carried out in different batches according to the type of genotype and all the fractions obtained in the milk coagulation process (whey, curd, rennet) were weighed separately for the milk samples from goats carrying the CSN1S1 AA and FF gene in order to establish the processing yield.

Figure 6. Whey expression and collection / Exprimarea și colectarea zerului

RESULTS AND DISCUSSION

In a study by Vacca et al., 2018, the overall effect of breed on cheese production traits was divided into direct effects of breed (independent of milk production and composition) and indirect effects of breed (mediated by production and composition milk). These results show that much of the total breed variation is attributable to milk yield and composition, and demonstrate that further investigation is needed into the actual effect of milk composition on goat milk cheesemaking ability.

Studies have demonstrated the existence of large differences between goat breeds in terms of milk composition (Lôbo et al., 2017), coagulation capacity (Pazzola et al., 2019; Vacca et al., 2018) and the efficiency of the manufacturing process cheeses (Soryal et al, 2005). Milk composition significantly influences the yield and sensory quality of cheese, and results from the dairy cow literature cannot be adapted to goats. There are large differences between species in coagulation, coagulation and syneresis properties (Bittante et al., 2012) and consequently in the recovery of milk nutrients in curd and cheese production.

The results regarding milk production/lactation are presented in table 1.

Table 1. Quantitative control of goat milk production (mean ± standard deviation of the mean) / Controlul cantitativ al producției de lapte de capra (medie±deviația standard a mediei)

Parameter	Control 1 Skimmed milk Kg/head/day	Control 2 Skimmed milk Kg/head/day	Control 3 Skimmed milk Kg/head/day	Total Kg milk/ lactation
Average amount of milk/goat	1,74 ±0,34211	2,22 ±0,5123	1,89 ±0,4873	239,28

Milk production on the control day sums up the amount of milk milked in the morning and in the evening for each goat, and the average milk production per lot represents the arithmetic mean of individual productions. The total amount of milked milk / lactation is calculated by the Fleishman formula:

$$SM = I_0 M_1 + I_1\left(\frac{M_1 + M_2}{2}\right) + I_2\left(\frac{M_2 + M_3}{2}\right) + \cdots + I_{n-1}\left(\frac{M_{n-1} + M_n}{2}\right) + I_n M_n$$

The results regarding the milk quality of the females taken in the study are shown in table 2.

Table 2. The physico-chemical and biochemical parameters of the milk from the studied farms / Parametrii fizico-chimici și biochimici ai laptelui din fermele luate în studiu

Parameter	Protein %	Casein %	Fat %	SU non-fat%	Lactose %	Total SU g/100g	pH
average	3,53	2,54	4,23	8,43	4,34	12,80	6,47
St dev	0,4947	0,3146	1,1649	0,4688	0,3337	1,3231	0,0979

SU – Dry substance

In the **Carpathian goat breed**, the average milk production is 200- 250 litres obtained in a lactation of 200-240 days, with a maximum of 800 litres and 4,5-6,5 % fat, with a maximum of 10% (Tafta, 2002). The average milk production/goat/lactation of 239,28 kg, calculated for the group of goats followed in the present study (n=175 heads) falls within the specifics of the breed. The percentage of fat in milk samples collected in the 5th, 6th month of lactation are lower, below the average indicated by the breed standard per lactation. This reduced value is explained by the fact that the analysis was performed at the end of the lactation period, at 15-180 days of lactation out of 210-240 days as recorded in the females of the monitored group. Another factor that contributes to the decrease in fat percentage is the low nutritional value of the pasture at the end of summer, the goats being exploited in the traditional system.

The results of the transformation into cheeses are shown in table 3.

The milk transformation yield differentiated into cheeses on the two batches of goats identified with the CSN1S1 AA and FF genotypes respectively, (64 typed with the **CSN1S1**AA genotype and 69 typed with the **CSN1S1** FF genotype) are recorded in table 3.

Table 3. Milk transformation yield into cheeses / Randamentul de transformare a laptelui în brânzeturi

Batch	Amount of milk milked (kg)	Amount of curd obtained (kg)	Yield transformation into cheese (%)	Amount of whey obtained (kg)	Wort amount (kg)	Yield transformation into wort (%)
Batch 1 (AA genotype) (n=64 goats)	36,55	7,02	**19,206**	28,2	1,385	**4,91**
Batch 2 (FF genotype) (n=69 goats)	41,55	6,73	**16,197**	34,1	1,360	**3,99**
Total	78,1	13,75		62,3	2,745	

As can be seen in table 3 and from the analysis of the statistical parameters highlighted in table 4, that the yield of transformation into cheese of milk from goats carrying the **CSN1S1**AA gene (batch 1) is much better than in the case of goats from group 2 (the goats carriers of the **CSN1S1**FF gene), although the average individual production in group 1 was lower (0,571 kg/head/milking) than in group 2 (0,602 kg/head /milking).

Table 4. Statistical parameters of milk transformation yield / Parametri statistici ai randamentului de transformare a laptelui

Descriptive Statistics 1		
	Cheese conversion yield %	**Conversion yield to wort %**
Valid	2	2
Missing	2	2
Mean	17.701	4.450
Std. Deviation	2.128	0.651
Minimum	16.197	3.990
Maximum	19.206	4.910

Descriptive Statistics 2	N	Mean	SD	SE	Coefficient of variation
Yield transformation into cheese	2	17.701	2.128	1.505	0.120
Yield transformation into wort	2	4.450	0.651	0.460	0.146

DE – standard deviation
SE – standard error

Both in terms of the yield of milk transformation into cheese and in obtaining the wort from the whey, superior results were obtained with the milk from the goats in batch 1, in which it was identified. genotype CSN1S1AA, from 36,55 kg of milk collected from 64 goats obtaining 7,02 kg of cheese and 1,385 kg of curd. In the case of batch 2, consisting of several goats (n=69 heads), at the morning milking, 41,55 kg of milk were collected, with 5 kg more, from which 6,73 kg of curd was obtained and 1,360 kg wort amount.

CONCLUSION

1. The yield of raw milk transformation into cheese, respectively of protein coagulation, is higher in the case of milk collected from females carrying the CSN1S1 AA genotype (19,206%) compared to milk collected from goats carrying the CSN1S1 FF genotype (16,197%). The same difference in yield was also obtained in the process of obtaining the wort from the whey collected after the coagulum was drained (4,91% vs 3,99%).

2. Farmers aiming to establish dairy processing plants should have as a priority objective the typing of females carrying the CSN1S1 AA genotype to be selected and promoted in breeding to increase their economic efficiency.

BIBLIOGRAPHICAL REFERENCES

1. Anghel A.H., Nadolu D., Marinică L., Ilișiu E., Anghel F., Bușuricu F.; Goat's milk proteins – hypoallergenic and therapeutic significance; 2022; Animal & Food Sciences Journal Iasi, 95-102.
2. Anghel A., Jitariu D., Nadolu Dorina**,** Zamfir Z., Ilişiu E.; Considerations on goat milk biochemical composition; 2021; Ovidius University Annals of Chemistry, Volume 32, Number 1, pp. 85 - 89, DOI: 10.2478/auoc-2021-0012, (2021) ISSN 1583-2430, DOI: 10,2478/auoc-2021-0012.
3. Balteanu V.A., Pascal C., Vlaic A.; Genetic polymorphisms of αs1-casein (*CSN1S1*) and β-casein (*CSN2*) genes in **Carpathian goat breed**; 2015; Scientific Papers - Animal Sciences Series: Lucrări Stiinţifice - Seria Zootehnie, 63: 193-198.
4. Bittante G., M. Penasa and A. Cecchinato; Invited review: Genetics and modelling of milk coagulation properties; 2012; J. Dairy Sci. 95:6843–6870. https://doi.org/10.3168/jds.2012-5507.
5. Lôbo A.M.B.O., Lôbo R.N.B., Facó O., Souza V., Alves A.A.C., Costa A.C., Albuquerque M.A.M.; Characterization of milk production and composition of four exotic goat breeds in Brazil; 2017; Small Rumin. Res. 153:9–16. https://doi.org/10.1016/j.smallrumres.2017.05.005 .
6. Pazzola M., Stocco G., Dettori M.L., Bittante G., Vacca G.M.; Effect of goat milk composition on cheesemaking traits and daily cheese production; 2019; J. Dairy Sci. 102:3947–3955. https://doi .org/10.3168/jds.2018-15397.
7. Soryal K.A., Zeng S.S., Min B.R., Hart S.P., Beyene F.A.; Effect of feeding systems on goat milk composition and yield Domiati cheese; 2004; Rumin mic. Res. 54, 121–129.
8. Taftă V.; Producția și Reproducția Caprinelor; 2002; Editura Ceres, București.
9. Vacca Giuseppe M., Stocco Giorgia, Dettori Maria L., Summer Andrea, Cipolat-Gotet Claudio, Bittante Giovanni, Pazzola Michele; Cheese yield, cheesemaking efficiency, and daily production of 6 breeds of goats; 2018; J. Dairy Sci. 101:7817–7832 https://doi.org/10.3168/jds.2018-14450.
10. Vintilă I, Vintilă C, Vintilă D.; Imunogenetica și imunomodularea producțiilor animale; 2005; Ed Orizonturi universitare.

RESEARCH ON COLOR SHADES TRANSMISSION IN GRAY LINE FROM KARAKUL OF BOTOŞANI

CERCETĂRI PRIVIND TRANSMITEREA NUANȚELOR DE CULOARE LA KARAKUL DE BOTOȘANI DIN LINIA SUR

NECHIFOR IONICĂ[1], FLOREA ALEXANDRU MARIAN[1], NECHIFOR BOGDAN-IOAN[1], ȚURCANU IOANA[1], BOLDIȘOR ANA[1], NECHIFOR DANIEL CONSTANTIN[1], PASCAL CONSTANTIN[1,2], MACIUC VASILE[1,2]

[1]Research and Development Station for Sheep and Goat Breeding ”Popăuți” – Botoșani
[2]University of Life Sciences, Iasi

Correspondence address: popauti@yahoo.com

Abstract

*In 2015-2022 period, on a representative flock of lambs, the data related to color shades transmission within Gray Line belonging to **Karakul of Botoșani** breed were processed. The biological material studied belongs to SCDCOC Popăuți and is registered in the Genealogical Register of **Karakul of Botoșani** breed, making it the object of improving productive performance. For each generation of lambs analyzed, the mode of color transmission and the proportion of shades obtained as a result of mating between parents belonging to the Gray Line were evaluated. Within the line, the color shades encountered are extremely varied, the most valuable being the silver one. One of the main objectives of the breeding the **Karakul of Botoşani** refers to the genetic diversification and strengthening of the specific curl colors. In accordance with this objective, at the end of the studied period, there is an increase in the number of individuals with a silver shade within the Gray Line, from 0.52% in 2016, to 18.47% in 2021 and respectively to 14, 82% in 2022. Although applied breeding is based on homogenous pairings, in the obtained lambs it is found that lambs that have a shade similar to that of the parents do not have the most numerous proportions, usually being in positions two or three. The diversity of shades within Gray Line, to which is added the small size of the population, but also the fact that it was recently homologated, makes the breeding activity to take place slowly, the expected results being more difficult to quantify in each generation of lambs.*

Key words: Karakul of Botoșani, Gray line, pelts, curls

Rezumat

*În perioada 2015-2022, pe un efectiv reprezentativ de miei, au fost prelucrate datele referitoare la transmiterea nuanțelor de culoare la Linia Sur aparținând rasei **Karakul de Botoșani**. Materialul biologic studiat aparține SCDCOC Popăuți și este înscris în Registrul Genealogic al rasei **Karakul de Botoșani**, făcând obiectul ameliorării performanțelor productive. Pentru fiecare generație de miei analizată a fost evaluat modul de transmitere a culorii și proporția nuanțelor obținute ca urmare a împerecherilor realizate între genitori aparținând Liniei Sur. În cadrul liniei nuanțele de culoare întâlnite sunt extrem de variate, mai valoroasă fiind cea argintie. Unul dintre obiectivele principale ale creșterii rasei **Karakul de Botoșani** se referă la diversificarea și consolidarea genetică a culorilor specifice buclajului. În acord cu acest obiectiv, la finalul perioadei studiate, se constată o creștere a numărului indivizilor de nuanță argintie aflați în cadrul Liniei Sur, de la 0,52% în anul 2016, la 18,47% în anul 2021 și respectiv la 14,82% în anul 2022. Cu toate că reproducția aplicată se bazează pe împerecherile omogene, la descendența obținută se constată că mieii care au o nuanță similară cu cea a părinților nu dețin proporția cea mai numeroasă, situându-se de obicei pe pozițiile doi sau trei. Diversitatea de nuanțe din cadrul Linie Sur, la care se adaugă mărimea redusă a populației, dar și faptul că aceasta a fost omologată recent, face ca activitatea de ameliorare să se deruleze lent, rezultatele așteptate fiind mai greu cuantificate în fiecare generație de miei.*

Cuvinte cheie: Karakul de Botoșani, linia sur, pielicele, buclaj

INTRODUCTION

Karakul of Botoșani breed is a unique breed worldwide because it presents a large palette of color varieties, called lines, respectively: black, greyish, brown, gray, white and pink. The Gray line is special because it has a particular feature: in this line, the color shade is dependent on the types of pigments at the wool thread base (from black and brown of various shades) and at the tip of the fibers in the curl (from yellow to clear white). Although the Gray Line within the **Karakul of Botoșani** breed was homologated in 2018, thanks to the improvement work carried out in order to consolidate and diversify the desired shades, seven color shades are currently in an advanced phase of genetic consolidation, respectively: antique, golden, copper, silver, tan, amber and platinum (figure no. 1).

The aesthetic and commercial value of skins obtained from lambs belonging to the Gray Line depends not only on the ratio and the intensity of the colors found at the base and respectively at the top of the fibers, but also on the length and highlighting of the light colored segment of the fiber, on the way the transition from the dark to the light shade is made from the tip of the fiber, by the general uniformity of the color at the level of the skin. Each shade is special and different, which makes the skins obtained from the lambs belonging to this line appreciated and requested by the processing industry.

Research completion had as its main activity the assessment of the way shades are transmitted in gray individuals, practicing homogenous pairings between gray breeders, as it is known that in the case of gray x gray mating (KS) a relatively high proportion of lambs with the same shade of color (73.5%) an obtained as an effect of the dominance of the genes involved in the transmission of this character (Nechifor et al. 2018).

Figure 1. Color shades within the Gray Line of Karakul of Botoșani breed / Nuanțele de culoare în cadrul Liniei Sur a rasei Karakul de Botoșani

MATERIAL AND METHOD

Research was carried out over a longer period of time, between years 2015 and 2022. During this period of time, a total flock of 1256 lambs belonging to Gray line (KS), resulting from the crossing of parents belonging to the same color line, were subjected to study. The resulting lambs were obtained from a total of 39 breeding rams and from 1152 KS ewes of various shades. The research included both a period before the homologation, and a rather significant period after the homologation of the Gray Line as a distinct line within the **Karakul of Botoșani** breed. The population under the study is registered in the Genealogical Register of the **Karakul of Botoșani** breed, a register managed by the Moldoovis Sheep and Goats Breeders' Association, a professional entity accredited by MADR, through ANZ Bucharest, since 2007, and is subject to selection and breeding activities through the official control program of productive performances. The evaluation of the productive performance was performed on recently born lambs, in the first 24-48 hours of life, that appreciates the shade of color among other characters.

From the processing of the data and the values obtained during the evaluation of the productive performances, the specific frequencies in the transmission of the color shades within the Gray Line were calculated.

All lambs were searched in the same place and under the same conditions during the morning of each day. The assessment was made by the same staff from the beginning to the end of the action, in order to maintain the same level of rigor.

RESULTS AND DISCUSSION

During the analyzed period, we survey the results obtained in the case of pairings between individuals from the Gray line:

- Rams of antique shade x ewes of different shades (table no. 1)
- Rams of copper shade x ewes of different shades (table no. 2)
- Rams of silver shade x ewes of different shades (table no. 3)
- Rams of golden shade x ewes of different shades (table no. 4)
- Rams of tan shade x ewes of different shades (table no. 5)
- Rams of platinum shade x ewes of different shades (table no. 6)

In the case of mating between males of antique shade with gray ewes of different shades, it can be observed, according to the data centralized in table no. 1, that tan lambs were the most frequently obtained. The highest frequency is recorded in the case of copper ewes, from which 76% of tan lambs were obtained, followed by golden ewes with 72.41% tan lambs. A single exception was recorded in the case of silver ewes, from which 50% antique lambs were obtained. In the case of homogenous pairings, between individuals of the same shade, the lambs of tan shade were the most frequently obtained, respectively 53.16%, followed by lambs of antique shade with 25.32%.

Table 1. Results of mating antique shade rams with ewes of different shades / Rezultatele împerecherii masculilor sur de nuanță antică cu femele sur de diferite nuanțe

Ewes shade	Ewes	Lambs' shades of color obtained in the period 2015-2022														Total lambs
		Antique		Copper		Silver		Golden		Tan		Amber		Platinum		
	n	n	%	n	%	n	%	n	%	n	%	n	%	n	%	
Antique	71	20	**25,32**	3	3,80	1	1,27	2	2,53	42	**53,16**	0	0,00	11	13,92	79
Copper	21	3	12,00	0	0,00	0	0,00	3	12,00	19	**76,00**	0	0,00	0	0,00	25
Silver	4	2	**50,00**	0	0,00	0	0,00	1	25,00	1	25,00	0	0,00	0	0,00	4
Golden	25	1	3,45	3	10,34	0	0,00	4	13,79	21	**72,41**	0	0,00	0	0,00	29
Tan	78	8	9,41	5	5,88	1	1,18	5	5,88	59	**69,41**	0	0,00	7	8,24	85
Platinum	35	12	31,58	0	0,00	0	0,00	1	2,63	23	**60,53**	0	0,00	2	5,26	38

In the case of pairings between copper rams and gray ewes of different shades, it can be observed, according to the centralized data in table no. 2, that the lambs with tan shade of color were obtained the most frequently. The highest frequency is recorded in the case of platinum ewes, from which 63.64% tan lambs were obtained, followed by tan females with 54.55% lambs of the same shade. Homogeneous mating resulted, most frequently, in tan lambs, respectively 40.00% followed by copper lambs with 30.00%. Antique lambs were obtained most frequently from the mating of antique rams with silver ewes, respectively 50.00%.

Table 2. Results of mating copper shade rams with ewes of different shades / Rezultatele împerecherii masculilor sur de nuanță arămie cu femele sur de diferite nuanțe

Ewes shade	Ewes	Lambs’ shades of color obtained in the period 2015-2022														Total lambs
		Antique		Copper		Silver		Golden		Tan		Amber		Platinum		
	n	n	%	n	%	n	%	n	%	n	%	n	%	n	%	
Antique	7	1	14,29	1	14,29	1	14,29	1	14,29	3	**42,86**	0	0,00	0	0,00	7
Copper	9	2	20,00	3	**30,00**	0	0,00	1	10,00	4	**40,00**	0	0,00	0	0,00	10
Silver	2	1	**50,00**	0	0,00	0	0,00	0	0,00	1	**50,00**	0	0,00	0	0,00	2
Golden	13	3	21,43	4	28,57	0	0,00	0	0,00	6	**42,86**	0	0,00	1	7,14	14
Tan	11	2	18,18	1	9,09	0	0,00	1	9,09	6	**54,55**	0	0,00	1	9,09	11
Platinum	9	2	18,18	2	18,18	0	0,00	0	0,00	7	**63,64**	0	0,00	0	0,00	11

In the case of pairings between silver rams with gray females of different shades, a wider distribution of frequencies can be observed, thus according to the centralized data in table no. 3 the most significant frequency is recorded in the case of tan lambs obtained from golden ewes, respectively 54.55%, followed by tan lambs obtained from platinum ewes with 40.76%. Fairly significant frequencies are also obtained in the case of other shades, namely golden and antique from copper shade ewes, with 33.33% each and silver shade lambs were obtained from antique shade ewes with a frequency of 27.27 %. In the case of homogenous mating, lambs of antique shade were most frequently obtained, namely 31.25% followed by lambs of platinum shade with 25.00%. As the silver shade is the most valuable at the moment, we can see that silver shade lambs are obtained with a rather encouraging frequency also from ewes of shades: tan (18.81%), silver (18.75%) and platinum (14.81%).

Table 3. Results of mating silver shade rams with ewes of different shades / Rezultatele împerecherii masculilor sur de nuanță argintie cu femele sur de diferite nuanțe

Ewes shade	Ewes	Lambs’ shades of color obtained in the period 2015-2022														Total lambs
		Antique		Copper		Silver		Golden		Tan		Amber		Platinum		
	n	n	%	n	%	n	%	n	%	n	%	n	%	n	%	
Antique	72	17	22,08	2	2,60	21	**27,27**	0	0,00	22	**28,57**	1	1,30	14	18,18	77
Copper	9	3	**33,33**	0	0,00	0	0,00	3	**33,33**	1	11,11	0	0,00	2	22,22	9
Silver	16	5	**31,25**	0	0,00	3	**18,75**	2	12,50	2	12,50	0	0,00	4	25,00	16
Golden	21	5	22,73	0	0,00	0	0,00	1	4,55	12	**54,55**	0	0,00	4	18,18	22
Tan	89	15	14,85	3	2,97	19	18,81	11	10,89	31	**30,69**	4	3,96	18	17,82	101
Platinum	51	9	16,67	0	0,00	8	14,81	3	5,56	22	**40,74**	0	0,00	12	22,22	54

The mating of golden rams with gray females of various shades, as also observed in the centralized data in table no. 4, had various results, the most frequently resulting in tan lambs from copper ewes, respectively 61.54% followed by antique lambs resulting from silver ewes, respectively 60.00%. From the homogenous pairings, between rams and ewes of a golden shade, the most frequent lambs of tan shade resulted in a proportion of 38.03%, followed by lambs with the same shade as the parents, respectively 26.76% golden and lambs of an antique shade with 22.54%. Golden lambs were the most frequently obtained from the mating of golden rams with tan ewes, respectively 43.48%.

Table 4. Results of mating golden shade rams with ewes of different shades / Rezultatele împerecherii masculilor sur de nuanță aurie cu femele sur de diferite nuanțe

Ewes shade	Ewes	Lambs’ shades of color obtained in the period 2015-2022														Total lambs
		Antique		Copper		Silver		Golden		Tan		Amber		Platinum		
	n	n	%	n	%	n	%	n	%	n	%	n	%	n	%	
Antique	10	4	**40,00**	0	0,00	0	0,00	3	30,00	3	30,00	0	0,00	0	0,00	10
Copper	10	1	7,69	0	0,00	0	0,00	4	30,77	8	**61,54**	0	0,00	0	0,00	13
Silver	4	3	**60,00**	0	0,00	0	0,00	1	20,00	0	0,00	1	20,00	0	0,00	5
Golden	63	16	22,54	2	2,82	0	0,00	19	**26,76**	27	**38,03**	1	1,41	6	8,45	71
Tan	22	2	8,70	0	0,00	0	0,00	10	**43,48**	8	34,78	1	4,35	2	8,70	23
Platinum	11	2	15,38	2	15,38	0	0,00	3	**23,08**	3	**23,08**	0	0,00	3	**23,08**	13

In table no. 5, the data related to the mating of tan rams with gray females of different shades are centralized, the most frequently obtaining lambs of antique shade, respectively 75.00% from ewes of antique shade. In the case of homogenous pairings, it is observed that the highest frequency is found in lambs of antique shade, respectively 44.44% followed by lambs of tan shade with 37.04%. Tan lambs were obtained most frequently from the mating of tan rams with copper ewes, respectively 40.00%.

In the case of pairings between platinum rams and gray ewes of different shades, it can be observed, according to the centralized data in table no. 6, that the tan shade lambs are most frequently obtained, respectively 46.61% from tan shade ewes, followed by those obtained from antique shade ewes, respectively 44.12%. In the case of homogenous pairings, it can be seen that lambs of antique shade color were the most frequently obtained, respectively 39.46%, followed by those of tan shade color with 34.69% and only in the third place are lambs of platinum shade color with 14.29%.

Table 5. Results of mating tan shade rams with ewes of different shades / Rezultatele împerecherii masculilor sur de nuanță bronzată cu femele sur de diferite nuanțe

Ewes shade	Ewes	Lambs’ shades of color obtained in the period 2015-2022														Total lambs
		Antique		Copper		Silver		Golden		Tan		Amber		Platinum		
	n	n	%	n	%	n	%	n	%	n	%	n	%	n	%	
Antique	3	3	**75,00**	0	0,00	0	0,00	0	0,00	0	0,00	0	0,00	1	25,00	4
Copper	5	0	0,00	2	**40,00**	1	20,00	0	0,00	2	**40,00**	0	0,00	0	0,00	5
Silver	1	0	0,00	0	0,00	0	0,00	0	0,00	0	0,00	0	0,00	1	100,00	1
Golden	15	4	25,00	4	25,00	1	6,25	1	6,25	5	**31,25**	0	0,00	1	6,25	16
Tan	22	12	**44,44**	1	3,70	0	0,00	2	7,41	10	**37,04**	0	0,00	2	7,41	27
Platinum	9	8	72,73	0	0,00	0	0,00	0	0,00	1	9,09	0	0,00	2	18,18	11

Table 6. Results of mating platinum shade rams with ewes of different shades / Rezultatele împerecherii masculilor sur de nuanță platinie cu femele sur de diferite nuanțe

Ewes shade	Ewes	Lambs’ shades of color obtained in the period 2015-2022														Total lambs
		Antique		Copper		Silver		Golden		Tan		Amber		Platinum		
	n	n	%	n	%	n	%	n	%	n	%	n	%	n	%	
Antique	65	13	19,12	1	1,47	1	1,47	7	10,29	30	**44,12**	3	4,41	13	19,12	68
Copper	23	6	23,08	0	0,00	0	0,00	8	30,77	11	**42,31**	0	0,00	1	3,85	26
Silver	18	7	**38,89**	0	0,00	1	5,56	2	11,11	3	16,67	1	5,56	4	22,22	18
Golden	84	22	25,58	3	3,49	4	4,65	11	12,79	36	**41,86**	1	1,16	9	10,47	86
Tan	110	28	23,73	4	3,39	8	6,78	11	9,32	55	**46,61**	1	0,85	11	9,32	118
Platinum	134	58	**39,46**	3	2,04	2	1,36	12	8,16	51	34,69	0	0,00	21	**14,29**	147

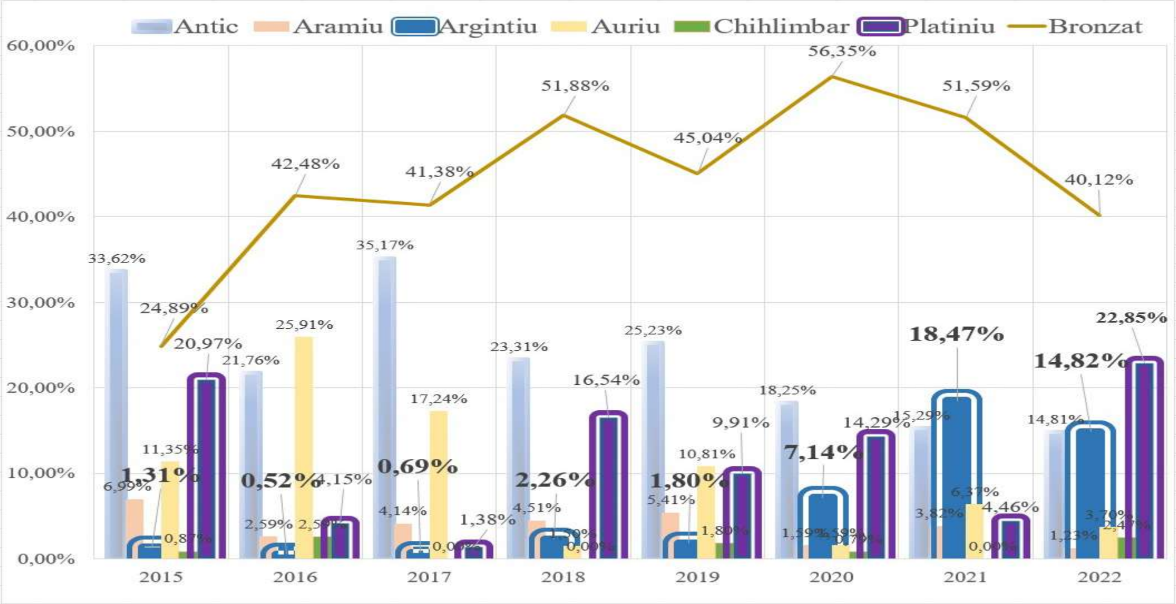


Figure 2. Graphical distribution of lambs according to color shade in each year analyzed / Distribuția grafică a mieilor în funcție de nuanța de culoare în fiecare an analizat

For each year taken into study, the frequency of color shades obtained in the lambs from the Gray Line was evaluated following the pairings made between parents from the same color line. Figure no. 2 shows the percentage values of lambs obtained, in each season, depending on the shade of color. It can be seen that the tan shade registers the highest numbers of lambs during the analyzed period, with a maximum of 56.35% in 2020. Follows by the antique shade lambs, which at the beginning of the period registered the highest values, respectively 33.62% in 2015 and 35.17% in 2017 and in 2022 they reached a minimum of 14.81%. Silver lambs shown the most spectacular growth, from a minimum of 0.52% in 2016 to a maximum of 18.47% in 2021 and 14.82% in 2022, and this fact is due to our desire to multiply individuals in the silver shade considering the special beauty found in this color shade.

CONCLUSIONS

1. Antique lambs are obtained the most frequently, respectively 75%, from mating tan rams with antique ewes, followed by those obtained from mating golden rams with silver ewes, respectively 60%.
2. Copper lambs are obtained the most frequently, respectively 40%, from mating tan rams with copper ewes, followed by those obtained from mating copper rams with copper ewes, respectively 30%.
3. Silver lambs are obtained most frequently, respectively 27.27%, from mating silver rams with antique ewes, followed by those obtained from mating silver rams with tan ewes, respectively 18.81%, and at a very short difference the lambs obtained from mating silver rams with silver ewes, respectively 18.75%.
4. Golden lambs are obtained the most frequently, respectively 43.48%, from mating golden rams with tan ewes, followed by those obtained from mating silver rams with copper ewes, respectively 33.33%.
5. Tan shade lambs are obtained the most frequently, respectively 76%, from mating antique shade rams with copper shade ewes, followed by those obtained from mating antique shade rams with tan shade ewes, respectively 69.41%.
6. Platinum lambs are obtained the most frequently, respectively 25%, from mating silver rams with silver ewes, followed by those obtained from mating golden rams with platinum ewes, respectively 23.08%.
7. Homogeneous mating the most frequent resulted in obtaining tan or antique lambs, respectively 53.16% tan lambs in the case of mating between individuals of the antique shade, 40.00% tan lambs in the case of mating between individuals of the copper shade, 31.25% antique shade lambs in the case of mating between silver shade individuals, 38.03% tan shade lambs in the case of mating between golden shade individuals, 44.44% antique shade lambs in the case of mating between individuals of tan shade and 39.46% antique shade lambs in the case of mating between platinum shade individuals.
8. The high degree of variability in the transmission of color shades within the Gray line shows us the fact that the genetic diversity has a fairly high coefficient, this is also due to the fact that there are quite a few color shades and thus explains why the frequencies of obtaining lambs of the same shade as the parents usually ranked second or third.
9. We believe that through the work of selection and breeding, the frequencies of lambs with the same shade in the case of homogenous pairings can be considerably improved with positive effects on the reproduction capacity of individuals within families of the same shades of color.
10. The tan shade is a reservoir of genetic resource, currently having the largest population within the Gray line.

ACKNOWLEDGMENTS

Part of the costs related to the research activities were provided by MADR through the financing of the ADER 5.1.4 project. (Contributions to the genetic consolidation and numerical increase of the **Karakul of Botoșani** sheep of the Gray variety with the aim to homologate them).

BIBLIOGRAPHICAL REFERENCES

1. Nechifor I., Pascal C., Florea M., Albată A., Crâșmaru A., Darie V., Onaciu P.; Cercetări privind transmiterea genetică a varietății de culoare sură în cadrul rasei **Karakul de Botoșani**; 2018; Editura Ceres, București, România.
2. Nechifor I., Pascal C., Florea Al. M., Albaţă A., Crâşmaru A., Darie V., Onciu O.; Research regarding quality of pelts obtained by cross-breading between genitors belonging to **Karakul of Botoşani** breed, grey variety; 2019; Revista de Zootehnie, Anul XVI, nr. 1-2/Ianuarie – Iunie 2019, Iaşi, România.
3. Nechifor I., Florea Al. M., Crîșmaru A., Pascal C.; Research on current evaluation stage of curl type improvement for **Karakul of Botoșani**; 2022; *Sci. Pap. Ser. D-Anim. Sci*. Vol. LXV, No. 2. p 291-297.
4. Pascal C., Nechifor I., Florea A.M., Crâșmaru A.; Genetic analysis and hereditary transmission of color at **Karakul of Botosani** sheep; 2022; *Anim.Food Scie. J. Iasi*, vol 77 (2), p. 34-43.
5. Florea Al. M., Nechifor I., Crâșmaru A., Pascal C.; Research regarding the youth female **Karakul of Botosani** reproductive function; 2020; Lucrări Științifice-Seria Zootehnie, vol. 74. p. 182-185.
6. Nechifor I., Florea M. Al., Pascal C.; Assessing the current state of curl type improvement for **Karakul of Botoşani** breed in relation to the color variety; 2019; Vol 76, No 2, p 87-93.

ACTA AGRICOLA ROMANICA, Volume 6, Year 6, No. 6.3.

RESEARCH REGARDING USING CICHORIUM INTYBUS IN ORDER TO IMPROVE THE PALATABILITY OF CATTLE FEEDSTUFF

CERCETĂRI PRIVIND UTILIZAREA CICORII FURAJERE ÎN VEDEREA ÎMBUNĂTĂȚIRII PALATABILITĂȚII FURAJELOR DESTINATE BOVINELOR

NEAMT RADU[1], NECIU FLORIN[1], SĂPLĂCAN GHEORGHE[1], MIHALI CIPRIAN[1], CZISZTER LUDOVIC[1,2], SĂPLĂCAN SILVIU[3], MIZERANSCHI ALEXANDRU[1]

[1]Research and Development Station for Bovine - Arad, Calea Bodrogului 32, CP 310059, phone: 0257/339130, fax. 0257/339131, e-mail: scdcbarad@gmail.com, Romania
[2]The Banat University of Agricultural Sciences and Veterinary Medicine "King Mihai I of Romania", Timisoara, in Timisoara, Faculty of Bioengineering of Animal Resources, Calea Aradului 119, CP 300645, phone: 0256/277099, fax. 0256/200296, e-mail: rectorat@usvt.ro, Romania
[3]University "Aurel Vlaicu" Arad, Faculty of Economics, Bulevardul Revoluției 77, CP 310130, phone: 0257/280679, fax. 0257/280679, e mail: stiinte.economice@uav.ro, Romania

Correspondence address: neamtr@yahoo.com

Abstract

The aim of the current study was to investigate the opportunity of including chicory in cows' diets based on its palatability compared to other usual feeds. A total of 1500 data were recorded from 150 ***Romanian Spotted*** *cows related to 6 forage structures: alfalfa (αα), chicory (C), mixed alfalfa and chicory (αC), mixed gramineous (G), mixed gramineous and alfalfa (Gα), mixed gramineous and chicory (GC). Data aimed at the total amount of forage consumption (TFC), according to forage structures and day of feeding. The palatability was assessed based on one-way analysis of variance protocol, with the categorical factor "feedstuff". The Chi-square test of independence was performed to determine if the botanical structure had an influence on palatability. No significant differences (p>0.05) were recorded related to αα (90.14%), C (87.56%), or αC (89.26%). The lowest palatability was recorded for G (71.35%) compared to αα, C, and αC (p≤0.001) or Gα and GC (p≤0.01). Current results suggest that the participation of C in botanical mixtures could improve the feedstuff palatability, with economic benefits.*

Keywords: cattle feeding, chicory, feeding efficiency, palatability, Romanian Spotted breed

Rezumat

Scopul studiului a fost de a investiga oportunitatea includerii cicorii furajere în alimentația vacilor de lapte, pe baza evaluării palatabilității acesteia în comparație cu alte sorturi furajere uzuale. Au fost înregistrate un total de 1500 de date de la 150 de vaci ***Bălțată Românească****, furajate alternativ cu 6 structuri furajere: lucernă (αα), cicoare (C), amestec de lucernă și cicoare (αC), amestec de graminee (G), amestec de graminee și lucernă (Gα), amestec graminee și cicoare (GC). Datele colectate și prelucrate au vizat cantitatea totală de furaje consumată (CTC), în raport cu structurile furajere administrate. Palatabilitatea a fost evaluată pe baza protocolului de analiză unidirecțională a varianței, cu factorul categoric „furaj". Testul Chi-pătrat al independenței a fost efectuat pentru a determina dacă structura furajeră a avut o influență asupra palatabilității. Nu au fost înregistrate diferențe semnificative (p>0,05) legate de αα (90,14%), C (87,56%) sau αC (89,26%). Cea mai scăzută palatabilitate a fost înregistrată pentru G (71,35%) în comparație cu αα, C și αC (p≤0,001) sau Gα și GC (p≤0,01). Rezultatele actuale sugerează că participarea C în amestecurile botanice ar putea îmbunătăți palatabilitatea furajelor, cu beneficii economice evidente.*

Cuvinte cheie: Bălțată Românească, cicoare furajeră, eficiența în furajare, furajarea bovinelor, palatabilitate

INTRODUCTION

Fodder chicory has been known since the Bronze Age. It presents great ecological plasticity, which is the reason it is found worldwide despite its Mediterranean origin. It has been used as a forage plant since 1915 [Leonard and Ebenezer, 2013]. Lancashire has classified it as an excellent ruminant feed since 1978 [Lancashire, 1978]. The Puna variety received the first approval for commercialization in 1985. Fodder chicory has grown significantly in popularity over the past ten years and now is utilized more frequently in animal feeding [Sanderson et al., 2003]. The characteristics of feed that induce an animal's sensory system to response are termed palatability. On a national level, there are no comparative studies regarding the palatability of chicory. At the international level, several studies were performed in this respect, the results being contradictory. In this sense, the studies performed by Neciu (2018) highlighted a higher palatability for chicory compared to alfalfa. As a result of chicory having less lignin than alfalfa, Terrill et al. (1992) also found greater palatability as well as greater digestibility values for chicory. According to many researchers, the chicory is considered a high-quality feedstuff, well-consumed by ruminants [Hopkins et al., 1995; Nwafor et al., 2017]. However, the taste, fragrance, and implicitly the palatability of chicory could change if lactones are present in high concentrations (0.8-1.5%) [Foster et al., 2006]. When administering a single feed variety, voluntary consumption or animal feeding behaviour are typically used to explain palatability [Baumont, 1996]. The use of some preference tests that reveal the animal's interest in a particular type of fodder is required in the case of fodder mixtures. In this sense, we can consider a previously acquired experience aimed at the taste, smell, or feed structure based on the animal's sensory analysis. The preferability is the animal's sensorial response, either with an acute character or determined by previous cognitive processes. As a result of their length and feed availability, the studies aimed at preferability provide inconsistent and occasionally incorrect results. Therefore, providing animals with a single variety of fodder, over a short period of time, will motivate them to consume all of it, in order to ensure their satiety state. Voluntary consumption will decrease if this period is extended, which serves as a warning that the fodder does not meet all quality requirements. Regardless of the length of the period, the animals get the opportunity to choose their preferred fodder when a mixed diet is provided. Generally, the preferability is assessed either on the voluntary consumption [Faverdin 1985] or feeding behaviour [Parsons et al., 1994]. The comparative voluntary consumption test related to six forage structures, was performed in the current research. More studies are required in order to determine the feasibility of using chicory on a large scale in cattle feeding, taking into accont that it is a plant with tremendous agro-technical potential in our country's increasingly prolonged drought circumstances. Also, there is a significant lack regarding the knowledge related to the palatability of chicory compared to other usually fodder species.

MATERIALS AND METHOD

All tests related to the current research were observational and non-invasive. Ethical issues of this study were evaluated according to the European Union's Directive for animal experimentation (Directive 2010/63/EU). The study was approved by the Scientific Council at the Research and Development Station for Bovine Arad through the Decision no. 51 issued on November 11, 2015.

Location: The study was performed at the Research and Development Station for Bovine Arad, Romania (location: 46° 10' 36" N, 21° 18' 4" E, 107 m altitude. The data recording was performed in September 2022. Cows included in the study were managed under a loose system with zero grazing and were between 1th and 5th lactation. Cows received daily feed rations made of 70 kg green fodder (one of the studied forage structures), 5 kg of alfalfa hay and 4 kg of concentrates. Cows were fed 3 times per day in order to benefit from fresh green fodder all day long and to stimulate the intake behaviour. A data set related to 6 forage structures (the forage structures were: alfalfa (aa), chicory (c), mixt alfalfa and chicory (ac), mixed gramineous (G), mixed gramineous and alfalfa (Ga), mixed gramineous and chicory (GC)) administrated continuously to 150 cows (25 cows /1 forage structure) for a period of 10 days was analysed in order to assess palatability in **Romanian Spotted breed**. The experimental period consisted of a 7-day diet adaptation phase, followed by a 10-day assess phase during which voluntary intake dynamics were recorded. The effects of forage structure were assessed using a one-way ANOVA protocol. Differences between means were tested with the posthoc qi square test and were defined as being statistically significant at $p<0.05$. The analysed data were expressed as least square means and standard errors of the mean. All the statistical inferences were carried out using the software package Statistica (StatSoft Inc., Tulsa, OK USA) [Hill and Lewicki, 2007].

The main objective of the present research was to establish the palatability of chicory compared to other forage structures used in cattle feeding, based on its voluntary consumption by cattle.

