AGRICULTURAL AND FORESTRY SCIENCES ACADEMY

"GHEORGHE IONESCU - SISESTI"

# ACTA AGRICOLA ROMANICA

## ANIMAL SCIENCES AND VETERINARY MEDICINE

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BUCHAREST

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**Academy of Agricultural and Forestry Sciences**
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# CONTENT



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# GENETIC VARIABILITY WITHIN THE BAZNA AND RED MANGALITSA BREEDS

# DIVERSITATEA GENETICĂ ÎN CADRUL RASELOR DE SUINE BAZNA ŞI MANGALIŢA ROȘIE

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***Abstract***

*The objective of this study is to determine genetic variability, within two indigenous swine breeds, **Bazna** and **Red Mangalitsa**. The biological samples were collected under sterile conditions from a number of 12 pigs from the red **Mangalitsa** breed and 15 pigs from the **Bazna** breed. It seems that at the mitochondrial DNA level, the genetic background on the maternal line of the **Mangalitsa** breed is European, arguing the formation of this breed on the territory of Europe. At the level of mitochondrial DNA, genetic diversity is reduced, thus having a high degree of inbreeding both within the **Red Mangalitsa** variety breed and within the wild boar. A number of 10 analyzed samples from the **Bazna** breed were grouped in the European genetic cluster, the other 5 samples being grouped in the Asian cluster (divided into two subclusters), together with the **Vietnamese** breed. The results of the genetic variability analysis based on SNP polymorphisms indicate that the swine breeds studied form several clusters with composed group of breeds or groups of breeds. We could conclude that the **Mangaliţa** breed of swine, the red variety, raised in the mother nucleus at RDSA Turda, presents a reduced genetic diversity at the level of mitochondrial DNA. On the other hand, the **Bazna** swine breed bred in the maternal nucleus from RDSA Turda, presents a fairly high genetic diversity at the level of mitochondrial DNA, which confirms the existence of a large number of maternal lines in this population.*

***Keywords: Bazna, Red Mangalitsa, mitochondrial DNA, SNP polymorphisms, genetic diversity***

***Rezumat***

*Obiectivul acestui studiu este de a determina variabilitatea genetică, în cadrul a două rase de porcine indigene, **Bazna** și **Mangaliţa Roșie**. Probele biologice au fost recoltate în condiții sterile de la un număr de 12 porci din rasa **Mangaliţa roșie** și 15 porci din rasa **Bazna**. Cel puțin la nivelul ADN mitocondrial, fondul genetic pe linia maternă a rasei **Mangaliţa** este european, argumentând formarea acestei rase pe teritoriul Europei. La nivelul ADN-ului mitocondrial, diversitatea genetică este redusă, având astfel un grad ridicat de consangvinizare atât în cadrul rasei de suine **Mangaliţa Roşie**, cât și în cadrul mistreţului. Un număr de 10 probe analizate din rasa **Bazna** au fost grupate în clusterul genetic european, celelalte 5 probe analizate fiind grupate în clusterul asiatic (împărţite în două subclustere), alături de rasa vietnameză. Rezultatele analizei variabilității genetice pe baza polimorfismelor SNP, indică faptul că rasele de porci studiate formează grupuri compuse de rase sau grupuri de rase. Am putea concluziona că rasa de porc **Mangaliţa**, varietatea roșie, crescută în nucleul mamă la SCDA Turda, prezintă o diversitate genetică redusă la nivelul ADN-ului mitocondrial. Pe de altă parte, rasa porcină **Bazna** crescută în nucleul matern de la SCDA Turda, prezintă o diversitate genetică destul de mare la nivelul ADN-ului mitocondrial, ceea ce confirmă existența unui număr mare de linii materne în această populație.*