RESULTS AND DISCUSSION

In order to establish the cows' preferences, different forage structures were tested. The primary requirement for achieving larger yields and, implicitly, improved efficiency of farms is a high fodder consumption rate [DeVries and von Keyserlingk, 2005]. Based on this study (Table 1), no significant differences were assessed regarding the palatability associated with αα and C (90.14 vs. 87.56%, $p>0.05$) related to Spadona variety. The αC mixture increased the palatability without inducing significant differences from the two species' pure cultures. The lowest palatability was associated with G (71.35%), the cows being reluctant to this forage structure compared to αα or C ($p\leq0.001$). The introduction of αα or C into the G mixture increased the palatability to the threshold of 76% and 76.6% respectively. These results are in accordance to those obtained by Niderkorn et al., (2019) for a mixture of chicory and gramineous. However, the differences related to αα and C remains significant ($p\leq0.01$). Moreover, the increased palatability due to the presence of chicory in the forage structure led to an increase in individually performance, according to Totty et al., (2013) and Roca et al., (2016). More often, the farmers agree that a forage would have to be nutritionally superior in order to be preferred by the animals. Even animals have preferences. When the animals have access to various forage structures, it is easiest to observe these. In this situation, the preferred forage will be decided by the animals. Hunt and Hay (1990) investigating the preferences of different species for various forages, observed that cattle prefer gramineous rather than legumes when grazing. The findings are in contradiction to ours, which revealed that cows prefer C and αα, rather than G. An average of 90.14% was found based on the palatability assesses, with extreme thresholds of 85.71% and 94.28%, respectively. The dynamics of αα palatability exhibited an increasing trend during the trial. Being a forage that has well-known high palatability, it achieves high values in this respect from the first two days of administration (between 88.57 and 88.73%), which translates to 93.94-94.11% of the peak palatability of 94.28% attained from the third day of the current study. In general, the values associated with palatability remain high, over 90%. These values proved to be higher than those reported by Atanas et al., (2016), who determined palatability of 73.5. According to Madruga et al., (2019), the addition of αα in G increased the palatability by 13%, also increasing growth rate by 12% in beef Simmental heifers.

Table 1. The daily and periodically average palatability and multiple comparison test results according to the forage structure / Palatabilitatea medie zilnică și periodică și rezultatele testelor de comparație multiplă în raport cu structura furajului

Parameters	Forage structure					
	αα	***C***	***αC***	***G***	***Gα***	***GC***
Day 1	88.57^{a}	77.14^{a}	88.57^{a}	72.85^{a}	81.42^{a}	81.42^{a}
Day 2	88.73^{a}	85.71^{b}	88.57^{a}	68.57^{a}	71.42^{b}	75.71^{b}
Day 3	94.28^{b}	90^{c}	90^{a}	70^{a}	70^{b}	84.28^{a}
Day 4	87.14^{a}	82.85^{b}	84.28^{a}	72.85^{a}	81.42^{a}	84.28^{a}
Day 5	85.71^{a}	82.85^{b}	87.14^{a}	68.57^{a}	78.57^{a}	84.28^{a}
Day 6	94.28^{b}	88.57^{b}	91.42^{a}	68.57^{a}	71.42^{b}	85.71^{a}
Day 7	90^{a}	90^{c}	91.42^{a}	72.85^{a}	72.85^{b}	85.71^{a}
Day 8	90^{a}	94.28^{c}	97.14^{c}	82.85^{b}	87.14^{c}	85.71^{a}
Day 9	94.28^{b}	92.85^{c}	92.85^{a}	74.28^{a}	75.71^{b}	87.14^{c}
Day 10	90^{a}	91.42^{c}	94.28^{b}	77.14^{b}	81.42^{a}	88.57^{c}
Average palatability	*90.14^{a}*	*87.56^{a}*	*89.26^{a}*	*71.35^{b}*	*76^{c}*	*76.7^{c}*

Different superscript in the same row significantly differ at $P \leq 0.05$

Related to chicory palatability, the current study found an average value of 87.56% with extreme thresholds of 77.14% and 94.28%, respectively. This average value related to Spadona variety is lower compared to Puna variety (92.4%), studied by Neciu (2018). The dynamic of C palatability, during the study interval, recorded an upward trend. The lowest value of palatability was recorded in the first day of the study, the highest value being reached in the 8th day. No decrease tendency was recorded related to C palatability during the studied period. These findings highlighted that cows pleasantly consume the C as green forage, despite the lack of previous experience with it. Daily feeding of C allows the cows to become accustomed and to express the preference related to this forage. These results are in accordance with the previous findings reported by Minneé et al., (2017), which recorded an increase in C palatability during a short period of time. Also, a positive effect on milk yield, average daily gain and a decrease in methane and ammonia in urine were observed.

The positive effects on milk yield and average daily gain were also confirmed in a previous study performed by Clark et al., (1990), Woodward et al., (2013) and Muir et al., (2014) for pure chicory. Including the C in cows feeding induced behavioural changes due to its morphological and chemical features according to Drescher et al. (2006). High leaves and low fibres content allow cows to take a big bite, increasing the number and time spent for mastication, according to Stewart (1996). Prolonging of mastication time correlates with a lower fibers content allow a rapid disintegration of chicory in rumen according to Hoskin et al., (1995), leading to a reduce time spent for rumination and increase time of idling [Gregorini et al., 2013]. Thus, a higher palatability combines with a higher digestibility rates and fewer rumination boluses led to a decreased amount of methane and ammonia.

The αC mixture recorded an average palatability of 89.26%, generated by the minimum threshold of 84.28% recorded in the 4th day and a maximum of 94.28% in the last day of the study period. The lag time until palatability approached maximum value is considered to be 2-5 days for an unfamiliar specie, according to Scharenberg et al., (2008).

The mixture palatability recorded an upward trend characterized by a slower pace compared with that associated with αα or C in pure culture. The synergistic effect of these two species proves to be present, the palatability of mixture being higher compared to C. Despite of this, the difference was not significant ($p>0.05$). The current situation was also found by Lombardi et al. (2015) which recorded an increased palatability for a mixture of chicory and legumes. Also, analysing the cows preferences related to these botanical species, the study recorded that cows spent 71% of their feeding time consuming legumes and just 23% consuming chicory [Lombardi et al., 2015].

The main goal of farmers is to increase production yields. This goal could be attain through increasing voluntary feed intake. In this respect, the biological value, the palatability and the dry matter yield become essential influencing factors in order to meet the cows' nutritional requirements. Mixed chicory with alfalfa or other legumes could lead to increases dry matter production, according to Guangdi et al., (2012). Similar findings were obtained also by Kunelis et al., (1999) which recorded an increased dry matter production in chicory – legumes mixtures compared to pure chicory. The presence of legumes species could fill the fed gapes in cold season when chicory is less productive. Also, the legumes provide N requested by chicory, through N fixation processes [Matthew et al., 2010].

Related to G palatability, the average value was 71.35%, significant less compared to αα, C or αC ($p \leq 0{,}001$). The dynamic of G palatability recorded a sinusoidal trend, which highlighted a reduced preference of cows for G. Voluntary intake seems to be influenced directly by the state of satiety and cows individuality. The current findings are consistent with those obtained by Bargo et al., (2002), who found that G palatability decreased over time even while its allowance met the nutritional requirements of cows. Previous studies that investigated the dynamic of G palatability observed an upward curvilinear trend up to a point but also a plateau phase. The moment of reaching this phase is highly variable between authors and depends on the herbage allowance. Thus, related to herbage allowance, the plateau phase occurs at an allowance of 33 kg DM/cow (Peyraud et al., (1996), 55 kg DM/cow Dalley et al., (1999) or 60 to 72 kg DM/cow according to Hodgson and Brookes (1999). The current results are contradictory, with no plateau phase being recorded in our study in terms of G consumption.

The minimal threshold of G palatability recorded in this study was 68.57%, associated to 30% of the period length, while the maximal one was 82.85% being observed just in the 8th day of the study. High palatability values were invariably followed by subsequent prolonged declines over 1-3 consecutive days. A possible explanation could be the unfamiliar character of the grasses, under the conditions of an exclusive αα feeding (fresh green fodder, hay, or alfalfa silage). The chemical and morphological structure of G could be another cause of reduced palatability compared to αα or C. The high fibre content of grasses reaches the 30% threshold at the beginning of flowering. Parga et al., (2002) recorded significant differences related to G palatability, the highest values being associated to young plants with lower lignin content. Comparing to C, the G highlighted a reduced palatability even in the case of C plants in the same phenotypic phase and of the same age. Moreover, the decreasing tendency of biological value represented by protein content proved to be accelerated in G compared to αα (0.5%/day vs. 0.3%/day) [Koldmae et al., 1999]. However, the G forages constitute the most prevalent botanical species used in cattle feeding due to large production, ecological plasticity, and the suitability of the forms of mixtures with other species. In this respect, the possibilities of increasing the G palatability have been extensively studied. The reduced G palatability may also be due to the digestive particularities of cattle. The palatability seems to be strongly dependent and could be increased by increasing the rumen rate of clearance of material which is highly correlated with the rate of degradation and passage rate of material. In this sense, these features are characteristic of some species with high sugar and low fibre content. A widely accepted method to make G species increasingly palatable is to breed them for sugar content [Lee et al., 2002; Taweel et al., 2005]. Additionally, research focused on developing particular agro - technical ways to improve the G palatability in order to maximize benefits from its high production of it. Improving the G qualities features could be achieved by applying N fertilization. The cows feeding with fertilized G showed desired behavioural changes such as increased bites rate and time spent feeding [Chiy and Phillips, 1999]. Cows' milk production, protein, fat, and lactose content improved as a result of boosting the G palatability [Chiy et al., 1993].

Including αα in the G mixture, increased palatability to an average of 76%, significantly higher ($p \leq 0.01$) compared to G. Comparing to αα, C or αC, the αG palatability proved to be significantly lower ($p \leq 0.001$). The dynamic of αG palatability highlighted a sinusoidal tendency in the experimental period. Sporadic growth of palatability proved to be followed by the prolonged decreasing intervals of it. High thresholds have been repeatedly attained on the day 1st, 4th, 8th, and 10th followed by a significant decrease of 8-12% during the next 2-3 days. The inclusion of C in the G mixture does not generate significant differences compared to αG, but neither does it reach the palatability associated with C or αα. The average palatability threshold was 76.7%. Compared to the αG mixture, the CG palatability recorded an upward trend even if the maximum threshold was reached on the 10th day of the study period. The cows' preference for CG mixture is evident and emerges from a prolonged plateau phase (between day 3rd and 8th) that continues with an upward phase associated with the end of the period. No palatability impairments were recorded during the study. Increasing the CG mixture palatability is dependent on several external influential factors.

The addition of C or αα to the G mixture could improve the botanical structure's ability to endure in culture and, in turn, increase the amount of dry matter yield over time. The αG or CG reported higher dry matter yield values from an economic standpoint. In addition, compared to pure culture, weather-related risks are decreased by up to 24%. Obtaining high yields results in an increased dry matter yield, which can improve the palatability of a feed due to availability of it [Sanderson et al., 2006]. Increasing the CG or αG palatability could also be done through increasing the nutritional value of the forage. Compared to pure G nutritional value, the CG mixture presented a greater accumulation of micro and macro elements with an essential role in increasing the nutritional value of the forage and implicitly in increasing of its palatability, according to Beleski et al., (1999). The presence in the G mixture of C or αα allows large accumulations of N in the soil, which allows high productions and a greater persistence of the culture over time, which leads to an increased availability of dry matter. With the inclusion of αα or C in the G mixture, the forage's palatability increased, which also resulted in an improvement in the rumen's ability to absorb nutrients. According to Garret K et al., (2021), the inclusion of αα or C in the G mixture led to an increase in gas and a reduction in NH_3 in the rumen, thus indicating greater fermentation and greater microbial protein synthesis providing nutrient supply to the cattle.

CONCLUSIONS

1. The dynamics of the forages palatability highlighted the increased interest of cows for pure αα, C or αC mixtures, which supports its implementation and unrestrained large-scale use in cows feeding;
2. Including C alongside G increased the palatability of its mixtures, thus making feeding more efficient.

ACKNOWLEDGEMENTS

This work was supported by a grant of the Romanian Ministry of Agriculture and Rural Development, throughout the project ADER 9.1.3. / 30.09.2019 project.

BIBLIOGRAPHICAL REFERENCES

1. Leonard C., Ebenezer T., URL:https://nzhistory.govt.nz/media/photo/leonard-cockayne-and ebenezer-teichelmann; 2013; (Ministry for Culture and Heritage), updated 15-Jul-2013.
2. Lancashire J.A.; Improved species and seasonal pasture production; 1978; Proceedings of Agronomy Society of New Zealand, 8, pp. 123 – 127.
3. Sanderson M.A., Labreveux M., Hall M.H., Elwinger G.F.; Nutritive value of chicory and english plantain forage; 2003; Crop Sci., 43, pp. 1797-1804.
4. Neciu F.C.; Cercetări privind agrobiologia și utilizarea cicorii furajere (*Cichorium intibus L.*); 2018; Universitatea de Științe Agricole și Medicină Veterinară a Banatului „Regele Mihai I al României" din Timișoara.
5. Terrill T.H., Rowan A.M., Douglas G.B., Barry T.N.; Determination of extractable and bound condensed tannin concentrations in forage plants, protein concentrate meals and cereal grains; 1992; Journal of Science Food and Agriculture, 58, pp. 321-329.
6. Hopkins D.L., Holst P.J., Hall D.G., Atkinson W.R.; Carcass and meat quality of second-cross crytorchid lambs grazed on chicory (*Cichorium intybus*) or lucerne (*Medicago sativa*); 1995; Australian Journal of Experimental Agriculture, 35, pp. 693 – 697.
7. Nwafor I.C., Karabo S.K., Chilaka M.A.; Chemical Composition and Nutritive Benefits of Chicory (*Cichorium intybus L.*) as an Ideal Complementary and/or Alternative Livestock Feed Supplement; 2017; The Scientific World Journal, Article ID 7343928, pp. 11.
8. Foster J.G., Clapham W.M., Belesky D.P., Labreveux M., Hall M.H., Sanderson M.A.; Influence of cultivation site an sesquiterpene lactone composition of forage chicory (*Cichorium intybus L.*); 2006; J. Agric. Food Chem, 54, pp. 1772 – 1778.

9. Baumont R., Palatability and feeding behaviour in ruminants. A review.; 1996; Annales de zootechnie, 45(5), pp. 385-400.
10. Faverdin P.; Regulation de l’ingestion des vaches laitibres en debut de lactation; 1985; These de doctorat, Ina-PG, France, pp. 131.
11. Parsons A.J., Newman J.A., Penning P.D., Harvey A., Orr R.J.; Diet preference of sheep: effect of recent diet, physiological state and species abundance; 1994; J. Anim. Ecol., 63, pp. 465-478.
12. Hill T., Lewicki P.; Statistics: Methods and Applications; 2007; Stat Soft, Tulsa, OK, USA.
13. DeVries T.J., M.A.G. von Keyserlingk; Time of feed delivery affects the feeding and lying patterns of dairy cows; 2005; J. Dairy Sci, 88, pp. 625-631.
14. Niderkorn V., Martin C., Bernard M., Le A., Morvan, Rochette Y., Baumont R.; Effect of increasing the proportion of chicory in forage-based diets on intake and digestion by sheep; 2019; Animal, 13(4), pp. 718-726.
15. Totty V., Greenwood S., Bryant R., Edwards G.; Nitrogen partitioning and milk production of dairy cows grazing simple and diverse pastures; 2013; Journal of Dairy Science, 96, pp. 141–149.
16. Roca-Fernández A., Peyraud J-L., Delaby L., Delagarde R.; Pasture intake and milk production of dairy cows rotationally grazing on multi-species swards; 2016; Animal, 10, pp. 1448–1456.
17. Hunt W.F., Hay J.M.; A photographic technique for evaluating the pasture species performance of grazing animals; 1990; Journal of New Zealand Grasslands, 51, pp. 191–195.
18. Atanas K., Ina S., Viliana V.; Palatability of Annual and Perennial Legumes; 2016; Nutri Food Sci Int J., 1(5), pp. 555-573.
19. Madruga A., Abril R.S., González L.A., Manteca X., Panella-Riera N., Gil M., Ferret A.; Using 19% of alfalfa hay in beef feedlot finishing diets did not modify meat quality but increased feed intake and ADG; 2019; J Anim Sci., 97(5), pp. 2076-2086.
20. Minneé E.M.K., Waghorn G.C., Lee J.M., Clark C.E.F; Including chicory or plantain in a perennial ryegrass/white clover-based diet of dairy cattle in late lactation: Feed intake, milk production and rumen digestion; 2017; Animal Feed Science and Technology, 227, pp. 52-61.
21. Clark D., Anderson C., Hongwen G.; Liveweight gain and intake of Friesian bulls grazing ‘Grasslands Puna’ chicory (*Cichorium intybus L.*) or pasture; 1990; New Zeal J Agric Res., 33, pp. 219–24.
22. Woodward S.L., Waugh C.D., Roach C.G., Fynn D., Phillips J.; Are diverse species mixtures better pastures for dairy farming?; 2013; Proc New Zeal Grassl Assoc., 75, pp. 79–84.
23. Muir S., Ward G., Jacobs J.; Milk production and composition of mid-lactation cows consuming perennial ryegrass-and chicory-based diets; 2014; J. Dairy Sci., 97, pp. 1005–15.
24. Drescher M., I. Heitkönig, J.G. Raats, H.H. Prins; The role of grass stems as structural foraging deterrents and their effects on the foraging behaviour of cattle; 2006; Appl. Anim. Behav. Sci., 101, pp. 10-26.
25. Stewart A.; Plantain (*Plantago lanceolata*)—A potential pasture species; 1996; Proc. N.Z. Grassl. Assoc., 58, pp. 77-86.
26. Hoskin S., Stafford K., Barry T.; Digestion, rumen fermentation and chewing behaviour of red deer fed fresh chicory and perennial ryegrass; 1995; The Journal of Agricultural Science, 124(2), pp. 289-295.
27. Gregorini P., Minnee E.M.K., Griffiths W., Lee J.M.; Dairy cows increase ingestive mastication and reduce ruminative chewing when grazing chicory and plantain; 2013; J. Dairy Sci., 96, pp. 7798-7805.
28. Scharenberg Anna, Arrigo Y., Gutzwiller A., Soliva C.R., Wyss U., Kreuzer M., Dohme-Meier F.; Palatability in sheep and *invitro* nutritional value of dried and ensiled sainfoin (*Onobrychis viciifolia*) birdsfoot trefoil (*Lotus corniculatus*), and chicory (*Cichorium intybus*); 2008; Archives of Animal Nutrition, 61(6), pp. 481-96.
29. Lombardi D., Vasseur E., Berthiaum R., DeVries T.J., Bergeron R.; Feeding preferences and voluntary feed intake of dairy cows: Effect of conservation and harvest time of birdsfoot trefoil and chicory; 2015; Journal of Dairy Science, 98(10), pp. 7238-7247.
30. Guangdi Li, Hayes R.C., Matthew Gardner, Jeff McCormick, Matthew Newell, Graeme Sandral, Richard Lowrie, Hao Zhang; Companion legume species maximise productivity of chicory (*Cichoruim intybus*). "Capturing Opportunities and Overcoming Obstacles in Australian Agronomy"; 2012; Edited by I. Yunusa. Proceedings of 16th Australian Agronomy Conference 2012, Armidale, NSW.
31. Kunelius H.T., McRae K.B.; Forage chicory persists in combination with cool season grasses and legumes; 1999; Can. J. Plant Sci., 79(2), pp. 197-200.
32. Matthew G., Jason Condon, Brian Dear, Mark Conyers, Guangdi Li; Chicory increases the level of N fixation by companion legumes; 2010; Food Security from Sustainable Agriculture, Proceedings of the 15th Australian Agronomy Conference, Lincoln, New Zealand 15-18 November 2010.
33. Bargo F., Muller L.D., Delahoy J.E.; Milk response to concentrate supplementation of high producing dairy cows grazing at two pasture allowances; 2002; Journal of Dairy Science, 85(7), pp. 1777-1792.
34. Peyraud J.L., Comeron E.A., Wade M.H.; The effect of daily herbage allowance, herbage mass and animal factors upon herbage intake by grazing dairy cows; 1996; Annales de Zootechnie, 45(3), pp. 201-217.

35. Dalley D.E., Roche J.R., Grainger C.; Dry matter intake, nutrient selection and milk production of dairy cows grazing rainfed perennial pastures at different herbage allowances in spring; 1999; Australian Journal of Experimental Agriculture, 39(8), pp. 923-931.
36. Hodgson J., Brooks I.M.; Nutrition of grazing animals; 1999; In: White, J. and Hodgson, J. eds. New Zealand pasture and crop science, Oxford University Press, Auckland.
37. Parga J., Peyraud J.L., Delagarde R.; Age of regrowth affects grass intake and ruminal fermentations in grazing dairy cows. In: Durand, J.L., Emile, J.C., Huyghe, C., et al. eds. Multifunction grasslands: quality forages, animal products and landscapes: proceedings of the 19th general meeting of the European Grassland Federation, La Rochelle, France; 27-30 May 2002; European Grassland Federation., Versailles, 256-257. Grassland Science in Europe no. 7.
38. Koldmae H, Kirsel R, Toelp J., Vadi M.; On the crude fibre content of grass feeds; 1999; Proceedings of the Institute of Animal Husbandry of the Estonian Agricultural University, 68, pp. 25-32.
39. Lee M.R.F., Harris L.J., Moorby J.M.; Rumen metabolism and nitrogen flow to the small intestine in steers offered *Lolium perenne* containing different levels of water-soluble carbohydrate; 2002; Animal Science, 74(3), pp. 587-596.
40. Taweel H.Z., Tas B.M., Smit H.J.; Improving the quality of perennial ryegrass (*Lolium perenne L.*) for dairy cows by selecting for fast clearing and/or degradable neutral detergent fiber; 2005; Livestock Production Science, 96 (2/3), pp. 239-248.
41. Chiy P., Phillips C.; The rate of intake of sweet, salty and bitter concentrates by dairy cows; 1999; Animal Science, 68(4), 731-740.
42. Chiy P.C., Phillips C.J.C., Bello M.R.; Sodium fertilizer application to pasture. II. Effect on dairy cow production and behaviour, Grass and Forage Science; 1993; 48, pp. 203–212.
43. Sanderson M.A., Corson M.S., Rotz C.A., Soder K.J.; Economic analysis of forage mixture productivity in pastures grazed by dairy cattle; 2006; Online. Forage and Grazinglands, doi: 10.1094/FG-2006-0929-01-RS.
44. Belesky D.P., Turner K.E., Fedders JM., Ruckle J.M.; Productivity, botanical composition, and nutritive value of forage chicory; 1999; Agron. J., 91, pp. 450-456.
45. Garrett K., Beck M.R., Marshall C.J., Logan C.M., Maxwell T.M.R., Greer A.W., Gregorini P.; Effects of incorporating plantain, chicory, and alfalfa into a ryegrass-based diet on in vitro gas production and fermentation characteristics; 2021; Applied Animal Science, 37(4), pp. 367-376.

RESEARCHES REGARDING THE OPTIMIZATION OF THE FODDERING TECHNOLOGIES FOR THE VARIOUS CATEGORIES OF SHEEP DEPENDING ON THE DIRECTION OF PRODUCTION

CERCETĂRI PRIVIND OPTIMIZAREA TEHNOLOGIILOR DE FURAJARE A DIFERITELOR CATEGORII DE OVINE ÎN FUNCŢIE DE DIRECŢIA DE PRODUCŢIE

ALINA NARCISA NICOLESCU[1], RĂDUCU RADU[1], NICOLAE CUTOVA[1], ZOIA CAMELIA ZAMFIR[1], ANA ENCIU[1], MARIA STANCIU[1]

[1] Institute of Research and Development for Sheep and Goats Breeding Palas,
Str. I.C. Brătianu no. 248, Telephone: 0241/639506,
Email: icdcocpalas@yahoo.com

Correspondence address: icdcoc.palas@asas.ro

Abstract

The research work aims to elaborate certain new profitable technologies, involving in the technological flux the mechanization of the food processing and supplying with the processed fodders, which assure their complete valuation comparatively to supply with unprocessed fodders (as they were obtained at gathering). The voluntary consumptions of various categories of fodders and the fodder losses were determinate. In the case of the fodders used as unique mixture the losses are of 2,33% till 4,53%. If unprocessed fodder were supplied, the losses were: at hay of alfalfa 15%, at hay of vetch 20,2%, at straws 47,5%, at corn cobs 4,7%. The average total milk production of sheep was determined through the bimonthly control (Nica-Dermengi method). The average production of milked milk, duration of lactation and the chemical composition of milk (fat %, protein %) were also determined. At the ewes of ***Palas Merino breed*** *from the experimental lot the total milk production was of 119,51±2,1 litres, the average production of milked milk was of 48,26±1,15 litres, in a lactation of 111,2 days; at the sheep from the control lot the total milk production was of 75,11±3,2 litres, the average production of milked milk of 23,05±1,01 litres, in a lactation of 92,4 days. At the ewes from* ***Palas Prolific Breed****, at the experimental lot the total milk production was of 151,25±2,1 litres, the average production of milked milk was of 82,75±1,63 litres, in a lactation of 131,8 days; at the control lot, the total milk production was of 101,25±2,1 litre, the average production of milked milk was of 42,75±1,63 litres, in a lactation of 156,3 days. At the sheep from Milk Breed–Palas, at the experimental lot, the total production of 211,3±7,81 litre, the average production of milked milk was of 120,6±2,8 litre, in a lactation of 205,6 days; at the control lot the total production 197,6 litres, the average production of milked milk was of 105,8±3,9 litres, in a lactation of 186,3 days. Regarding the chemical composition of milk, higher values were determined at the experimental lots, comparatively with those from the control lots both at fat and protein; at the sheep of* ***Palas Merino Breed*** *from the experimental lot, fat of 6,96%, protein of 6,47% were registered; at control lot fat of 6,90%, protein of 6,61% were recorded; at the sheep from* ***Palas Prolific Breed****, at the experimental lot, fat and protein were 5,59%, and respectively 5,40%; at the control lot fat were of 5,56%, and protein of 5,37%; at the sheep from* ***Palas Milk Breed****, at the experimental lot fat of 6,41%, and protein of 6,32% were registered and at the control lot fat of 6,46%, protein of 6,27% were measured.*

Key words: unique mixture; fodders, grinding; chopping.

Rezumat

Lucrarea îşi propune elaborarea unor tehnologii noi rentabile, implicarea în fluxul tehnologic al mecanizării și pregătirii hranei, administrarea furajelor prelucrate, care asigură valorificarea lor completă comparativ cu administrarea furajelor în stare neprelucrată (cum au fost obţinute la recoltare). S-au determinat consumurile voluntare pe diferite categorii de furaje şi piederile de furaje. În cazul furajelor administrate sub formă de amestec unic pierderile sunt de la 2,33% la 4,53%. Dacă s-au administrat furaje neprelucrate, pierderile au fost: la fân de lucernă 15%, la fân de borceag 20,2%, la paie 47,5%, la porumb ştiuleţi 4,7%. S-a determinat producţia medie totală de lapte la oi prin controlul bilunar (metoda Nica-Dermengi). S-a determinat şi producţia medie de lapte muls, durata lactaţiei, compoziţia chimică a laptelui *(grăsime%, proteină%). La oile de rasă* ***Merinos de Palas*** *din lotul experimental, producţia totală de lapte a fost de 119,51±2,1 litri, producţia medie de lapte muls de 48,26±1,15 litri, într-o lactaţie de 111,2 zile; la oile din lotul martor producţia totală de lapte a fost de 75,11±3,2 litri, producţia medie de lapte muls de 23,05±1,01 litri, într-o lactaţie de 92,4 zile. La oile din* ***Rasa Prolifică Palas****, la lotul experimental, producţia totală de lapte a fost de 151,25±2,1 litri, producţia medie de lapte muls de 82±1,63 litri, într-o lactaţie de 131,8 zile; la lotul martor producţia totală de lapte a fost de 101,25±2,1 litri, producţia medie de lapte muls de 42,75±1,63 litri, într-o lactaţie de 156,3 zile. La oile din* ***Rasa de lapte Palas****, la lotul experimental, producţia totală 211,3±7,81 litri, producţia medie de lapte muls de 120,6±2,8 litri, într-o lactaţie de 205,6 zile; la lotul martor producţia totală 197,6 litri, producţia medie de lapte muls de 105,8±3,9 litri,într-o lactaţie de 181,3 zile. În ce privește compoziția chimică a lapelui s-au determinat valori mai mari la loturile experimentale, comparativ cu cele de la loturile martor atât la grăsime, cât şi la proteină: la oile de rasă* ***Merinos de Palas*** *din lotul experimental grăsimea a fost de 6,96%, proteina de 6,47%, la lotul martor grăsimea a fost de 6,9 %, proteina de 6,61%; la oile din* ***Rasa Prolifică Palas****, la lotul experimental, grăsimea a fost de 5,59%, proteina de 5,40%, la lotul martor grăsimea a fost de 5,56%, proteina de 5,37%,; la oile din* ***Rasa de lapte Palas****, la lotul experimental grăsimea a fost de 6,41%, proteina de 6,32%, la lotul martor grăsimea a fost de 6,46%, proteina de 6,27%.*

Cuvinte cheie: amestec unic; furaje, măcinare; tocare.

INTRODUCTION

The research work aims to elaborate new profitable technologies performed in order to improve and optimize the technological sequences, to involve the mechanization of food processing and administering it in the technologies of sheep breeding and exploitation.

Administering the unprocessed fodders to sheep, as they were obtained at gathering does not assure their complete valuation.

To prevent the waist of fodders and to increase their value, the fibrous and gross fodders should be previously prepared, so that the animal could convert them with higher efficiency into animal products of high biological value.

There is an increased concern to find the most efficient methods to valuate fibrous and gross fodders as unique mixture at the international level.

The nutritive contribution of the gross fodders, at a content of circa 85% SU, can be appreciated as moderately energetic (0,3-0,4 UN/kg), poor in protein (3 g/kg PBD at straws, 18 g/kg PBD at corn cobs and 35 g/kg PBD at stems of leguminous plants) and rich in raw cellulose (37-42%, out of which, 9-12% lignin). Regarding the mineral part, the content is low in P, moderate in Ca (2-4 g/kg), but it is high in silicic salts. Digestibility of the organic substance from the gross fodders has resulted to be less than 50%. The low digestive valorification of the nutritive substances from the gross fodders is an impediment in using them in the food of animals. That is why there is a permanent concern of the nutritionists to find processing methods and techniques through which these fodders to become an usual component of the fodder ratios for rumminators.

The improvement of the foddering technologies for sheep aims to introduce certain methods of improving the foddering of sheep by chopping, grinding, mixing and processing the fodders, operations which determine the increase the nutritional value of the fibrous and gross fodders (fodders from secondary production, obtained from cereal crops), the productive increasing rate achieved through this activity justifying the made expenses (the productive increasing rate can be of 20-25%, especially if the animals are from improved breeds).

By processing the fodders, by chopping, grinding and administering them as unique mixture, the selective consumption of fodders decreases and their palatability increases, is assuring the increase of the degree of consumption and assimilation, and influences directly the quantity and the quality of the obtained products (the fan eliminates the spores of saphrofite or parasite mushrooms), at the same time reducing the losses produced by manipulating the fodders. The administration of chopped and grinded fodders can be done mechanically (in the sheep exploit the distribution of fodders in the stable period needs approximately 61% of the daily operations of sheep care) reducing a lot the volume of transported fodders, fact that consequently determines the reduction of the transport expenses and the possibility of the supply with fodders for a longer period of time (minimum 2 days).

The mechanical processing is done with the purpose to facilitate the digestive labor of the animals (prehension, mastication, digestion), and also for an ulterior processing and manipulation of the fodders. The following can be achieved through mechanical processing: the chopping of fodders, which is done with specific mills providing the main size of the fodder particles of under 10 mm; the chopping is done with choppers and the usual size of the fodder particles is of 10-60 mm; defibration is regularly done simultaneously with chopping and has as effect the long way cutting of the silicous cuticle from the stems of the corn cobs; the pounding is done in the case of the stagger weeds or corn cobs when specific tools (pounder) are used.

The stable period of sheep in Romania is between 90 and 160 days (depending on the climatic conditions in the breeding area), and during this period the females are in the last period of gestation and in the first months of lactation, when it is necessary to equilibrate the negative energetic balance through a balanced alimentation, so that healthy and well developed lambs are obtained, and their females have appropriate productions of milk (during milking and sucking periods).

In the sheep exploitations the use of the choppers and universal mills for processing the fibrous and gross fodders and for chopping the cereals added to the fodder mixtures to increase the energetic value of the ratios (these depending on the physiological estate of the sheep), the capacity of the exploitations, the consumption of energy and quality of the obtained fodder mixtures must take into account.

MATERIAL AND METHOD

The researches have been carried on at ICDCOC Palas. The maintenance of the sheep was done in stable, for 150-160 days and 205-215 days in the pasture. The foddering for the stable period was done at the experimental lots with a unique mixture, based on fibrous fodders: 52,5% hay, 32% gross fodders and 15,5% concentrated fodders, all chopped and homogenised, and at the control lots the same fodders were administered, but unprocessed. The sheep grazed on pastures of a mixture of 70-75% grain plants and 25-30% annual leguminous plants, with a high degree of consumption of 94,12%, during this period also being administrated a mixture of 0,5-0,7 kg, consisting in chopped and concentrated hays.

The research works were done on sheep of **Palas Merino Breed**, **Palas Milk Breed** and **Palas Prolific Breed**, organized in experimental lots and control lots. To administrate the unique mixtures into the sheep food, a technology of mechanization of preparing these mixtures was established. This technology consists in more lines of processing the fodders that are in the composition of the unique mixtures. For chopping the concentrated fodders, including the corn cobs, a cob pounder and a snail transporter that takes the chopped product and carries it in a MC-3mill are provided in the technological flux. The chopped product is pneumatically guided to a bunker with 2 compartments for deposit. The line for chopping the fibrous and gross fodders is provided with a chopping mill fed with a transporting band and the product is pneumatically carried to a fan in the assuaging cyclone, which guides it in the depositing bunkers.

The degree of fodders chopping was: 1-3 mm at cobs and barley; 2-10 mm at stagger weed; 2-8 mm at straws and hay. For a better chopping of the fibrous and gross fodders, a hammer mill of high productivity, was used. The construction and functional parameters which are at the base of the working process of the mill are as follows:

- peripheral speed of hammers...90 m/s.
- distance between the point of hammers and strainer ....15 mm
- number of hammer rows 4
- number of hammers on rotor 72

- the angle of coverage of the strainer...175

Unlike the type of hammer mills for chopping concentrated fodders, the mill used for chopping the fibrous and gross fodders has on its rotor two hammers between every two consecutive discs, fact that improves the process of chopping the fodders. Also for the improvement of the chopping process, on the superior part of the chopping room two jagged tracks were mounted.

To chop the concentrated fodders, the corn cobs and the stagger weeds, the mill is equipped with a bunker provided with a pounder at its inferior part.

The administration of sheep unique mixtures was done at the experimental lots, and at the control lots received the same fodders, but unprocessed. During the entire experimental period both the fodder consumptions and losses were noted.

The animals involved in the researches were individually observed in terms of their own performances, registering data concerning the following: control of milk production and quantitative determinations of milk. The control of milk production was made on the basis of the Romanian method of the control coefficient (Nica-Dermengi), a method which is based on the proportion between the daily milk production and the quantity on a single milking in the same day and it can be applied during the whole period of lactation, once or twice a month.

For the quantitative determinations of milk the following methods were used: Gerber method for the fat percentage; Kjeldahl method for the protein percentage; method of thermosetting, for the percentage of dry substance.

To calculate and systematize the data, the usual statistical methods were used.

RESULTS AND DISCUSSIONS

A fodder ratio of 3,5 kg, with a content of 1,71 SU, 1,71 UNL, 169 g PDIN and 166 g PDIE was provided for the sheep on gestation.

Through weighing all fodders at administration and the rests of fodders it was noted that in the case of administered fodders after chopping, grinding and mixing, as unique mixture, the losses ranged between 2,33% and 4,53%.

If the fodders are given unprocessed, as they were gathered, the losses were as follows: at hay of alfalfa 15%, at hay of vetch 20,2%, at straws 47,5%, at corn cobs 4,7%.

To get the sheep used to the unique mixture and to avoid certain gastrointestinal disorders there was a period of 8 days for accommodating (fodders as unique mixture and unprocessed fodders as hays of alfalfa and vetch were simultaneously given, in separate feeders).

The average total milk production of sheep was determined by bimonthly control, after Nica - Dermengi method (table no.1). The average production of milked milk, the duration of lactation and the chemical composition of the milk (dry substance %, fat% and protein %), were also determinate.

At the sheep of **Palas Merino Breed** from the experimental lot the total milk production was of 119,51±2,1 litres, with an average production of milked milk of 48,26±1,15 litres, in a lactation which lasted for 111,2 days. At the control lot the total milk production was of 75,11 ±3,2 litres, with an average production of milked milk of 23,05±1,01 litres, in a lactation which lasted for 92,4 days.

At the sheep from **Palas Prolific Breed**, at the experimental lot, the total milk production was of 151,25 ± 2,1 litres, with an average production of milked milk of 82,02 ± 1,636 litres, in a lactation which lasted for 131,8 days; at the control lot the total milk production was of 101,25 ± 2,1 litres, with an average production of milked milk of 42,75 ± 1,63 litres, in a lactation which lasted for 186,3 days.

At the sheep from the **Palas Milk Breed**, from the experimental lot, the total milk production was of 211,3 ± 7,81 litres, with an average production of milked milk of 81,6 ± 2,8 litres, in a lactation which lasted for 205,6 days, at the control lot the total milk production was of 197,6 litres, with an average production of milked milk of 105,8±3,9 litres, in a lactation which lasted for 186,3 days.

Table 1. The total average milk production, the average production of merchandised milk, the duration of lactation and the chemical composition of milk at the sheep being given the unique mixture and at the control lots / Producția medie totală de lapte, producția medie de lapte marfă, durata lactației și compoziția chimică a laptelui la oile la care s-a administrat amestec unic și la loturile martor

Breed or population of sheep/batch	n	Average total milk production (litres)		Average milk production (litres)		Duration of lactation (days)	Chemical composition		
		$X \pm S_x$	V%	$X \pm S_x$	V%		Dry substance %	Fat %	Protein %
Palas Merino Breed - Experimental batch	64	119,51±2,1	14,05	48,26±1,15	19,06	111,2	16,62	6,96	6,47
Palas Merino Breed - Control lot	49	75,11±3,2	29,82	23,05±1,01	30,67	92,4	16,02	6,90	6,61
Palas Prolific Breed -Experimental batch	46	135,2±4,6	23,07	56,2±2,6	31,37	131,8	16,75	5,59	5,40
Palas Prolific Breed - Control lot	25	101,25±2,1	10,37	42,75±1,63	19,06	156,3	16,22	5,56	5,37
Palas Milk Breed - Experimental batch	64	211,3±7,81	29,56	81,6±2,8	27,45	205,6	16,53	6,41	6,32
Palas Milk Breed - Control batch	25	197,6±1,32	3,34	72,8±3,9	26,78	161,5	6,12	6,46	6,27

Regarding the chemical composition of the milk higher values were determined at the experimental lots, comparatively to the values from the control lots at the dry substance, fat, and protein as follows: at the sheep of **Palas Merino Breed** from the experimental lot the dry substance was of 16,68%, fat was of 6,96% and protein of 6,47%, and at the control lot the dry substance was of 16,02%, fat was of 6,90% and protein of 6,61%; at the sheep from **Prolific Population of Palas**, at the experimental lot, the dry substance was of 16,75%, fat was of 5,59% and protein of 5,40%, at the control lot the dry substance was of 16,22%, fat was of 5,56% and protein of 5,37%; at the sheep from the **Palas Milk Breed**, from the experimental lot the dry substance was of 16,53%, fat was of 6,41% and protein of 6,32%, and at the witness lot the dry substance was of 16,12% fat was of 6,47% and protein of 6,27%.

CONCLUSIONS

1. The administration of fodders as unique mixture in the food of sheep contributes to the efficient use of fodders, by reducing the losses and increasing the degree of consumption of fibrous and gross fodders.
2. At the administration of unprocessed fodders, as they were at gathering, the losses were the following: at hay of alfalfa 15%, at hay of vetch 20,2%, at straws 47,5%, at corn cobs 4,7%.
3. The administration of fodders after chopping, grinding and mixing, as unique mixture the losses are between 2,33% and 4,53%.
4. The administration of fodders as unique mixture allows the mechanization of food processing and distribution, reducing the expenses needed for sheep caring and in the end the increase of economic efficiency of the sheep farms.
5. The administration of fodders as unique mixture determined higher milk productions.

BIBLIOGRAPHICAL REFERENCES

1. Bria N.; The System of Machines and Installations designed for the Agricultural Production. Mechanization of Agriculture Magazine, Bucharest; 2001; nr. 12, pag.15, ISSN 1011-7296.
2. Dragomirescu I.T., Paula Mariana Marica; Machines and Installations for Animal Food Processing and Preparation; 1987; "Ceres" Publishing House, Bucharest.
3. Mitroi A.; Machines and Installations for Animal Breeding; 1998; Ed. Foundation of Farmer's House, Bucharest.

4. Moise S.T., Comșa I, Ionescu A.; Economic Efficiency of Preparing and Administering the Fodders as Unique Mixture at Sheep, Central Station of Researches for Sheep Breeding; 1977; vol. III Publisher: Centre of Didactic Material and Agricultural Propaganda, Bucharest.
5. Raba T., Nicolescu I.; Mechanization of Works in Sheep Breeding; 1975; "Ceres" Publishing House Bucharest, 1975.
6. Stoica I., Liliana Stoica; Preparation of Gross Fodders, the Way to Their Superior Valuation in the Food of Ruminators; 2001; Magazine of Animal Breeding and Veterinary Medicine, Bucharest, nr.1, page 39, ISSN 120-8507.

IMPROVING THE KINEMATIC PARAMETERS OF RAM EPIDIDYMAL SPERM USING A COCONUT WATER-BASED EXTENDER

ÎMBUNĂTĂȚIREA PARAMETRILOR KINETICI AI SPERMEI EPIDIDIMALE DE BERBEC CU AJUTORUL UNUI DILUANT PE BAZĂ DE APĂ DE NUCĂ DE COCOS

PALL EMOKE[1,2], CENARIU MIHAI[1], GROZA IOAN[1], DUCA GHEORGHITA[2], SPINU MARINA[1,2], SANDRU CARMEN DANA[1,2], BORZAN MIHAI[1], CIUPE SIMONA[1]

[1]University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, str. Manastur no. 3-5, Cluj-Napoca, Romania
[2] Research and Devepoment Institute for Montanology, Cristian – Sibiu, Str. XIII No.147, Cristian, Sibiu, Romania

Corespondence address: emoke.pall@usamvcluj.ro

Abstract

The sperm quality plays a crucial role in reproductive success, being of paramount importance in assisted reproduction programs in domestic animals. Epididymal sperm cells are characterized by a unique maturation process within the epididymis. Unlike ejaculated sperm, epididymal sperm undergo a series of biochemical and physiological changes during their transit through the epididymal duct, which are essential for acquiring motility and fertilization competence. The aim of our study focused on improving the kinetic parameters of ram epididymal sperm using a coconut water-based extender. The ram epididymal sperm was collected after slaughter, followed by the evaluation of various sperm kinetic parameters using an automated analysis system. Subsequently, after dilution, the effects of the coconut water-based extender were compared with those of a commercial diluent. Our results demonstrate that the coconut water-based extender significantly improved the kinetic parameters of ram epididymal sperm compared to the commercial diluent used in the study. Sperm motility was remarkably improved, with increased velocity and improved linearity observed in samples treated with the coconut water-based extender. Our findings suggest the potential use of natural sources such as coconut water as an effective diluent for improving seminal material quality in sheep breeding programs.

Keywords: ram, epididymal sperm, kinematic parameters, extender

Rezumat

Calitatea materialului seminal joacă un rol crucial în succesul reproducției, cu importanță majoră în programele de reproducție asistată la animalele domestice. Celulele spermatice de la nivel epididimar sunt caracterizate printr-un proces unic de maturare în epididim. Spre deosebire de spermatozoizii din ejaculat, spermatozoizii epididimari suferă o serie de modificări biochimice și fiziologice în timpul tranzitului lor prin canalul epididimar, care sunt esențiale pentru dobândirea motilității și a competenței de fertilizare. Scopul studiului nostru s-a axat pe îmbunătățirea parametrilor kinetici ai spermei epididimare de berbec, folosind un diluant pe bază de apă de nucă de cocos. Sperma epididimară de a fost colectată după abatorizarea berbecilor, urmată de evaluarea diverselor parametrii kinetici spermatici cu ajutorul unui sistem automatizat de analiză. Ulterior, după diluare, s-au comparat efectele diluantului pe bază de apă de nucă de cocos cu cel al unui diluant comercial. Rezultatele noastre demonstrează că diluantul pe bază de apă de nucă de cocos a îmbunătățit semnificativ parametrii kinetici ai spermei epididimare de berbec comparativ cu diluantul comercial utilizat în studiu. Motilitatea spermatozoizilor a fost îmbunătățită în mod remarcabil, cu o creștere a velocității și o îmbunătățire a linearității observate în probele tratate cu diluantul pe bază de apă de nucă de cocos. Rezultatele noastre indică potențialul utilizării surselor naturale, cum ar fi apa de nucă de cocos, ca diluant eficient pentru îmbunătățirea calității materialului seminal în programele de reproducere a oilor.

Cuvinte cheie: berbec, spermă epididimară, parametrii kinetici, diluant

INTRODUCTION

The post-mortem collection of spermatozoa is a vital technique and final opportunity for obtaining germplasm reserves from genetically valuable domestic animals as well as endangered wild animal species (Kaabi et al., 2003, Podico et al., 2022). Harvesting epididymal semen from domestic animals occurs following castration, death, or euthanasia (Podico et al., 2022). The suitable storage and temperature differ among species (Lago-Alvarez et al., 2020). In addition to storage temperature, the preservation of epididymal sperm cells fertility is influenced by other factors such as the time of collection after euthanasia/death/slaughter of the animals, the conditions under which the testicles are handled post-collection, the transportation environment, the collection time from epididymis, and the duration of collection (Abella et al., 2014). Results from different studies demonstrate that various strategies have been developed for preserving epididymal sperm, such as refrigerating the entire epididymis or storing undiluted epididymal semen or storing it after dilution (Tamayo-Canul et al., 2011a, Tamayo-Canul et al., 2011b). It is imperative that epididymal semen be collected promptly following the death/euthanasia/slaughter of males, and subjected to dilution, refrigeration, freezing, or used for insemination. However, this is not always feasible; instances may arise where males are not promptly identified, or extended transportation to the laboratory is required (Tamayo-Canul et al., 2011b). According to the study conducted by Kaabi and colleagues (2003), the collection and maintenance of post-mortem epididymis at 5 °C resulted in satisfactory sperm parameters after 48h, but the fertility capacity decreased. In this context, it is important to identify suitable extenders for dilution, which have the potential to maintain the fertility potential. After collection and during the various stages of dilution and preservation, spermatozoa are exposed to artificial conditions, which can lead to thermal, biochemical, and osmotic stress, respectively (Gibb et al 2015, Pini et al., 2018, Neila-Montero et al., 2024). All these conditions lead to cytomorphological changes and consequently to the decrease in sperm kinetic parameters (Rizkallah et al., 2022). Thus, selecting appropriate extenders along with adhering to cooling times and equilibration can lead to reducing the potential negative effects of various exogenous factors (Neila-Montero et al., 2024). In this context, the aim of the present study was to assess the protective potential of a coconut water-based extender. The selection and utilization of natural constituents for diluting ram semen play a crucial role (Ilas et al., 2018, Odrada et al., 2023). Coconut water emerges as a promising natural alternative for the refrigeration and cryopreservation of ram seminal material. With its rich composition of salts, sugars (sucrose, sorbitol, fructose, glucose) (Prades et al., 2012), vitamins, proteins, coconut water has the potential to sustain the viability and fertility of sperm cells (Moreira et al., 2021, Odrada et al., 2023). In addition to these components, coconut water is also rich in nicotinic acid, pantothenic acid, thiamine, biotin, riboflavin, and pyridoxine, as well as phytohormones such as auxin, cytokinin, and gibberellin (Yong et al., 2009, Odrada et al., 2023).

MATERIALS AND METHODS

The biological material for the study was collected after the slaughter of the **Țurcana** rams (n=5, 4-5 years old) at a locally authorized slaughterhouse. The transport of the collected testicles was done in polystyrene boxes. Sample processing was conducted 4.5 h after slaughter. After dissection and removal of connective tissue and surface blood vessels, epididymal semen was harvested at 4°C using the retrograde flushing method from the distal portion of the tail of the epididymis using an intravenous catheter (22G). The harvested samples were centrifuged at 500g, 7 minutes, pooled and split into two aliquots (two experimental groups). The first experimental group was diluted (1:10) with a commercial extender Triladyl® (Minitübe) based on glycerol, Tris, citric acid, fructose, tylosin, gentamicin, lincomycin, and spectinomycin. The second experimental group was diluted with a coconut water-based extender (1.355g Tris, 0.7g citric acid, 0.5g fructose in 50 ml distilled water supplemented with 20%

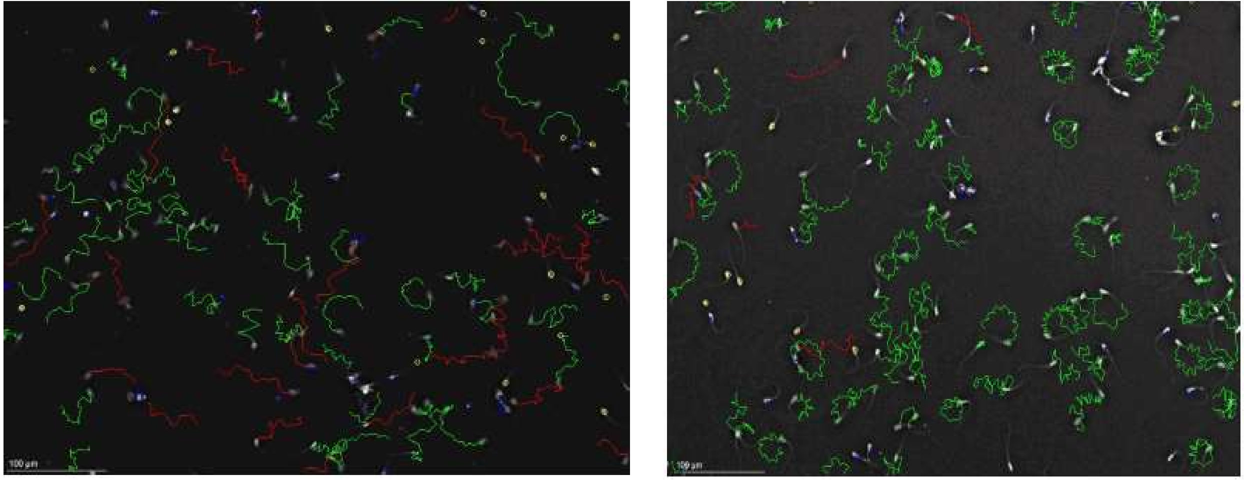
coconut water (Nutricost, Organic Coconut Water), 1% antibiotic –antimycotic (Gibco). After 4.5 h of equilibration at 4ºC, sperm motility and kinetic parameters were assessed with Computer-Assisted Sperm Analysis (CASA) equipped with Sperm Class Analyzer® (SCA) software version 6.3.0.59 (Microptic S.L., Barcelona, Spain). Subsequently, the concentration and kinetic parameters of sperm (including total motility and progressive motility, curvilinear velocity (VCL, µm/s), straight-line velocity (VSL, µm/s), average path velocity (VAP, µm/s), linearity (LIN, %), amplitude of lateral sperm head displacement (AHL, µm), straightness (STR, %), oscillation (WOB, %), tail beat frequency (BCF, Hz)), as well as cytomorphological characteristics were assessed. Epididymal semen vitality was determined using the FluoVit kit (Microptic, S.L., Barcelona, Spain). Data analysis was performed using GraphPad Prism 6 software (GraphPad Software, Manual Graph Pad Prism 5.0), with statistical significance set at a p-value of ≤ 0.05. After CASA assessment, diluted epididymal sperm samples were stored at 4°C and subsequently re-evaluated at 24 h.

RESULTS AND DISCUSSIONS

The epididymal sperm was collected from sexually mature rams, which were slaughtered. The method used for collection was retrograde flushing with a flushing medium based on isotonic saline supplemented with 2.9% sodium citrate buffer, 1% antibiotic-antimycotic (100%). The collected samples underwent macroscopic and microscopic examination. The semen samples contaminated with blood were excluded from the experiment. For retrograde flushing, a total volume of 0.75 ml of flushing medium was used, and the average of the recovered samples was 1.36±0.42. Following macroscopic evaluation (volume, color), concentration, sperm morphology, vitality, and kinetic parameters were assessed in each experimental group (EG1 and EG2). The results of the kinetic parameters are presented in Table 1.

Table 1. Results of kinetic parameters / Rezultatele parametrilor kinetici

Kinetic parameters	EG1	EG2
VAP, µm/s	20.41±3.09	35.52±0.39
VSL, µm/s	13.05±3.20	16.14±0.38
VCL, µm/s	38.86±8.60	76.92±3.45
ALH, µm/s	2.2±0.49	4.09±0.11
BCF, Hz	6.51±0.53	6.47±0.09
STR, %	69.57±3.94	48.57±0.11
LIN, %	32.37±3.99	22.12±0.22
WOB, %	53.21±2.97	45.67±1.05



EG1- experimental group 1 (epididymal sperm sample diluted with Triladyl), EG2 – experimental group 2 (epididymal sperm sample diluted with coconut water based extender), VAP-average path velocity, VSL- straight-line velocity, VCL- curvilinear velocity ALH -lateral sperm head displacement, BCF-tail beat frequency, STR-straightness, LIN – linearity, WOB-oscillation index

The higher mean values of VCL, ALH, and BCF in samples diluted with coconut based extender indicate a higher energetic value of the spermatozoa, compared with the commercial extender. The differences are statistically significant for all kinetic parameters, $p \leq 0.05$. These results indicate high motility of the spermatozoa. It should be noted that the values of VCL are much higher than those of VAP, which correspond to lower values of the LIN percentage. These results indicate a high degree of lateral deviation of the spermatozoa heads from their direction of movement. In the sample diluted with commercial medium, the kinetic sperm parameters indicated lower values. The mean values of VCL (38.86 µm/s ±8.60), ALH (2.2 µm/s ±0.49), and BCF (6.51 Hz ±0.53) suggest a slight decrease in the energetic capacity of sperm cells collected from the epididymal level. Notably, also in the EG2 the substantially higher values of VCL in comparison to VAP, coupled with lower LIN percentages, highlight a pronounced lateral deviation of spermatozoa heads from their direction of movement.

Consequently, the values of total and progressive mobility also indicated a lower percentage compared to the medium based on coconut water. As observed in Figure 1, the parameters of mobility of ram epididymal sperm cells exhibited notable fluctuations. Specifically, the rapid progressive mobility observed in samples diluted with coconut water-based extender displayed an average of 57.23% ± 4.12 in group 1 and 71.43% ± 10.21 in group 2.

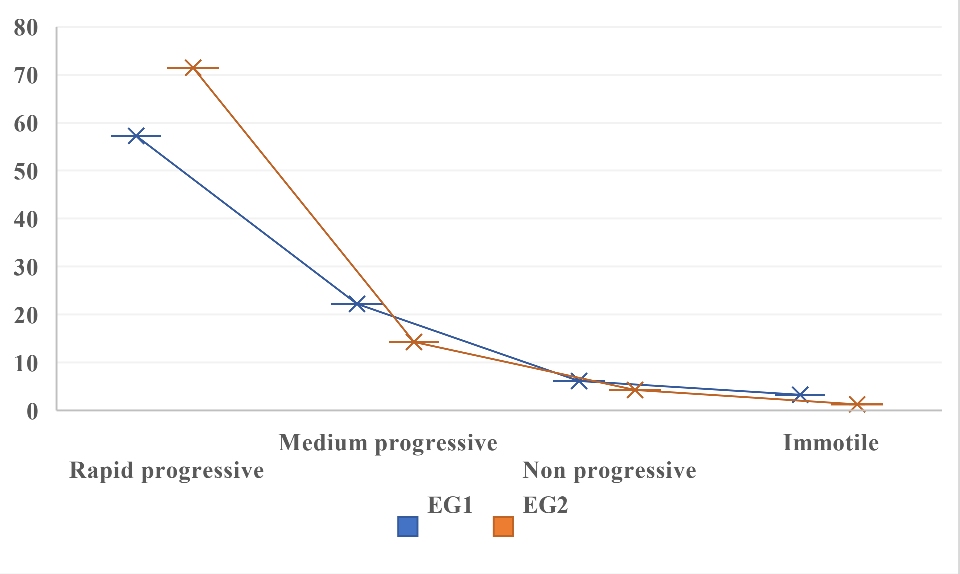


Figure 1. The parameters of sperm mobility evaluated using the CASA system / Mobilitatea spermei determinată cu sistemul CASA

Mobility serves as the primary and most important indicator of seminal material quality. In our study, total and progressive motility, as well as kinetic sperm parameters such as VAP and VSL, were significantly higher in samples diluted with coconut water-based extender. Apart from minerals and vitamins, coconut water harbors phenolic compounds and flavonoids with notable antioxidant potential, which mitigate lipid peroxidation and the generation of free radicals (Lima et al., 2015).

Consequently, extenders supplemented with coconut water may mitigate the incidence of morphological anomalies and reduce the alterations in semen kinetic parameters induced by reactive oxygen species (Ahmadip et al., 2016; Banday et al., 2017). After 24 hours of incubating the samples at 4ºC, a reassessment of the kinetic parameters was conducted, and the findings are depicted in Figure 2.

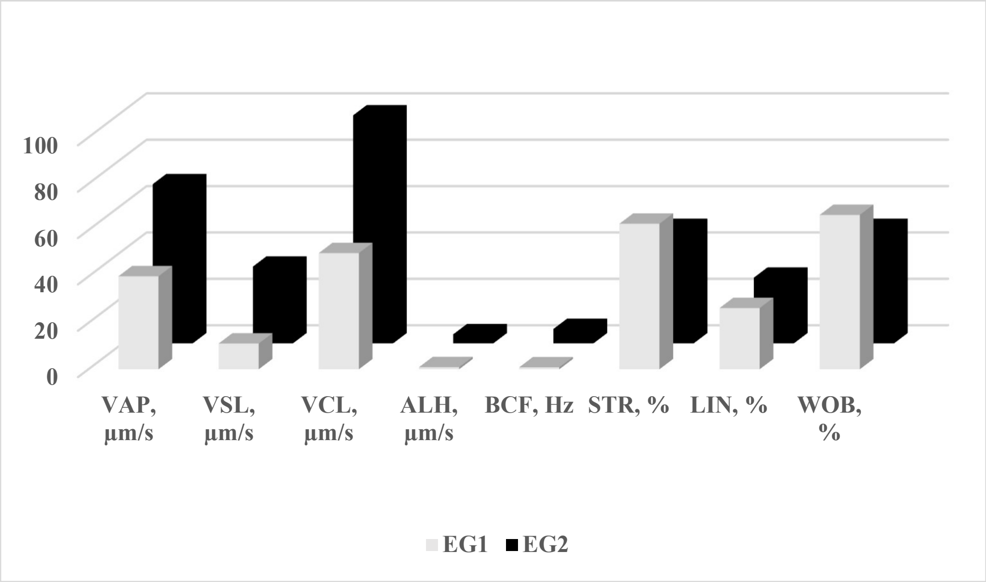
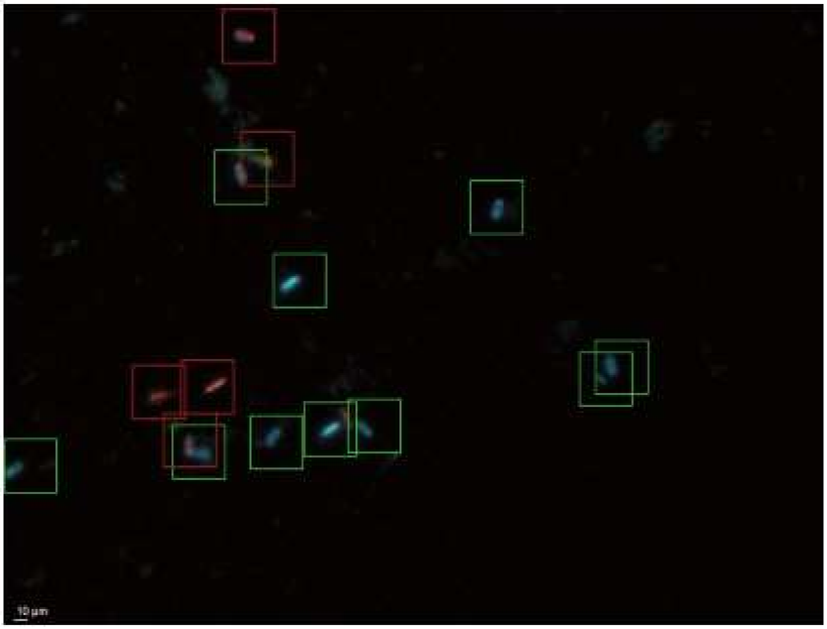


Figure 2. Results of the kinetic parameters evaluated after 24 hours / Evaluarea parametrilor kenetici după 24 de ore

After 24 hours, a slight decrease in kinetic parameters was observed in EG1, a trend not identified in EG2. Furthermore, analyses of cytomorphological attributes revealed minor variances (Table 2), but these differences are not statistically significant.

Table 2. The results of sperm cytomorphological analysis / Rezultatele analizeilor crio-morfologice ale spermei

Parameters	EG1	EG2
Normal %	56.23±1.01	60.1±3.09
Head anomalies%	6.70±0.11	3.4±0.12
Intermediate piece %	5.40±0.12	2.60±3.36
Tail anomalies%	8.60±3.13	8.34±1.93
Protoplasmic droplet%	1.20±0.45	0.98±0.16
		

Primary and secondary anomalies were observed in the both experimental group and predominantly at the tail level, but did not exceed 10%. Abnormalities affecting sperm head morphology entail atypical or irregular alterations in their form and structure. Within this framework, the elevation in EG1 anomalies compared to the EG2 suggests a small decline in seminal material quality. The teratozoospermia index, signifying the ratio between the total count of abnormal spermatozoa and the overall spermatozoa count, measured 2.09 in EG1 and 1.70 in EG2. The calculated sperm deformity index reflects the comprehensive extent of sperm cell deformations, registering 3.35 in EG1 2.09 in EG2. Meanwhile, the index of multiple anomalies denotes the prevalence of simultaneous anomalies, with values of 3.71 in EG1 and 2.13 in EG2. These indices provide quantitative metrics for assessing seminal material quality degradation, which may have implications for its fertilization capacity. These findings suggest that EG1 may be more susceptible to changes over time compared to EG2, highlighting the importance of monitoring storage conditions closely. In addition to the observed differences in kinetic parameters and cytomorphological attributes, it may be worthwhile to investigate potential factors contributing to the variation between EG1 and EG2 after 24 hours. Factors such as temperature fluctuations during storage, differences in extender composition or quality, and individual variability among samples could all play a role in influencing the observed trends. Further analysis and exploration of these factors could provide valuable insights into optimizing sample storage conditions and preserving sperm quality effectively. Indeed, coconut water has demonstrated its efficacy as a semen extender in various studies (Odrada et al., 2023). Its components create a conducive environment and can maintain viability throughout the cooling, freezing (Barbosa et al., 2020) and post-thawing processes, especially when combined with other substances such as egg yolk and glycerol (El-Sheshtawy et al., 2017).

Similar studies have been conducted in dogs (Cardoso et al., 2005) and stallions (Sampaio-Neto et al., 2002). In equines, the emphasis has been on spermatozoa retrieved from the epididymis, whereas in canines, seminal material has mainly been obtained via electroejaculation, manual methods, or employing an artificial vagina. The evaluation of material quality is crucial for predicting male fertility potential and serves as an essential component of clinical diagnostics. Another noteworthy observation from these results is the potential influence of extender selection on sperm quality and motility.

CONCLUSIONS

The enhanced performance of the coconut water-based extender, indicated by elevated kinetic parameters and mobility percentages, suggests its efficacy in maintaining sperm viability and function during storage. This highlights the importance of carefully choosing extenders in sperm preservation procedures, with coconut water-based extenders emerging as a promising choice.

Furthermore, the observed fluctuations in sperm mobility parameters between different extenders highlight the need for careful consideration when choosing extender formulations. Factors such as extender composition and osmolality may significantly influence sperm quality and should be tailored to the specific requirements of each reproductive program or research protocol. Overall, these conclusions underscore the importance of optimizing sperm preservation techniques to maintain high-quality sperm samples, ultimately contributing to improved reproductive outcomes in both clinical and research settings.

BIBLIOGRAPHICAL REFERENCES

1. Abella D.F., Da Costa M., Guérin Y., Dacheux J.L.; Fertility of undiluted ram epididymal spermatozoa stored for several days at 4°C; 2015; Animal. 9(2):313-9.
2. Ahmadip S., Bashirip R., Ghadiri-Anarip A., Nadjarzadehp A.; Antioxidant supplements and semen parameters: An evidence-based review; 2016; International Journal of Reproductive Biomedicine, 14(12): 729.
3. Banday M.N., Lone F.A., Rasool F., Rashid M., Shikari A.; Use of antioxidants reduce lipid peroxidation and improve quality of crossbred ram sperm during its cryopreservation; 2017; Cryobiology 74: 25–30.

4. Barbosa B de S., Izzo R.G., Silva H.V.R., Nunes T.G.P., Brito B.F., da Silva T.F.P., Silva L.D.M.; Recovery and cryopreservation of epididymal sperm from domestic cat using powdered coconut water (ACP-117c) and TRIS extenders; 2020; Cryobiology 92: 103–108.
5. Cardoso R.C.S., Silva A.R., Silva L.D.M.; Use of the powdered coconut water (ACP106®) for cryopreservation of canine spermatozoa; 2005; Anim Reprod 2: 257e62.
6. El-Sheshtawy R.I., Walid Said El-Nattat, Gamal Abdel Daiem Ali; Cryopreservation of cattle semen using coconut water extender with different glycerol concentrations; 2017; Asian Pacific Journal of Reproduction, 6(6): 279-282.
7. Gibb Z., Lambourne S.R., Quadrelli J., Smith N.D., Aitken R.J.; L-Carnitine and Pyruvate Are Prosurvival Factors during the Storage of Stallion Spermatozoa at Room Temperature; 2015; *Biol. Reprod.* 2015; 93:104.
8. Ilas V., Pall E., Tripon, Mirela Sinteonean, Lavinia Cerbu, Constantin Groza, I.S.; Effect of different concentrations of coconut water on ram sperm kinematic parameters; 2018; Annals of Phytomedicine 7(2): 170-173.
9. Kaabi M., Paz P., Alvarez M., Anel E., Boixo J.C., Rouissi H., Herraez P., Anel L.; Effect of epididymis handling conditions on the quality of ram spermatozoa recovered post-mortem. Theriogenology; 2003; 60(7):1249-59.
10. Lago-Alvarez Y., Podico G., Segabinazzi L.G., Cunha L.L., Barbosa L., Arnold C.E., Lima F.S., King L.T., McLean A.K., Canisso I.F.; Donkey epididymal transport for semen cooling and freezing; 2020; Animals 2020; 10:2209.
11. Lima E.B., Sousa C.N.S., Meneses L.N., Ximenes N.C., Santos Júnior M.A., Vasconcelos G.S., Lima N.B.C., Patrocínio M.C.A., Macedo D., Vasconcelos S.M.M.; *Cocos nucifera* (L.) (Arecaceae): A phytochemical and pharmacological review; 2015; Brazilian Journal of Medical and Biological Research, 48(11): 953.
12. Moreira S.S.J., da Silva A.M., Souza A.L.P., Praxedes E.C.G., de Souza Junior J.B.F., Pereira A.F., Silva A.R.; Cryopreservation of Spix's yellow-toothed cavy epididymal sperm using Tris- and coconut water-based extenders supplemented with egg yolk or Aloe vera; 2021; Cryobiology 99: 40-45.
13. Neila-Montero M., Alvarez M., Riesco M.F., Soriano-Úbeda C., Montes-Garrido R., Palacin-Martinez C., de Paz P., Anel L., Anel-Lopez L.; The Adaptation Time to the Extender as a Crucial Step for an Accurate Evaluation of Ram Sperm Quality during the Liquid Storage; 2024; Vet Sci. 16, 11(3):132.
14. Odrada P.M.S., Purnamasari L., Cruz J.F.D.; The Effects of Water-Based Coconut Extenders on Semen Preservation: A review; 2023; Jurnal Sain Peternakan Indonesia Volume 18 Issue 1.
15. Pini T., Leahy T., de Graaf S.P.; Sublethal Sperm Freezing Damage: Manifestations and Solutions; 2018; Theriogenology, 118, 172–181.
16. Podico G., Canisso I.F.; Retrograde Flushing Followed by Slicing Float-Up as an Approach to Optimize Epididymal Sperm Recovery for the Purpose of Cryopreservation in Equids; 2022; Animals (Basel). Jul 14,12(14):1802.
17. Prades A., Dornier M., Diop N., Pain J.P.; Coconut water uses, composition and properties: a review; 2012; Fruits 67(2): 87–107.
18. Rizkallah N., Chambers C.G., de Graaf S.P., Rickard J.P.; Factors Affecting the Survival of Ram Spermatozoa during Liquid Storage and Options for Improvement; 2022; Animals, 12:244.
19. Sampaio-Neto J.C., Salgueiro C.C.M., Mateos R.E., Nunes J.F.; Utilizaçao do diluente ~ ACP-105® na refrigeraçao do semen eqüino; 2002; Ver Bras Reprod Anim Supl, 5:137e9.
20. Tamayo-Canul J., Alvarez M., López-Urueña E., Nicolas M., Martinez-Pastor F., Anel E., Anel L., de Paz P.; Undiluted or extended storage of ram epididymal spermatozoa as alternatives to refrigerating the whole epididymis; 2011; Anim Reprod Sci. 126(1-2):76-82.
21. Tamayo-Canul J., Alvarez M., Mata-Campuzano M., Alvarez-Rodríguez M., de Paz P., Anel L., Martínez-Pastor F.; Effect of storage method and extender osmolality in the quality of cryopreserved epididymal ram spermatozoa; 2011; Anim Reprod Sci. 129(3-4):188-99.
22. Yong J.W.H., Ge L., Ng Y.F., Tan S.N.; The chemical composition and biological properties of coconut (*Cocos nucifera* L.) water; 2009; Molecules 14 (12): 5144–5164.

ACTA AGRICOLA ROMANICA, Volume 6, Year 6, No. 6.3.

ASSESSMENT OF THE PROLIFERATIVE POTENTIAL OF SHEEP ENDOMETRIAL MESENCHYMAL STEM CELLS

EVALUAREA POTENȚIALULUI PROLIFERATIV AL CELULELOR STEM ENDOMETRIALE DE OAIE

PALL EMOKE[1,2], CENARIU MIHAI[1], DUCA GHEORGHITA[2], SPINU MARINA[1,2], SANDRU CARMEN DANA[1,2], CIUPE SIMONA[1], BORZAN MIHAI[1]

[1]University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, str. Manastur no. 3-5, Cluj-Napoca, Romania
[2] Research and Development Institute for Montanology, Cristian – Sibiu, Str. XIII No.147, Cristian, Sibiu, Romania

Correspondence address: emoke.pall@usamvcluj.ro

Abstract

In the recent development of veterinary medicine, stem cell therapy has become increasingly utilized, both in companion animals and livestock. Among the types of stem cells that have garnered researchers' attention are hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs). Growing interest is evident in the investigation of the presence and functionality of endometrial cells in farm animals, given their significant potential in the field of reproduction and the treatment of uterine pathologies. Endometrial MSCs it is estimated to have a significant impact on various aspects of veterinary medicine, including transplantation therapy and regenerative medicine. To explore the therapeutic potential of these cells, our study focused on isolating, cultivating, and characterizing endometrial MSCs in sheep. This research makes a significant contribution to an emerging and promising field, providing important insights into the physiology and biology of these cells and contributing to the understanding of reproductive disorders in production animals and the development of new therapeutic modalities.

Keywords: endometrial stem cells, multipotency, proliferative potential, regenerative therapy

Rezumat

În recenta dezvoltare a medicinei veterinare, terapia cu celule stem a devenit din ce în ce mai frecvent utilizată, atât în cazul animalelor de companie, cât și la animalele de rentă. Printre tipurile de celule stem care au captat atenția cercetătorilor, se numără celulele stem hematopoietice (HSCs) și celulele stem mezenchimale (MSCs). Un interes crescând se manifestă în cercetarea prezenței și funcționalității celulelor endometriale la animalele de fermă, datorită potențialului lor deosebit de important în domeniul reproducției și al tratamentului unor patologii uterine. Se estimează că MSCs endometriale ar putea avea un impact semnificativ și în alte aspecte ale medicinei veterinare, inclusiv terapia transplantului și medicina regenerativă. În vederea investigării potențialului terapeutic al acestor celule, studiul nostru s-a concentrat pe izolarea, cultivarea și caracterizarea MSCs endometriale de oaie Această cercetare aduce un aport semnificativ într-un domeniu emergent și promițător, oferind perspective importante asupra fiziologiei și biologiei acestor celule, și contribuind la înțelegerea fiziopatologiei afecțiunilor reproductive la animalele destinate producției și la dezvoltarea unor noi modalități terapeutice.

Cuvinte cheie: celulele stem endometriale, multipotență, potential proliferative, terapia regenerativă

INTRODUCTION

The growing interest within the medical community regarding regenerative therapy utilizing adult stem cells is notable, propelled by numerous recent achievements. Across both human and veterinary medicine, whether employing autologous, heterologous, or xenogeneic approaches, the efficacy of regenerative therapies remains indisputable (Volk et al., 2013). In veterinary medicine, stem cell therapy has emerged as a clinical reality since the early 2000s, particularly in addressing equine tendon injuries

(Smith et al., 2003). Subsequently, numerous companies worldwide have offered services for isolating mesenchymal stem cells (MSCs) from bone marrow or adipose tissue. These MSCs undergo characterization, preservation, and subsequent application in companion animals or livestock (Volk et al., 2013). The creation of tissues, organs, or even organisms by human hands has long been considered a myth or a dream throughout the evolution of medicine. Currently, it is becoming a tangible reality. Tissue engineering and regenerative medicine are the terms used today to describe the initiative of generating complex tissues and organs from basic components. Both are evolving branches of biotechnology and medicine aimed at improving patient treatments by generating and regenerating tissues and organs as part of their therapy. These techniques hold great promise, with high expectations (Barrett, 2016). These techniques aim to improve treatment possibilities and enhance the quality of life for patients. It is also anticipated that this biotechnology will have a significant economic impact in clinical medicine. To meet these expectations, several challenges need to be addressed, including technological, scientific, clinical, ethical, and even social issues. Basic research still requires the evaluation and development of fundamental processes and procedures in many research areas. A few centuries ago, before the development of medical specializations, physicians treated both humans and their animals. Recently, the synergy between veterinarians, medical doctors, and other medical and environmental sciences has been promoted as a concept called "One Health," aimed at improving the lives of all species through the involvement of both human and veterinary medicine (Barret, 2016). The importance of this concept is a global strategy supported by a large number of organizations. "One Health" aims to merge human and animal medicine, particularly in the field of regenerative medicine, by restoring cells, tissues, or organ function after injuries, aging, or illness (Polak et al., 2008).The efficient potential of stem cells in healing must be maximized while the side effects of using this therapy should be minimal, both in veterinary and human medicine applications. The few obstacles encountered in regenerative medical product therapy in veterinary medicine, compared to human medicine, have encouraged the use of stem cell therapy in veterinary clinical practice (Volk et al., 2013). Endometrial cells meeting the criteria outlined for MSCs have already been harvested and characterized from women, rodents, pigs, dogs, and sheep. The therapeutic potential of endometrial MSCs has already been demonstrated in relation to ovarian insufficiency, Parkinson's disease, and pelvic organ prolapse (Emmerson et al., 2016). The ovine endometrium is highly dynamic, undergoing cyclic regeneration, suggesting the presence of an active population of mesenchymal precursor cells (Letouzey et al., 2015). To ultimately explore the therapeutic potential of these cells, our study focused on isolating, cultivating, and characterizing ewes endometrial stem cells.

MATERIALS AND METHODS

The genital tracts (n=3) were harvested from clinically healthy ewes post-slaughter. Following collection, the genital tracts were immersed in sterile physiological saline supplemented with 1% antimycotic antibiotic (Gibco) and transported to the laboratory for processing. In the laboratory, the external surface of the uterine tissues was washed with sterile physiological saline. From the three collected samples, the genital tract from a 4-year-old clinically healthy ewe was selected. The remaining samples were excluded from the study due to various pathologies and contamination. The collected samples were immersed in sterile phosphate buffer saline (PBS), after which the endometrium was separated from the myometrium. The three endometrial samples were cut and then introduced in Dulbecco Modified Eagle Medium (DMEM/F12, Gibco®) medium supplemented with 10% FBS (fetal bovine serum, Gibco). The samples were incubated at 4°C for 24 hours. Subsequently, the samples were minced and treated with 1% type I collagenase (Sigma-Aldrich) and incubated at 37°C for 45 minutes. The resulting cell suspension was filtered using 70µm nylon filters to remove debris. To neutralize the effect of collagenase, the cell suspension was diluted with fetal bovine serum culture medium and centrifuged at 1200 rpm for 10 minutes. The supernatant was discarded, and the cell pellet was resuspended with saline solution and centrifuged. This step was repeated three times. Then, the cell pellet along with some residual tissue explants were resuspended in propagation medium DMEM/F12 (Gibco) supplemented with 10% FBS, 1% glutamine (Gibco), 1% non-essential amino acids (Sigma-Aldrich), and 1% antibiotic-antimycotic (Gibco) and incubated at 37°C, 5% CO2, and 90% humidity. After 72 hours, non-adherent cells were removed by changing the propagation medium while preserving the tissue explants. At 24, 48, and 72 hours, the degree of cell attachment to the culture substrate was evaluated, and the medium was changed every 2 days. The morphology of adherent cells was assessed using an inverted phase microscope. Cell passaging was performed at 70% confluence by treating the cultures with trypsin/EDTA, and after 6 successive passages, the cultures were characterized immunophenotypically and functionally.

The clonal capacity of the isolated cells was assessed by quantifying fibroblast colony-forming units (CFU-F). Mesenchymal cells harvested from ewe (eMSCs) endometrium (passages 5, 6) were seeded at a density of $1x10^2/100cm^2$ onto T-25 plates (NuncTM). The plates were then incubated at 37°C, 5% CO2, 60-90% humidity. The propagation medium was changed every 2-3 days. After 10 days, the cultures were fixed and stained with a 3% alcoholic solution of crystal violet for 10 minutes. CFU-F colonies were microscopically assessed, clusters of 20-25 cells considered as a colony. Results were quantified using the following formula: CFU-F% =number of colonies/number of cells initially seeded x 100.

For evaluating the migration potential of eMSCs, a concentration of 2.5×10^2 cells were aggregated in standard culture medium using hanging drops method. After 48 hours, the formed aggregates (spheroids) were harvested and added onto 0.1% gelatin-coated Petri dishes. After 24 hours, the migration potential was assessed by measuring the migration area. The evaluation was performed in triplicate. The following formula was used for calculation: migration capacity = migration area - aggregate size. In order to evaluate the proliferation potential of the isolated cell line the cell doubling time (DT) were calculated. DT was calculated between passages 5 and 6 using the following equation:

DT = T ln2 / ln(Xe/Xb), where T is the incubation time in days, and Xb and Xe represent the number of cells at the beginning and end of the incubation period, respectively. Evaluations were performed in triplicate. For assessment of the proliferation potential of eMSCs, the colorimetric CCK8 assay (Sigma-Aldrich) was used. For this purpose, cells from passage 6 were cultured at a concentration of $5x10^3$ cells/ml in 24-well plates. After 24, 48 hours, and 5 days, the CCK8 assay was applied. For assessing the proliferation degree, 10 µl of CCK-8 solution was added to the culture medium, and the plates were incubated at 37°C for 4 hours. Absorbance was recorded using a BioTek Synergy 2 microplate reader set at a wavelength of 450 nm. The amount of formazan generated by the cells' dehydrogenase activity is directly proportional to the number of viable cells. Evaluations were performed in triplicate.

For the immunophenotypic characterization of stem cells derived from endometrial tissue collected from mares, monoclonal antibodies CD44, CD90, CD105 (BD Biosciences, San Jose, CA, USA) were used. The labeling protocol followed the manufacturer's recommendations. Immunophenotypic characterization was performed after passage 6. Samples were evaluated using the FACS Canto II flow cytometer (BD Biosciences). Instrument calibration was performed automatically using BD Cytometer Setup & Tracking Beads (BD Biosciences) and the CS&T application included in the FACS Diva program. Compensation was performed for the fluorochromes PerCP (peridinin chlorophyll), APC (Allophycocyanin), FITC (fluorescein isothiocyanate), and PE (phycoerythrin). Data were processed using the FACS Diva program (BD Biosciences), and results were presented as histograms. Fluid pressure was set to the lowest level to ensure an appropriate acquisition speed. Fluorescence detection was performed with a 488 nm blue laser and a 530/30 filter for FITC and 575/26 for PE, PerCP. 10.000 events were recorded for each labeled sample. For data interpretation, expression levels were considered as follows: high (> 80%), low (<70%), and very low or negative (<2%).

RESULTS AND DISCUSSIONS

To identify an alternative source of mesenchymal stem cells (MSCs) in ewes, cells were isolated from the endometrium of a clinically healthy ewe. For cell isolation, enzymatic treatment and co-cultivation with residual tissue explants were chosen. Morphological examination and the degree of cell adhesion of the isolated cells were performed daily through microscopic examination. The degree of cell adhesion was examined in primary culture within the first 72 hours. At 24 hours after adding the cells derived from the ewe endometrium, moderate cellular heterogeneity was observed, with the average degree of attachment being 25% ± 5 (Figure 1).

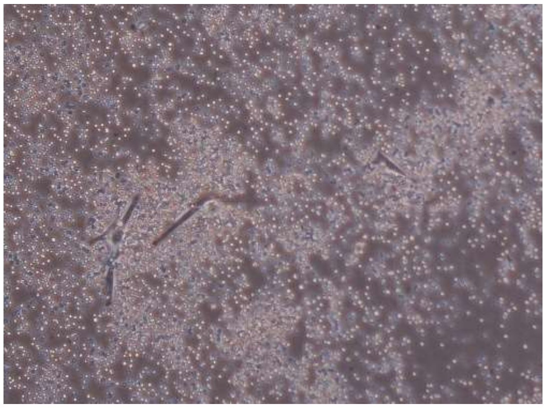


Figure 1. Heterogeneous cell population derived from ewe endometrial tissue, attached cells (24h) have a fusiform appearance (× 10) / Populația celulară heterogenă derivată din țesut endometrial de oaie, celulele atașate (24h) au aspect fuziform (× 10)

After 48 hours, the mean degree of cell attachment reached 53.3% ± 4.37, while after a period of 72 hours, it increased by almost 20% ± 2.4 compared to the previous day. To evaluate the clonogenic capacity of ewe endometrium-derived cells, the CFU-F test was conducted. Cells from passages 5 and 6 were assessed, during which cultures displayed a high degree of homogeneity, characterized by predominance of spindle-shaped cells and pronounced bipolarity. For CFU-F calculation purposes, cultures were fixed after 10 days of cultivation, stained with crystal violet, and microscopically evaluated (Figure 2). The presence of cells with fibroblastic morphology, grouped in cellular colonies (clusters), is observed. The nucleus occupies a large portion of the cellular diameter, predominantly located centrally.