***Cuvinte cheie: Bazna, Mangalita roșie, AND mitocondrial, polimorfisme SNP, variabilitate genetică***

## INTRODUCTION

The domestication of animals is a revolutionary event that has profoundly influenced human history (Huang et al., 2020). The swine (*Sus scrofa*) was independently domesticated in Europe and Asia about 10,000 years ago (Larson et al., 2005). On the European continent there are small populations belonging to the transboundary breed **Mangalitsa** (in Austria, Germany, Romania, Serbia and Hungary), or to the local breeds **Turopolje** (in Croatia) and **Bazna** (in Romania) (Zahan, 2017). The importance of local breeds as valuable genetic reservoirs is well established (Zorc et al., 2022). The **Red Mangalitsa** descends directly from the wild boar and is a rustic breed of swine from the Carpathian Basin (based in Hungary and Romania) (Oroian and Petrescu., 2014). The **Bazna** is a breed originating in Romania, formed in 1872 from a cross between **Mangalitsa** sows and **Berkshire** boars (Draganescu et al., 2008).

Genetic diversity of farm animals is necessary to meet current production needs in different environments, to enable sustained genetic improvement, and to facilitate rapid adaptation to changing breeding goals (Noter., 1999). Maintaining a sufficient diversity of animal genetic resources is necessary to ensure the sustainability of agricultural production, especially under conditions of climate change (Boettcher et al., 2015). The way to improve sustainability is a low-input system based on selecting animals that are more robust to climate change and are better adapted to convert low-quality feed (local feed, feed by-products and food waste) into meat, but it is feasible if this is combined with a reduction in food waste (Rauw et al., 2020).

Aspects of domestication have often been investigated by studying mitochondrial DNA, while genetic diversity was initially studied using simple sequence repeat (SSR) and amplified fragment length polymorphism (AFLP) in intensively selected breeds but also in autochthonous populations with limited diffusion (SanCristobal et al., 2006).

Mitochondrial (mt) DNA is haploid, non-recombining and almost exclusively maternally inherited (Fajardo et al., 2008). The advantage of mitochondrial DNA analysis derives from the fact that there are many mitochondria per cell and many DNA molecules in each mitochondrion, making mtDNA a naturally amplified source of genetic variation (Girish et al., 2004). In addition, mitochondrial genes evolve much faster, thus, contain more sequence diversity, facilitating the identification of phylogenetically related species (Mortiz et al., 1987).

Microsatellites and SNP-type polymorphisms have been mainly used for traceability purposes, the latter currently predominates over the former, presenting many advantages such as easier manipulation in the laboratory, low mutation rate and better suitability for standardization (Fries and Durstewitz, 2001). SNP-type polymorphisms provide the opportunity for genome-wide comparisons between individuals and races (Dadousis et al., 2022). The development of SNP-type polymorphism panels with genome-wide distributed SNPs has provided new opportunities to investigate and decipher the complex relationship between indigenous pig breeds (Lukic et al., 2020).

Preserving the diversity of indigenous pig breeds (*Sus scrofa*) is a key factor for sustaining the pork chain (both locally and globally), including the production of high-quality branded products, the enrichment of animal biobanking and the progress of conservation policies. In addition, local pig farming is closely linked to high-quality niche products that contribute to the development and sustainability of the local economy (Picardy et al., 2019). No less important is the growing demand for organic and high welfare animal-based food products, which has led consumers to prefer local breed products that are considered more nutritious, tasty, healthy and safe, and because animals are usually grown freely and outdoors (Garcia-Gudino et al., 2021).

## MATERIAL AND METHOD

*Biological samples*

The biological samples were collected under sterile conditions from a number of 12 pigs from the red **Mangalitsa** breed and 15 pigs from the **Bazna** breed. The Vietnamese breed was chosen as a swine breed with an Asian background, and hair samples were collected from 9 individuals. Wild boar hair samples were collected from 22 individuals during the hunting season. Genomic SNP data available in genebanks were used to perform preliminary studies on genetic differentiation from the **Mangalitsa** breed red variety from Hungary and other commercial pig breeds (**Landrace, Great White, Hampshire**).