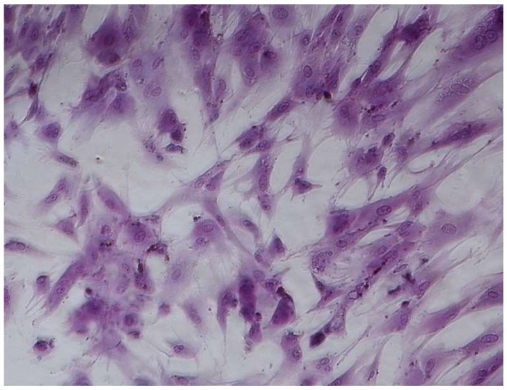


Figure 2. Representative photomicrographs - cellular clusters stained with crystal violet / Fotomicrografii reprezentative - clustere celulare colorate cu cristal violet

After evaluating the results, the average number of CFU-F was determined to be 31 ± 9.16 colonies/100 cm^2 at passage 5 and 37.66 ± 4.10 at passage 6 (Figure 3).

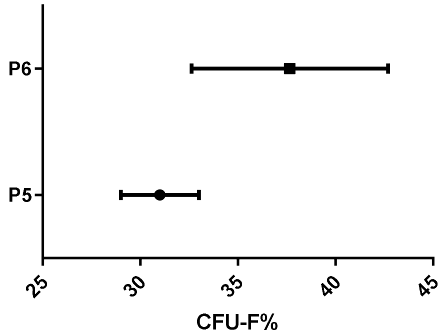


Figure 3. Results of the CFU-F test - eMSCs passages 5 and 6 / Rezultatele testului CFU-F - pasajele 5 și 6 ale eMSC

For the analysis of migration potential, sheep endometrial mesenchymal stem cells were initially aggregated in hanging drops, followed by their cultivation on gelatin-precoated plates. The migration capacity obtained for passage 5 demonstrated an average of 0.26 mm^2/24 h ± 0.05, with a range from 0.21 mm^2/24h ± 0.05 to 0.34 mm^2/24h ± 0.05. In passage 6, the average migration capacity was 0.277 mm^2/24h ±0.03, with limits ranging from 0.22 mm^2/24h ±0.03 to 0.31 mm^2/24h ± 0.03.

For evaluating the proliferation potential of cells derived from the mare's endometrium, the cell doubling time was calculated. In the case of the first passage, non-adherent cells exhibited a high percentage, which can be explained by the reduction in the number of attached round cells. The calculated cell doubling time averaged 4.16 ± 0.5. At passage 5, the doubling time showed stagnation (4.5 ± 0.2), while at passage 6, the recorded tD was 3.7 ± 0.1 (SE) (Figure 4). At passage 5, the culture was homogeneous, with spindle-shaped cells predominating with multiple extensions.

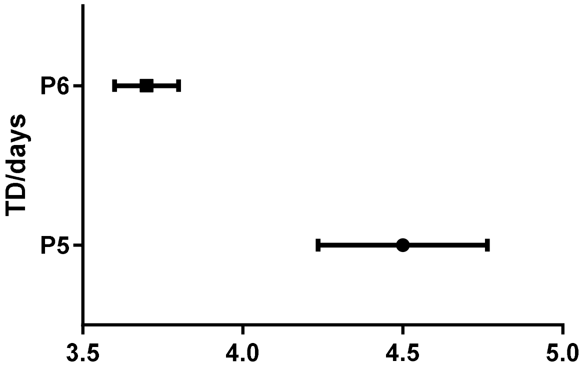
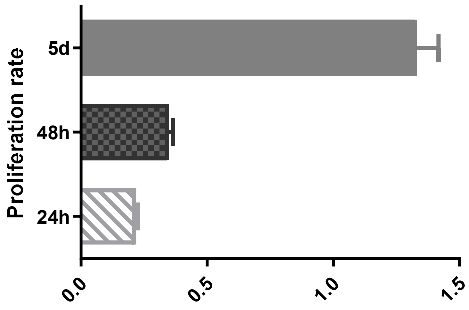


Figure 4. Cell doubling time evaluated in two separate passage / Timpul de dublare celulară evaluat în două pasaje separate

The proliferation rate of endometrial stem cells derived from ewe endometrium was assessed using the CCK8 colorimetric assay. After 24 hours, the recorded absorbance mean was 0.219±0.005, showing a slight increase after 48 hours to 0.347±0.01, and a significant growth recorded after 5 days with 1.332±0.08 (Figure 5). The differences were statistically significant.



ANOVA summary	
F	454.3
P value	< 0.0001
P value summary	****
Are differences among means statistically significant? (P < 0.05)	Yes
R square	0.9934

Figure 5 Results of the proliferation test – 24, 48 hours, and 5 days / Rezultatele testului de proliferare – 24, 48 de ore și 5 zile

Immunophenotypic analysis was conducted to assess the expression of cell markers CD44, CD90, CD105. Thus, the density of membrane markers within the sample was determined using linear algebra elements. Sample compensation was performed for fluorochromes PerCPCy5, ALP, FITC, and PE. Following evaluation, a positivity of 98.9% was obtained for the CD44 marker. Sample compensation was performed for the FITC fluorochrome. According to the International Society for Cellular Therapy, mesenchymal stem cells should express the surface marker CD44, and the results obtained are in line with the minimum requirements for defining multipotent stem cells (Dominici et al., 2006). Following the evaluation of cellular response, 99.2% of eMSCs express positivity for the surface marker CD90. PerCP Cy5 fluorochrome was used for sample compensation. The cellular population analyzed for the evaluation of the cellular response to CD105 (Endoglin) demonstrated a positivity rate of 95.7%. Our study confirms the mesenchymal nature of the stem cells isolated from the endometrium, as evidenced by their specific characteristics and the expression of cellular markers CD44, CD90, CD105. We postulate that mesenchymal stem cells play a role in tissue homeostasis, remodeling, and repair by replenishing lost mature cells resulting from physiological wear, senescence, or joint injury (Sekiya et al., 2012). The involvement of endometrial stem cells in the spontaneous repair of uterine lesions lays the foundation for exploring the role of endometrial mesenchymal stem cells in maintaining homeostasis in the female genital tract.Recent findings suggest that the endometrium possesses regenerative potential, largely attributed to the presence of these cells with unique capabilities. While most research on mesenchymal stem cell biology focuses on those derived from bone marrow, emerging evidence indicates the regenerative capacity of endometrial MSCs. Studies in rats with severe uterine lesions demonstrate the efficacy of mesenchymal cells in uterine regeneration, promoting microvascular restoration and facilitating subsequent pregnancies (Song et al., 2021). Due to their potential for self-renewal, multiple differentiation capacities, and unique immunomodulatory characteristics, MSCs represent promising agents for the regenerative therapy of various conditions such as ovarian, oviductal, uterine, tendinous, ligamentous, neurodegenerative processes, burns, wounds, etc. Of course, these require a team of well-trained practicing physicians, specialized equipment for the isolation and cultivation of stem cells, patients, trust, and a good relationship between the physician and the animal owner. This study contributes valuable insights into the potential applications of endometrial mesenchymal stem cells in regenerative medicine, highlighting their significance in tissue repair and regeneration across various physiological and pathological contexts.

CONCLUSIONS

The study demonstrates the robust clonogenic capacity, migration potential, and proliferative capability of stem cells isolated from ewe endometrium, indicating their promise for regenerative therapies. Immunophenotypic analysis confirmed the mesenchymal nature of these cells, validating their suitability for therapeutic applications. The regenerative potential of endometrial mesenchymal stem cells suggests their promising role in tissue repair, particularly within the female reproductive system. However, successful translation of stem cell-based therapies into clinical practice requires well-trained medical personnel, specialized equipment, patient cooperation, and effective communication between healthcare providers and animal owners. Overall, this study sheds light on the therapeutic potential of endometrial stem cells and underscores the importance of further research in regenerative medicine.

BIBLIOGRAPHICAL REFERENCES

1. Barret J.G.; A Set of Grand Challenges for Veterinary Regenerative Medicine; 2016; Front Vet Sci.; 3-20.
2. Dominici M., Le Blanc K., Mueller I., Slaper-Cortenbach I., Marini F., Krause D., Deans R., Keating A., Prockop Dj., Horwitz E.; Minimal criteria for defining multipotent mesenchymal stromal cells; 2006; The International Society for Cellular Therapy position statement. Cytotherapy. 2006;8(4):315-7.
3. Emmerson S.J., Gargett C.E.; Endometrial mesenchymal stem cells as a cell based therapy for pelvic organ prolapse; 2016; World J Stem Cells. 26;8(5):202-15.
4. Letouzey V., Tan K.S., Deane J.A., Ulrich D., Gurung S., Ong Y.R., Gargett C.E.; Isolation and characterisation of mesenchymal stem/stromal cells in the ovine endometrium; 2015; PLoS One. 18; 10(5):e0127531.
5. Polak Julia M., Mantalaris S.; Stem Cells Bioprocessing, An Important Milestone to Move Regenerative Medicine Research Into the Clinical Arena; 2008; 4-8.
6. Sekiya I., Larson B.L., Smith J.R., Pochampally R., Cui J.G., Prockop D.J.; Expansion of human adult stem cells from bone marrow stroma: conditions that maximize the yields of early progenitors and evaluate their quality; 2002; Stem cells, 20(6), 530-541.
7. Smith R.K.W., Korda M., Blunn G.W., Goodship A.E.; Isolation and implantation of autologous equine mesenchymal stem cells from bone marrow into the superficial digital flexor tendon as a potential novel treatment; 2003; Equine Veterinary Journal, vol. 35, no. 1, 99–102.
8. Song Y.T., Liu P.C., Tan J., Zou C.Y., Li Q.J., Li-Ling J., Xie H.Q.; Stem cell-based therapy for ameliorating intrauterine adhesion and endometrium injury; 2021; Stem Cell Res Ther. 30;12(1):556. doi: 10.1186/s13287-021-02620-2. PMID: 34717746; PMCID: PMC8557001.
9. Volk S.W., Theoret C.; Translating stem cell therapies: the role of companion animals in regenerative medicine; 2013; Wound Repair and Regeneration, vol. 21, no. 3, 382–394.

ASSESSMENT OF GROWTH INTENSITY AND PRODUCTION PERFORMANCE IN THE ROVASI SHEEP BREED

EVALUAREA INTENSITĂȚII DE CREȘTERE ȘI A PERFORMANȚELOR DE PRODUCȚIE LA RASA DE OVINE ROVASI

PASCAL CONSTANTIN[1], COSTICĂ CRISTIAN[2], BULMAGĂ DRAGOȘ[2]

[1] University of Life Sciences Iași, Faculty Food and Animal Sciences; Phone 0744863347;
[2]Research and Development Station for Sheep and Goat Breeding Secuieni-Bacău

Correspondence address: constantin.pascal@iuls.ro

Abstract

The biological material subjected to research was represented by the main categories of sheep undergoing growth and exploitation, forming the livestock of the ***Rovasi breed****. The working methods applied in the research were adequate and adapted to the specificity of this type of technical activities. For the evaluation and analysis of development degree, weight and conformation body measurements were performed for adult categories; to these aspects, some growth measurements were added for the youth categories. The analysis of growth intensity based on the data obtained from weighing at weaning indicates that the generation of* ***Rovasi*** *lambs obtained in the 2023 season has a higher live weight by 3.72% compared to the 2020 generation and by approximately 17% compared to the level recorded for* ***Tigaie*** *breed lambs. The statistical difference between the live weight of* ***Rovasi*** *lambs in 2023 and that of* ***Tigaie*** *lambs expressed in absolute values was +4.39 kg, being highly significant for $P \leq 0.01$. The evaluation of specific productivity level of the basic production indicates that the milk production obtained in one season is strongly influenced by the applied technologies; also, the degree of ensuring nutritional requirements capable of supporting a high level of productivity has a great influence. In the case of batch L3, the determined average production (274.26 kg) confirms that the new population of sheep has a superior lactogenic potential, much higher than that determined for other local breeds. The high values of variability, combined with the intensification of selection work, can contribute to increasing the productivity level, and based on productive control activities, valuable breeding stocks can be formed.*

Key-words: milk sheep, Rovasi, body condition, sheep

Rezumat

Materialul biologic supus cercetărilor a fost reprezentat de principalele categorii de ovine aflate în creștere și exploatare la SCDCOC Secuieni-Bacău, formând stocul viu al rasei ***Rovasi****. Metodele de lucru aplicate în cercetare au fost adecvate și adaptate pe specificul acestui gen de activități tehnice. Pentru evaluarea și analiza gradului de dezvoltare au fost efectuate cântăriri și măsurători corporale de masă și de conformație pentru categoriile adulte; la acestea s-au adăugat și unele măsurători de creștere efectuate la categoriile de tineret. Pe baza datelor obținute la cântăririle efectuate la înțărcare, analiza intensității de creștere indică faptul că generația de tineret ovin de rasă* ***Rovasi*** *obținută în sezonul 2023 are o greutate vie mai mare cu 3.72% față de generația din anul 2020 și cu aproximativ 17% față de nivelul înregistrat la mieii de rasă* ***Ţigaie****. Diferența statistică dintre greutatea vie a mieilor* ***Rovasi*** *din 2023 și cea a mieilor* ***Ţigaie****, exprimată în valori absolute, a fost de +4,39 kg și foarte semnificativă din punct de vedere statistic ($P \leq 0.01$). Evaluarea nivelului specific producției de bază, indică faptul că cantitatea de lapte obținută într-un sezon este puternic influențată de tehnologiile aplicate și de gradul de asigurare a cerințelor nutriționale. În cazul lotului L3 producția medie determinată a fost de 274,26 kg, confirmând faptul că noua populație de ovine are un potențial lactogen superior, mult peste cel determinat pentru alte rase locale. Valorile mari ale variabilității, coroborate cu intensificarea lucrărilor de selecție, pot contribui la creșterea nivelului productiv, iar pe baza activităților de control productiv se poate trece la constituirea de nuclee de elită.*

Cuvinte cheie: Rovasi, lapte, dezvoltare corporală, ovine

INTRODUCTION

The youth belonging to species of zootechnical interest must be subjected to growth technologies that highlight all their biological peculiarities. In order to assess the direction and manner in which body development occurs, periodic activities of analysis, control, and evaluation must be carried out. The purpose of these evaluations is particularly important because the determined average values provide valuable insights into how the growth process has unfolded, and the accumulated body mass increments certify whether the applied technology was adequate and contributed to harnessing the specific potential of the individual or population to which it belongs.

For adult categories, body development represents an internal environmental factor that exerts its influence on productive potential intensely and directly. Even though both live weight and milk production are considered to be more influenced by practicing a stimulating feeding level, there is a direct correlation between these two traits. Some studies conducted in this regard scientifically demonstrate that while sheep with a body weight of 35 kg can produce up to 2.75 kg of milk per unit of live weight, those weighing 60 kg produce only 1.7 kg per unit of live weight (Taftă, 1983). From these considerations, both characteristics represent important criteria for selection practiced in the case of sheep, regardless of their growth direction, as body weight has a significant influence on the level of milk production, although smaller than age.

Furthermore, in animals with a larger live mass, as body weight increases, so does the volume of the abdomen, and thus the capacity to ingest forages. In sheep, there is a very close correlation between abdominal volume and rumen volume ($r = 0.97$), but the correlation between body weight and milk production is much lower ($r = 0.34$), confirming the hypothesis that individual genetic basis has a stronger influence on the expression of this trait (Mohnacs et al., 1973). Many authors and researchers consider that in both small ruminant species, namely sheep and goats, body weight only has about a 10% influence on milk production, and its influence on fat content is very weak, but significant (Sandu, 1993; Taftă et al., 1998; Pascal, 2007).

MATERIAL AND METHOD

The biological material subjected to research was represented by the main categories of sheep comprising the livestock of the **Rovasi breed**, formed at SCDCOC Secuieni-Bacău. Since the research objectives were diverse and associated with various activities, all categories of young sheep from the current year and the previous year, as well as adult sheep forming the active breeding nucleus, were the subject of research in order to achieve the goals.

In order to obtain an accurate picture of the degree of body development, analyses, research, and determinations were carried out on all age categories, including rams, adult ewes, lambs, ram lambs, and neonatal sheep.

The working methods applied in the research were appropriate to the pre-established objectives. For the evaluation of the current situation and the determination of weight and degree of development, weighing and body measurements for mass and conformation were performed for adult categories, supplemented by some growth measurements for the young categories.

Live weight for the categories represented by adult ewes and the previous year's youth was determined by weighing immediately after shearing. Weighing was done with electronic scale-type balances that record minimal deviations (±100g).

The estimation of milk production for the suckling period was carried on using control weighing of lambs, performed at birth, at 28 days, at 56 days, and at weaning, using the transformation coefficient method.

Performance evaluation for milk production was based on the application of successive productive controls at 30-day intervals. At each control interval, the standard method was used, namely AT4, in accordance with the technical specifications recommended by the International Committee for Animal Recording and detailed by Tangorra et al., 2008.

At each applied control, the amount of milked milk from each female was determined by weighing using instrumentation with a precision of ±5 g, and the estimation of the total average milk production obtained over the lactation considered was carried on using *the method described by Fleischmann.*

$$\text{Milk production [kg]} = \mathrm{L}_1.\text{int}_1 + \sum_{i=2an} \left(\frac{\mathrm{L}_i + \mathrm{L}_{i-1}}{2}.\text{int}_i \right) + \mathrm{L}_n.14$$

where:

L1 = the milk quantity in the first control;
Li = the milk quantity in other monthly tests (i = 1,..., n);
Ln = the milk quantity in the last control;
int_1 = the number of days from the weaning to the first control;
int_i = the number of days between the monthly tests (i-1) and (i = 1,...,n);
n = the total number of monthly tests for a certain animal.

All the obtained data were evaluated and subjected to statistical processing using the **REML** (Restricted Maximum Likelihood) algorithm, which allows the analysis of statistical parameters within normal limits.

RESULTS AND DISCUSSIONS

1. Evaluating the body development in suckling lambs was a primary objective of the research. Its importance stems from the fact that correctly applied experimental techniques yield highly relevant data regarding the growth capacity of current-year ovine youth. The bodily development of lambs in the neonatal period is primarily dependent on how gestation progressed, with reference to meeting the specific nutritional requirements for pregnant ewes.

The body weight of lambs at birth is crucial in sheep farming, especially for meat production, aiming for lambs to have a higher birth weight. Recommended values typically fall between 4 and 5.5 kg in specialized literature. This recommendation is supported by the fact that lambs with such birth weights show higher daily increments during growth due to their higher consumption capacity, typically 3-5 times higher compared to underdeveloped lambs. Those born with weights below 2 - 2.5 kg may face a survival rate of only about 65% (Mochnacs et al., 1997).

Birth weight reflects fetal development throughout gestation and is considered an index of prenatal growth. This has been intensively studied with attempts to find mathematical terms for its optimization. Researchers have concluded that to obtain accurate information about maternal effects on lamb birth weight, research should be intensified both before and after birth (Stephenson and Lamborne, cited in Mochnacs et al., 1978).

During the research, birth weights were determined within the first two days after birth when lambs were individually identified. Delaying lamb weighing can decrease data accuracy due to heightened environmental influences, notably maternal milk production impacting the weight change in early postpartum periods.

To properly evaluate the dynamics of bodily development during the suckling period, separate weighing was conducted for females and males. Subsequently, the data were centralized and processed separately by sex and total number of individuals obtained in that particular lambing season, as will be synthetically presented further.

Table 1 provides a summary of how body weight evolved for male lambs. Intermediate weighing was conducted at 20 days, at 56 days, and weaning (90 days), to better assess maternal lactogenic capacity.

Birth weight was determined by individually weighing lambs born in the lambing season in 2023. Lambs were weighed within the first 24 hours after birth, after the drying of their wool and gaining their physical stability. High-precision decimal instrumentation was used for weighing, following accepted scientific research techniques.

After the lambing season ended, the obtained data were subjected to statistical processing to compare differences and highlight their statistical significance. Since the primary objective of the research is to develop improved technologies for enhancing the productive performance of the new sheep population formed at SCDCOC Secuieni-Bacău, with direct impact on milk production, it was deemed unnecessary to evaluate body development in suckling lambs by sex.

However, to highlight how specific parameters of the suckling period have changed, data from this year were compared with those from 2020 and with those obtained by analyzing the same traits in lambs obtained in the same season, but from the Romanian **Tigaie** rusty variety breed.

At birth, it can be observed that the specific body weight data are relatively close in value. Nevertheless, the differences recorded between birth weights in the **Tigaie** lamb group and those in the new population from this year's mating season had significant statistical significance at the $p \leq 0.01$ level. In practice, this significance is due to the fact that lambs belonging to the new milk type formed in Bacău had a higher live weight at birth by 12.11% compared to the value determined for lambs belonging to the rusty variety of the **Tigaie** breed.

2. Analyzing live weights at 28 and 56 days is extremely useful not only to accurately analyze the growth intensity of youth in the first part of the suckling period, but also to more precisely determine the amount of milk consumed by lambs during this time interval.

Just like in other situations, differences are recorded between the batches of lambs in this case as well. At age of 28 days, the body weight of lambs from the new dairy breed was superior by 5.74% compared to the average value determined for lambs of the same population, though conducted in the 2020 season. However, compared to lambs belonging to the **Tigaie** breed, that characteristic had a lower value by 4.32%. Based on these data, it can be concluded that the main factor contributing to these differences was the lactogenic capacity of the ewes. Similar data are recorded in the specialized literature, where lambs of the **Tigaie** or **Țurcană** breed have recorded higher values of live weight during the birth-weaning interval. Thus, in a study conducted in our country, differences were reported between the live weight of **Tigaie** breed lambs compared to lambs belonging to the **Palas Merino breed** (Călin, 2012; Pădeanu, 2012; Pascal, 2015; Taftă et al., 1996).

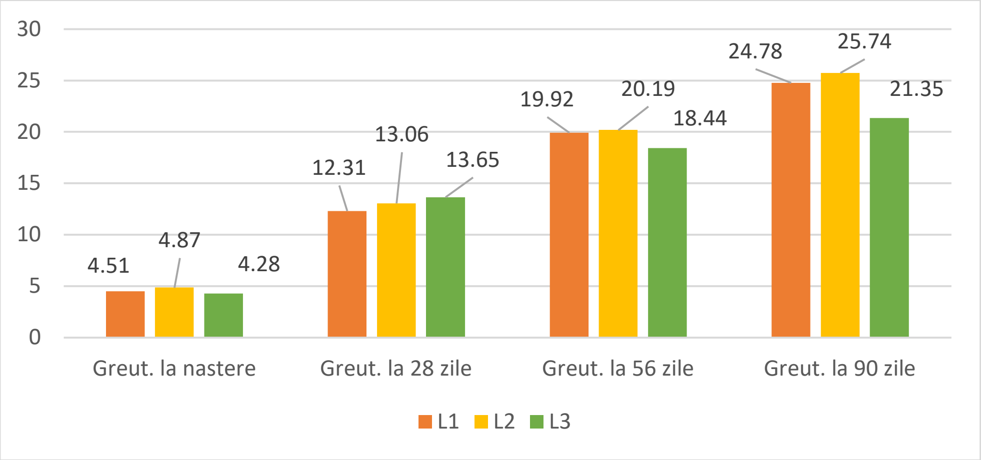
Table 1. The analysis of body condition evolution in suckling lambs / Analiza evoluției greutății corporale la mieii aflați în perioada de alăptare

The analysed character	ROVASI				Tigaie breed, brown variety (L3)		Difference L2-L3	
	2016 (L1)		2020 (L2)				±	The difference of significance
	$\overline{X}$	$\pm s_{\overline{X}}$	$\overline{X}$	$\pm s_{\overline{X}}$	$\overline{X}$	$\pm s_{\overline{X}}$		
The body mass at birth (kg)	4.51	0.14	4.87	0.15	4.28	0.11	+0.59	**
The body mass at 28 days (kg)	12.31	0.21	13.06	0.22	13.65	0.14	-0.59	*
The body mass at 56 days (kg)	19.92	0.29	20.19	0.41	18.44	0.22	+1.75	***
The body mass at weaning (90 days)	24.78	0.65	25.74	0.32	21.35	7.25	+4.39	***

The evaluation of live weight determined at the age of 56 days indicates the existence of differences between batches. The average value of live weight determined for the two batches of lambs belonging to the **Rovasi breed**, assessed in the seasons of the year 2020 and 2023 respectively, is extremely close and without any statistical significance. On the other hand, analyzing the differences regarding the average body weight determined for the lambs of the new population in the 2024 season, compared to the live weight recorded in the batch consisting of **Tigaie** breed lambs, the absolute difference was +1.75 kg and was very significant for $p \leq 0.01$.

The assessments made based on the analysis of the recorded data from weighing the lambs at 90 days of age confirm the higher growth rate of lambs from the new dairy population; this fact is obvious from the dynamics of live weight determined at various intervals throughout the entire suckling period depicted in Figure 1.

In the batch consisting of lambs from the new population, following the conducted weighing, the average weights were approximately 17% higher compared to the level recorded for **Tigaie** breed lambs. The absolute difference between these batches was +4.39 kg and highly significant for $p \leq 0.01$.



Note: *L1= youth sheep* ***Rovasi*** *of the generation 2020; L2= youth sheep* ***Rovasi*** *of the generation 2023;*
L3= youth sheep ***Rovasi*** *of the* ***Tigaie breed***

Figure 1. The dynamics of living weight in suckling lambs (kg) / Dinamica evoluției greutății vii la mieii aflați în perioada de alăptare (kg)

Typically, under farm conditions, most sheep breeds raised for milk production wean their lambs at around the age of 70 days, except for those retained for replacement, which are weaned at 90 days of age. This explains why the research team chose to analyze the values recorded at these moments to better characterize the new sheep population.

Analyzing the growth intensity of lambs during the suckling period is an important indicator that influences the growth rate and body development in subsequent periods. In this case, the most conclusive data for analysis and evaluation are provided by the average values determined for the daily weight gain over certain periods of time or growth and fattening phases.

Considering the complex nature of the research and the technical specifications outlined in the implementation plan, this indicator, namely body weight, was analyzed over the three periods corresponding to the duration of lamb suckling. Statistical processing of the data confirms the existence of differences between the average values determined for each of the three batches, and Table 2 provides a summary of these statistical data, with differences between batches easily identifiable from the graphical representation of the mean values in Figure 2.

For the first 28 days corresponding to the suckling period, the average daily gain registers a higher value on average for lambs of the **Tigaie breed**, which had an average daily gain of 333.09 ± 0.035 g. This value was approximately 43 g higher compared to the average value determined for the same trait but for lambs belonging to the new dairy population obtained in the current year, with the difference between batches being significant for the statistical threshold considered.

Table 2. The analysis of average daily gain evolution in suckling lambs / Analiza evoluției sporului mediu zilnic de creștere la mieii aflați în perioada de alăptare

The analyzed character	ROVASI BREED				Tigaie breed, brown variety (L3)		Difference L2-L3	
	2020 (L1)		2023 (L2)				±	The difference of significance
	$\bar{X}$	$\pm s_{\bar{x}}$	$\bar{X}$	$\pm s_{\bar{x}}$	$\bar{X}$	$\pm s_{\bar{x}}$		
Average daily gain between 0-28 days (g/zi)	280.2	0.47	290.02	0.61	333.09	0.35	-0.43	*
Average daily gain between 28-56 days (g/zi)	271.2	0.65	250.12	0.24	171.03	0.25	+79.09	**
Average daily gain between birth and weaning (g/cap)	225.2	0.15	231.8	0.37	189.66	0.28	+42.14	*
Average daily gain on the whole suckling period (kg/cap)	20.27	0.87	20.87	0.18	17.07	0.05	+3.80	***

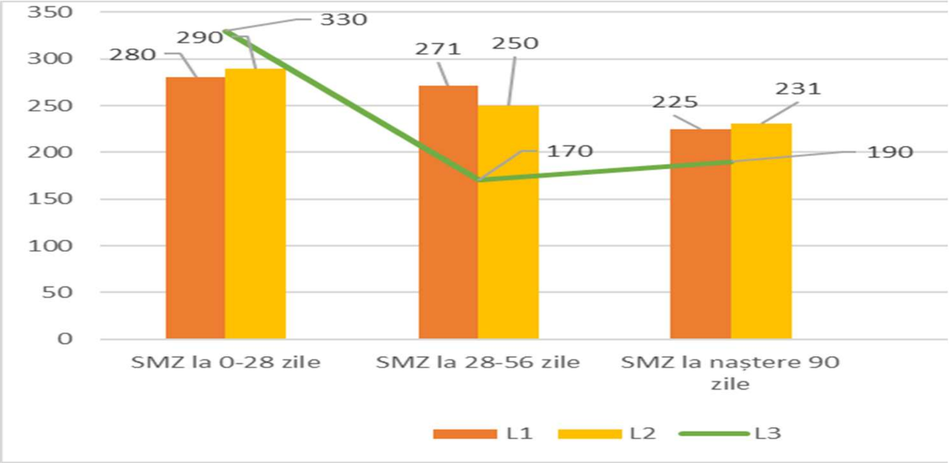


Figure 2. The dynamics of average daily gain evolution in suckling lambs (g/day) / Dinamica evoluției sporului mediu zilnic la mieii aflați în perioada de alăptare (g/zi)

3. ***Conducting growth measurements*** aims to highlight the growth intensity of a body area or the whole animal and is performed at different age periods. In the case of sheep and goats, to characterize growth intensity, it is recommended that these measurements be performed at specific age periods, typically at 3, 6, 12, and 18 months, and then at 2 and 3 years, with the specification that after one year old, all determinations will be made only after shearing.

Table 3. The body measurements determined in lambs at weaning (cm) / Dimensiunile corporale determinate la momentul înțărcării mieilor (cm)

Specification	**ROVASI** (L2)		**The Tigaie breed,** Brown variety (L3)	
	$\bar{X}$	$\pm s_{\bar{x}}$	$\bar{X}$	$\pm s_{\bar{x}}$
The trunk weight	61.07	1.55	55.04	1.87
The trunk length	64.0	1.74	59.0	2.17
The trunk width between shoulders	14.8	0.52	14.4	0.92
The trunk width at the last rib	18.4	0.91	17.4	1.6
The croup width at the coxal-femural joint	11.8	0.48	12.1	0.83
The perimeter of the gizzard in the upper third	32.1	0.54	30.8	0.66
The internal length of the muscle between the hock and perineum	20.3	0.73	21.8	0.96
The circumference of the hock	9.6	0.25	8.4	0.24

Typically, measurements are taken to determine body heights, lengths, widths, depths, and circumferences. Throughout the research conducted in this stage, the most important measurements specific to the lambs' growth period were taken (the statistically processed data are presented in Table 3). From the analysis of these measurements, it is observed that lambs from the new sheep population had a superior growth rate and, also, a better intensity of body development.

Figure 3. Rovasi lambs at weaning / Mieii de rasă Rovasi aflați la momentul înțărcării

Regarding height and trunk length, it can be observed that lambs from both batches have a rectangular body shape because, in both cases, the length has higher average values compared to trunk height. However, the fact that both batches have average values close to 60 cm for both traits indicate very good body development from the early stages of life.

Overall, in the case of conducting all specific body measurements during the growth period, it can be said that lambs belonging to the new population had higher average values for all categories of body measurements, indicating better growth intensity and achieving a superior level of body development.

4. Evaluation of quantitative milk production during the suckling period was done through control weighing of the lambs conducted at birth, after 56 days, and at weaning (90 days), and based on these data, the average quantity of milk consumed by the lambs during that time interval was calculated.

Typically, the quantity of milk consumed by lambs is estimated through methods that may have some degree of subjectivity. Therefore, in scientific research, certain values of the amount of milk needed to achieve one kilogram of body weight are established and followed. Moreover, for more objectivity, the respective value has different levels depending on the age of the lambs at the time of assessment. This is necessary because in the early part of the suckling period, lambs accumulate body mass primarily based on the intake of maternal milk.

Estimating milk production during the suckling period was done through control weighing of the lamb at birth, after 28 days, and at weaning. Regarding this aspect, out an international level, in order to assess the quantity of milk consumed by lambs in the first 56 days, it is considered that a lamb needs a total milk quantity of 3.0 kg to achieve one kilogram of body weight gain. At an older age, the requirement for achieving 1 kg of weight gain decreases to 2.0 kg of milk.

After weaning, and based on the statistical processing of the values obtained from the control weighing applied to the suckling lambs, the total quantity of milk consumed by the lambs in this time interval was determined.

In line with the results obtained regarding the total quantity of milk sucked, very significant differences are observed between the two batches of adult ewes. Additionally, the statistical processing of the data highlights the fact that ewes from the new sheep population have a higher suckling capacity compared to the local **Tigaie breed** (Table 4). Due to this fact, lambs belonging to the new sheep population had a greater suckling capacity by 18.35%, and at the time of weaning, the average body weight was higher by over 11 kg. Understanding these aspects is of significant practical importance both biologically and economically.

Table 4. The milk production obtained in suckling period (kg) / Producția de lapte obținută în perioada de alăptare (kg) (n=60)

Genotype	The quantity of milk based on the age interval				The difference	
	at 56 days		for the suckling period			
	$\overline{X} \pm s_{\overline{X}}$	V %	$\overline{X} \pm s_{\overline{X}}$	V %	Absolute (±)	Its significance
The Tigaie breed lambs	48.65±0.36	11.21	51.12±0.23	8.58	+11.49	***
The lambs from the new population	52.35±0.30	12.33	62.61±0.54	6.96		

The biological importance stems from the fact that during the first 15-20 days of a lamb's life, milk represents its exclusive source of nutrition and possesses remarkable toning, immunological, and nutritional qualities. Colostrum, due to its rich content of fats, proteins, immunoglobulins, vitamins, salts etc., plays a crucial role in the lamb's subsequent development and in enhancing organic resistance.

The economic importance is determined by the value of milk as a commodity production, as well as its influence on the future individual's meat and wool production. Therefore, conducting milk production control aims primarily at identifying good milk-producing ewes, which will subsequently constitute future breeding nuclei to achieve offspring with superior productivity compared to the paternal forms from which they originate.

In summary, it can be said that lambs from the new sheep population, benefiting from a higher quantity of maternal milk, recorded a more intense rate of total gain accumulated between birth and weaning.

Evaluating milk production based on the conducted controls represented an important activity, as this production plays both a biological and economic role, and in our country's conditions, sheep are primarily raised for milk production. In a farm setting, the size of milk commodity production largely depends on the efficiency of sheep rearing for milk production, given that this production is primary while others (meat, wool) are secondary.

There are multiple methods for estimating milk production from sheep, some of which have a high degree of subjectivity. However, regardless of the accuracy level specific to each method, each is inseparably linked to sheep rearing activity. In the Romanian sheep farming community, several methods for evaluating milk commodity production are encountered, such as based on the quantity of milked milk or based on the quantity of semi-matured cheese resulting from milk processing. Both procedures are used in traditional sheep farming. However, considering the destination and the necessary accuracy regarding the average values of milk production obtained from this new sheep population, recognized methods of scientific research techniques were applied.

Regarding this aspect, several milk production controls were scheduled at 30-day intervals over the specific lactation period of this year. The table below presents the average values, minimum and maximum limits, and variability recorded for the ewes subjected to the first productivity control (Table 5).

To enhance the value of reference data, performance controls were applied to three herds located in Bacău County, for which the experimental treatment consisted of local natural conditions and factors. Regarding the technology applied during the period in which the sheep were subjected to performance control, it was traditional, with elements reflecting the specific microclimate conditions of hill and sub-mountainous areas.

Table 5. Average values of milk production at the first control (kg/day) / Valorile medii a producției de lapte la controlul 1 (kg/zi)

Specification		**Batches**		
		L1	**L2**	**L3**
n		60	60	60
$\overline{X}$		0.938	1.032	1.113
$\pm s_{\overline{X}}$		0.025	0.063	0.053
V %		15.1096	33.867	26.526
s		0.141	0.349	0.295
Limits	**Minimum**	0.72	0.55	0.66
	Maximum	1.1	1.57	1.57

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.456727	2	0.228363	2.984867	0.055748	3.101296
Within Groups	6.656113	87	0.076507			
Total	7.11284	89				

Statistical data processing confirms the existence of differences that also acquire a certain statistical significance through the application of the ANOVA test. It can be observed that the highest quantity of milk obtained on the control day was from batch coded L3, consisting of sheep under rearing and management at the Research and Development Station for Sheep and Goats in Secuieni-Bacău. The performance of this batch determined on the day of the first control was superior by 7.27% compared to the level reached by the sheep in L2 and by 8.43% compared to the production on the control day resulting from L1.

On the other hand, the batch from which the most quantity of milk was obtained has a more limited variability for this characteristic in terms of the extent of the mean values. Overall, it can be said that even though the conditions and technological factors were different, the quantity of milk obtained on the day planned for the first control has high values for each of the three analyzed batches.

After an interval of 30 days, the second control of productive performances was scheduled. To eliminate deviations, the sheep that provided a quantity of milk less than 0.2 kg on that day were removed. This requirement is in accordance with the methodological specifications regarding this aspect imposed by the International Committee for Animal Recording.

Table 6. Average values of milk production at the second control (kg/day) / Valorile medii ale producției de lapte la controlul 2 (kg/zi)

Specification		**Batches**		
		L1	**L2**	**L3**
n		50	50	50
$\overline{X}$		0.540	0.911	0.986
$\pm s_{\overline{X}}$		0.018	0.064	0.056
V %		18.498	38.840	31.226
s		0.101	0.353	0.306
Limits	**Minimum**	0.39	0.45	0.52
	Maximum	0.71	1.42	1.42

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3.296687	2	1.648343	21.65165	2.34E-08	3.101296
Within Groups	6.623323	87	0.07613			
Total	9.92001	89				

Figure 4. The control of milk production on the scheduled day / Efectuarea controlului prin mulsul oilor în ziua planificată

Statistical processing of raw data highlights a decrease in the quantity of milk from the control day for each batch. The greatest decrease was approximately 0.4 kg of milk and was recorded in the case of batch L1, which belonged to a farmer in this county.

Detailing the data obtained at the second control, we can observe that the highest level from the control day was still at batch L3. At this control, the performance of this batch was superior by over 40% compared to batch L1. One explanation for this difference could be that batch L3 was in an area more affected by this year's drought, and consequently, the decrease in the quantity and quality of pasture grass influenced the level reached at that control.

At the end of August, the third control (C3) was scheduled. In this month, the effects of the drought become evident. Practically, the drought and early heat have affected not only tens of hectares of crops but also large areas of pasture and hayfields in Bacău County. Obviously, against this background, by reducing the quantity of grass and also its modifications regarding nutritional value, the sheep reacted by decreasing the quantity of milk.

On the day of the third control, it is observed that in the batches located in hill areas, the quantities of milk were higher on the control day, being 0.750±0.054 kg at the sheep stock located at Secuieni-Bacău Station, and also 0.670±0.064 kg of milk at the nominated nucleus from the herd of a farmer located near the pre-mountainous area.

Table 7. The values of milk production at the third control (kg/day) / Valorile medii ale producției de lapte la controlul 3 (kg/zi)

Specification		**Batches**		
		L1	**L2**	**L3**
n		30	30	30
$\overline{X}$		0.46	0.67	0.75
$\pm s_{\overline{X}}$		0.032	0.064	0.054
V %		38.571	52.517	39.297
s		0.176399	0.349588	0.29368
Limits	**Minimum**	0.22	0.21	0.24
	Maximum	0.75	1.23	1.23

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.341722	2	0.670861	8.400598	0.000462	3.101296
Within Groups	6.94771	87	0.079859			
Total	8.289432	89				

However, in the case of the performance determined for L1, it can be observed that the average value of this characteristic is 31.3% lower compared to that determined for L2 and 38.66% lower compared to the performance on the control day determined for L3.

The last production control was scheduled for the last decade of September. The effects generated by the drought intensify, and the high temperatures limit the time in which the sheep can consume food from pasture. We did not intend to establish at a concrete level what its effect is because it can be observed that this phenomenon has become a constant presence every year. Against this background, we believe that research is needed to identify how climate change affects the production and reproduction capacity of farm animals.

As can be seen from Table 7, statistical data for milk production obtained at this control are missing for L1. The explanation is that the production level has decreased significantly in all the controlled sheep, and the farmer has decided to prepare them for a new breeding season.

In the case of the L2 and L3 batches, the productions are acceptable, being higher than 0.5 kg. The owners of these batches intervened in the feeding of the sheep by administering a supplement consisting of a mixture of bran and wheat bran in quantities of approximately 0.3 kg per individual and per day.

To determine the total milk production, the AT4 estimation method was used, conducting four controls during lactation according to the methodology. On control days, the quantity of milk was determined both in the morning and in the evening. The method used to determine the quantity of milk was gravimetric, using an electronic scale and standard milking cups. The interval between control days was established so as to comply with the official methodology recommended for the application of the milk production center for sheep.

Table 7. The average values of milk production in the fourth day (kg/day) / Valorile medii ale producției de lapte la controlul 4 (kg/zi)

Specification	**Batches**		
	L1	**L2**	**L3**
n	-	30	30
$\overline{X}$	-	0.51	0.56
$\pm s_{\overline{X}}$	-	0.049	0.042
V %	-	52.979	40.686
s	-	0.270	0.231

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.053402	1	0.053402	0.84278	0.362405	4.006873
Within Groups	3.675097	58	0.063364			
Total	3.728498	59				

Table 8. The average values of milk production per lactation (kg/lactation) ⁄ Valorile medii ale producției de lapte pe lactație (kg/lactație)

Specification		**Batches**		
		L1	**L2**	**L3**
n		30	30	30
$\overline{X}$		136.95	173.78	188.84
$\pm s_{\overline{X}}$		3.563	12.297	10.480
V %		14.250	38.759	30.396
s		19.514	67.354	57.399
Limits	**Minimum**	108.75	85.785	106.89
	Maximum	173.01	274.26	274.26

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	42763.81	2	21381.91	7.811226	0.000759	3.101296
Within Groups	238147.8	87	2737.331			
Total	280911.6	89				

After completing the specific activities for applying the last milk production control for the current years' lactation, the data were subjected to statistical processing. Based on the obtained values, differences between the three batches can be observed, ensured by the statistical thresholds considered.

The highest quantity of milk was obtained from the sheep forming batch L3. The level determined for this batch was at an average of 274.26±10.84 kg of milk. At this production level, it can be stated that the new population of sheep has a superior lactogenic potential, far exceeding that determined for other local breeds. The high variability values allow for the intensification of selection work, based on performance control and the establishment of elite stocks.

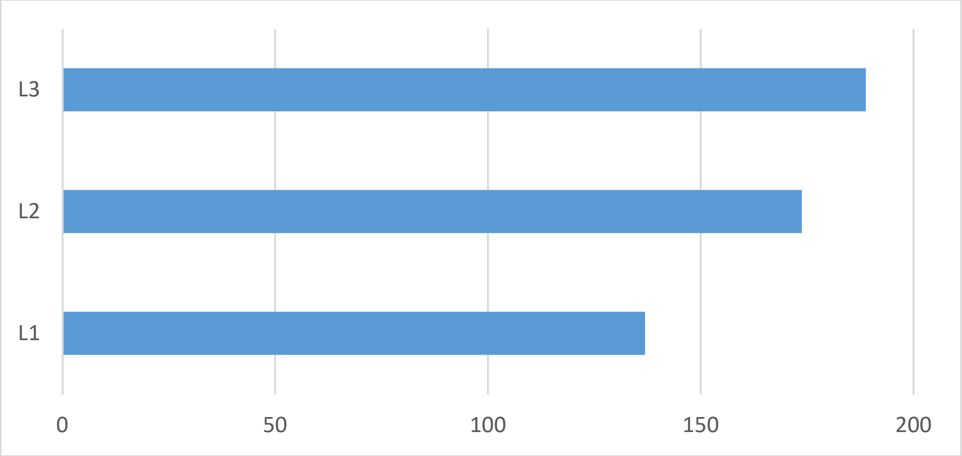


Figure 5. The representation of milk production of researched batches (kg) / Reprezentarea grafică a producției de lapte obținută la loturile supuse cercetării (kg)

The differences between the batches are easier to observe from the graphical representation of the levels of this characteristic determined for each of the batches that were the subject of research in this stage.

Figure 6. The mammary gland development in the ewes from the new population / Dezvoltarea glandei mamare la oile din noua populație

It's important to emphasize once again that this activity will be extened into the following lactation periods, which coincide with the length of funding and implementation of these research activities. This ongoing effort ensures that we will ultimately achieve the most accurate characterization possible of the lactogenic capacity and specific productive potential of this sheep population, which has developed and adapted to the specific conditions of the Central Moldavian Plateau.

CONCLUSIONS

Regarding the birth, the results obtained highlight that the average values for this trait exhibit certain differences with different statistical significances.

1.1. The differences recorded between birth weights in the **Tigaie** lamb batch and that of the new population from this years' breeding season were statistically significant for the threshold $p \leq 0.01$.

1.2. Essentially, this significance is due to the fact that at birth, lambs belonging to the new dairy type developed in Bacău had a higher live weight by 12.11% compared to the value determined for lambs of the rust variety within **the Tigaie breed**.

At 28 days of age, the body weight of lambs from the new dairy population was superior by 5.74% compared to the average value determined for lambs of the same population, though conducted in the 2016 season. However, compared to lambs belonging to **the Tigaie breed**, that characteristic had a lower value by 4.32%.

2.1. Based on these data, it can be concluded that the main factor contributing to these differences was the lactogenic capacity of the ewes.

In the batch consisting of lambs from the new population, following weighing conducted in the current year, the average weights were approximately 17% higher compared to the level recorded for **Tigaie breed** lambs.

3.1. Between these batches, the absolute difference expressed was +4.39kg and was highly significant for $p \leq 0.01$.

Evaluating the growth intensity of lambs during the suckling period is an important indicator that influences the rate of growth and body development in subsequent periods.

4.1. For the first 28 days corresponding to the suckling period, the average daily gain registers a higher value on average for lambs of **the Tigaie breed**, which had an average daily gain of 333.09 ± 0.035 g.

4.2. This average value was approximately 43 g higher compared to the average value determined for the same trait for lambs belonging to the new dairy population obtained in the current year, with the difference between batches being significant for the statistical threshold considered.

4.3. Upon weighing at the age of 56 days, it is observed that the average daily gain realized over the 28-day and 56-day intervals was higher by 79 g compared to that determined for **Tigaie breed** lambs, and statistically, this difference was distinctly significant for $p \leq 0.01$.

4.4. Regarding the total mass accumulated by the lambs from the constituted batches, there are different values; the analysis of this trait shows that over the 90-day suckling period, lambs from the new population had a higher average value by 2.87 g compared to lambs from the same population obtained during the lambing season of 2016.

4.5. Compared to the batch consisting of **Tigaie breed** lambs in 2020, lambs from the new ovine population recorded a higher total growth of 3.80 kg during the same period, representing a superior average value by approximately 18.20%.

4.6. The difference between these average values was highly significant for the statistical threshold considered.

The height and length of the trunk confirm that the lambs from both batches have a rectangular body shape due to the fact that, in both cases, the length has higher average values compared to the height of the trunk.

5.1. However, the fact that both batches have average values close to 60 cm for both traits indicates very good body development from the early stages of life.

The highest amount of milk was obtained from the ewes forming batch L3. The level determined for this batch was on average 188.84 ± 10.84 kg of milk.

ACKONWLEDGMENTS

The research was financed by The Minister of Agriculture and Rural Development through the research programs ADER Sectoral Plan (the projects' code is 817).

BIBLIOGRAPHICAL REFFERENCES

1. Abdullah M.B. and Tabbaa J.M.; Comparison of body weight and dimensions at birth and weaning among Awassi and Chios sheep breeds and their crosses; 2011; Jordan Journal of Agricultural Sciences, Volume 7, No.4.
2. Atanasiu T., Pascal C., Iftimie N., Cristian C., Crăciun Otilia; *S*tudy performance of hybrid sheep obtained from crossing of **Awassi** rams with ewe **Tigaie**; 2010; Lucrari Ştiinţifice-Seria Zootehnie, Electronic ISSN 2067-2330, Print ISSN 1454-7368, p 427-430.
3. Călin I., Pădeanu I., Pascal C., Dărăban S.V.; Results concerning the fattening of half breeds stemming from the crossing of **Ţurcana bred** with **Ile de France breed**; 2010; Vol Lucrări Ştiinţifice, Seria D. Vol LIII, USAMV Bucureşti, p 454-460.
4. De La Fuente F.L., Gabina D., Carolino N., Ugarte E.; **The Awassi** and **Assaf** breeds in Spain and Portugal; 2006; Book of Abstracts of the 57-th Annual Meeting of the European Association for Animal Production (EAAP). Book of abstracts, No 12(2006), 17-20 September, Antalya, Turkey.
5. Epstein H.; **The Awassi** sheep with special reference to the improved dairy type; 1985; FAO Animal production and health paper 57. Rome, Italy.
6. Pădeanu I., Voia S., Gavojdian D., Pascal C., Olaru N., Frăţilă I.; Researches regarding growth speed in crossbreeds lambs **Lacaune** x **Tigaie** reared in the westen part of Romania; 2010; Lucrari Ştiinţifice-Seria Zootehnie, Electronic ISSN 2067-2330, Print ISSN 1454-7368, p 431-433.
7. Pacinovski N., Elena Eftimova, Gievski M.; Comparison of milk production ability between **Awassi** and **East-Friesian** sheep in Macedonia; 2007; Proceedings of the III Symposium of livestock production with international participation, p 65-72.
8. Pascal C., Cristian C.; The evaluation of milk production for the **Tigaie breed** which is raised in different traditional parts of Romania; 2014; Lucrări Ştiinţifice, Seria Zootehnie, ISSN L 1454-7368, vol 61, 2014, p 3-10.
9. Pascal C.; Tratat de creștere a ovinelor și caprinelor; 2015; Ion Ionescu de la Brad Publ., Iași, Romania, ISBN 978-973-147-196-9, p 325.
10. Pascal C.; Creşterea ovinelor şi caprinelor; 2007; PIM Publ., Iaşi. ISBN 978-973-716-502-2.
11. Taftă V., Vintilă I., Zamfirescu S.; Producția, ameliorarea și reproducția ovinelor; 1996; Ceres Publ. Bucharest-Romania. ISBN 973-40-0408-5.
12. http://statistici.insse.ro/shop/index.jsp?page=tempo3&lang=ro&ind=AGR202A. Accessed on 12/02/2016.
13. http://www.madr.ro/cresterea-animalelor/ovine-si-caprine.html. Accessed on 12/02/2016.

RESEARCH ON THE OPTIMIZATION OF FISH FEEDING STRATEGY IN DIFFERENT TYPES OF PRODUCTION SYSTEMS USING ALTERNATIVE, LESS USED AND SUSTAINABLE FEED ADDITIVES AND FEEDS

CERCETĂRI PRIVIND OPTIMIZAREA STRATEGIEI DE HRĂNIRE A PEȘTILOR ÎN DIFERITE SISTEME DE PRODUCȚIE FOLOSIND ADITIVI ȘI FURAJE ALTERNATIVE MAI PUȚIN UTILIZATE ȘI SUSTENABILE

RADU DANIELA, RADU SILVIA, MARICA NINO, COSTACHE MIOARA, BARBU ALIN, ARCADE MARIANA CRISTINA, GANCEA MARINELA

Fish Culture Research and Development Station Nucet, Romania 549 Principala, 137335, Nucet, Romania, scp_nucet@yahoo.com

Correspondence address: scdp.nucet@asas.ro

Abstract

Fish feed accounts for more than 50% of the total cost of production in intensive aquaculture. Feeding fish with poor quality feed or adopting inappropriate feeding strategies not only causes food wastage and subsequent loss of income, but also leads to water pollution. Feed additives are considered an essential component in fish diets. Additives are substances that are added in small amounts to improve the nutrient content and extend the shelf life of the forage administered in the feed. The addition of additives to fish feed is essential in stimulating growth and supporting fish immunity. Among the additives frequently used in aquaculture are: preservatives, binders, feeding stimulants and food colorings. Optimizing the feeding strategy of fish reared in different types of production systems can be achieved by finding alternative feed sources and/or by adding additives to feed recipes. Ingredients already used as alternatives include soybean, barley, rice, peas, lupine, canola, wheat gluten, corn gluten, various other plant proteins, yeast, insects and algae.

Keywords: additives, alternative feed sources, fish feeding, optimization

Rezumat

Furajele pentru pești reprezintă mai mult de 50% din costul total de producție în acvacultura intensivă. Hrănirea peștilor cu hrană de proastă calitate sau adoptarea de strategii de hrănire inadecvate nu numai că provoacă risipa de alimente și pierderea ulterioară a veniturilor, dar duce și la poluarea apei. Aditivii furajeri sunt considerați o componentă esențială în dietele pentru pești. Aditivii sunt substanțe care sunt adăugate în cantități mici pentru a îmbunătăți conținutul nutrienților și pentru a prelungi durata de valabilitate a furajului administrat în hrană. Adăugarea de aditivi în hrana peștilor este esențială în stimularea creșterii și pentru a susține imunitatea peștilor. Printre aditivii folosiți frecvent în acvacultură se numără: conservanții, lianții, stimulentele alimentare și coloranții alimentari. Optimizarea strategiei de hrănire a peștilor crescuți în diferite tipuri de sisteme de producție se poate realiza prin găsirea de surse alternative de hrană și/sau prin adăugarea de aditivi la rețetele de furaje. Ingredientele folosite deja ca alternative includ soia, orzul, orezul, mazărea, lupinul, rapița, glutenul de grâu, glutenul de porumb, diverse alte proteine vegetale, drojdia, insectele și algele.

Cuvinte cheie: aditivi, surse alternative de furaj, hrănirea peștilor, optimizare

INTRODUCTION

In aquaculture, nutrition plays a key role mainly due to feed cost accounting for approximately 50% of production costs. Most aquaculture production is carried out in semi-intensive and intensive farming systems, which requires a considerable input of fertilizers and feed. Therefore, fish feeding and feeding issues have become some of the most important research concerns in the aquaculture sector. The administration in fish feeding of inadequate quality feed from a quantitative and qualitative point of view, as well as the adoption of inadequate feeding strategies produce not only food waste and pollution of the culture environment, but also the subsequent loss of income.

In order to ensure sustainable growth, the aquaculture industry needs to improve the sustainability of the feed administered. The current trend aims to improve fish feed recipes by replacing fishmeal (which constitutes a main source of protein) with other alternative sources such as: protein concentrates with a 90% protein content (casein, gelatin), krill meal, poultry by-product meal, blood meal or meat and bone meal from animal slaughterhouses, insect meal or other plant-based protein ingredients (soybean, pea, rapeseed etc.) because they are cheaper compared to fish meal.

According to the Strategic Plan for the development of the Aquaculture and Fishing sector in our country, optimizing the technology of intensive growth of fish with economic value is a priority. Optimizing production in fish farms can also be achieved through the use of additives in feed recipes. Preservation and improvement of feed quality in fish feed can be successfully achieved by adding additives, substances that can also be growth promoters, binders, dyes etc. Additives can combat antimicrobial resistance, contribute to animal welfare, mitigate the effects of climate change and environmental degradation (Angelis and Carreño 2021). Interest is growing in additives that are able to reduce the production of organic waste that can harm the environment.

European legislation Regulation (EC) no. 1831/2003 classifies feed additives in the following categories: technological, sensory, nutritional, zootechnical, coccidiostatic and histomonostatic additives. Additives with nutritional functions and pharmacological effects should be selected to improve the production performance of aquatic animals; natural green extracts should be selected to effectively improve the balance of flora in the intestines of aquatic animals, reduce the occurrence of intestinal diseases in aquatic animals and improve feed digestibility; Additives with immunoregulatory effects should be selected to maintain the body's normal immune function, improve disease resistance and ensure the health of aquatic animals.

MATERIALS AND METHODS

An important bibliographic resource was consulted, specialized works published in national and international journals, which address topics related to the optimization of feeding strategies in different culture systems, alternative feeds, the use of additives in aquaculture.

RESULTS AND DISCUSSION

Aquaculture systems in Romania, depending on the degree of production intensification, can be classified into intensive, semi-intensive and extensive. Proper nutrition and feeding are of utmost importance in aquaculture because of their influence on fish growth and health, production costs, and profitability. Nutrition and feeding strategies are also interrelated with nutrient utilization, waste production, and the effect of these on water quality. Because prepared diets constitute the largest variable cost in intensive fish production, reducing that cost by increasing the efficiency of diet utilization can make aquaculture more profitable. The development of refined diet formulations and the implementation of optimal feeding strategies will also reduce the environmental impact of intensive aquaculture.

Several aspects of nutrition and feeding in intensive aquaculture have not been adequately investigated under conditions comparable to commercial production. Factors such as diet composition and feeding practices deserve further consideration because of their influences on production efficiency and nutrient utilization.

In order to establish an adequate feeding regimen, knowledge of the basic nutritional and feeding requirements of the fish corresponding to each species and life stage is essential. Nutrient requirements change with age and according to circumstances and are affected by culture system, water temperature and diet. The refinement of feeding strategies in aquaculture (Gross, A., et.al 1999) - in particular the feeding time, frequency and rate - also can improve production efficiency and minimize the excretion of undigested nutrients.

In recent decades, aspects of farmed fish nutrient requirements, feed formulation and feeding management have been the subject of research. Standard nutrient requirements for fish are based on the minimum amount of nutrients needed to achieve good growth and maintain health. These requirements vary by species and age. In order to fully meet the physiological requirements of the fish, the feed must contain in a reduced proportion a series of ingredients such as: vitamin premixes, zooforts, enzyme preparations, hormones, antioxidants, antimicrobial agents, flavoring substances, binders. The list of additives that can be incorporated into food for farmed animals is strictly established by European legislation. The maximum authorized doses and conditions of use are also specified.

Knowledge of the nutritional requirements of fish is necessary to identify suitable feed (including food additives and supplements to ensure optimal digestibility), to reduce negative effects on fish and the environment, and to help reduce feed costs. Proteins, carbohydrates, and lipids are distinct groups of nutrients that the body metabolizes to produce the energy it needs for numerous physiological processes and physical activities. The ability of fish species to use energy-producing nutrients varies considerably, being associated with their natural feeding habits (herbivorous, omnivorous or carnivorous species). There is a close relationship between natural feeding habits and protein requirements. Herbivorous and omnivorous species have lower protein requirements than carnivorous fish species (NRC, 1993). The efficient use of protein for energy is largely attributed to the way ammonia from deaminated proteins is excreted through the gills with limited energy expenditure.

Food additives are substances added to food to improve the taste, appearance, durability or other characteristics of the products. They can be of natural or synthetic origin. The addition of feed additives presents a number of benefits that cost both the improvement of the growth yield and the health status of the fish.

The main role of additives in aquaculture is to support faster growth and maintain good health factors that can ensure better production results.

The types of additives used for the purpose of fish feed production are: essential additives, non-essential additives and auxiliary additives.

I. Essential additives are added in small amounts to fish feed, to nutritionally balance the recipe, to ensure good growth and health. The most important essential additives are: vitamins, minerals, fish oils, fatty acids, phospholipids, cholesterol.

- Vitamins are organic compounds required in relatively low concentrations to support certain structural or metabolic functions. Fifteen vitamins are essential for terrestrial animals and several fish species that have been examined to date (Halver, 2002). Vitamin premixes are available to add to diets so that fish receive adequate levels of each vitamin. This gives producers a margin of safety for losses associated with processing and storage. The stability of vitamins during feed manufacture and storage has been improved over the years, this is particularly evident in the development of various stabilized forms of the highly labile ascorbic acid (Halver, 2002). Therefore, vitamin deficiencies are rarely observed in commercial production.

- **The essential minerals** in the fish diet are: calcium, phosphorus, sodium, potassium, iron, manganese, magnesium, copper, chloride, iodine, cobalt and zinc. Trace elements such as copper, zinc, cobalt, iodine and manganese added to the feed contribute to the improvement of fish growth and development. Calcium and phosphorus are found in the bones and exoskeleton, sodium, potassium, magnesium and chloride are associated with osmoregulation. Magnesium, manganese and zinc are found as co-factors of metabolic enzymes. There are phases of growth and development, especially in the first summer, when an intake of trace minerals induces better growth and greater resistance to the aggression of medial factors.

- **Fish oils**, namely cod liver oil, sardine oil, squid oil and clam oil are rich in PUFA. Addition of fish oil (2-3%) to the diet improves growth rate and feed conversion rate.

- **Unsaturated fatty acids** (eicosatetraenoic acid and docosahexaenoic acid) added to the diet (1%) contribute to improving the growth rate.

- **Phospholipids** such as phosphatidylcholine are essential in fish diets for growth and survival rate. Soybean lecithin (1-2%) added to fish diet promotes faster growth, improves feed conversion rate.

- **Cholesterol** is a nutritionally essential steroid in the diet of fish that added to the diet (0.1-0.5%) contributes to improved growth and development and survival rate.

II. Non-essential additives are plant and animal derived materials, single cell proteins and some synthetic substances that are added as additives to the fish diet for faster growth and higher production. They are represented by hormones, enzymes, antibiotics, drugs, probiotics, prebiotics, symbiotics, phytobiotics.

- Natural and/or synthetic hormones are used in aquaculture to stimulate reproduction, reverse sex, produce monosex populations, improve growth. The most important hormones used are growth hormone, thyroid hormone, gonadotropin, prolactin, insulin and various steroids. Steroid hormones (androgens, estrogens and progestogens) and non-steroidal hormones (thyroxine) are used as growth promoters.

- Enzymes added to the feed administered in fish feed contribute to improve digestion. Enzyme preparations can be introduced into feed recipes to accelerate the hydrolysis processes, in order to increase the availability of nutrients. They have a positive influence on the utilization of food and on the synthesis of nutrients in the body. Added to feed, these products allow an increase in the digestibility index and at the same time a reduction in the conversion coefficient (Oprea L. et al, 2000).

- Antibiotics are used for therapy, prevention of bacterial diseases or as performance enhancers. Antibiotics play a vital role in the aquaculture industry and are even more indispensable for specialty aquaculture. However, long-term use of antibiotics, drug residues and bacterial resistance problems are becoming more serious. The widespread use of feed antibiotics and the emergence of bacterial resistance are impact in food safety and human health.

- Medicines added to feed help to improve growth and ensure well-being and general appearance.

- Probiotics are foods or supplements that contain live microorganisms intended to maintain or improve the "good" bacteria (normal microflora) in the body. Bacteria are present in culture systems and can be beneficial or malignant (pathogenic) to the culture organisms. Beneficial bacteria have been studied and used as probiotics in aquaculture, given that they can favor intestinal microbial balance, inhibit the growth of pathogens, and improve immune response and digestion through host enzymatic activities, among others (Saravanan et al. 2021).

- Prebiotics are foods (usually high fiber foods) that act as food for the intestinal microflora.

- Symbiotics refers to the food supplement that includes in a synergistic relationship both prebiotics and probiotics (Butt et al. 2021). Symbiotics help enhance the positive effects of prebiotics and probiotics in host organisms.

- **Phytobiotics** present a wide range of characteristics: antioxidants, antimicrobials, anticarcinogenics, analgesics, insecticides, antiparasitics, anticoccidials, growth promoters, they increase appetite, stimulate bile secretion and the activity of digestive enzymes. Phytobiotics have laxative and antidiarrheal or hepatoprotective properties.

III. Auxiliary additives

Certain components added to the feed help in better feed utilization, resulting in feed efficiency. Such additives can be considered auxiliary additives (feed dyes, binders, molasses).

- Colorants. More than 300 pigments have been identified and are found in various plants and animals. Carotenoids and astaxanthins are either present in some natural substances or as synthetic substances. Xanthophylls and carotenoids are the most important classes of pigments. Most xanthophylls are found in plants, and carotenoid pigments are found in crustaceans and fish. They are also improving fish growth and survival. The main function of these colors is to correct pigmentation of the crop species.

- Binders – have great importance for fish feed because they can avoid the feed disintegration during handling and transport and offer an increased stability in water. Materials such as agar-agar, carboxymethylcellulose (CMC), bentonite, guar gum, lignin sulfate, plaster of Paris, polyvinyl alcohol, sodium alginate, and wheat gluten are used as binders to provide greater stability to the pellets.

- Molasses added to feed contributes to improving palatability. It is also a good source of energy in feed.

The most effective feed additives currently used in aquaculture are probiotics, prebiotics, phytogenic constituents, immunological stimulants, enzymes, hormones, mycotoxin binders, organic acids and others (Cano-Lozano, 2022; de Araújo, 2018). Probiotics, prebiotics, phytogenic ingredients, immunostimulants, enzymes, hormones, mycotoxin binders, organic acids and other functional feed additives are the optimal feed additives used in aquaculture today.

One of the main challenges facing aquafeed sustainability is its dependency on raw materials (fish oil and fishmeal), increasingly scarce ingredients that call into question the ecological sustainability of the aquafeed sector. Fishmeal and fish oil continue to be strategic feed components used in aquaculture, but at lower levels than decades ago, due to high prices, limited availability, variability in composition and nitrogen pollution can be considered potential challenges faced by rich forages. Fishmeal can be largely substituted, but at a cost (enzyme and amino acid supplementation) bringing the final cost in line with fishmeal diets. There is a pressing need to look into the use of non-conventional feed ingredients, but there is limited knowledge or information on possible alternatives and their availability.

A global research effort has been made over the past three decades to search for complementary ingredients in aquafeeds. According to Ytrestøyl et al. 2015, for the main crop species, other types of feed containing reduced amounts of fishmeal have already appeared. Zarantoniello et. al. 2022, found that the replacement of fishmeal with ingredients rich in vegetable proteins has negative effects that are identified by reduced intake, low digestibility of nutrients and energy, and poorer physical and organoleptic quality of the fillets, but which can be counteracted by the addition of additives fodder According to Bélanger et al. 2021, the lack of certain nutrients present in fish meal or fish oil can be supplemented with the addition of micro and macroalgae extracts.

Until a few years ago, antibiotics were used as a feed additive to help improve most of the quality of fish feed, but now the use of antibiotics as a growth promoter has been banned in the EU. As an alternative to the use of antibiotics, medicinal plants, plant extracts, phytochemicals, plant secondary metabolites, immunostimulators, probiotics etc. have been proven that they can replace antibiotics in feed recipes.

Additives added to fish feed contribute to the control of pathogenic microorganisms, inhibiting the development of toxins in feed, increasing the activity of digestive enzymes, strengthening the immune system, with the effect of improving growth performance and disease resistance. The use of additives increases nutrient use efficiency, helps reduce the waste load in the environment, can support best aquaculture practices in the long term.

Refinement of aquaculture feeding strategies – particularly the timing, frequency and rate of feeding, can also improve production efficiency and minimize the excretion of undigested nutrients.

In order to meet the physiological requirements of the fish, the feed must contain in a reduced proportion a series of ingredients such as: premixes, zooforts, enzyme preparations, hormones, antioxidants, antimicrobial agents, flavoring substances, binders. Good quality feed is key to fish production as it improves the yield or quality of fish products, which in turn helps increase income. It is not possible to make a profit without using feed that has a direct impact on the growth rate, productivity and health of the fish. Optimizing the feeding strategy in different types of production systems can be achieved by using alternative feed sources: proteins of plant origin (soybean, barley, rice, peas, lupine, rapeseed, wheat gluten, corn gluten) and proteins from from land animals (blood meal, feather meal, poultry by-products, pig by-products). According to Ghisaura et al., 2014, terrestrial animal protein sources used together with vegetable protein sources, and/or dietary additive additions can provide the necessary nutrients to fish that are not present in vegetable meals. Currently, it is believed that the most viable alternative sources may be insects and fishery and aquaculture by-products.

In aquaculture, feed technologies and various technical measures can be applied in the energy and food conversion process to improve substantially the conversion ratio and food utilization, so that the food source for terrestrial plants can be introduced into the production of aquatic products. Sustainable aquaculture development can be achieved by applying technologies that consume less energy and maximize production.

CONCLUSIONS

The purpose of using feed additives in aquaculture is to promote faster and healthier fish growth. Feed recipes must include the appropriate amounts of additives for each type of feed. A review of the current status and trends in administered feed and feeding strategies is required for the inclusion of additives in fish feed and sustainable production.

BIBLIOGRAPHICAL REFERENCES

1. Bélanger A., Sarker P.K., Bureau D.P., Chouinard Y., Vandenberg G.W.; Apparent digestibility of macronutrients and fatty acids from Microalgae (*Schizochytrium sp.*) fed to rainbow trout (Oncorhynchus mykiss): a potential candidate for fish oil substitution; 2021; Animals 11(2):Art. 2. https://doi.org/10.3390/ani11020456.
2. Butt U.D., Lin N., Akhter N., Siddiqui T., Li S., Wu B.; Overview of the latest developments in the role of probiotics, prebiotics and symbiotics in shrimp aquaculture; 2021; Fish Shellfish Immunol 114:263–281. https://doi.org/10.1016/j.fsi.2021.05.003.
3. Cano-Lozano J.A., Villamil Diaz L.M., Melo Bolivar J.F., Hume M.E., Ruiz Pardo R.Y.; Probiotics in Tilapia (*Oreochromis niloticus*) culture: Potential probiotic *Lactococcus lactis* culture conditions; 2022; Journal of Bioscience and Bioengineering, 133(3), 187-94. https://doi.org/10.1016/j.jbiosc.2021.11.004 , PMID 34920949.
4. De Araújo E.R.L., Barbas L.A.L., Ishikawa C.M., de Carla Dias D., Sussel F.R., de Almeida Marques H.L.; Prebiotic, probiotic, and synbiotic in the diet of Nile Tilapia post- larvae during the sex reversal phase; 2018; Aquaculture International, 26(1), 85-97. https:// doi.org/10.1007/s10499-017-0201-7.
5. Ghisaura S., Anedda R., Pagnozzi D., Biosa G., Spada S., Bonaglini E., Cappuccinelli R., Roggio T., Uzzau S., Addis M.F.; Impact of three commercial feed formulations on farmed gilthead sea bream (*Sparus aurata, L*); 2014.
6. Gross A., C.E. Boyd and Lovell. R.T.; Effects of feed protein concentration and feeding rate combinations on quality of pond water and effluent in channel catfish culture; 1999; Israeli Journal of Aquaculture. Bamidgeh 51:47-57.
7. Halver J.E. and Hardy R.W.; Fish nutrition; 2002; Academic Press.
8. Oprea L., Georgescu R.; Nutriția și alimentația peștilor; 2000; Editura Tehnică.
9. Saravanan K., Sivaramakrishnan T., Praveenraj J., Kiruba-Sankar R., Haridas H., Kumar S., Varghese B.; Effects of single and multi-strain probiotics on the growth, hemato-immunological, enzymatic activity, gut morphology and disease resistance in Rohu. *Labeo rohita*; 2021; Aquaculture 540:736749. https://doi.org/10.1016/j.aquaculture.2021.736749.
10. Ytrestøyl T., Aas T.S., Åsgård T.; Utilization of feed resources in production of Atlantic salmon (*Salmo salar*) in Norway; 2015; Aquaculture 448:365–374. https://doi.org/10.1016/j.aquaculture. 2015.06.023.
11. Zarantoniello M., Pulido Rodriguez L.F., Randazzo B., Cardinaletti G., Giorgini E., Belloni A., Secci G., Faccenda F., Pulcini D., Parisi G., Capoccioni F., Tibaldi E., Olivotto I.; Conventional feed additives or red claw crayfish meal and dried

microbial biomass as feed supplement in fish meal-free diets for rainbow trout (*Oncorhynchus mykiss*): possible ameliorative effects on growth and gut health status; 2022; Aquaculture 554:738137. https://doi.org/10.1016/j.aquaculture.2022.738137.
12. National Research Council, NRC. Nutrient requirements of fish; 1993; National Academy of Science of Washington, Washington, USA.

ACTA AGRICOLA ROMANICA, Volume 6, Year 6, No. 6.3.

INFECTIOUS MYCOTIC DISEASES OF *BOMBYX MORI* L. SILKWORMS – REVIEW

BOLILE INFECȚIOASE MICOTICE ALE VIERMILOR DE MĂTASE *BOMBYX MORI L.* – REVIZUIRE

VASILICĂ SAVU[1], AGRIPINA ŞAPCALIU[1]

[1]Research Station for Sericulture Băneasa Bucharest, Romania, București-Ploiești no. 69, District 1,
e-mail: secretariat@scsbaneasa.ro

Correspondence address: agripina.sapcaliu@scsbaneasa.ro;
vasilica.savu@scsbaneasa.ro

Abstract

The silkworm can be affected in all stages of development (larva, chrysalis, butterfly) by the evolution of some diseases (mycotic, bacterial, viral, parasitic). Mycotic diseases of silkworms (muscardina, aspergillosis) are caused by fungi that parasitize the body of larvae and chrysalises and can seriously affect silkworm populations, causing significant production losses. To investigate the influence and evolution of fungal diseases on silkworms, we consulted the publications available in scientific databases, covering the period 2000-2024. Information from more than 52 publications was collected, and finally 23 relevant studies were analyzed. Statistical data indicate that temperature and humidity influence the development of these diseases. A relative humidity between 90 and 100% favors the appearance of diseases, while a level of 70% does not allow them to develop. Also, a temperature between 15 and 18°C is extremely favorable for the development of fungi, while spore germination is not recorded at 33°C. Proactive monitoring of silkworm health to prevent fungal diseases and prompt intervention when signs of disease appear are essential to maintaining healthy and efficient silk production.

Keywords: silkworms, contagious mycotic diseases.

Rezumat

Viermele de mătase poate fi afectat în toate stadiile de dezvoltare (larvă, crisalidă, fluture) de evoluția unor boli (micotice, bacteriene, virale, parazitare). Bolile micotice ale viermilor de mătase (muscardina, aspergiloza) sunt produse de către fungi care parazitează corpul larvelor și crisalidelor putând afecta serios populațiile de viermi de mătase, determinând pierderi semnificative ale producției. Pentru a investiga influența și evoluția bolilor micotice asupra viermilor de mătase, am consultat publicațiile disponibile în bazele de date științifice, acoperind perioada 2000-2024. S-au adunat informații din mai mult de 52 de publicații, iar în cele din urmă au fost analizate 23 de studii relevante. Datele statistice indică faptul că temperatura și umiditatea influențează dezvoltarea acestor boli. O umiditate relativă între 90 și 100% favorizează apariția bolilor, în timp ce un nivel de 70% nu permite dezvoltarea acestora. De asemenea, o temperatură cuprinsă între 15 și 18°C este extrem de propice pentru dezvoltarea fungilor, în timp germinarea sporilor nu se înregistrează la 33°C. Monitorizarea proactivă a stării de sănătate a viermilor de mătase pentru prevenirea bolilor micotice și intervenția promptă în cazul apariției semnelor de boală sunt esențiale pentru menținerea unei producții sănătoase și eficiente de mătase.

Cuvinte cheie: viermi de mătase, boli contagioase micotice.

INTRODUCTION

The silkworm can be affected in all stages of development (larva, chrysalis, moth) by the evolution of some diseases (fungal, bacterial, viral, parasitic). Fungal diseases of silkworms (muscardina, aspergillosis) are caused by fungi that parasitize the body of larvae and chrysalises and can seriously affect silkworm populations, causing significant production losses (5). Fungi that infect the body of silkworm larvae and pupae can lead to the development of fungal diseases.

The term "*muscardine*" generally attributed to mycoses affecting silkworms comes from the fact that the larvae, when they die of this disease, become rigid and are covered by a mycelium, which, depending on the pathogenic fungus responsible, can be of various colors (16). There are four types of muscardine, named after the color the larva's body acquires as a result of the disease, each caused by a specific fungus: white muscardine (*Beauveria bassiana*), green muscardine (*Spicaria prasina*), yellow muscardine (*Isaria farinosa*) and brown or black muscardine (*Aspergillus spp*). These are fungal pathogens that can affect various insect pests, and some of them are used in the biological control of these pests. Among these mycoses, white muscardina or "lime disease" is the most widespread, caused by *Beauveria bassiana*, a fungus that goes through three stages in evolution: conidia, vegetative mycelium and aerial mycelium. The conidia, which have an oval or globular shape and are visible as a white powder, penetrate the integument of the larva after germination. *Beauveria bassiana* spores can survive in the environment for several months to a year. *Beauveria bassiana* is a facultative parasite, being able to develop in different sources of infection (19, 22, 23). The main source of infection is diseased corpses and droppings that contaminate various other insects with which they come into contact. Contamination occurs through the growth of vegetative hyphae and small hyphae, which consume nutrients and water from the silkworm's body. This is the main cause of the formation of crystals in the hemolymph, disrupting its functions and circulation. The vegetative mycelium produces toxins which are the main cause of poisoning and death (28). Other sources of contamination include: transmission of spores through all surfaces that come into contact with diseased larvae, direct contact with persons carrying the disease, use of non-disinfected premises for rearing silkworms (29).

The route of infection in primary infection is based on contact with the infected environment. The disease is transmitted orally through food, the main routes of contamination being: digestive, respiratory, skin pores (32).

Predisposing factors are primarily related to the age of the larvae. In older ages (III-V), worms are much more sensitive than in younger ages (I, II). They are also sensitive during larval sleep, especially during the sleep exit phase. Statistics show that this disease is influenced by temperature and humidity. The conditions favoring the appearance of the disease are low temperature (15-18^{0}C) and a humidity of 90-100%, when the fungus develops rapidly (8). The main source of spreading the disease is the corpse of the sick larva on which the spores of the fungus are produced. Also, the temperature of 24-28^{0}C seems quite favorable for the development of the fungus, but the spores do not germinate at 33ºC, it being impetuously necessary to control the microclimate factors, as a first measure to prevent and combat mycoses.. The disease frequently occurs in spaces where ventilation is not adequate or when these spaces are located near dirty water. The presence of elastic corpses to the touch of silkworms can be a first element in establishing the diagnosis. If these corpses, in humid conditions, develop conidia and become hard, with a chalky appearance, the diagnosis is certain. In the case of an asymptomatic evolution, the laboratory examination will highlight the presence of vegetative hyphae, useful for specifying the diagnosis.

The clinical signs of evolution of muscardine that affect silkworm larvae are: inappetence of the larvae (lack of appetite or refusal of food), the color of the larvae takes on a slightly red hue, the abdominal legs turn black, a black line appears on the line separating the head from the thorax - collar, after 10 days the larva's body becomes rigid and a white powder appears on its surface, giving the larvae a chalky appearance (chalky or matte surface), dark or black spots appear on the larvae's body (common sign of muscardina), loss of mobility and elasticity and the appearance of immobility (they lose mobility and elasticity, becoming immobile), and death occurs in 6-12 hours from the first signs of infection. Blackening of the abdominal legs in the terminal part occurs, suggesting a burn, corpses are stiff red-white, then white dust-like (red-white stiffness), which later turns into a white dust-like texture, with the death of the embryo in the inside of the egg. These clinical signs may vary depending on the severity of the infection and the fungal species involved. After a few days of infection, spots similar to small drops of oil appear on the larva's body, initially with reddish hues, and before they die, the larvae present soft excrement and vomit. After death, the body which is soft and elastic becomes rigid, its color changing to pink-red. After 1-2 days, the appearance of the corpse changes and aerial hyphae appear, which develop on the intersegmental membranes and become covered with a white powder consisting of conidia. The number of conidia that appear on the body of the fifth-stage larva can be several million (35).

Infection in the chrysalis stage produces mummification of the chrysalis. The reaction to external stimuli is slow, the thorax shrinks, the abdomen is wrinkled, and hyphae and conidia grow between the intersegmental limbs.

In the adult stage, the body of the moth hardens and the wings become very fragile, with frequent breaks. The time from infection to death is generally 2-3 days for first-instar larvae, 3-4 days for second-instar larvae, 4-5 days for fourth-instar larvae, and 5-6 days for those in the fifth stage. These periods can vary depending on temperature, humidity and the general health of the silkworms.

Aspergillosis is a mycotic disease of silkworms that is produced by the etiological agent represented by fungi of the genus *Aspergillus sp.*, being a strongly saprophytic fungus, able to live in a saprophytic state on wood, tools, in bird feed, where there are humid conditions. Because of these varied sources of fungal presence, the spread is high.

Conidia are large, spherical in shape, 3-7 µ in diameter, with one surface smooth and the other rough, bright yellow in color that gradually changes to brown. Germination occurs at a temperature of 30-35ºC, and this disease generally occurs in the first and second instars, with morbidity decreasing as the larvae age (13). After infection, in the 1st larval instar there is an interruption of mulberry leaf consumption, the larvae retreating under the leaf in a lethargic state. The corpses are covered by hyphae at the site of invasion, after a day the aerial hyphae also appear, and later, yellow and brown conidia cover the entire body of the larva. During the disease, by chance, large spots may appear on the body of the larva, and before death, the head and thorax relax and vomiting occurs. Characteristic in this disease is the fact that, in the place where the fungus has penetrated, the corpses are hard, and the other parts begin to blacken and rot. The larvae affected by the disease in the first age die during sleep, the disease appears in the adult stages, the presence of spots is sometimes observed, which may disappear during sleep and the silkworm recovers.

The infection of the mature larvae leads to the appearance of the disease in the chrysalis, manifested by its hardening, the color becoming dark brown. Increased humidity causes an abundant proliferation of aerial hyphae, the chrysalis becoming blackish. Silkworm eggs kept in a humid environment tend to be parasitized by *Aspergillus sp.*, the symptom of this parasitism being their darker than normal color and under these conditions, the embryo suffocates and dies (15).

Germination of conidia and invasion of the body is achieved slowly and only at the site of infection, when the fungus begins to secrete protease and aflatoxin, and symptoms of intoxication and mass mortality appear rapidly. Suffocation of the embryo in the egg is caused by the obstruction of the pores on the surface of the egg by the growth of aerial hyphae on its exterior.

MATERIAL AND METHOD

To explore new data on fungal infectious diseases of the silkworm *Bombyx mori* L., we considered publications accessible in the published scientific databases (2000-2024) Elsevier, MDPI, PubMed, Web of Science, Research Gate, Springer and Wiley Online Library. To search for articles on this topic, we used the following keywords: silkworms, muscardine, aspergillosis and infectious mycotic diseases. The criteria for selecting the publications consisted of the topic of the papers, years of study, articles in English that met the eligibility criteria. Data were collected from over 52 publications, considering 39 major studies, from which 23 relevant studies were finally selected. In the future, it is possible to witness the development of new investigative methods and methodological approaches in our country, which could significantly contribute to the understanding and management of fungal infectious diseases in silkworms.

RESULTS AND DISCUSSION

Epidemiological investigation based on laboratory diagnosis is an essential component of efforts to prevent and control mycotic diseases in silkworms and can significantly contribute to maintaining the health and productivity of silkworm populations (Figure 1, 2, 3). By using modern techniques and appropriate equipment, laboratories can provide important information for managing the health and productivity of silkworm populations, thereby helping to maintain the sustainability of the sericulture industry (18).