*DNA purification from the collected samples*

DNA purification from hairs was performed using the ZR Genomic DNA II Miniprep kit (Zymo Research). The bulbs of the harvested hairs (10/individual) were transferred into sterile 1.5 ml Eppendorf tubes, appropriately marked. Tissues were lysed in 400 µl of lysis buffer and then incubated for 20 min at room temperature. The obtained lysate was transferred to a purification column, and the resulting eluate in the collection tube by centrifuging the columns for 1 minute at 10,000 rpm was removed. The DNA retained in the filters of the purification columns was washed twice with 400 µl wash buffer from the kit (wash buffer) and re-centrifuged. The purified DNA from the filter was eluted in 50 µl of elution buffer by centrifugation at 18,000 rpm. These DNA samples were later used for cytochrome B sequencing in the study subjects.

*PCR amplification and sequencing of the cytochrome B (Cyt B) gene from mitochondrial DNA*

A reference sequence available in GenBank (Acc. Number AJ002189) was used for the design of the primers required for the amplification and sequencing of the cytochrome B gene coding region from pig and wild boar mitochondrial DNA. The PCR amplification mixture (Taq polymerase, Buffer, MgCl2, dNTPs, primers) DNA was made using a commercial kit 2X PCR Master mix Bioline (table 1).

**Table 1. Preparation of the reaction mix for PCR amplification / Prepararea mixului de reacţie pentru amplificarea PCR**

| Component | Volume for 1 sample (µl) |
|---|---|
| sterile milli Q water | 8,5 |
| master mix 2X | 12,5 |
| primer forward | 1 |
| primer revers | 1 |
| **Total reaction mix** | **25** |

A 23 µl volume of the amplification mixture was transferred into 0.2 ml tubes labeled with the sample number. 2 µl of DNA from each sample was then added to each tube.

PCR amplification was performed in the thermal cycler according to the program:

- DNA predenaturation at 95°C/3 minutes → 1 cycle
- denaturation at 94°C/1 minute
- fixing the primers at 58°C/1 minute } 35 cycles
- extension (Elongation) at 72°C/1 minute
- final extension at 72°C/3 minutes → 1 cycle

Amplification quality was achieved electrophoretically by migrating 5 µl of PCR product in 2% agarose gel in 1X TBE buffer at 70 V for two hours.

Sequencing PCR amplification of purified PCR products was performed in separate reactions with PCR primers using the BigDyeTerminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, USA), according to the instructions.

Sequencing products were purified using the BigDyeXterminator kit (Thermo Scientific) and subsequently migrated to a SeqStudio sequencer (Thermo Scientific).

*Analysis procedure using the DNA microarray technique*

The analysis procedure followed a work protocol and steps established by the manufacturers of the genotyping arrays. In the case of pigs, an array of 60,000 SNPs was used.

Genotyping by the DNA microarray technique is based on the use of a matrix on which specific allele DNA probes are fixed, positioned in the vicinity of some predetermined SNPs from the pig genome. These SNPs can be located in exons, introns or intergenic regions. A certain amount of the purified DNA samples is injected onto the genotyping arrays. Amplification takes place simultaneously in the tens of thousands of positions (polymorphic loci) represented on the array, each with a fluorescently labeled nucleotide (A, T, G or C). These incorporated nucleotides are complementary to the SNPs in the template DNA hybridized to the probes on the array.

The detection of fluorescent nucleotides incorporated in each investigated position represented on the genotyping array and the identification of SNPs is done with the help of a high-resolution scanner.

*Bioinformatics interpretation of data*

The analysis of the obtained sequencing chromatograms was performed by visual inspection using the BioEdit program. The obtained sequences were aligned using the ClustalW2 program. The calculation of the differentiation / genetic diversity indices and the preparation of the phylogenetic trees was carried out with the help of the MEGA and DnaSP programs.

The bioinformatics analysis of the data (identification of the SNPs, respectively of the genotypes from the 60,000 investigated loci) was carried out with the help of specific software: e.g. Plink, Admixture etc.

## RESULTS AND DISCUSSIONS

### Analysis of genetic diversity and phylogenetic relationships based on mitochondrial DNA sequences.

Within the phylogenetic tree (figure 1) obtained through the bioinformatics analysis of the variability of the nucleotide sequence of the Cyt B gene obtained by sequencing this region in the three breeds of domestic pigs and wild boar, two main clusters were highlighted: the European and the Asiatic ones.