Figure 1. Larvae with clinical signs of muscardine (Pau, 2000) / Larve cu semne clinice de muscardină (Pau, 2000)

Figure 2. Biological cycle I of *Beauveria bassiana*. Outside the body (vegetative mycelium, aerial mycelium, conidia, germinal spore, small mycelium). Inside the body (integument, middle intestinal wall, adipose layer, musculature) (Pau, 2000) / Ciclul biologic I al *Beauveria bassiana*. În afara corpului (miceliu vegetativ, miceliu aerian, conidii, spor germinal, miceliu mic). În interiorul corpului (tegument, peretele intestinal mediu, stratul adipos, musculatură) (Pau, 2000)

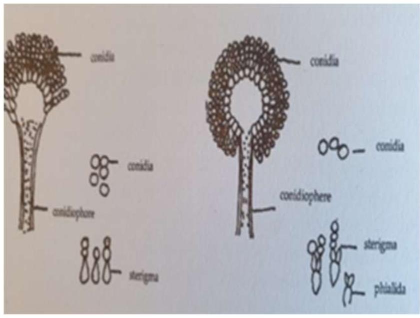


Figure 3. The species that produce aspergillosis; a) *Aspergillus flavus*; b) *Aspergillus orzyae* (Pau, 2000) / Speciile care produc aspergiloza; a) *Aspergillus flavus*; b) *Aspergillus orzyae* (Pau, 2000)

Identification of mycotic diseases of silkworms by modern diagnostic technologies

Advanced molecular technologies, namely polymerase chain reaction (PCR) and DNA sequencing, are an essential component in the diagnosis of silkworm diseases (2, 5), presenting significant advantages in terms of sensitivity, specificity and diagnostic accuracy (37). PCR is a molecular technique that allows the selective amplification of specific DNA sequences, thus facilitating the detection and identification of the pathogen even in very small amounts of biological samples (6). This method is highly sensitive and can identify even incipient or subclinical infections (12).

PCR can be adapted for the detection of different pathogens (bacteria, viruses, fungi and parasites), which makes it a highly effective method in the diagnosis of silkworm diseases. DNA sequencing can provide essential information about the structure and evolution of pathogens, as well as the mechanisms of resistance to the treatments used. By using these advanced molecular technologies, laboratories can achieve accurate and rapid disease diagnosis in silkworms (40), thus contributing to the management of the health and productivity of silkworm populations.

Bioinformatics in the diagnosis of fungal diseases in silkworms represents a modern and efficient approach for the analysis of genetic data and for the rapid identification of specific genetic sequences associated with various conditions. The method consists of analyzing genetic data, identifying specific genetic sequences associated with different diseases, rapidity and efficiency of identification, personalization of treatments and disease management strategies (24). Bioinformatics involves the use of computers and specialized algorithms to analyze genetic data (DNA sequences, transcriptomes and proteomes) obtained from silkworm samples. This analysis can reveal important information about the silkworm genome and its interactions with pathogens. By applying advanced bioinformatics techniques, such as genomic and transcriptomic sequence analysis, specific genetic sequences that are associated with various diseases in silkworms can be identified. By identifying the fungal pathogen and analyzing the molecular mechanisms involved in disease detection in silkworms we can gain significant insights into how diseases evolve and affect the health and productivity of these insects. This deep understanding of diseases can serve as the basis for developing effective prevention and control strategies, helping to maintain the health and stability of silkworm populations and protect the sericulture industry. Bioinformatics enables rapid and efficient identification of specific genera associated with silkworm diseases compared to traditional genetic analysis methods and can facilitate rapid implementation of control and treatment measures in silkworm farms (3). By applying advanced genetic data analysis techniques, bioinformatics can help identify relevant genetic markers for specific diseases (38), understand the molecular mechanisms involved in their pathogenesis, and develop personalized diagnostic and treatment strategies.

Immunodiagnosis of fungal diseases in silkworms involves the development and use of immunoassays and immunodetection technologies for the identification of disease-specific antigens and antibodies. This approach relies on the host's immunological reactions to the presence of a pathogen and can provide accurate and sensitive information about infection or exposure to a particular pathogen (35). Immunodiagnostics includes the development of immunological tests and their optimization such as ELISA (*Enzyme-Linked Immunosorbent Assay*) or agglutination tests for the detection of specific antigens or antibodies of certain diseases in silkworms and the evaluation of the effectiveness of treatments. These tests are designed to provide fast and accurate results and can be adapted to detect various pathogens (bacteria, viruses, fungi and parasites) (37). By detecting and quantifying these antigens in tissue or excrement samples, the presence of the pathogen can be confirmed and the degree of infestation in the silkworm population can be assessed. Immunological tests can also be used to detect specific antibodies produced by the host organism in response to the presence of a pathogen. This detection can provide information about the previous exposure of silkworms to certain diseases (carriers of pathogens) and can indicate the immunity status of the respective population. By monitoring the levels of antigens or antibodies before and after treatment, one can assess the host's response and determine whether the treatment has been effective in reducing the incidence of the disease (34).

The use of nanotechnology in the diagnosis of fungal diseases in silkworms represents an innovative and promising approach that may provide more sensitive and accurate methods of pathogen detection (9). Nanotechnology in this context can be integrated by using nanomaterials with unique properties for sensitive and specific detection of pathogens at the molecular level, developing nanosensor-based diagnostic systems, amplifying detection signals, and implementing automation in diagnostic and analysis processes (10, 11, 20). For example, functionalized nanoparticles can be designed to selectively interact with certain specific pathogen molecules, facilitating their identification in tissue or excreta samples (26). Nanotechnology allows the construction of advanced diagnostic systems (nanosensors), which can detect and quantify the specific molecules of pathogens with extremely high sensitivity. These nanosensors can be integrated into handheld or automated devices, enabling rapid (39) and on-site diagnosis of silkworm diseases (20). Nanotechnology can also be used to amplify detection signals, thus improving the sensitivity and specificity of diagnostic tests (27). For example, functionalized nanoparticles can amplify fluorescence or electrochemical signals generated during interaction with pathogens, facilitating the detection of even very small amounts of pathogens in biological samples, thereby facilitating real-time disease diagnosis and management (19). By integrating nanotechnology into silkworm disease diagnosis, more efficient, faster and more affordable diagnostic methods can be achieved, which can contribute to improving the health and productivity of silkworm populations and supporting the sericulture industry (26).

Early and preventive diagnosis of fungal diseases in silkworms requires the use of sensitive and specific technologies that can detect even incipient or subclinical infections at an early stage. Key aspects of early diagnosis in this context include the use of sensitive and specific technologies (regular and systematic monitoring of silkworm health), using sensitive and specific technologies that can detect even small amounts of pathogens or early signs of disease. Early diagnosis involves establishing a program of regular and systematic monitoring of the health of silkworms to detect any early signs of disease or changes in their health status by periodic examination of tissue or excrement samples using appropriate diagnostic techniques (36). Automatic and digital health monitoring of silkworms is a modern and effective technique for monitoring their populations and quickly detecting any abnormalities or changes in their behavior or health, which can be achieved by: using specialized digital devices and sensors to constantly monitor various aspects of worm health, collect real-time data and transmit it to a centralized monitoring system, quickly detect anomalies through automatic analysis of collected data, automatically generate alerts and notifications for farmers when a problem is identified, and analyze data to make more informed and effective management decisions (33). Early and preventive diagnosis of diseases in silkworms can ensure effective and sustainable management of their health, helping to maintain the sustainability and success of the sericulture industry. Digital sensors can transmit the data collected in real time to a centralized monitoring system (sudden changes in temperature or humidity, or unusual behavior of the worms), allowing farmers to obtain up-to-date and detailed information about the health of the silkworm population and environmental conditions on their farms, which require rapid intervention. It can also help prevent serious complications or mortality associated with untreated or undiagnosed diseases (14). Early diagnosis can help reduce the economic impact of diseases on the sericulture industry by minimizing production losses and costs associated with disease treatments and management.

The integration of artificial intelligence technologies in the diagnosis of mycotic diseases in silkworms is an advanced and promising approach that facilitates efficient data analysis and provides accurate and rapid disease diagnosis using modalities such as data analysis, rapid and accurate diagnosis, identification of subtle signs of diseases, personalizing treatments and optimizing health management (33). Artificial intelligence can be used to analyze and interpret data collected from various sources (laboratory test results, silkworm health monitoring data or epidemiological information), and artificial intelligence algorithms can identify patterns, correlations and trends in the raw data, thereby facilitating the identification of risk factors and early signs of diseases (25). With the help of predictive models and machine learning algorithms, artificial intelligence can generate accurate and rapid diagnoses of silkworm diseases based on available data and previously accumulated knowledge. These diagnoses can be obtained in real time and can serve as a guide for the immediate establishment of treatment and control measures. Some silkworm diseases can develop without clinical signs or be difficult to diagnose in the early stages, and artificial intelligence can be trained to recognize these subclinical signs and associate them with certain conditions, thus enabling early disease identification and rapid intervention. Based on the individual data and medical history of each silkworm lots, artificial intelligence can recommend personalized treatments tailored to the specific needs of each case, helping to improve the effectiveness of treatments and minimize the risk of treatment resistance. By continuously analyzing health and performance data, artificial intelligence can help optimize silkworm health management and identify best practices for disease prevention and control, significantly improving the ability to diagnose and manage their health.

Personalized medicine in the treatment of mycotic diseases of silkworms adapts to individual characteristics (including genome sequencing, assessment of the growth environment, diagnostic tests and personalized treatments), as well as continuous monitoring and adjustment of therapy, tailored treatments, continuous monitoring and adjustment (1). DNA sequencing can identify genetic variations and genetic markers relevant to the health and disease susceptibility of each silkworm lots, information that can then be used to identify potential disease risks and develop diagnostic and treatment strategies adapted (7). By evaluating the specific growth environment of each silkworm lots, environmental factors that could contribute to disease risk can be identified and measures can be implemented to minimize these risks.

Based on genetic and environmental information, personalized diagnostic tests can be developed to specifically detect pathogens or risk factors for each individual silkworm batch, after which personalized treatment strategies tailored to the individual characteristics of the silkworm can be developed. to each batch of worms. These treatments may include the use of specific drugs, changes in diet or management of the rearing environment, and other tailored interventions (25). Personalized medicine involves continuously monitoring the health status of silkworms and adjusting diagnostic and treatment strategies according to their evolution (31).

Prophylactic and necessary measures in mycotic diseases of silkworms

Prophylactic measures

Cleaning and disinfection of silkworm rearing areas, inventory and litter at each rearing series**.** Solutions recommended according to the legislation in force can be used for the premises and the inventory. During the rearing of silkworms, the following measures are necessary: collection of diseased or suspected diseased larvae and carcasses followed by their destruction by burning; carrying out daily disinfection in the growth spaces with recommended antimycotic solutions; disinfection of the larval body with an antifungal solution, finely sprayed from a distance of 30-40 cm above the rearing beds, at the beginning of each evening and once at each larval stage, after emerging from sleep. If necessary, the application can be done therapeutically every day. Maintaining a suitable microclimate for the larvae, is recommended for the prevention on the development of fungi (temperature to be between 22-26°C, humidity below 75%, ensuring adequate ventilation, hygiene of premises and equipment). Protecting doors and windows against biological vectors or drafts have to be used. Periodic application of phytosanitary treatments to mulberry plantations, using antifungal substances with low remanence are also necessary.

If a pathogen is detected at an early stage or if there is an increased risk of infection, preventive treatments (antiparasitic or antimicrobial drugs, according to the recommendations of veterinary specialists) can be given to prevent the occurrence or spread of the disease in the silkworm population (30). If the presence of a pathogen is identified in a silkworm lot it is important to take immediate measures to isolate the affected lot in order to prevent the spread of the disease to other lots and limit the negative impact on the entire silkworm population. Early identification of diseases can lead to the implementation of changes in farm management (improving hygiene, adjusting rearing conditions or applying more rigorous biosecurity measures), which can help reduce the risk of infection and maintain the health of silkworms. After implementing preventive measures, it is important to continuously monitor the health status of the silkworm population and evaluate the effectiveness of the measures taken by performing periodic diagnostic tests and carefully observing signs of improvement or worsening of the disease over time. By implementing preventive measures following an early identification of diseases, their negative impact on the health and productivity of silkworms can be significantly reduced.

Control and prevention measures in fungal diseases

The measures to combat and prevent mycotic diseases are carried out by: isolating the lots and shelters where the disease has appeared and the lots where the disease has appeared massively will be burned; collecting diseased larvae, corpses and litter in containers with antiseptic solutions or plastic bags and destroying them by burning; decontamination with disinfectants recommended according to the legislation in force of the woody parts of the shoots, together with the change of the litter and its evacuation to the platform; daily disinfection in the growing spaces with antifungal solutions; disinfection of the body of the larvae with antimycotic solution sprayed on their body and on the litter by applying it once a day before feeding; ensuring microclimate conditions, food and premises hygiene; reducing excess moisture in growing spaces by regulating ventilation and using chlorinated lime as a powder spread under bedding, under racks and on indoor walkways and using protecting doors and windows from growing spaces with gauze curtains to prevent pathogenic fungi from entering silkworms.

CONCLUSIONS

1. After exploring and comparing classical and modern diagnostic methods for mycotic diseases in silkworms, it can be concluded that technological progress has brought a series of innovations that improve the efficiency, accuracy and speed of diagnosis.
2. From traditional methods, such as microscopic examination, to advanced technologies (genome sequencing and the use of artificial intelligence), there are a variety of tools available for the detection and management of fungal diseases in silkworms.
3. The integration of these classical and modern techniques can help to improve the health and productivity of these valuable insects, having a positive impact on the sericulture industry.
4. In the future, a diagnostic laboratory may move from using simpler diagnostic techniques to more advanced and sophisticated technologies due to the rapid advancement of technology and the increasing availability of equipment and resources. This may involve purchasing specialized equipment, such as PCR or other molecular diagnostic technologies, and training specialized personnel to use it effectively.
5. Thus, in a relatively short period of time, the laboratory can adopt more advanced techniques for the diagnosis of mycotic diseases in silkworms.

ACKNOWLEDGEMENTS

This research was supported by the Ministry of Agriculture and Rural Development through the ADER Program within the projects ADER 24.1.1./2023, with the title "*Family sericulture farm model for the innovative capitalization of secondary sericulture productions obtained from sericulture and sericulture in the Farma-Farming system*".

BIBLIOGRAPHICAL REFERENCES

1. Atoe B., Ifijen I.H., Okiemute I.P., Emmanuel O.I. and Maliki M.; Silk Biomaterials in Wound Healing: Navigating Challenges and Charting the Future of Regenerative Medicine; 2024; In TMS Annual Meeting & Exhibition (pp. 904-920). Cham: Springer Nature Switzerland.
2. Bebitha B., Mohanra J.P., Manimegalai S., Mahalingam C.A.; Silkworm disease diagnosis through molecular approach and their management. International Journal of Plant Protection; 2016; 9(1), 343-352.
3. Brahma D., Bora N.R., Narzary P.R., Chakraborty R., Sarma J. and Rajkhowa A.; Tools and Applications of Bioinformatics in Sericulture: A Review; 2024; Asian Journal of Current Research, 9(1), pp.86-94.
4. Bura M., Acatincăi S., Pădeanu I.; Viermii de mătase, biologie și creștere; 1995; Editura Helicon, Timisoara, România.
5. Chen K. & Lu Z.; Immune responses to bacterial and fungal infections in the silkworm, *Bombyx mori*; 2018; Developmental & Comparative, Immunology, 83, 3–11.
6. Dai W., Qi J., Chen H., Zhang Z., Shang R., Zhang Y., Tang S. and Shen Z.; Rapid and sensitive detection of Nosema bombycis using loop-mediated isothermal amplification and colorimetric nanogold; 2019; Science Asia, 45(3).
7. Deepika I., Ramesh K.V., Kumar I., Singh A., Debnath R., Dubey H., Shukla P., Ponnuvel K.M., Moorthy S.M. and Subrahmanyam G.; Molecular diagnostics in sericulture: A paradigm shift towards disease diagnosis in silkworms; 2024; *Entomologia Experimentalis et Applicata*.
8. Doliș M.; Sericicultura; 2008; Editura ALFA, Iași, România.
9. Hassan S., Karaila G.K., Singh P., Meenatchi R., Venkateswaran A.S., Ahmed T., Bansal S., Kamalraj R., Kiran G.S. and Selvin J.; Implications of fungal nanotechnology for sustainable agriculture-applications and future perspectives; 2024; Biocatalysis and Agricultural Biotechnology, p.103110.
10. Huang W., Ling S., Li C., Omenetto F.G. and Kaplan D.L.; Silkworm silk-based materials and devices generated using bio-nanotechnology; 2018; Chemical Society Reviews, *47*(17), pp.6486-6504.

11. Kaman P.K., Das A., Verma R., Narzary P.R., Saikia B. and Kaman N.; The Importance of Nanotechnology on Sericulture as a Promising Field; 2023; Agriculture Association of Textile Chemical and Critical Reviews Journal (2023) 40-46.
12. Kang G.P., Guo X.J.; Overview of silkworm pathology in China; 2011; African Journal of Biotechnology. 10(79): 18046-18056.
13. Kumari K.M., Rajan R.K., Himantharaj M.T., Nataraj B., Rekha M.; Influence of temperature and relative humidity on the rearing performance and disease incidence in CSR Hybrid Silkworms, *Bombyx mori L.*; 2001; International Journal of Industrial Entomology, 3(2): 113-116.
14. Liu Y., Deng S., Li Y., Zhang Y., Zhang G. and Yan H.; Fast identification of the BmNPV infected silkworms by portable NIR spectroscopy and chemometrics; 2024; Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, p.124158.
15. Majima H., Arai T., Kamei K. and Watanabe A.; *In vivo* efficacy of pitavastatin combined with itraconazole against *Aspergillus fumigatus* in silkworm models; 2023; Microbiology Spectrum, 11(5), pp. e02666-23.
16. Manjunatha S.R., Narayanaswamy K.C., Gowda M., Chandrashekar S., Kumar M.P. and Benherlal P.S.; Studies on the Effect of White Muscardine Infection on Growth and Yield Performance of Bivoltine Silkworm, Bombyx mori Breeds; 2023; Mysore Journal of Agricultural Sciences, 57(2).
17. Matei A.; Creșterea viermilor de mătase; 2002; Editura ALEX and ALEX.
18. Nagashetti S.M., Biradar S., Dambal S.D., Raghavendra C.G. and Parameshachari B.D.; Detection of disease in *Bombyx mori* silkworm by using image analysis approach; 2021; IEEE Mysore Sub Section International Conference (MysuruCon) (pp. 440-444). IEEE.
19. Pandey K. and Kachhwaha N.; Effects of green nanoparticles on the pathogenic fungi of silkworm: a review; 2021; Uttar Pradesh J Zool, 42(21), pp.28-38.
20. Park S.K., Kwon H.G., Kang S.K. and Kim S.R.; Isolation and identification of insect pathogenic fungus from silkworms with suspected white muscardine disease; 2024; International Journal of Industrial Entomology, 48(1), pp.42-47.
21. Pau E.; Bolile și dăunătorii viermilor de mătase; 2000; S.C. Sericarom, Filiala de cercetare, București, România.
22. Praveena M.S., Rani A.S. and Savithri G.; Day-to-day analysis of nitrogenous products in Different Mulberry Silkworm Breeds during the Progressive infection of fungal pathogen *Beauveria bassiana*; 2024; Korean Journal of Physiology and Pharmacology, 28(1), pp.107-112.
23. Puneet C., Raghavendra Cg., Mohana Ks., Bhaskar R.N.; Assessment of diseases in *Bombyx mori* silkworm – A survey, Global Transitions Proceedings; 2021; 2 (1): 133-136, ISSN 2666-285X.
24. Punyavathi H.B.M.; Seri-bioinformatics: emerging trends and challenges in silkworm research; 2013; Advances in Biochemistry, 1(2), pp.33-42.
25. Raju C.G., Sarkar S., Canamedi V., Parameshwaranaik J. and Sarkar S.; A Review of Silk Farming Automation Using Artificial Intelligence, Machine Learning, and Cloud-Based Solutions; 2022; In International Conference on Data Analytics & Learning (pp. 101-116). Singapore: Springer Nature Singapore.
26. Ramos N., Miranda M.S., Franco A.R., Silva S.S., Azevedo J., Dias I.R., Reis R.L., Viegas C. and Gomes M.E.; Toward spinning greener advanced silk fibers by feeding silkworms with nanomaterials; 2020; ACS Sustainable Chemistry & Engineering, 8(32), pp.11872-11887.
27. Rohela G.K., Srinivasulu Y. and Rathore M.S.; A review paper on recent trends in bio-nanotechnology: implications and potentials; 2019; Nanoscience & Nanotechnology-Asia, 9(1), pp.12-20.
28. Sahana K.P., Gowda M., Narayanaswamy K.C. and Prasanna Kumar M.K.; Characterization of *Beauveria bassiana* isolate SHDL: a white muscardine-causing fungus in silkworm, *Bombyx mori L.* from Karnataka, India; 2023; Journal of Experimental Zoology India, 26(1).
29. Saharia S., Nath M., Kalita S., Kalita D. and Bardoloi S.; Identification and Toxicity Evaluation of Beauveria sp. Associated with White Muscardine Disease in Muga Silkworm Antheraea assamensis Helfer; 2024; Indian Journal of Entomology, pp.1-5.
30. Şapcaliu A., Savu V., Mihalcea T., Gheorghe A., Hăbeanu M., Măicănescu M.; Rolul diagnosticului de laborator în controlul bolilor contagioase la viermii de mătase, starea actuală, perspective și impactul asupra sericiculturii; 2023; Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca.
31. Schaly S., Ghebretatios M. and Prakash S.; Baculoviruses in gene therapy and personalized medicine; 2021; Biologics: Targets and Therapy, pp.115-132.
32. Shanmugam V, Seethapathy P.; Isolation and characterization of white muscardine fungi *Beauveria bassiana* (Bals.) Vuill. A causative of mulberry silkworm; 2017; J. Entomol. Zool. Stud; 5: 512-515.
33. Shi H., Xiao W., Zhu S., Li L. and Zhang J.; CA-YOLOv5: Detection model for healthy and diseased silkworms in mixed conditions based on improved YOLOv5; 2024; International Journal of Agricultural and Biological Engineering, 16(6), pp.236-245.
34. Sivaprasad V., Rahul K. and Makwana P.; Immunodiagnostic of silkworm diseases; 2021; In Methods in Microbiology (Vol. 49, pp. 27-46). Academic Press.

35. Subrahmanyam G., Kalita M., Krondashree D., Chutia M. & Das R.; Isolation and morphological characterization of a fungal isolate obtained from muscardine diseased Muga silkworm *Antheraea assamensis* Helfer (Lepidoptera: Saturniidae); 2018; International Journal of Microbiology Research, 10, 1435–1440.
36. Subrahmanyam G., Ponnuvel K.M., Arunkumar K.P., Rahul K., Moorthy S.M. and Sivaprasad V.; Molecular methods for diagnosis of microbial pathogens in *Muga silkworm*, *Antheraea assamensis* Helfer (Lepidoptera: Saturniidae); 2023; International Journal of Industrial Entomology, 47(1).
37. Vankadara S., Kamidi R., Pooja M.; Chapter 2-Immunodiagnosis of silkworm diseases, Editor(s): Volker Gurtler, Gangavarapu Subrahmanyam, Methods in Microbiology; 2021; Academic Press, 49: 27-46, ISSN 0580-9517, ISBN 9780128211458.
38. Yashaswini B., Madhusudhan N., Suresh D.; Automated smart sericulture based on IoT and image processing technique; 2020; 10 (6), ISSN 2321(3361).
39. Zhao P., Hong S., Li Y., Chen H., Gao H. and Wang C.; From phyllo sphere to insect cuticles: silkworms gather antifungal bacteria from mulberry leaves to battle fungal parasite attacks; 2024; Microbiome, 12(1), p.40.

CONTROL OF *BALANTIDIUM COLI* INFECTION IN SWINE WITH HYDROALCOHOLIC EXTRACTS OF WORMWOOD (*Artemisia absinthium* L.) AND TAGETES (*Tagetes patula*)

CONTROLUL INFECȚIEI CU *BALANTIDIUM COLI* LA SUINE, CU EXTRACTE HIDROALCOOLICE DE PELIN (*Artemisia absinthium* L.) ȘI CRĂIȚĂ (*Tagetes patula*)

TIȘE NICOLAE[2], BĂIEȘ HORIA-MIHAI[2], COZMA-PETRUȚ ANAMARIA[3*], MILITARU DUMITRU[1], GROZA IOAN[1,2], GYÖRKE ADRIANA[2], SZAKÁCS ANDREI[2], SPÎNU MARINA[1,4], COZMA VASILE[1,2]

[1]Academy of Agricultural and Forestry Sciences Gheorghe Ionescu-Șișești, 61 Mărăști Boulevard, 011464, Bucharest, Romania;
[2]Department of Parasitology and Parasitic Disease, Faculty of Veterinary Medicine, University of Agricultural Sciences, and Veterinary Medicine of Cluj-Napoca, Calea Mănăştur 3-5, 400372 Cluj-Napoca, Romania;
[3]"Iuliu Haţieganu" University of Medicine and Pharmacy, Faculty of Pharmacy, 8 V. Babeș Street, 400010, Cluj-Napoca, Romania;
[4]Institute of Research and Development for Agro Montanology - Cristian, Sibiu, Romania;

*Correspondence address: anamaria.cozma@umfcluj.ro

Abstract

Experimental research on the effect of hydroalcoholic extracts of antiparasitic wormwood (Artemisia absinthium L.) and tagetes (Tagetes patula) in B. coli infection in pigs, carried out during March and June 2020 on a total of 64 pigs aged 3 to 4 months, male and female, from actual maintained extensive, low input systems, have led to the following:
In the case of the hydroalcoholic extract of wormwood (Artemisia absinthium L.), B. coli infection extensity decreased from 60% to 30% after 7 days post-treatment and 20% respectively at 14- and 28-days post-treatment, and the values of CPG decreased from 350(±111.80) at 266,67(±16.67); 175(±25) at 50 after 7, 14- and 28-days post-treatment.
In the case of the hydroalcoholic extract of tagetes (Tagetes patula) B. coli infection extensity increased from an initial value of 50 % (23.7-76.3) to 60% (31.3-83.2) after 7- and 14-days post-treatment and 70% respectively on day 28, and the values of CPG dropped from 240 (±119.79) (pre-treatment) to 78.57 (±14.87), at the end of the experiment.
In the control group, the extensity B. coli infection ranged between 58.3 (32-87.7) and 66.7 (39.1-86.2) %, and infection intensity ranged between 263 (±71.18) and 363 (±88.01) CPG.

Keywords: Balantioides coli, wormwood, tagetes, pigs

Rezumat

Studiile experimentale privind acțiunea antiparazitară a extractelor hidroalcoolice de pelin (Artemisia absinthium L) și crăiță (Tagetes patula) în infecția cu B. coli la porci, derulate în perioada martie –iunie 2020, pe 64 animale, în vârstă de 3 - 4 luni, masculi și femele, provenind dintr-o fermă în sistem de creștere cu aport redus, au relevat următoarele:
La extractul hidroalcoolic de pelin (Artemisia absinthium L), prevalenta infecției cu B. coli și intervalul său de încredere de 95% au scăzut de la 60% la 30%, după 7 zile post-terapeutic și respectiv la 20%, la 14 și 28 zile post-terapeutic, iar valorile CPG au scăzut de la 350 (±111.80) la 266,67 (±16.67); 175 (±25) și 50 după 7, 14 și 28 zile, post-terapeutic.
Extractul hidroalcoolic de crăiță (Tagetes patula) a determinat creșterea prevalenței infecției cu B. coli și intervalului său de încredere de 95% de la valorile inițiale de 50 % (23.7-76.3) la 60% (31.3-83.2) după 7 și 14 zile post-terapeutic și respectiv la 70% (39.7-89.2) în ziua 28 a experimentului, iar valorile CPG au scăzut de la 240 (±119.79) (ante-terapeutic) la78.57 (±14.87), la finalul experimentului.
La lotul martor, prevalența infecției cu B. coli și intervalul său de încredere de 95% au avut valori cuprinse între 58,.3 (32-87.7) și 66.7 (39.1-86.2) %, iar CPG au prezentat valori cuprinse între 263 (±71.18) și 363 (±88.01).

Cuvinte cheie: Balantioides coli, pelin, crăiță, suine

INTRODUCTION

Balantidiosis, caused by *Balantidium coli* (syn. *Neobalantidium coli* or *Balantioides coli*) represents a neglected parasitic infection of zoonotic significance affecting a variety of hosts, including domestic pigs that are the main reservoir. *Balantidium coli* is an intestinal protozoan parasite that causes an infection called balantidiosis in humans. Other mammals can become infected by ingesting infectious cysts from food and water contaminated with feces. *Balantioides coli* is an enteric protozoan parasite of public veterinary health relevance and concern in animal production and food safety (João Mega et al., 2022).

Usually asymptomatic, *Balantidium coli* infection can cause symptoms such as diarrhea and abdominal pain. *B. coli* occurs worldwide but is most prevalent in tropical and subtropical regions as well as developing countries. Since pigs are an animal reservoir, human infections occur more frequently in areas where pigs are raised, especially if hygienic conditions are not respected (CDC-Balantidiasis).

Since 2014, *B. coli* has been considered, both by the Food and Agriculture Organization of the United Nations and the World Health Organization, as an emerging pathogen and a foodborne parasite that should be included in specific control guidelines (Bouwknegt et al., 2016).

This parasite is frequently found in the gastrointestinal tract of mammals, namely domestic and production animals, such as pigs (the primary reservoir host), sheep, and horses. *Balantioides coli* is the only ciliated protozoan capable of infecting humans, albeit with low prevalence the damage being sometimes associated with symptoms as diarrhea and abdominal pain. In most mammals, including humans, *B. coli* infection is asymptomatic and causes no significant damage to the gastrointestinal tract (Schuster et al.,2008). However, if the host is immunocompromised, acute or chronic clinical manifestations may appear (Vasilakopoulou et al., 2003).

The aim of the experimental research was to establish the antiparasitic effect of the hydroalcoholic extracts of wormwood (*Artemisia absinthium* L.) and tagetes (*Tagetes patula*) in the infection with B. coli in pigs from low input rearing systems.

MATERIALS AND METHODS

The experimental protocol

The study was carried out between March and June 2020 on a total of 64 pigs, aged 3 to 4 months, male and female, from the Bistriţa-Năsăud department. The distribution of animals per batch is presented in Table 1.

The classic conditions of extensive exploitation are preserved in the pig herds studied, with some differences from one area to another. The shelters provide microclimatic conditions based solely on the animals' biological heat, without mechanical ventilation; there are no special provisions for feeding or watering, and work in the shelters is done manually.

In total, 64 samples were collected from households per stage, representing 100% of the herds studied. Hermetically sealed plastic packages (boxes) that were properly labeled for identification were used to collect the samples.

Table 1. Groups of pigs taken in the study / Loturile de porci luate în studiu

Group no.	*Locality*	*Number of pigs*	*Number of samples*	*Plant extract*
1	VM	20	20	*Artemisia absinthiumL.*
2	C1	20	20	*Tagetes patula*
3	C2	24	24	Control group
Total		**64**	**64**	

Two commercial products for human use, HYDROALCOHOLIC EXTRACTS, Wormwood (*Artemisia absinthium*), Pure Health Botanicals and *Tagetes patula,* 50ml, HOFIGAL, with a composition for 100 g of solution: tagetes flowers (*Tagetes* flos) 20g, ethyl alcohol 70% v/v were used in the experiment.

Group 1 was treated with hydroalcoholic extract of wormwood - *Arthemisia absinthium* L., at a dose of 10 ml per animal, for 7 days. Group 2 was treated with the hydroalcoholic extract of sedge - *Tagetes patula*, the whole plant, administered for 7 days, at a rate of 20 ml to each animal. Group 3, control, did not receive any treatment. On day 0 of the experiment, fecal samples were collected individually from the three batches and examined using the McMaster quantitative flotation method. After establishing batches, treatments were carried out, and samples were collected at 7, 14, and 28 days. The coproparasitological examination was carried out at the Discipline Laboratory of Parasitology and Parasitic Diseases, FMV Cluj Napoca.

Statistical analysis. Prevalence and its 95% confidence interval of *Balantidium coli* infection in pigs were calculated for each group and time point before (day 0) and after treatment (day 7, 14, and 28 after treatment). Chi-square test was used to analyze the differences among groups and different time points using Epitools (Sergeant, 2018).

Then, the mean value and standard error of the mean were calculated for the number of *B. coli* cysts per gram faeces. The Kruskal-Wallis test was used to find differences within treatment at different exposure time with Conover as a post-hoc test, and the Friedman test was used to compare different treatments at specific times. A value of $p \leq 0.05$ was considered statistically significant. Statistical analysis was performed with MedCalc Statistical Software version 19.0.4.

RESULTS AND DISCUSSION

The results of coproscopic examinations of *B. coli* infection in pigs from batch 1 treated with hydroalcoholic extract of wormwood (*Artemisia absinthium*) are presented in Table 2, with the evolution of the dynamics of parasite extensibility.

Table 2. Prevalence and its 95% confidence interval of *Balantidium coli* infection in untreated and treated pigs with hydroalcoholic extract of *Artemisia absinthium L.* and *Tagetes patula* / Prevalența și intervalul de confidență de 95% al infecției cu Balantidium coli la porcii netratați și tratați cu extract hidroalcoolic de *Artemisia absinthium L.* și *Tagetes patula*

Groups	Day 0	Day 7	Day 14	Day 28	p
Control	66.7 (39.1-86.2)	66.7 (39.1-86.2)	58.3 (32-87.7)	66.7 (39.1-86.2)	0.97
Artemisia absinthium L.	60 (31.3-83.2)	30 (10.8-60.3)	20 (5.7-51)	20 (5.7-51)	0.08
Tagetes patula	50 (23.7-76.3)	60 (31.3-83.2)	60 (31.3-83.2)	70 (39.7-89.2)	0.84
p	0.73	0.93	0.12	0.04	

Table 3. Number of *Balantidium coli* cysts per gram faeces in untreated and treated pigs with hydroalcoholic extract of *Artemisia absinthium L.* and *Tagetes patula* (mean±standard error of the mean) / Numărul de chisturi Balantidium coli per gram de fecale la porcii netratați și tratați cu extract hidroalcoolic de *Artemisia absinthium L.* și *Tagetes patula* (media ± eroarea standard a mediei)

Groups	Day 0	Day 7	Day 14	Day 28	*p*
Control	363 (±88.01)	306 (±79.30)	271 (±70.48)	263 (±71.18)	0.86
Artemisia absinthium L.	350 (±111.80)	266.67 (±16.67)	175 (±25)	50	0.10
Tagetes patula	240 (±119.79)	191.67 (±43.62)	166.67 (±52.70)	78.57 (±14.87)	0.92
p	0.90	0.50	0.22	0.00008	

Evolution of *Balantidium coli* infection in the group treated with hydroalcoholic extract of *Artemisia absinthium L.*

At the pre-treatment level, the extent of infection was 60%, and the average intensity value was 350 ± 273.86 (50-750) CPG.

The harvest carried out 7 days after treatment with the hydroalcoholic extract of wormwood revealed the following changes: the decrease in the average intensity value to 266.6 ± 28.86 (0-300) OPG, and 30% extensibility.

At 14 days post-therapeutic, following coproparasitological examinations, the following data were recorded: the extent decreased by up to 20%, and the intensity also made a descending cube, reaching the average value of 175 ± 35.35 (0-200) CPG.

In the last sample taken 28 days post-therapy, the extent of *B. coli* infection was 20%, and the average intensity value was 50 (0-50) CPG.

Evolution of infection with *B. coli* in swine group treated with extract of *Tagetes patula*

At the pre-treatment level, the extent of *B. coli* infection in pigs in group 2 was 50%, and the average value of the intensity was 240 ± 267.66 (50-700) CPG.

At 7 days after administration of *Tagetes patula* extract, the extent of *B. coli* infection was increased to 60%, while the intensity slightly decreased to 191.66 ± 106, 84 (50-300) CPG.

The extension zone remained the same, and the collection zone was carried out 14 hours after treatment, at 60%, and the extension zone was mediated using an intensity of 166.6 ± 129.09 (50-400) CPG.

The last collection carried out on the 28th of each week of treatment with an extract of your cream allows an intensity of B. coli infection to continue in a recording scale of 78.57 ± 39.33 (50-150) CPG, peak extension with 10 percent value and 70% value.

Evolution of *Balantidium coli* infection in the control group

In the experiment, the prevalence of *B. coli* infection ranged from about 58.3 to 66.6%, and the intensity of infection ranged from 262.5 to 362.5 CPG (Table 2; 3).

Comparative dynamics of *B. coli* infection intensity in the 3 groups of pigs

It was observed that at the beginning of the experiment, the extent of infection with *B. coli* in the 3 groups of pigs taken in the study had values between 50-66.6%. At the end of the experiment, the lowest extent (20%) was recorded in the case of the group of pigs treated with *Artemisia absinthium* extract. Also, at the end of the experiment, the lowest intensity (50 CPG) was recorded in the group treated with *Artemisia absinthium.*

On the international level, the aspects related to medicinal chemical pollution are increasingly being studied through the accumulation of residues with active potential (mutagenic, carcinogenic, teratogenic) in the products and by-products from animals subjected to antiparasitic treatments (Cernea et al., 2006). The way to avoid these inconveniences could be to prioritize the use of pharmaceutical products of an organic nature from the plant kingdom. Plant extracts can be an alternative source of materials used in the control of parasitosis in animals, because they contain large amounts of bioactive chemicals and are often used as flavorings or spices in the food industry (Cicevan et al., 2016).

It is evident that to combat parasitic diseases properly, the judicious combination of several methods must be considered, and, therefore, the application of only some methods cannot be fully effective. At the same time, non-chemical control will eliminate selection pressure for resistance and help maintain the parasite's sensitivity to synthetic compounds (Cernea et al., 2006).

Balantidiosis, a digestive zoonosis, produced by the ciliate *Balantidium coli,* is found in humans, pigs, laboratory animals etc. In piglets and young pigs, it is manifested by diarrheal syndrome, growth retardation, and in adult pigs the disease evolves subclinical. The access of the balantids to the depth of the intestinal mucosa is possible when the populations are very numerous or when there are changes in the intestinal chemistry, with the fragility of the cell membranes. The disease progresses clinically in piglets. After an incubation of 10-15 days, the following was observed: the appearance of diarrhea, watery feces with mucus, grayish yellow, sometimes hemorrhagic; capricious appetite, emaciation, and bodily underdevelopment. Symptoms can remit spontaneously after 6-8 days, with healing or severe relapse after a few days (Şuteu and Cozma, 2004).

Different studies conducted to determine the extent of infection with *B. coli,* show different values in humans and animals.

Balantidiosis represents a neglected parasitic infection of zoonotic significance affecting a variety of hosts, including domestic pigs, that are the main reservoir. *B. coli* has a direct life cycle with a fecal-oral route transmission, mainly by ingesting food and water contaminated with cysts. The ingestion of meat contaminated during inappropriate slaughtering processes may represent a new potential route of transmission. Only a few studies have investigated the prevalence of *B. coli* in domestic pigs in Italy despite its high prevalence and zoonotic significance. This study aimed to improve the knowledge of *B. coli* prevalence in domestic swine reared both in intensive and "en plein air" breeding systems in the south of Italy (Giarratana et al., 2021).

A cross-sectional survey was performed on 20 pig breeding farms in southern Hesse, central Germany, to evaluate the prevalence and age-dependent occurrence of intestinal protozoan parasites in unweaned piglets. Faecal samples of clinically unaffected piglets of different age (< 1 to 5-7 weeks) were examined. Infections with the following protozoan species were detected: *Balantidium coli* (16 of 20 farms), *Entamoeba sp.* (15), *Jodamoeba sp*. (14), *Isospora (I.) suis* (9), *Chilomastix sp.* (6) and *Eimeria spp*(6) (Damriyasa and Bauer, 2006).

In Romania, Băieș et al., 2021, 2022 say that the zoonotic protozoan *B*. *coli* was the most frequent parasite in all categories, with the highest prevalence (72.5%) in fattening pigs, while in weaners and sows, the values were somewhat lower (70.31% and 62.19%, respectively. Similar infection rates were diagnosed in Kenya (69.6% in sows, 69.2% in fatteners, and 66.7% in sows) and Greece (13.5% in weaners, 54.3% in fatteners, 81.3% in sows) (SYMEONIDOU et al., 2020). The differences in prevalence could be due to some extrinsic factors such as the detection procedures, sampling sizes, farm management, climate differences, geographical separation, and intrinsic ones such as the breed, immune status, or other intercurrent diseases (Ichim, O*.,* 2012; Jankowska-Makosa and Knecht, 2015*;* Kochanowski et al., 2017; Li et al., 2020).

As the most common medicinal and aromatic plants from Europe that can be used on pigs the following were listed: *Allium sativum* (garlic), *Artemisia absinthium* (Wormwood), *Calendula officinalis* (marigold), *Coriandrum sativum* (coriander), *Cucurbita pepo* (pumpkin) and *Satureja hortensis* (summer savory, bean herb, garden savory). These plants have several medicinal properties, such as anti-inflammatory, anti-tumoral, antibacterial, antiviral, and antiparasitic effects in domesticated animals (Pop and Olos, 2004; Băies et al., 2021).

In this experiment, the evaluation of the antiparasitic efficacy of two plants from the Romanian flora (*A. absinthium and T. patula*) was carried out successfully on a low-input farm. The efficacy of plants in *B. coli* infections in swine was estimated by calculating the difference between the parasitic prevalence and intensity before and after the wormwood and tagetes therapy. *Balantioides coli* (former *Balantidium coli*) was diagnosed in all swine groups. *A. absinthium* were both effective against balantidiosis. *Tagetes patula* had a lower antibalantidian activity.

CONCLUSIONS

Balantioides coli (former *Balantidium coli*) was diagnosed in all swine groups. *A. absinthium* were both effective against balantidiosis. *Tagetes patula* had a lower antibalantidian activity.

The results obtained from our research have practical implications for low-input livestock systems, as well as the welfare and health of pigs. Moreover, considering the zoonotic potential of *B. coli*, disseminating such studies is of utmost importance.

BIBLIOGRAPHICAL REFERENCES

1. Băieş M.H., Boros Z., Cozma-Petrut A., Duca G., Spînu M., Cozma V.; Medicinal plants with antiparasitic potential in swine (*Sus scrofa domesticus*); 2021; Scientia Parasitologica 22, 112-119.
2. Băieş M.H., Boros Z., Gherman C., Spînu M., Mathe A., Cozma V.; Co-infections with gastrointestinal parasites in a free-range swine farm from the Transylvania area; 2021; Scientia Parasitologica, 22, 50-57.
3. Băieş M.H., Boros Z., Gherman C.M., Spînu M., Mathe A., Pataky S., Cozma V.; Prevalence of swine gastrointestinal parasites in two free-range farms from Nord-West region of Romania; 2022; Pathogens 11, 954.
4. Bouwknegt M., Devleesschauwer B., Graham H., Robertson L.J., van der Giessen J.W.; Prioritisation of foodborne parasites in Europe; 2016; the Euro-Fbp Workshop Participants. Eurosurveillance 2018, 23, 17–161. https://doi.org/10.2807/1560.
5. Cernea Laura Cristina, Şuteu E., Cernea M., Lefkaditis M., Cozma V.; Realizarea unui model experimental de testare *in vitro* a efectului acaricid al extractelor vegetale; 2006; Sc. Parasitol., 1-2, 35-40.
6. Cicevan R.Al., Hassan M., Sestras AF, Prohens J., Vicente O., Sestras R.E., Boscaiu M.; Screening for drought tolerance in cultivars of the ornamental genus *Tagetes* (Asteraceae); 2016; PeerJ 4:e2133 https://doi.org/107717/peerj.2133.
7. Damriyasa I.M., C. Bauer; Prevalence and age-dependent occurrence of intestinal protozoan infections in suckling piglets; 2006; Berl Munch Tierarztl Wochenschr. Jul-Aug;119(7-8):287-290.
8. Giarratana F., Nalbone L., Napoli E., Lanz V., Panebianco, A.; Prevalence of *Balantidium coli* (Malmsten, 1857) infection in swine reared in South Italy: A widespread neglected zoonosis; 2021; Veterinary World, 14(4): 1044-1049. www.doi.org/10.14202/vetworld.2021.1044-1049.
9. Ichim O.; An overview of organic pig farming in Romania; 2012; Porcine Research, 2(2), 50-65.
10. Jankowska-Makosa A., Knecht D.; Prevalence of endoparasites infection in fatteners depending on maintenance system and season; 2015; Veterinarija ir Zootechnika 70(92):29-36
11. Kochanowski M., Karamon J., Dąbrowska J., Dors A., Czyżewska-Dors E. & Cencek T.; Occurrence of intestinal parasites in pigs in Poland-the influence of factors related to the production system; 2017; Journal of veterinary research, 61(4), 459-466.
12. Li J., Wang Z., Karim M.R., Zhang L.; Detection of human intestinal protozoan parasites in vegetables and fruits: A review; 2020; Parasit. Vectors 2020, 13, 380.
13. Mega J., Santos-Silva S., Loureiro A., Palmeira J.D., Torres R.T., Rivero-Juarez A., Carmena D., Mesquita J.; *Balantioides coli* Fecal Excretion in Hunted Wild Cervids (*Cervus elaphus* and *Dama dama*) from Portugal; 2022; Pathogens 11, 1242. https://doi.org/10.3390/ pathogens11111242.
14. Pop M., Olos E.; Remedii din farmacia naturii; 2004; ediţia a 2-a, Edit. Fiat lux. 148(7):357-64.
15. Schuster F.L., Ramirez-Avila L.; Current world status of *Balantidium coli*; 2008; Clin. Microbiol. Rev. 21, 626–638. [CrossRef].
16. Sergeant E.S.G.; Epitools Epidemiological Calculators; 2018; Ausvet. Available at: http://epitools.ausvet.com.au.
17. Şuteu I., Cozma V.; Parazitologie clinică veterinară, vol. 1; 2004; Colecţia veterinaria. Ed. Risoprint, Cluj-Napoca.
18. Symeonidou I., Panagiotis Tassis, Athanasios I. Gelasakis Eleni, D. Tzika, Elias Papadopoulos; Prevalence and Risk Factors of Intestinal Parasite Infections in Greek Swine Farrow-To-Finish Farms, Pathogens, 2020, 9, 556; doi:10.3390/pathogens9070556.
19. Vasilakopoulou A., Dimarongona K., Samakovli A., Papadimitris K., Avlami A.; *Balantidium coli* pneumonia in an immunocompromised patient; 2003; Scand. J. Infect. Dis. 2003, 35, 144–146. 917.ES.2018.23.9.17-00161.
20. https://www.cdc.gov/balantidium/about/index.html: CDC-Balantidiasis.

PREDICTIVE VALUE OF CIRCULATING IMMUNE COMPLEXES' LEVELS AND LESIONAL SCORES IN CONTAGIOUS AGALACTIA OF SHEEP

VALOAREA PROGNOSTICĂ A NIVELELOR COMPLEXELOR IMUNE CIRCULANTE ȘI A SCORURILOR LEZIONALE ÎN AGALAXIA CONTAGIOASĂ A OVINELOR

VASIU AUREL [1], PALL EMOKE[1,2], DUCA GHEORGHITA[,2], SPINU MARINA[1,2], SANDRU CARMEN DANA [1,2], OLAH DIANA[1]

[1] Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, Str. Manastur no. 3-5, Cluj-Napoca, Romania
[2] Institute of Research and Development for Montanology Str. XIII No. 147, Cristian, Sibiu, Romania

Correspondence address: marina.spinu@gmail.com

Abstract

Contagious agalactia of sheep and goats, induced by Mycoplasma agalactiae represents an infectious condition often accompanied by septicemia, arthritis and keratoconjunctivits. The outcome of the interaction between micoplasmae and the immune system plays an important role in the development of the disease, the severeness of lesions and even the survival rate. The present experiment sought the quantification of circulating immune complexes, quantified by 4.2% PEG precipitation test in a flock of 827 privately owned ***Turcana*** *sheep with contagious agalactia, in correlation with the severity of the gross lesions, vaccination status and therapy, quantified by scores. The levels of circulating immune complexes in animals with clinically apparent contagious agalactia did not differ significantly before and after the implementation of the therapy. The calculation of the correlation coefficient r between the lesion scores and the CIC levels demonstrated that the dependence of the two studied parameters was direct but not very close, lacking statistical assurance. The levels of CIC increased in relation to the increase of the time elapsed since vaccination and along with the aggravation of the lesions. Meanwhile, the long-term treatment caused a decrease in the levels of circulating immune complexes, positively influencing the remediation of lesions, probably also due to the reduction of CIC concentrations.*

Key words: sheep, contagious agalactia, lesional scores, circulating immune complexes, predictive relevance

Rezumat

Agalactia contagioasă a oilor și caprinei, indusă de Mycoplasma agalactiae reprezintă o afecțiune infecțioasă adesea însoțită de septicemie, artrită și keratoconjunctivite. Rezultatul interacțiunii dintre micoplasme și sistemul imunitar joacă un rol important în dezvoltarea bolii, severitatea leziunilor și chiar rata de supraviețuire. Experimentul de față a urmărit cuantificarea complexelor imune circulante, evaluate prin testul de precipitare PEG 4,2% într-un un efectiv de 827 de oi ***Țurcană*** *proprietate privată cu semne de agalactie contagioasă, în corelație cu gravitatea leziunilor macroscopice, statusul vaccinare și terapie, cuantificate prin scoruri. Nivelurile complexelor imune circulante la animalele cu agalactie contagioasă evidentă clinic nu au diferit semnificativ înainte și după implementarea terapiei. Calculul coeficientului de corelație r între scorurile lezionale și nivelurile CIC a demonstrat că dependența celor doi parametri studiați a fost directă, dar nu foarte apropiată, lipsită de asigurare statistică. Nivelurile de CIC au crescut în raport cu creșterea timpului scurs de la vaccinare și odată cu agravarea leziunilor. Tratamentul de lungă durată a determinat scăderea nivelurilor complexelor imune circulante, influențând pozitiv remedierea leziunilor, probabil și datorită reducerii concentrațiilor de CIC.*

Cuvinte cheie: ovine, agalaxie contagioasă, scoruri lezionale, complexe imune circulante, valoare predictivă

INTRODUCTION

Contagious agalactia of sheep and goats, induced by *Mycoplasma agalactiae* represents an infectious condition often accompanied by septicemia, arthritis and keratoconjunctivits.

The outcome of the interaction between mycoplasma and the immune system plays an important role in the development of the disease, the severeness of lesions and even the survival rate. Thus, the quantification of the functional status of certain immunological effectors could endorse the predictive value of clinical findings (Cole and Atkin, 1991) In the current frame of infectious diseases, the control of notifiable diseases represents an essential scope. Effective epidemiological surveillance requires the detection of animals without evident clinical signs of the disease and their elimination from the herd as soon as possible, thus interrupting the epidemiological cycle. Outlining the immunological profile of a disease for which the carrier state represents an important link in the progression of the disease is extremely useful for diagnostic purposes (Vior et al., 1982).

Mycoplasma diseases are typical examples of multifactorial diseases, where factors such as concurrent infections, overcrowding, changes in climatic conditions, age, genetic constitution, transportation stress and handling represent important determinants in the success of the infection (Stuart et al., 2002).

The role of the host's immune reactions during *M. pneumoniae* infection is supported by numerous arguments, such as: the existence of more serious symptoms during secondary infections; the presence of autoantibodies; the gross lesions with the presence of perivascular and peri-bronchial lymphocyte and plasma cell infiltrates (Almeida et al., 1992).

The immune prevention of mycoplasma induced diseases also raises certain problems (Barbosa et al., 2024). Although passing through the infection has a protective effect, the immunity is short-lived in mastitis. The ability of some anti-mycoplasma vaccines to induce immunosuppression has already been mentioned. One of the major goals of vaccination in this disease is to preserve the protective side of the immune response and to remove the hypersensitivity side (known feature in natural infections) (Roitt et al., 1989).

The elimination of mycoplasmas through milk by animals without clinical infections, as well as the presence of mycoplasmas at ear level and in parasites of the genera *Psoroptes* and *Railletia* in healthy carriers, favors their dissemination during transhumance. The contamination of naïve herds implies the broadening of the transmission framework and the increase of economic importance of the disease. The data related to the epidemiological surveillance by establishing the immunological profile of the disease is limited to signaling the presence of anti-mycoplasma antibodies.

Contagious agalactia, an infectious-contagious disease specific to sheep and goats, is widespread in many countries in Europe, Africa and North America causes an important economic impact, especially due to the high morbidity in lactating sheep and compromising milk production for the respective season, or for the entire economic life of the ewe. Some of the newborn lambs die either of starvation, or due to serious eye and joint injuries that impede on movement.

Mycoplasma agalactiae, a polymorphic germ, persists in the environment, as well as, in the mammary gland in a latent form, after healing of the animal, which could lead to further episodes and spread of the disease (Kumar et al., 2014). Sheep and goats are naturally susceptible, especially lactating ones, while young, barren sheep and males are much less susceptible. The main sources of infection are represented by sick or diseased animals, which eliminate germs through: milk, feces, urine, nasal, ocular and genital secretions (Vasiu, 2001).

The introduction of the disease into disease-free herds is usually done by the acquisition of germ-carrying and excreting animals, the infection spreading easily through milking, evolves as an endemic or sometimes as an epidemic. Another route of infection is the digestive one, through contaminated food and water. The infection can be achieved by respiratory, conjunctival or cutaneous route (Hegde et al., 2016).

The multiplication of the germ produces an irritation and consequently the exudative inflammation complicated by secondary bacterial infections which can lead to the appearance of pussy-necrotic complications (pussy mastitis - mammary sclerosis, pussy arthritis - ankyloses, pan-ophthalmia)

(Hegde et al., 2018, Chen et al., 2021). Animals that have gone through the disease become less receptive to a new infection.

Estimating the significance of the autoimmune component through CIC dosing is an important element in completing the immuno-pathological profile of the disease.

The present experiment sought the quantification of circulating immune complexes in sheep with contagious agalactia, in correlation with the severity of the gross lesions. The correlation of the gross lesions with the immunological status of the animals (vaccination or absence of vaccination) and with the therapeutic results was also followed.

MATERIAL AND METHOD

a. Biological material. Experimental protocol

The experiment was carried out in a flock of 827 privately owned **Turcana** sheep. The animals were aged 2 to 8 years, the herd being composed of 21 rams and 806 lactating ewes in different stages of pregnancy. In the flock, vaccinations against mycoplasma disease were performed on regular basis, but stopped two years before the disease episode.

The investigations were carried out on 18 sick animals, 2 rams and 16 ewes being tested; some of the animals were treated during the experiment, while others were not.

Both vaccinated and non-vaccinated animals were subjected to investigations, all with clinical signs of contagious agalactia, with different lesions: joint, ocular or mixed.

Blood was sampled twice, 18 days apart. The blood was allowed to clot at 37°C, the sera were centrifuged at 2500 rpm for 10 minutes, then separated, transferred into Eppendorf tubes and kept in the freezer at -20°C until testing.

b. Methods

b1. Circulating immune complexes were evaluated by precipitation with PEG 4.2% (Ghergariu S., 2000). Measurement of the level of circulating immune complexes (CIC) allows evaluation of the molecular clearance capacity at a particular moment. A 4.2% polyethylene glycol (PEG) solution was prepared in borate buffer and was used as the precipitating agent. The control sere were treated with the buffer alone and served as controls for borate-induced precipitation. The reaction was performed in a 96-well-plate and red spectophotometrically. For that, aliquots of 196.7µl of borate buffer and PEG solution, respectively, were mixed with 3.3µl samples of the serum, for each sample, in parallel wells. The samples were allowed to precipitate at room temperature (22–23°C) for 60·min, then read spectrophotometrically at a wavelength of 450nm in the test plate (d=0.5·cm) (multichannel spectrophotometer SUMAL PE2, Karl Zeiss, Jena, Germany). Circulating immune complexes' concentrations, expressed in optical density units (ODU) were calculated by subtracting the value of the control (serum + buffer) from that of the PEG precipitate (Klokhlova et al., 2004).

b2. Scores were given for the gross lesions, by location (joint location = 1, ocular location = 2, mixed location = 3), for the presence or absence of vaccination (non-vaccinated = 1, vaccinated = 2) as well as the presence or absence of the treatment (untreated=1, treated=2). These scores were correlated with the levels of circulating immune complexes for the both samplings, in order to evaluate the interference of CIC and their circulation in the blood during the pathogenic process.

b3. The vaccination score was established by simply ranking the non-vaccinated (score 1) and vaccinated animals (score 2), which showed clinical signs of the disease. These values were included in table 3 along with the CIC values to investigate the significance of the correlation levels between the two parameters. Similarly, scores were allocated for therapy implemented (score 2) or not implemented (score 1).

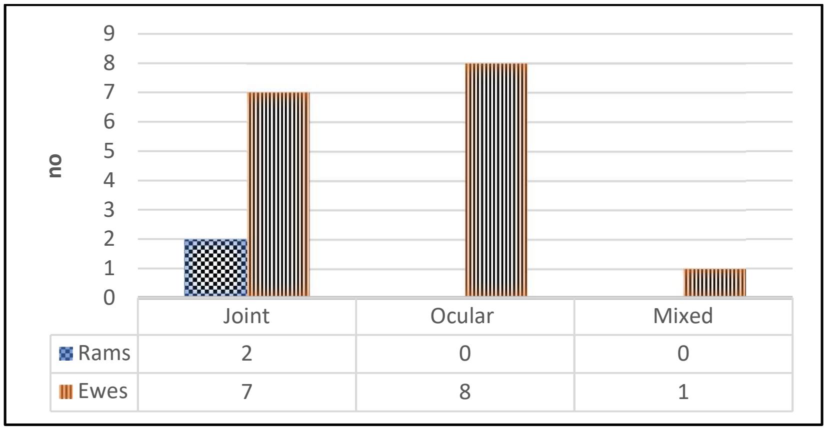
b4. All the obtained results were processed statistically to obtain the mean values, standard deviations and variance using the usual formulas. The significance of the differences was assessed by the Student t-test using the Microsoft Excel program.

RESULTS AND DISCUSSION

The evaluation of the dynamics of immune status during a disease where the asymptomatic carriers allow its propagation and persistence in naïve livestock in conditions of transhumance, brings an element of novelty. The assessment of certain tests used to investigate the *in vitro* non-specific and/or specific response significantly contributes to the characterization of some particular aspects related to the pathogenesis of contagious agalactia in sheep, in connection with the animals' immune profile. Changes in the functional levels of specific effectors during the course of the disease allow the clarification of some aspects related to the recrudescence of the disease under constant vaccination against the disease in areas of high epidemiological risk (Franzoso et al.,1992).

The characteristic condition induced by mycoplasmas in sheep and goats is the mycoplasma mastitis, produced by *M. agalactiae* which is often accompanied by septicemia, arthritis, keratoconjunctivitis. This mycoplasma is adapted to the mammary gland, but can also be present in the digestive tract. The interaction of mycoplasmas with the immune system is significant and plays a key role in the establishment and evolution of mycoplasma infection. The correlation of the presence of antibodies with the synthesis of immune complexes could be responsible for the extrapulmonary lesions associated with the infection: lesions of the nervous system, myocarditis, arthritis.

The experiment sought a better knowledge of humoral reactivity, which can contribute to the elucidation of pathogenic mechanisms and the establishment of more effective countermeasures.



	Joint	Ocular	Mixed
▩ Rams	2	0	0
▥ Ewes	7	8	1

Figure 1. The recording of the lesions' distribution by gender of the animals and anatomical region / Distribuția leziunilor micoplasmice la berbeci și oi în funcție de zona anatomică

Figure 1 illustrates the distribution of the lesions by gender of the animals and anatomical location. Most of the affected animals were females (the number of males in the herd being smaller), the lesions being mostly ocular ones, while the males presented exclusively joint lesions.

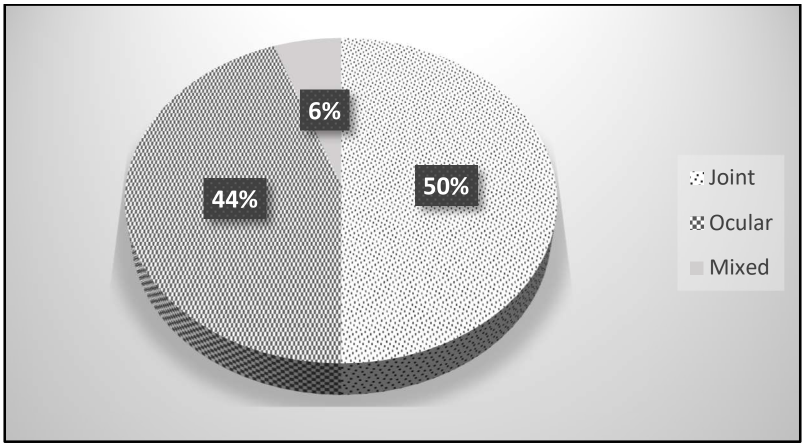


Figure 2. The percentages of the gross lesions by anatomical site / Distribuția procentuală a leziunilor micoplasmice în funcție de localizare

In fig.2 the percentages of the lesions by location are represented in the tested group.

The lesion scores given and the CIC levels read must also be correlated with the nutritional status of the tested animals, being known that environmental factors influence with different intensity the response to *Mycoplasma* (Lai et al., 1991).

The individual values obtained at the first sampling fall between 0.002 and 0.016 UDO, while at the second sampling they are between 0 and 0.029 UDO, a slight increase being observed at the second sampling (table 1).

Table 1. Individual values and statistical data on the CIC values in sheep with contagious agalactia / Valorile individuale şi medii ale CIC la ovine cu agalaxie contagioasă

Sample No.	**Sampling I**			**Sampling II**			
	PEG	**borate buffer**	**CIC**	**Sample No.**	**PEG**	**borate buffer**	**CIC**
1	0.047	0.031	**0.016**	1	0.029	0.029	**0**
2	0.031	0.016	**0.015**	2	0.046	0.037	**0.009**
3	0.038	0.032	**0.006**	3	0.023	0.016	**0.007**
4	0.047	0.036	**0.011**	4	0.083	0.083	**0**
5	0.035	0.033	**0.002**	5	0.036	0.025	**0.011**
6	0.032	0.022	**0.01**	6	0.046	0.021	**0.025**
7	0.032	0.028	**0.004**	7	0.016	0.015	**0.001**
8	0.042	0.035	**0.007**	8	0.031	0.03	**0.001**
9	0.022	0.016	**0.006**	9	0.032	0.025	**0.007**
10	0.033	0.022	**0.011**	10	0.051	0.022	**0.029**
11	0.05	0.04	**0.01**	11	0.033	0.023	**0.01**
12	0.042	0.032	**0.01**	12	0.03	0.023	**0.007**
13	0.031	0.021	**0.01**	13	0.038	0.032	**0.006**
14	0.034	0.028	**0.006**	14	nt	nt	**nt**
15	0.049	0.04	**0.009**	15	nt	nt	**nt**
16	0.05	0.041	**0.009**	16	0.061	0.06	**0.001**
17	0.038	0.033	**0.005**	17	0.054	0.034	**0.02**
Average	**0.0384**	**0.0297**	**0.0086**	**Average**	**0.0406**	**0.0316**	**0.0089**
Stdev	0.0082	0.0079	0.0036	Stdev	0.0168	0.0178	0.0090
Variance	6.726	6.365	1.355	Variance	0.0003	0.00031	8.265

When the presence of pathogenic mycoplasma is demonstrated in healthy goats in the ear canal and when these animals can cohabit with sheep in extensive breeding and transhumance, the role of the bacteria in causing the disease can be relatively easily demonstrated and the presence of an immune response to those is not surprising (Cottew and Yeats, 1982).

The calculation of the correlation coefficient between the lesion scores and the CIC levels demonstrates that the dependence of the two studied indicators is direct but not very close, lacking statistical assurance. It can be concluded that, although the appearance of lesions can be influenced by the synthesis of circulating immune complexes, this influence is not major or mandatory (table 2).

The effect of different adjuvants in increasing protection against mycoplasmas mastitis (*Mycoplasma agalactiae*) is demonstrated by numerous researches. Immunization against *agalactia contagiosum* specifically involves the protein Hsp60 (Glew et al., 2000, Ron et al., 2002, Scherm et al., 2002). For example, it is known that polyinosinic-polycytidylic acid leads to the conversion of IgG2a synthesis to IgG3, by modifying the B isotype in a suitable cytokine environment (Avramidis et al., 2002). The effect of vaccination *per se*, by increasing the titers of antibodies, can lead to an increase in the concentration of circulating immune complexes. Sometimes they can have unwanted effects, such as the aggravation of the lesions appearing during the course of the disease, when the animals are insufficiently protected, due to the long time that elapsed since the last vaccination.

Table 2. The individual arrays of CIC and lesional scores used for calculation of r for the two samplings / Şirurile de valori utilizate pentru evaluarea corelaţiei dintre scorurile lezionale acordate şi nivelele complexelor imune circulante la cele două recoltări

Sample No	Sampling I		Sampling II	
	CIC	Score	CIC	Score
1	0.016	1	0	1
2	0.015	3	0.009	3
3	0.006	1	0.007	1
4	0.011	1	0	1
5	0.002	2	0.011	2
6	0.01	1	0.025	1
7	0.004	1	0.001	1
8	0.007	1	0.001	1
9	0.006	1	0.007	1
10	0.011	2	0.029	2
11	0.01	2	0.01	2
12	0.01	2	0.007	2
13	0.01	2	0.006	2
14	0.006	2	0.0089	2
15	0.009	2	0.0089	2
16	0.009	1	0.001	1
17	0.005	1	0.02	1
18	0.0086	2	0.0089	2
r	**0.27227**	**P NS**	**0.213351**	**P NS**

Table 3. The individual arrays of CIC and immunisation levels used for calculation of r for the two samplings / Şirurile de valori utilizate pentru evaluarea corelaţiei dintre nivelele imunizării şi concentraţiile complexelor imune circulante la cele două recoltări

Sample No	Sampling I		Sampling II	
	CIC	Vaccination status	CIC	Vaccination status
1	0.016	1	0	1
2	0.015	1	0.009	1
3	0.006	1	0.007	1
4	0.011	1	0	1
5	0.002	1	0.011	1
6	0.01	2	0.025	2
7	0.004	2	0.001	2
8	0.007	2	0.001	2
9	0.006	2	0.007	2
10	0.011	2	0.029	2
11	0.01	2	0.01	2
12	0.01	2	0.007	2
13	0.01	2	0.006	2
14	0.006	2	0.0089	2
15	0.009	2	0.0089	2
16	0.009	2	0.001	2
17	0.005	2	0.02	2
18	0.0086	2	0.0089	2
r	**-0.24275**	**P NS**	**0.272895**	**P NS**

The results presented in table 3 indicated a reversed non-significant correlation between the two parameters for the first sampling, while the correlation was direct for the second sampling. The last, although statistically non-significant, indicated a potential increase in CIC subsequent to vaccination which could negatively influence the lesion scores, in case the disease appeared. Meanwhile, further estimations are required to evaluate the clearance of the CIC (CIC dynamics) in order to rank the normal functioning of the immune system.

Even for this parameter, the r values were not ensured statistically. It is interesting that for the first sampling, CIC concentrations decrease with vaccination status, while during the second sampling, which was carried out at an interval of 18 days, CIC levels increased in relation to the increase in the time interval elapsed since vaccination and aggravation of injuries.

Table 4. The individual arrays of CIC and therapy implementation used for calculation of r for the two samplings / Șirurile de valori utilizate pentru evaluarea corelației dintre scorurile terapeutice acordate şi nivelele complexelor imune circulante la cele două recoltări

Sample No	Sampling I		Sampling II	
	CIC	Therapy	CIC	Therapy
1	0.016	2	0	2
2	0.015	2	0.009	2
3	0.006	2	0.007	2
4	0.011	2	0	2
5	0.002	2	0.011	2
6	0.01	1	0.025	1
7	0.004	1	0.001	1
8	0.007	1	0.001	1
9	0.006	1	0.007	1
10	0.011	1	0.029	1
11	0.01	1	0.01	1
12	0.01	1	0.007	1
13	0.01	1	0.006	1
14	0.006	1	0.0089	1
15	0.009	1	0.0089	1
16	0.009	1	0.001	1
17	0.005	1	0.02	1
18	0.0086	1	0.0089	1
r	**0.242748**	**P NS**	**-0.2729**	**P NS**

The situation is reversed in the case of investigating the correlation between the severity of the lesions and the implementation and non-implementation of the therapy, the long-term treatment leading to a decrease in the levels of circulating immune complexes. Without going into details about the CIC reduction mechanism (decreasing synthesis or intensifying of the clearance from the blood stream), the beneficial influence of the therapy on the repair of injuries is obvious, probably also due to the reduction of CIC concentrations.

CONCLUSIONS

The levels of circulating immune complexes in animals with clinically apparent contagious agalactia did not differ significantly before and after the implementation of the therapy. The calculation of the correlation coefficient r between the lesion scores and the CIC levels demonstrated that the dependence of the two studied parameters was direct but not very close, lacking statistical assurance. The levels of CIC increased in relation to the increase of the time elapsed since vaccination and along with the aggravation of the lesions. Meanwhile, the long-term treatment caused a decrease in the levels of circulating immune complexes, positively influencing the remediation of lesions, probably also due to the reduction of CIC concentrations.

BIBLIOGRAPHICAL REFERENCES

1. Almeida R.A., Wannemuehler M.J., Rosenbusch R.F.; Interaction of *Mycoplasma* with alveolar macrophages; 1992; Infect. Immun. 60:2914-19.
2. Avramidis N., Victoratos P., Yiangou M., Hadjipetrou-Kourounakis L.; Adjuvant regulation of cytokine profile and antibody isotype of immune responses to *Mycoplasma agalactiae* in mice; 2002; Vet Microbiol.; 88 (4) :325-38.

3. Barbosa M.S., Sampaio B.A., Spergser J., Rosengarten R., Marques L.M., & Chopra-Dewasthaly R.; *Mycoplasma agalactiae* Vaccines: Current Status, Hurdles, and Opportunities Due to Advances in Pathogenicity Studies; 2024; Vaccines, 12(2), 156. https://doi.org/10.3390/vaccines12020156.
4. Chen Y., Wu Y., Qin L., Zhu C., You X.; Infection Strategies of Mycoplasmas: Unraveling the Panoply of Virulence Factors; 2021; Virulence, 12, 788–817.
5. Cole B.C., Atkin C.L.; The *Mycoplasma arthritidis* T-cell mitogen, MAM: A model superantigen; 1991; Immunol. Today, 12:271-76.
6. Cottew GS, Yeats FR.; Mycoplasmas and mites in the ears of clinically normal goats; 1982; Aust Vet J. Sep; 59(3):77-81.
7. Franzoso G., Dimitrov D.S., Blumenthal R., Barile M.F., Rottem S.; Fusion of *Mycoplasma fermentans* strain incognitus with T-lymphocytes; 1992; Fed.Eur.Biol.Soc. Lett., 303:251-54.
8. Glew M.D., Papazisi L., Poumarat F., Bergonier D., Rosengarten R., Citti C.; Characterization of a Multigene Family Undergoing High-Frequency DNA Rearrangements and Coding for Abundant Variable Surface Proteins in *Mycoplasma agalactiae*; 2000; Infect Immun.; 68 (8): 4539–4548.
9. Hegde S., Zimmermann M., Flöck M., Brunthaler R., Spergser J., Rosengarten R., Chopra-Dewasthaly R.; Genetic Loci of *Mycoplasma agalactiae* Involved in Systemic Spreading during Experimental Intramammary Infection of Sheep; 2016; Vet. Res. 47, 106.
10. Hegde S., Zimmermann M., Rosengarten R., Chopra-Dewasthaly R.; Novel Role of Vpmas as Major Adhesins of *Mycoplasma agalactiae* Mediating Differential Cell Adhesion and Invasion of Vpma Expression Variants; 2018; Int. J. Med. Microbiol. 308, 263–270.
11. Kumar A., Rahal A., Chakraborty S., Verma A.K., Dhama K.; *Mycoplasma agalactiae*, an Etiological Agent of Contagious Agalactia in Small Ruminants: A Review; 2014; Veterinary medicine international, 286752. https://doi.org/10.1155/2014/286752.
12. Lai W.C., Bennett M., Lu Y-S., Pakes S.P.; Vaccination of Lewis rats with temperature-sensitive mutants of *Mycoplasma pulmonis:* Adoptive transfer of immunity by spleen cells but not by sera; 1991; Infect. Immun., 59:346-50.
13. Roitt I., Brostoff J., Male D.; Immunology; 1989; Gower Medical Publishing.
14. Ron Y., Flitman-Tene R., Dybvig K., Yogev D.; Identification and characterization of a site-specific tyrosine recombinase within the variable loci of *Mycoplasma bovis*, *Mycoplasma pulmonis* and *Mycoplasma agalactiae*; 2002; Gene, 292(1-2):205-11.
15. Scherm B., Gerlach G.F., Runge M.; Analysis of heat shock protein 60 encoding genes of mycoplasmas and investigations concerning their role in immunity and infection; 2002; Vet.Microbiol.;89(2-3):141-50.
16. Stuart P.M., Cassell G.H., Woodward L.J.; Induction of class II MHC antigen expression in macrophages by *Mycoplasma* species; 1989; J. Immunol., 142:3392-409.
17. Vior C., Răducănescu H., Manolescu N., Popoviciu A.; Imunitate şi imunoprofilaxie la animale; 1982; Ed. Ceres, București.

STAPHYLOCOCCAL MASTITIS IN SHEEP: A SOLVED OR A PENDING PROBLEM?

MASTITA STAFILOCOCICĂ LA OVINE: O PROBLEMĂ REZOLVATĂ SAU ÎN AȘTEPTARE?

VASIU AUREL[1], PALL EMOKE[1,2], DUCA GHEORGHITA[2], SPINU MARINA[1,2], SANDRU CARMEN DANA[1,2], RUSU MARIANA[2], OLAH DIANA[1]

[1]Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, Str. Manastur no.3-5, Cluj-Napoca, Romania
[2]Institute of Research and Development for Montanology Str. XIII No.147, Cristian, Sibiu, Romania

Correspondence address: marina.spinu@gmail.com

Abstract

Raising sheep in different technological systems represented one of the benchmarks of human civilization. One of the most impacting diseases at herd level, from both health and welfare point of view and in all milk producing species is mastitis, leading to severe economic loss. This research aimed to describe a case study of staphylococcal mastitis in a sheep herd, with an atypical course where the cases recorded developed in an endemic episode in spite of previous vaccinations and to evaluate the impact of therapy in diminishing casualties. Epidemiological, clinical and bacteriological investigations were carried out to diagnose the inducive agent. The clinical diagnosis of the gangrenous mastitis was confirmed by the bacteriological investigation while it also supported the increased virulence of the isolated *S. aureus* strain and explained the inadequacy of the previous therapy. The increased virulence of the strain also stood for its pathogenicity in leading to an outbreak in spite of the vaccination of the animals before. The laboratory results suggested that a rapid intervention by therapy, based on the antibiogram, where amoxicillin was recommended in cases of newly detected disease in this particular flock. Vaccination performed constantly, based on the preventive program of the flock enhances the animals' response to bacterial challenge along with adequate veterinary management measures.

Key words: Staphylococcus aureus, sheep, mastitis, epidemiology, vaccination, therapy

Rezumat

Creşterea oilor în diferite sisteme tehnologice a reprezentat unul dintre reperele civilizației umane. Una dintre cele mai importante boli la nivelul efectivului, atât din punct de vedere al sănătății, cât și al bunăstării la toate speciile producătoare de lapte este mastita, care duce la pierderi economice grave. Această cercetare și-a propus să descrie un studiu de caz al mastitei stafilococice într-o turmă de ovine, cu un curs atipic în care cazurile înregistrate au progresat într-un episod endemic în ciuda vaccinărilor anterioare și să evalueze impactul terapiei în diminuarea pierderilor. Au fost efectuate investigații epidemiologice, clinice și bacteriologice pentru diagnosticarea agentului inductor. Diagnosticul clinic al mastitei gangrenoase produse de S. aureus a fost confirmat de investigația bacteriologică în timp ce aceasta a subliniat virulența crescută a tulpinii izolate și a explicat lipsa de eficacitate a terapiei anterioare. Virulența crescută a tulpinii a susținut patogenitatea sa, dând naștere unui focar de boală în ciuda vaccinării anterioare a animalelor. Rezultatele de laborator au sugerat că o intervenție rapidă prin terapie, bazată pe antibiogramă, în care amoxicilina a fost recomandată în cazurile de boală nou depistate reprezintă o soluție validă a problemei. Vaccinarea efectuată în mod constant, pe baza programului preventiv al efectivului, îmbunătățește răspunsul animalelor la provocarea bacteriană, împreună cu măsuri adecvate de management veterinar.

Cuvinte cheie: Staphylococcus aureus, ovine, mastită, epidemiologie, vaccinare, terapie

INTRODUCTION

Raising sheep in different technological systems represented one of the benchmarks of human civilization. It is spread all over the world nowadays, since this species is highly adaptable to different climates. From an economic point of view, sheep farming or traditional raising provides especially in the developing and undeveloped countries food and jobs to millions of people worldwide also helping progression of the economy and income increase in numerous countries (Skapetas and Kalaitzidou, 2017). The record holder, with reference to sheep numbers is China, with a total of over 173 million sheep.

The highest sheep productions (more than 34% of milk and 25% of meat) come from less-favored areas, particularly from the mountainous areas of different countries, i.e., in Europe including, countries like Greece, Spain, Romania, France, Italy and Croatia (https://www.euromontana.org/decline-in-sheep-and-goat-farming-in-the-eu-calls-for-more-ambitious-policy-for-the-sector/). In Romania, according to the 2024 statistics the number of animals is of 11.1 million.

One of the most impacting diseases at herd level, from both health and welfare point of view and in all milk producing species is mastitis, leading to severe economic loss (Gonzalo et al., 2002; Leitner et al., 2008). In sheep, bacterial mastitis is a significant financial issue especially for low input, transhumance flocks (Gelasakis et al., 2015) – impeding on animal health but also welfare of the individuals (European Food Safety Authority, 2014).

Along time, numerous means including antimicrobial therapy, biosecurity measures (management of the shelters, pasture, milking parlous, preventive antibiotic treatment at the end of the milking season - Petridis and Fthenakis, 2014, etc.) and also vaccination (Lacasta et al., 2015) were implemented to control the onset but also the dynamics and eventual outcome of bacterial mastitis in sheep (Gelasakis et al., 2015; Vasileiou et al., 2018).

Different research groups working on isolated episodes concluded that not only coagulase positive or negative staphylococci could be the causative agents of ovine mastitis, but diverse bacteria such as *Escherichia coli*, *Mannheimia haemolytica*, *Pseudomonas* spp. and various streptococci could induce alterations of the mammary gland morphology and its function (Dore et al., 2016). Nevertheless, two of the most common mastitis causative agents in Romania are *Staphylococcus aureus* during isolated episodes and *Mycoplasma agalactia*, inducing endemic outbreaks (Menzies and Ramanoon, 2001; Gonzalo et al., 2004; Mørk et al., 2005; Mavrogianni et al., 2011). The pathogenicity of these agents is related to their extended virulence potential and also toxicity (Chua et al., 2014).

In general, an infectious disease is the consequence of the encounter between the causative agent and the defense capacity of the host, mainly relying on its immunity. The proper functioning of the non-specific innate protective barriers (skin, mucosae) and the integrity of the leukocyte pool and of its phagocytic function restrict the clearance or successful multiplication of the bacteria (Murray et. al., 2008). Anti-staphylococcal antibodies have their part in more efficiently clearing the bacteria from the host (Vasileiou et al., 2019, Vasileiou et al., 2019a).

On those farms where the hygiene measures applied to the animals are scarce – especially when animals live in transhumance, the potential of the vaccine to control the onset and spread of the infection could be beneficial. Nevertheless, this procedure alone is not sufficient, while no other biosecurity measures (milking routine, disinfection of the milking devices, elimination of chronically infected animals, antibiotic treatment at the end of the lactation period) are applied. Therefore, the udder management proves its usefulness along with the vaccination procedure.

This research aimed to describe a case study of staphylococcal mastitis in a sheep herd, with an atypical course where the cases recorded developed in an endemic outbreak in spite of previous vaccinations and evaluate the impact of therapy in diminishing casualties.

MATERIAL AND METHOD

1. Materials

a. *Biological material.*

In a Lacaune sheep flock of 300 animals a disease episode was reported, involving mammary symptoms in lactating ewes, which reduced the milk production a rapidly progressed towards gangrene.

On the farm, the animals were fed with hay and concentrates according to the technology, with access to the pasture. They were milked in a mobile parlour benefited of 18 positions, while the teeth cups would not automatically disconnect when the mammary gland would be empty.

The animals were vaccinated within the frame of the national disease prevention program against anthrax and also, according to the preventive program of the farm – against small ruminant mastitis.

b. Sampling. For diagnostic purpose, the animals showing signs of the disease were milked from the healthy half; sampling from the diseased half failed. The samples were collected on cotton swabs in sterile conditions and transported to the laboratory for further investigations, being kept at +4°C till testing.

2. Methods

c. Epidemiological data. The epidemiological investigation was carried out in the flock about one month after the start of the outbreak, establishing the course and spread of the episode, the number of involved animals, the disease dynamics in the flock, the number of diseased and dead animals etc.

d. Clinical examination. The clinical examination of the affected animals started with an individual presented to the Infectious diseases clinic, which was examined as general state and the condition of the mammary gland. The mammary gland shows polymastia, one of its halves was increased in volume and hardened. The skin covering most of the mammary gland showed congestion, being in some places slightly cyanotic, with an increased local temperature and high sensitivity to palpation. In-depth of the mammary parenchyma, hard nodules of various sizes could be detected.

e. Bacteriological examination. The milk samples were inseminated without centrifugation on simple broth (Nutrient Broth, Tulip Diagnostics, Verna, India), simple agar (Nutrient Agar, Tulip Diagnostics, Verna, India) and chromogenic agar (Chromogenic UTI Medium, Oxoid, Hampshire, England). Subsequently, the tubes and the plates were incubated at 37°C for 24h. At the end of the incubation period a primary evaluation of the cultures was performed by evaluating and recording the bacterial colonies by their morphology. Smears of each obtained colony were stained by Gram method and the further processing protocol was decided. The microscopy indicated the presence of staphylococci, involving the use of API STAPH (BioMerial, France) and the interpretation of the results after a 24 h incubation of the galleries. Antibiotic susceptibility of the isolated strains was performed by use of antibiotics commonly involved in the therapy in small ruminant diseases (Trimethoprim/sulfamethoxazole, Amoxicillin, Streptomycin, Amoxicillin/ clavulanic acid, Enrofloxacin, Marbofloxacin, Gentamicin, Lincomycin, Cephalexin, Cefquinome, Tulathromycin, Tylosin, Oxytetracycline). The inhibition diameters were measured and the results were interpreted according to the CLSI classification in sensitive (S), resistant (R) and intermediate (I).

e. Therapeutic protocol. The animal presented to the clinic was hospitalized and a long duration treatment (6 weeks) was applied. The animal received amoxicillin (15mg/kg body weight) combined with marbofloxacin (2 mg/kg body weight), amoxicillin also being administered by intramammary infusion 1/1 with saline. The animal also received Dexamethasone (3 mg/animal), Novasul (7 ml/animal), Duphalyte (2 ml/kg body weight), glucose (1 ml/kg to be given by slow intravenous injection), vitamin C (800-1200 mg/ animal/per day). Local drainage was performed daily and warm water compresses were placed after disinfection with betadine. The animal also received saline by iv infusion for two days (10 ml/kg).

RESULTS AND DISCUSSION

The genus *Staphylococcus* represents a group of pathogenic bacteria with an increasing role in human and animal pathology, including coagulase positive but lately also coagulase negative germs. Some of its species considered harmless before proved to be aggressive mainly against immune suppressed patients, in hospital settings.