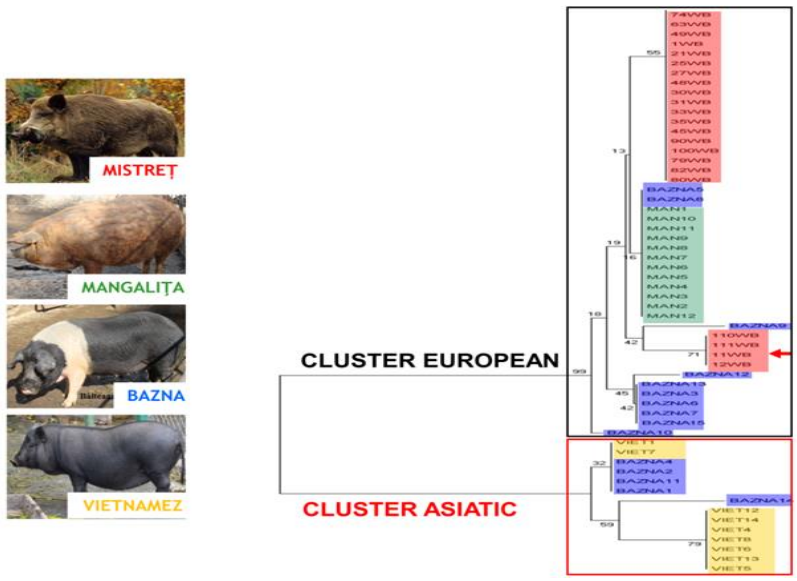


**Figure 1. Phylogenetic tree obtained based on polymorphism of the Cyt B gene nucleotide sequence from mitochondrial DNA / Arbore filogenetic obținut pe baza polimorfismului secvenței nucleotidice a genei Cyt B din ADN-ul mitocondrial**

Looking at the European cluster, we notice that there are samples obtained from the **Mangalitsa** swine breed, the Red variety, and from wild boars. It follows from this that, at least at the mitochondrial level, the genetic background on the maternal line of the **Mangalitsa** breed is European, arguing the formation of this breed on the territory of Europe.

At the level of mitochondrial DNA, genetic diversity is reduced, thus having a high degree of inbreeding both within the **Red Mangalitsa** variety breed and within the wild boar (table 2).

Only one haplotype was identified, both in the case of the **Mangalitsa** breed and in the wild boar (table 2). In the case of the **Red Mangalitsa** breed, this can be explained by the lack of new individuals from other populations that can be used to increase genetic diversity and reduce inbreeding.

**Table 2. Genetic diversity at the level of mitochondrial DNA / Diversitatea genetică la nivelul ADN-ului mitochondrial**

| Species / Breed | **Cytochrome B (Cyt B) – mitochondrial DNA** | | | |
|---|---|---|---|---|
| | Analyzed sequences (no.) | Nucleotide diversity | The diversity of haplotypes | Haplotypes identified (no.) |
| **Wild boar** | 18* | 0,0000 | 0,000 | 1 |
| **Red Mangalitsa** | 12 | 0,0000 | 0,000 | 1 |
| **Bazna** | 15 | 0,008 | 0,857 | 7 |
| **Vietnamese pork** | 9 | 0,004 | 0,511 | 2 |

Within the wild boars analyzed, 18 of the 22 were grouped in a subcluster resulting in a reduced genetic diversity and therefore a high inbreeding (figure 1). The other 4 individuals from whom samples were taken for analysis grouped in a subcluster different from the base population, being closer to the **Bazna** breed (figure1) (these 4 analyzed samples were excluded from the analysis due to the possibility of be hybrids with the domestic swine).