Recently available high performance techniques allowed for the investigation of population dynamics of staphylococci, as well as, their genetic changes (genome sequencing), revealing the possibilities for change of the virulence from generation to generation of the bacteria. One of the most relevant examples for genetic changes within the genus is represented by the emergence of methicillin-resistant *S. aureus* and its evolution of virulence, for which the horizontal gene transfer could provide the frame for the increase in virulence and antibiotic resistance. Population genetics for staphylococci could fundament new strategies to support prevention or treatment within the genus (Chua et al., 2014).

Considered to be a separate disease, the "Milk-drop syndrome of ewes" was thought to be a flock-pathology, and was well-defined by reduction in milk yield, with no other clinical signs. A sharp decrease of the milk production (over 30% in average for the flock and over 25%/animal) with an increased percent (over 25% of ewes) of affected animals could raise the suspicion of the disease. A study performed in Greece indicated that a reduction in milk production in the investigated flocks varied from 30% to 90%, while the infected animals could range from 27% to 100%. In 11 flocks subclinical mastitis was the only cause of milk yield decrease, while in 3 other it co-existed with various other causes. Contagious agalactia was indicated as another important cause of the syndrome, but at a much lesser extent (3 flocks with *M. agalactiae* infection). Factors such as inappropriate feeding regime, lentivirus infections, metabolic changes (acidosis) or intestinal parasitism could intervene in conditioning the outcome of the bacterial agent/host interaction, thus the value of certainty diagnosis is utmost important (Giadinis et al., 2012).

Clinical examination represented the fundamental method to identify mastitis in case of the presence or symptoms (Mavrogianni et al., 2011). On the other hand, *Staphylococcus aureus* as the inductive agent is identified by means of classical bacteriology, but also using molecular methods (Viguier et al., 2009, Achek et al., 2020).

The investigated episode was recorded during January 2024, three weeks after the booster vaccination against contagious agalactia and gangrenous mastitis. The morbidity was of 14.33%, while the mortality reached 6% and the lethality 41.86% by the time of diagnosis. In 25 of the animals, the mammary gland was compromised leading to their elimination from the flock.

Figures 1 and 2 show the result of the clinical examination in the investigated flock, with the most severe lesions revealed.

Figure 1. Oedemic of the mammary gland with nodules in the mammary tissue (case 1) / Edemul glandei mamare cu noduli subcutanați (caz 1)

Figure 2. Necrotic lesion of the mammary gland tissue, with pussy discharge on the sides (case 2) / Leziune necrotică a glandei mamare cu exsudat purulent pe margini (caz 2)

The severity of the clinical signs encountered was variable, ranging from oedemic and congestion of the mammary gland with subcutaneous nodules (Fig.1) as in the hospitalized case to extended necrotic and pussy lesions which led to detachment of necrotized tissue (Fig.2).

Subsequent to therapy, the lesions' gravity decreased, the healing process progressing slowly (Fig.3). From the moment of starting this stage in the treatment, only 3 animals suffered irreparable damage to the mammary gland, and the outbreak was subsequently stopped. The variety of gross lesions in the same flock could be explained by the different outcomes of the encounter between the bacterial strain and the host resistance.

Figure 3. Post therapeutic reaction: the necrotic tissue almost fully eliminated, the initial lesion diminished in size, the skin shows a regenerative trend (case 2) / Reacție post-terapeutică: țesutul necrotic este eliminat aproape integral, leziunea inițială redusă ca dimensiune, pielea prezintă o tendință regenerativă (caz 2)

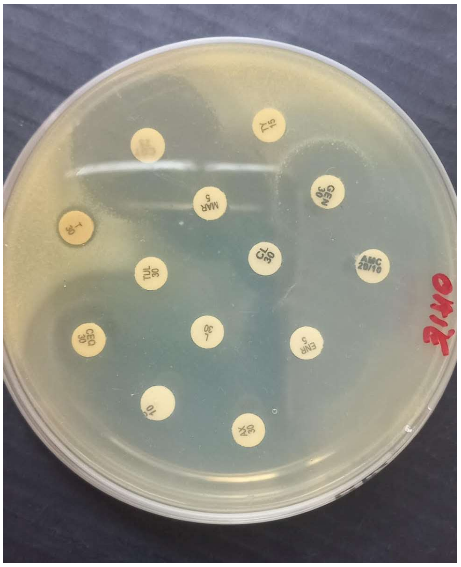
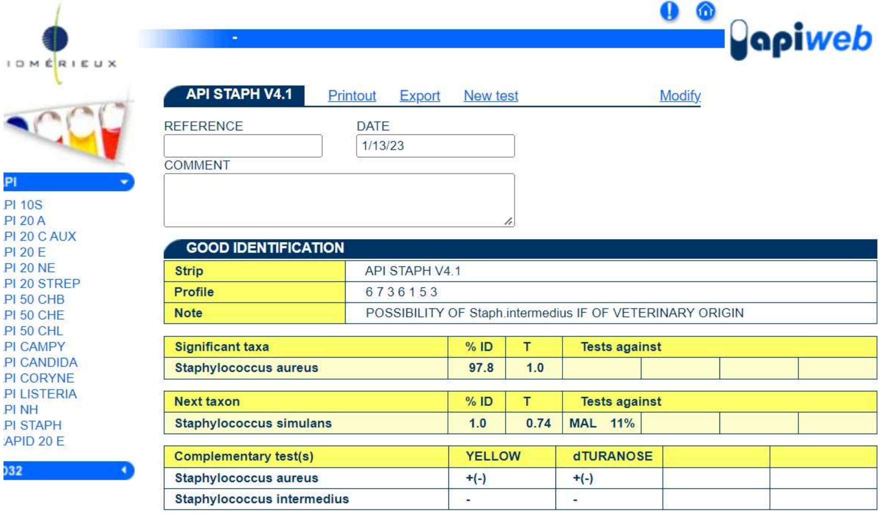


Figure 4. Antibiogram performed to identify the most efficient therapeutic agent (case 2) / Antibiogramă efectuată pentru identificarea celui mai potent agent terapeutic (caz 2)

Nevertheless, genotyping of *S. aureus isolates* is important for studying the dissemination of this pathogen in flocks (Porrero et al., 2012; Smith et al., 2014; Azara et al., 2017).



REFERENCE:

DATE: 1/13/23

COMMENT:

GOOD IDENTIFICATION

Strip	API STAPH V4.1
Profile	6 7 3 6 1 5 3
Note	POSSIBILITY OF Staph.intermedius IF OF VETERINARY ORIGIN

Significant taxa	% ID	T	Tests against			
Staphylococcus aureus	97.8	1.0				

Next taxon	% ID	T	Tests against			
Staphylococcus simulans	1.0	0.74	MAL 11%			

Complementary test(s)	YELLOW	dTURANOSE		
Staphylococcus aureus	+(-)	+(-)		
Staphylococcus intermedius	-	-		

Figure 4. The evaluation of biochemical traits of the isolated strain for its identification by API / Evaluarea caracterelor biochimice ale tulpinii isolate pentru identficarea prin API

The bacteriological examination led to a unanimous conclusion of the presence of *S. aureus* in the flock, in spite of the vaccination performed. The aggressiveness of the strain was supported by the results of antimicrobial testing, interpreted by the CLSI criteria (2023), as mentioned in the Methods (Table 1).

It became obvious that the strain had a high (0.46) MAR index, while the resistance was present to almost half of the tested antibiotics (6 of 7, 46.15%). Similarly, considering the definitions provided by Magiorakos et al., (2012) multi drug resistance (MDR) – „the antimicrobial resistance shown by a species of microorganism to at least one antimicrobial drug in three or more antimicrobial categories", it could be seen that the isolated strain of *S. aureus* fulfilled the criteria. Further, while defining xeno drug resistance (XDR) as "non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories), it could be observed, that in spite of a high MAR and MDR, the strain could not be included in the XDR category of agents.

Table 1. Elements confirming the virulence of *S. aureus* isolated from gangrenous mastitis of sheep during the investigated episode / Elemente de confirmare a virulenței tulpinii de *S. aureus* izolate de la animalele cu semen clinice pe parcursul episodului investigat

	Antibiotic	Inhibition diameter	CLSI ranking	Antibiotic class
1	Trimethoprim/sulfamethoxazole	29 mm	S	Sulfonamide
2	Amoxicillin	25 mm	S	Penicillin
3	Streptomycin	24 mm	S	Aminoglycoside
4	Amoxicillin/ clavulanic acid	20 mm	S	Penicillin/beta-lactamase inhibitors
5	Enrofloxacin	19 mm	S	Fluoroquinolone
6	Marbofloxacin	18 mm	S	Fluoroquinolone
7	Gentamicin	16 mm	S	Aminoglycoside
8	Lincomycin	13 mm	R	Lincosamide
9	Cephalexin	12.5 mm	R	First-generation cephalosporin
10	Cefquinome	12 mm	R	4th-generation cephalosporin
11	Tulathromycin	8 mm	R	Macrolides
12	Tylosin	6 mm	R	Macrolides
13	Oxytetracycline	6 mm	R	Tetracycline
	MAR index	0.46		

The therapy during the disease is complex, antibiotics being used to control the microbial agent. Nevertheless, the non-discriminatory use of antibiotics, without performing an antibiogram in advance could enhance the emergence of multi drug resistant or even pan-drug resistant strains (Kennedy et al., 2008), as it happened following the administration of oxytetracycline by the owner. Numerous studies deal with this phenomenon in sheep (Mørk et al., 2005; Lollai et al., 2008; Carfora et al., 2016). Probably coming from environmental sources, other animals on the farm or even caretakers, methicillin-resistant *S. aureus* (MRSA) was found in sheep milk (Carfora et al., 2016) and occasionally, in sheep mastitis (Mørk et al., 2005; Azara et al., 2017).

Oxytetracycline and penicillin or streptomycin are frequently used for the treatment of sheep mastitis (Attili et al., 2016; Lollai et al., 2016). In this study, although streptomycin was effective oxytetracycline proved to be non-effective, probably due to its frequent use on the farm. Although tetracycline could be an option, because of its broad spectrum (Ardic et al., 2005), this antibiotic was not evaluated in this study.

In the study areas, uncontrolled access to antibiotics led to inappropriate and abusive use of those especially in animals. Antibiotics from the tetracycline group are in extended use in the treatment/prevention of various infections, including the respiratory ones or lameness. Numerous researchers report and increased resistance of staphylococci to oxytetracycline in small ruminants in Italy and Brazil (Lollai et al., 2016, Achek 2020).

A well-founded treatment protocol is needed in case of endemic gangrenous mastitis involving a high number of animals in order to avoid the spread of antimicrobial resistance and also preserve the possibility of antimicrobial therapy in the flock.

CONCLUSIONS

The clinical diagnosis of the gangrenous mastitis was confirmed by the bacteriological investigation, while it also supported the increased virulence of the isolated *S. aureus* strain and explained the inadequacy of the previous therapy. The increased virulence of the strain also stood for its pathogenicity in leading to an outbreak in spite of the vaccination of the animals before. The laboratory results suggested that a rapid intervention by therapy, based on the antibiogram, where amoxicillin was recommended in cases of newly detected disease in this particular flock. Vaccination performed constantly, based on the preventive program of the flock enhances the animals' response to bacterial challenge along with adequate veterinary management measures.

BIBLIOGRAPHICAL REFERENCES

1. Achek R., El-Adawy H., Hotzel H., Tomaso H., Ehricht R., Mossadak Hamdi T., Azzi O., Monecke S.; Short communication: Diversity of staphylococci isolated from sheep mastitis in northern Algeria; 2020; Journal of Dairy Science, 103(1): 890-897.
2. Ardic N., Ozyurt M., Sareyyupoglu B., and Haznedaroglu T.; Investigation of erythromycin and tetracycline resistance genes in methicillin-resistant staphylococci; 2005; Int. J. Antimicrob. Agents 26:213–218.
3. Attili A.R., Preziuso S., Ngwa V.N., Cantalamessa A., Moriconi M., and Cuteri V.; Clinical evaluation of the use of enrofloxacin against *Staphylococcus aureus* clinical mastitis in sheep; 2016; Small Rumin. Res. 136:72–77.
4. Azara E., Piras M.G., Parisi A., and. Tola S.; Antimicrobial susceptibility and genotyping of *Staphylococcus aureus* isolates collected between 1986 and 2015 from ovine mastitis; 2017; Vet. Microbiol. 205:53–56.
5. Carfora V., Giacinti G., Sagrafoli D., Marri N., Giangolini G., Alba P., Feltrin F., Sorbara L., Amoruso R., Caprioli A., Amatiste S., and Battisti A.; Methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* in dairy sheep and in-contact humans: An intra-farm study; 2016; J. Dairy Sci. 99:4251–4258.
6. Chua K.Y., Howden B.P., Jiang J.H., Stinear T., and Peleg A.Y.; Population genetics and the evolution of virulence in *Staphylococcus aureus*, 2014; Infect. Genet. Evol. 21:554–562.
7. Dore S., Liciardi M., Amatiste S., Bergagna S., Bolzoni G., Caligiuri V., Cerrone A., Farina G., Montagna C.O., Saletti M.A., Scatassa M.L., Sotgiu G., Cannas E.A.; Survey on small ruminant bacterial mastitis in Italy, 2013–2014; 2016; Small Rumin. Res. 141:91–93.
8. European Food Safety Authority; 2014; Scientific opinion on the welfare risks related to the farming of sheep for wool, meat and milk production. EFSA J. 12:3933–4060.
9. Gelasakis A. I., Mavrogianni V.S., Petridis I.G., Vasileiou N.G.C., and Fthenakis G.C.; Mastitis in sheep – The last 10 years and the future of research; 2015; Vet. Microbiol. 181:136–146.
10. Giadinis N.D., Arsenos G., Tsakos P., Psychas V., Dovas C.I., Papadopoulos E., Karatzias H., and Fthenakis G.C.; "Milkdrop syndrome of ewes": Investigation of the causes in dairy sheep in Greece; 2012; Small Rumin. Res. 106:33–35.
11. Gonzalo C., Ariznabarreta A., Carriedo J.A., and San Primitivo F.; Mammary pathogens and their relationship to somatic cell count and milk yield losses in dairy ewes, 2002; J. Dairy Sci. 85:1460– 1467.
12. Gonzalo C., Tardaguila J.A., De La Fuente L.F., and San Primitivo F.; Effects of selective and complete dry therapy on prevalence of intramammary infection and on milk yield in the subsequent lactation in dairy ewes; 2004; J. Dairy Res. 71:33–38.
13. Kennedy A.D., Otto M., Braughton K.R., Whitney A.R., Chen L., Mathema B., Mediavilla J.R., Byrne K.A., Parkins L.D., Tenover F.C., Kreiswirth B.N., Musser J.M., and DeLeo F.R.; Epidemic community-associated methicillin-resistant *Staphylococcus aureus*: Recent clonal expansion and diversification; 2008; Proc. Natl. Acad. Sci. USA 105:1327–1332.
14. Lacasta D., Ferrer L.M., Ramos J.J., Gonzalez J.M., Ortin A. and Fthenakis G.C.; Vaccination schedules in small ruminant farms; 2015; Vet. Microbiol. 181:34–46.
15. Leitner G., Silanikove N. and Merin U.; Estimate of milk and curd yield loss of sheep and goats with intrammamary infection and its relation to somatic cell count; 2008; Small Rumin. Res. 74:221– 225.
16. Lollai S.A., Ziccheddu M., Di Mauro C., Manunta D., Nudda A. and Leori G.; Profile and evolution of antimicrobial resistance of ovine mastitis pathogens (1995–2004); 2008; Small Rumin. Res. 74:249– 254.
17. Lollai S.A., Ziccheddu M., Dupre I. and Piras D.; Characterization of resistance to tetracyclines and aminoglycosides of sheep mastitis pathogens: Study of the effect of gene content on resistance; 2016; J. Appl. Microbiol. 121:941–951.
18. Mavrogianni V.S., Menzies P.I., Fragkou I.A., and Fthenakis G.C.; Principles of mastitis treatment in sheep and goats; 2011; Vet. Clin. North Am. Food Anim. Pract. 27:115–120.
19. Menzies P.I. and Ramanoon S.Z.; Mastitis of sheep and goats; 2011; Vet. Clin. North Am. Food Anim. Pract. 17:333–358.

20. Mørk T., Tollersrud T., Kvitle B., Jorgensen H.J. and Waage S.; Genetic diversity of *Staphylococcus aureus* isolated from ovine intramammary infections in Norway; 2005; Vet. Microbiol. 106:265–273.
21. Murray R.J., Pearson J.C., Coombs G.W., Flexman J.P., Golledge C.L., Speers D.J., Dyer J.R., McLellan D.G., Reilly M., Bell J.M., Bowen S.F. and Christiansen K.J.; Outbreak of invasive methicillin-resistant *Staphylococcus aureus* infection associated with acupuncture and joint injection; 2008; Infection Control and Hospital Epidemiology 29, 859–865.
22. Petridis I.G., Fthenakis G.C.; Administration of antibiotics to ewes at the beginning of the dry-period; 2014; J. Dairy Res. 81:9–15.
23. Porrero M.C., Hasman H., Vela A.I., Fernandez-Garayzabal J.F., Dominguez L. and Aarestrup F.M.; Clonal diversity of *Staphylococcus aureus* originating from the small ruminants goats and sheep; 2012; Vet. Microbiol. 156:157–161.
24. Skapetas B., Kalaitzidou M.; Current status and perspectives of sheep sector in the world; 2017; Livestock Research for Rural Development. Volume 29, Article #21. Retrieved May 15, 2024, from http://www.lrrd.org/lrrd29/2/skap29021.html.
25. Smith E.M., Needs P.F., Manley G. and Green L.E.; Global distribution and diversity of ovine-associated *Staphylococcus aureus*; 2014; Infect. Genet. Evol. 22:208–215.
26. Vasileiou N.G.C.; Mastitis in Ewes associated with *Staphylococcus spp*.: New Clinical, Epidemiological, Management, Microbiological and Zoonotic Findings and Evaluation of a Novel Vaccine against the Disease (PhD thesis); 2019; University of Thessaly.
27. Vasileiou N.G.C., Chatzopoulos D.C., Cripps P.J., Ioannidi K.S., Gougoulis D.A., Chouzouris T.M., Lianou D.T., Calvo Gonzalez-Valerio T., Guix Vallverdu R., Argyros S., Cesio M., Font I., Mavrogianni V.S., Petinaki E., Fthenakis G.C.; Evaluation of efficacy of a biofilm-embedded bacteria-based vaccine against staphylococcal mastitis in sheep — A randomized, placebo-controlled field study; 2019; Journal of Dairy Science, 102(10):9328-9344.
28. Vasileiou N.G.C., Cripps P.J, Ioannidi K.S., Chatzopoulos D.C., Gougoulis D.A., Sarrou S., Orfanou D.C., Politis A., Calvo Gonzales Valerio T., Argyros S., Mavrogianni V.S., Petinaki E., Fthenakis G.C.; Extensive countrywide field investigation of subclinical mastitis in sheep in Greece; 2018; Journal of Dairy Science 101, 7297–7310.
29. Viguier C., Arora S., Gilmartin N., Welbeck K. and O'Kennedy R.; Mastitis detection: Current trends and future perspectives; 2009; Trends Biotechnol. 27:486–493.
30. Magiorakos A.P., Srinivasan A., Carey R.B., Carmeli Y., Falagas M.E., Giske C.G., Harbarth S., Hindler J.F., Kahlmeter G., Olsson-Liljequist B., Paterson D.L., Rice L.B., Stelling J., Struelens M.J., Vatopoulos A., Weber J.T., & Monnet D.L.; Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance; 2012; Clinical microbiology and infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases, 18(3), 268–281.
31. https://www.euromontana.org/decline-in-sheep-and-goat-farming-in-the-eu-calls-for-more-ambitious-policy-for-the-sector/.

ACTA AGRICOLA ROMANICA, Volume 6, Year 6, No. 6.3.

CREATION BY CROSSING OF A NEW MEAT BREED OF SHEEP WITH HIGH GROWTH SPEED, HIGH YIELD AT SACRIFICE AND QUALITY CARCASSES, REQUESTED BY THE E.U. MARKET

CREAREA PRIN ÎNCRUCIŞARE A UNEI NOI RASE DE OVINE DE CARNE, PERFORMANTĂ, CU VITEZĂ MARE DE CREŞTERE, RANDAMENT RIDICAT LA SACRIFICARE ŞI CARCASE DE CALITATE, SOLICITATE PE PIAŢA U.E.

GABRIEL PETRU VICOVAN[1], ALEXANDRU GABRIEL VARTIC[1], RĂDUCU RADU[1], ADRIANA VICOVAN[1], CORNELIU ION NEACȘU[1], ALINA NICOLESCU[1], MARIA STANCIU[1]

[1]Institute of Research and Development for Sheep and Goat Breeding Palas, Constanta, I.C. Bratianu, 248, Constanta, 0241.639.506, icdcocpalas@yahoo.com

Correspondence address: agvicovan@yahoo.com

ABSTRACT

The creation of a new breed of sheep specialized for meat production has an important role in our country, thus obtaining breeds that can improve the quantitative and qualitative production of meat in sheep farms. For this reason, within R.D.I.S.G.B. Palas Constanta, the creation of a new breed of sheep with pronounced traits for meat production is underway. The breeds used for this purpose are ***Texel****,* ***Rouge de L'Ouest****,* ***Palas Prolific Breed****. The results regarding the main morphoproductive indices obtained showed the achievement of an average daily gain from calving to the age of 1 year of 123.25 – 132.73g, being higher in mixed F1* ***Rouge de L'Ouest*** *x* ***Palas Prolific Breed*** *females by 7.69% compared with contemporaries from* ***Palas Merino Breed****. Also, the mixed F1* ***Texel*** *x* ***Palas Prolific Breed*** *females achieved, in the same period of time, an average daily gain of 123g, which is similar to that of contemporaries from the* ***Palas Merino Breed****. The yield at slaughter was between 44.46% and 49.84%, this being higher in the F1* ***Rouge de L'Ouest*** *x* ***Palas Prolific Breed*** *and the F1* ***Texel*** *x* ***Palas Prolific Breed*** *crossbreds compared with the* ***Palas Merino Breed****. The tissue structure of the carcasses showed the following values: muscle – 58.09 – 61.96%, bones – 24.68 – 25.83%, fat – 12.92 – 16.72%. The best carcasses, classified according to conformation and fattening stage respectively, were obtained from the F1* ***Texel*** *x* ***Palas Prolific Breed*** *crossbreds, these being distributed in classes U and R according to conformation and respectively in class 3 according to fattening stage. The obtained results show the superiority of the crossbreeds obtained compared with the* ***Palas Merino Breed*** *and the* ***Palas Prolific Breed****.*

Keywords*:* ***hybrids, yield, classification, breed.***

REZUMAT

Crearea unei noi rase de ovine specializată pentru producția de carne are un rol important în țara noastră, obținându-se astfel reproducători care pot ameliora producția cantitativă și calitativă de carne din cadrul exploatațiilor de ovine. În acest sens, în cadrul ICDCOC Palas Constanța este în curs de derulare obținerea unei noi rase de ovine cu aptitudini pronunțate pentru producția de carne. Rasele folosite în acest scop sunt: ***Texel, Rouge de L'Ouest, Rasa Prolifică Palas****. Rezultatele privind principalii indici morfoproductivi obținuți au arătat obținerea unui spor mediu zilnic de la fătare la vârsta de 1 an de 123,25 – 132,73 g, fiind mai mare la femelele metise* F_1 ***Rouge de L'Ouest*** *x* ***Rasa Prolifică Palas*** *cu 7,69% comparativ cu contemporanele din rasa* ***Merinos de Palas****. De asemenea femelele metise* F_1 ***Texel*** *x* ***Rasa Prolifică Palas****, au realizat în aceeași perioadă de timp un spor mediu zilnic de 123 g acesta fiind asemănător cu al contemporanelor din rasa* ***Merinos de Palas****. Randamentul la sacrificare a fost cuprins între 44,46% și 49,84%, acesta fiind superior la metișii* F_1 ***Rouge de L'Ouest*** *x* ***Rasa Prolifică Palas*** *și metișii* F_1 ***Texel*** *x* ***Rasa Prolifică Palas*** *comparativ cu rasa* ***Merinos de Palas****. Structura tisulară a carcaselor a prezentat următoarele valori: mușchi – 58,09 – 61,96%, oase – 24,68 – 25,83%, grăsime – 12,92 – 16,72%. Cele mai bune carcase, clasificate după conformație respectiv după stadiul de îngrășare s-au obținut de la metișii* F_1 ***Texel*** *x* ***Rasa Prolifică Palas****, acestea fiind repartizate în clasele U și R după conformație și respectiv în clasa 3 după stadiul de îngrășare. Rezultatele obținute arată superioritatea metișilor obținuți comparativ cu rasa* ***Merinos de Palas*** *și rasa* ***Prolifică Palas****.*

***Cuvinte cheie**: metiși, randament, clasificare, rasă.*

INTRODUCTION

There are many high performing meat sheep breeds in the world with high growth rates, high slaughter yield and quality carcasses.

In Romania there is only one breed of meat sheep, performing, competitive with the best meat breeds in the EU.

The creation of a new meat breed is welcomed as it provides the necessary breeding rams and ewes much in demand by sheep farms that intend to increase their income and become profitable.

MATERIAL AND METHOD

Presentation of the breeds used:

1. Texel breed (origin Great Britain)

Texel sheep were brought to the island of Texel in the North Sea off the Northwest coast of Great Britain by the occupying Roman legions. From here the breed spread throughout the world due to its exceptional meat production qualities. Use and distribution:

- The sheep of this breed are used due to their special qualities in industrial crossbreeding to improve the qualities of the products of rustic breeds and dairy breeds.
- The **Texel** rams pass on the development of muscle mass through crossbreeding to produce hybrid lambs with local breeds classified in classes U (very good carcasses) and R (good carcasses) according to the EUROP grid.

The effectives of the breed. The breed has a flock of 115,000 sheep, of which 5,000 heads are engaged in the breed selection program.

Maternal qualities:

- Prolificity: 185-200 %
- High lactation capacity (the ewes easily nurse 2-3 lambs)

Ability for meat production:

- Weight of rams: 110 – 140 kg/cap
- Sheep weight: 70 - 90 kg/head
- Average daily gain 30 - 70 days: 295 g/head

The origin of the breed. The breed originates from the island of the same name belonging to the Netherlands. It was formed by mating local sheep with rams from the Leicester, Lincoln, Romney Marsh and Southdown breeds in the middle of the last century. The breed was exported to Great Britain, France, Ireland, USA, Australia, New Zealand. In our country, it was used for crossbreeding with native breeds at R.D.I.S.G.B. Palas and the Institute of Montanology – Cristian – Sibiu.

The Nord Sheep Selection Body is in the town of Verdilly, Aisne district, which deals with the selection of the **Texel** and **Ile de France** breeds and with the dissemination of genetic progress in the kennels of the two breeds. Effective for the **Texel** breed – 300000 sheep of which 6028 sheep under official control.

Maternal traits:

- Average prolificacy: 179%
- Good lactation capacity expressed by a growth spurt in twin lambs of 12.7 kg/head in the first month of lactation.

Characteristics for meat production:

- Body weight: Rams – 115-130 kg/head, Sheep – 80-90 kg/head.
- Average daily growth rate of single male lambs from 30 to 70 days: 327 g/head

Traits. A meat breed par excellence, the **Texel** combines meat qualities with very good maternal qualities. The excellent meat qualities of the breed allow the production and delivery of 45 kg live weight lambs with good quality carcasses without excess fat. The carcasses are wide, compact with a very good conformation, fine skeleton, and low amounts of fat. In the carcasses of **Texel** lambs, the jig, the back, and the saddles (the most valuable butchering regions) have a weight of over 53%. By crossing with other breeds, the **Texel** breed faithfully transmits meat traits. The **Texel** breed is raised in the summer on pasture without supplement of concentrates and in the winter in the shelter, with hay and a small supplement of concentrates. The breed is used to improve the conformation of the carcasses of the British breeds (without this qualification) especially for the musculature of the back, shoulders, and jig and for lean meat with little fat.

Morphoproductive indices and reproductive indices:

- Lamb prolificacy: 130%
- Primiparous fertility: 160%
- Multiparous prolificacy: 180%
- Carcass weight of fattened lambs: 18 - 21 kg/head
- Slaughter yield: 60% (highest value encountered)
- Adult weight: Sheep – 85 kg/head; Rams – 120 kg/head

Other qualities of the breed: Exceptional muscularity and docility.

The British **Texel** Association is the largest in the UK.

2. Rouge de L'Ouest breed

It is a breed with an average body development, without excessive skeletal development. The name of the breed is given by the color of the embers. The head is hornless, with embers of variable color, from pink to red (rust) of different intensities.

Origin and skills. The breed is the result of crosses between local breeds from the west of France with some English breeds. The breed comes from the Loire area and was originally bred as a milk breed to produce Camembert cheese, being recognized for the high fat content of the milk.

French breeders reoriented and transformed the breed into a meat breed with high prolificacy and high milk production with a high percentage of fat (characteristics rarely found in a single breed).

The sheep of this breed have a prolificacy of 185-200% and due to the high milk production, the lambs produce carcasses at the age of 100 days with an average weight of 19 kg, twin lambs having a growth rate of over 300g/day and head (twins exceed the weight of 27 kg/head at the age of 70 days).

The breed has fine bones, so the lambs are free of dystocia, both in the pure breed and in the F1 hybrid sheep, obtained from the crossing of the Rouge de L´Ouest breed with other breeds.

Due to the fine skeleton the breed has a high yield in lean meat.

Breed selection. In the ram sector, a rigorous selection is carried on for the meat production skills, at the Skills Evaluation Station, where 150 rams are selected annually based on their own performance.

The top ten best are tested by progeny for "meat skills" to establish breeding rams - "AMBO" meat. These rams are intensively used for reproduction through artificial insemination, in order to improve body conformation and other attributes on which meat production depends.

In the sheep sector, the best ewes designated as "mothers of rams" and "mothers of lambs" represent the sheep on which the rams are tested on farms according to their offspring for "maternal skills". The best rams tested in this way are designated as "AMEL" rams. Those who meet these 2 qualifications are designated "ELITE" rams and are used for artificial insemination.

The calving takes place in the spring in February - April and the lambs are weaned at the age of 110 days, to be sold in July - August at a carcass weight of 22 kg.

The traits tracked on the maternal line are prolificacy and lactation capacity and on the paternal line are growth speed, development and body conformation and carcass quality indices.

Every year 150 male lambs from the best selected on their own performance and ancestry are brought to the testing station in Epieds in the Aisne district, where they are evaluated for meat skills. The best 12 heads are used for artificial insemination and tested for progeny.

The indexing of rams according to meat skills is done by the "Insem Ovin" body, which calculates the growth potential and carcass qualities for the offspring of each ram.

The daughters of the 12 rams are controlled in the farms they belong to for two years in a row, allowing indexing of the father rams according to their prolificacy and lactation capacity.

The best rams tested for meat traits are qualified "AMBO".

After the test in the breeders regarding the performance of the daughters, the best rams are qualified "AMEL".

Rams that combine the two qualifications are designated "ELITE".

3. Palas Prolific Breed

The breed was formed at R.D.I.S.G.B. Palas - Constanta starting in 1972 by the complex crossbreeding of the **Palas Merino breed** with the **Romanov**, **Ostfriesian**, **Border Leicester** and **Finnish Landrace** breeds.

After obtaining the F1 generations from the 4 breed combinations and the R1 and R2 generations from the **Romanov** x **Palas Merino** combination, the F1, R1 and R2 sheep (**Romanov** x **Palas Merino**) and F1 (**Finnish Landrace** x **Palas Merino**) were mated with **Ostfriesian** rams and F1 sheep (**Border Leicester** x **Palas Merino**) with **Romanov** rams.

Females obtained from the first combination were mated with rams from the second combination and vice versa. Starting from 1983, the reproductive isolation of the obtained population was carried out, aiming at maintaining the prolificacy at over 160% and a high lactating capacity.

Morphoproductive and reproductive indices:

- body weight - rams - 70 - 80 kg; ewes – 45 - 55 kg.
- wool production - rams - 3.5 - 4.5 kg; ewes – 2.5 - 3.5 kg.
- fiber fineness - 28 – 32 microns.
- lamb growth supplements – 170 - 190 g/head/day.
- total milk production – 158-181 liters/sheep.

Reproduction indices:

- fecundity - 94-96%.
- prolificacy - 160-180%.
- weaned lambs/weaned ewe – 1.5 - 1.6 heads.

The breed has evolved reproductively isolated since 1993, when the coefficient of reproductive isolation was + 0.52. After this date, the value of the coefficient of reproductive isolation increases to + 1 in 1998, a value that is still maintained today.

The breed is 45% genetically like the **Romanov** breed, 28.9% to the **Palas Merino**, 17.4% to the **Ostfriesian breed**, 4% to the **Ile de France**, 2.9% to the **Border Leicester** and 1.8% to the **Finish Landrace**.

RESULTS AND DISCUSIONS

Presentation of morphoproductive indices in the precursors of the new breed of meat sheep:

- Establishing growth dynamics from birth to 1 year of age in F_1 **Rouge** x **Palas Prolific Breed** female youth compared to contemporaries with **Palas Merino breed**.

Table 1. Body weight and weight gain in F₁ Rouge x Palas Prolific breed compared to Palas Merino breed / Greutatea coporală și sporul de creștere în greutate la hibridele F₁ Rouge x Prolifică Palas cu Merinos de Palas

No.	Specification		Body weight Kg/cap	Average daily gain from birth to 1 year (g/head)	Average age at last weighing (days)
			X± s_x	X ± s_x	X ± s_x
1.	F_1 **Rouge** x **Palas Prolific breed**		52.27 ± 0.6586	132.73 ± 1.9179	364.95±1.9360
2.	**Palas Merino**		50.50 ± 0.9395	123.25 ± 2.9566	379.55±3.6226
3.	The difference between genotypes and significance	± M.U.	+ 1.77	+ 9.48	- 14.6
		± %	+ 3.50 p > 0.05 insignificant	+ 7.69 p < 0.05 significant	

The table shows that in terms of body weight there are no significant differences between the genotypes. But if the weight is corrected according to age, then it turns out that the hybrids should have 54.42 kg / head being heavier by 3.91 kg (+ 7.8%) compared to the **Palas Merino**, the difference being statistically significant.

Regarding the increase in weight, the hybrids had a higher increase in weight by 7.69%, the difference being significant.

Establishing growth dynamics from birth to 1 year of age in F_1 **Texel** x **Palas Prolific Breed** hybrid female youth compared with contemporary **Palas Merino**, can be made based on the data within in table 2.

Table 2. Body weight at 1 year and average daily gain from birth to 1 year / Greutatea coporală la 1 an și sporul mediu zilnic de la naștere la 1 an

No.	Specification		Body weight Kg/cap	Average daily growth from birth to 1 year (g/head)	Average age at last weighing (days)
			X± s_x	X ± s_x	X ± s_x
1.	F_1 **Texel** x **Palas Prolific breed**		51.68 ± 0.6961	123.63 ± 1.7978	389.12 ± 0.8287
2.	**Palas Merino**		50.50 ± 0.9395	123.25 ± 2.9566	379.55 ± 3.6226
3.	The difference between genotypes and significance	± UM	+ 1.18	+ 0.38	+ 9.57
		± %	+ 2.34 p > 0.05 insignificant	+ 0.31 p > 0.05 insignificant	

Table 2 shows that there were no significant differences between genotypes both in terms of body weight and average daily weight gain.

Slaughter yield values for hybrids versus the **Palas Merino** are presented in table 3.

Table 3. Slaughter yield in hybrids compared with Palas Merino / Randamentul la sacrificare la hibrizi comparativ cu Merinosul de Palas

No.	Specification	Slaughter yield (%)		Differences between genotypes (± percentage points)			
		R_1	R_2	Between Rouge de L'Ouest and Merino		Between Texel and Merino	
		X ± s_x	X ± s_x	R_1	R_2	R_1	R_2
1	F_1 **Rouge** x **Palas Prolific Breed**	46.87 ± 0.0055	54.41 ± 0.0042	+ 2.41 Insignificant	+ 3.87 Insignificant	+ 5.38 Significant	+ 9.26 Significant
2	F_1 **Texel** x **Palas Prolific Breed**	49.84 ± 0.0064	59.80 ± 0.0136				
3	**Palas Merino**	44.46 ± 0.0098	50.54 ± 0.0113	-		-	

Table 3 shows that the F₁ **Texel** x **Palas Prolific breed** hybrids had the best yield at slaughter, the differences between the hybrids and the **Palas Merino** being statistically significant.

Thigh Muscularity Index (T.M.I.), the index values faithfully reflect the musculature of the jig and carcass and values of the index according to the genotype are presented in table 4.

Table 4. Thigh muscularity index (T.M.I.) values according to genotype / Valorile indicelui de muscularitate a coapsei (I.M.C.) în funcție de genotip

No.	Genotype	T.M.I. values	± Differences between genotypes			
		$X \pm s_x$	Between Rouge de L'Ouest and Palas Merino breed		Between Texel and Palas Merino breed	
			± M.U	± %	± M.U	± %
1	F₁ **Rouge** x **Palas Prolific breed**	0.480 ± 0.0220	+ 0.0347	+ 7.79	-	-
2	F₁ **Texel** x **Prolific Palas breed**	0.479 ± 0.0243	-	-	+ 0.0337	+ 7.57
3	**Palas Merino breed**	0.445 ± 0.0207	-		-	

From the table 4 it can be seen that between F₁ **Rouge de L'Ouest** x **Palas Prolific breed** and **Palas Merino** hybrids in terms of thigh muscularity index (T.M.I.) there is a difference of 7.79% and between F ₁ **Texel** x **Palas Prolific Breed** and **Palas Merino** there is a difference of 7.75%, both differences being statistically significant.

The tissue structure of carcasses is presented in table 5.

Table 5. Tissue structure of carcasses according to genotype / Structura tisulară a carcaselor în funcție de genotip

No.	Genotype	Tissue structure (%)			
		Muscle	Bone	Fat	Total
		$X \pm s_x$	$X \pm s_x$	$X \pm s_x$	-
1	F₁ **Rouge** x **Palas Prolific breed**	61.96 ± 1.6200	24.68 ± 0.3154	13.37 ± 1.3500	100.00
2	F₁ **Texel** x **Palas Prolific Breed**	58.09 ± 2.1700	25.19 ± 0.6822	16.72 ± 2.1500	100.00
3	**Merino de Palas**	61.36 ± 0.9168	25.83 ± 0.5646	12.92 ± 0.3001	100.00

Table 6 shows the differences between the genotypes and the significance.

Table 6. Differences between genotypes and significance of differences / Diferențe între genotipuri și semnificația diferențelor

No.	Specification	Differences ± percentage points		
		Muscle	Bones	Fat
1	Between F₁ **Rouge** x **Palas Prolific and Merino**	+ 0.6	- 1.15	+ 0.45
2	Between F₁ **Texel** x **Palas Prolific and Merino**	- 3.27	- 0.64	+ 3.8
3	Between F₁ **Rouge** x **Palas Prolific** and F₁ **Texel** x **Palas Prolific breed**	+ 3.87	- 0.51	- 3.35

The data shows that there are no significant differences between the hybrids F₁ **Rouge** x **Palas Prolific Breed** and the **Palas Merino** regarding the tissue structure of the carcasses.

Between the F₁ **Texel** hybrids and the **Palas Merino** there are also no significant differences, although it can be shown that the hybrids have a lower muscle content in the carcass by 3.27 percentage points and a higher fat content by 3.8 percentage points.

There are no significant differences between the two hybrids, however, it is observed that the **Texel** hybrids have 3.87 percentage points less muscle and 3.35 percentage points more fat in the carcass structure.

Classification of carcasses according to the EUROP grid is presented in table 7.

Table 7. Carcass classification / Clasificarea carcaselor

No.	Specification	Conformation Class (%)					Class according to degree of fattening (%)		
		E	U	R	O	P	lean	medium fat	fat
1	F_1 **Rouge de L'Ouest** x **Palas Prolific breed**	-	-	100.00	-	-	100.00	-	-
2	F_1 **Texel** x **Palas Prolific breed**	-	33.00	67.00	-	-	-	100.00	-
3	**Palas Merino breed**	-	-	100.00	-	-	100.00	-	-

The table shows that, from the point of view of conformation, the carcasses of the F_1 **Rouge** x **Palas Prolific Breed** hybrids are completely in class R (good carcasses), and in terms of fattening grade, all the carcasses were from class 2 (poor carcasses) requested on the E.U. market.

The carcasses of the F1 **Texel** x **Palas Prolific Breed** from the point of view of conformation are 33% in the U class (very good carcasses) and 67% of the carcasses were in the R class (good carcasses).

All carcasses from the point of view of the degree of fattening fell into class 3 (medium fat carcasses) being requested on the European Union market.

CONCLUSIONS

The obtained results show the superiority of the obtained hybrids compared with the **Palas Prolific Breed** and the **Palas Merino breed**.

The two F_1 **Rouge de L'Ouest** x **Palas Prolific Breed** and F_1 **Texel** x **Palas Prolific Breed**, will mate each other resulting in the "desired type" that has in the genome: 25% **Rouge de L'Ouest** genes, 25% **Texel** genes and 50% **Palas Prolific Breed** genes.

The obtained population will reproduce itself for at least 3 generations (12 years), after which it can be approved as a new breed of meat sheep.

BIBLIOGRAPHICAL REFERENCES

1. British Sheep, Ninth edition, Published by the National Sheep Association, July 1998.
2. Vicovan P.G., Radu R., Enciu A., Vicovan A.; New Breeds of Sheep Created by Scientific Research from ICDCOC PALAS – CONSTANȚA; 2020; Ed. Celebris, Constanța, Romania.
3. Vicovan P.G., Vicovan A., Radu R., Ida A., Zamfir C., Enciu A.; Genetic history of **Palas Merino** breed during 1930 – 2003; Archive Zootehnica volume 11, no. 4, 86-89, 2008.