Considering the formation of the **Bazna** breed, the Cyt B analysis showed different results compared to those obtained in the **Red Mangalitsa** variety breed. Thus, within the **Bazna** breed, a number of 10 analyzed samples were grouped into the European genetic cluster (figure 1). Here we have a great genetic diversity due to the fact that 8 of the 10 analyzed samples formed 4 different subclusters. Two of the analyzed samples were grouped in the subcluster of the **Mangalitsa** breed, a fact due to the maternal line contribution of this breed in the formation of the **Bazna** breed. This positioning of the samples analyzed in the European cluster is not surprising if we look at the past where we observe a concordance between the formation of the breed and its first European origin on the maternal side.

However, a number of 5 analyzed samples coming from the **Bazna** breed were grouped in the Asian cluster, in two subclusters alongside the **Vietnamese** breed (figure 1). This is not surprising, thus confirming its Asian origin on the maternal line, this positioning being consistent with the formation of the **Bazna** breed and the majority of British swine breeds (**Wessex, Large White** and **Hampshire**) that were massively introgressed in the 19th century with swine breeds from the Far East, for reproductive maturity and to increase the amount of fat in the carcass.

Within the **Bazna** breed, a great diversity of nucleotides was found in the analyzed Cyt B sequence, corresponding to the 7 distinct haplotypes highlighted (table 2). This fact is correlated with the multiracial maternal origin of the **Bazna** breed.

Based on the mitochondrial sequences obtained, the genetic distances (Fst index) were also calculated between the three breeds of domestic swine analyzed, respectively the wild boar (table 3). The Fst index is calculated to highlight the degree of genetic differentiation between populations / breeds / species. The values of the Fst index can be between 1 and -1. Values below 0.05 indicate little genetic differentiation between populations. Values between 0 and -1 indicate weak or non-existent differentiation between populations. Populations show an average degree of differentiation when Fst

values are between 0.05 - 0.5. Values between 0.5 and +1 indicate significant genetic differentiation between populations/breeds.

**Table 3. Genetic distances revealed by the Fst index calculated based on the nucleotide diversity of the CytB gene / Distanțele genetice evidențiate de indicele Fst calculat pe baza diversității nucleotidice a genei CytB**

| Species / Breed | **Bazna** | **Vietnamese pork** | **Red Mangalitsa** | **Wild boar** |
|---|---|---|---|---|
| **Bazna** | - | - | - | - |
| **Vietnamese pork** | 0,410*** | - | - | - |
| **Red Mangalitsa** | 0,272*** | 0,873*** | - | - |
| **Wild boar** | 0,933*** | 0,970*** | 0,981*** | - |

*** - P < 0,001

A significant difference can be observed between the wild boar and the three domestic swine breeds with an Fst index above 0.9 (table 3). There is also a significant difference between the **Red Mangalitsa** breed and the **Vietnamese** breed with an Fst index of 0.873, a fact due to the lack of the gene pool of breeds of Asian origin when the **Red Mangalitsa** breed was formed. Due to the maternal line contribution of the **Mangalitsa** breed to the formation of the **Bazna** breed, we have an average genetic differentiation index (0.272). Also due to the Asian gene pool in the background of the **Bazna** breed, we have an average genetic differentiation between the **Bazna** breed and the **Vietnamese** breed with an Fst index of 0.410.

**Analysis of genetic diversity and phylogenetic relationships based on SNP polymorphisms.**

In order to investigate the phylogenetic relationships and genetic distances between the studied pig breeds and wild boar, a 3D MDS (multidimensional scaling plot) diagram was constructed with the help of the PLINK program (figure 2). A first analysis of the results indicates that the swine breeds analyzed form several clusters composed of breeds or groups of breeds (fig. 1). We observe in the MDS plot that the **Mangalitsa** swine breeds from Romania and **Mangalitsa** from Hungary were positioned at a relatively appropriate distance due to their common genetic background. This positioning indicates a degree of differentiation between the two populations, this differentiation being auspicious as it allows for the genetic survival of the breed and the reduction of inbreeding through the exchange of breeding stock between the two populations.

The **Bazna** breed clustered close to the **Hampshire breed**, which is not surprising given the significant contribution of this breed to the formation of the **Bazna** breed.

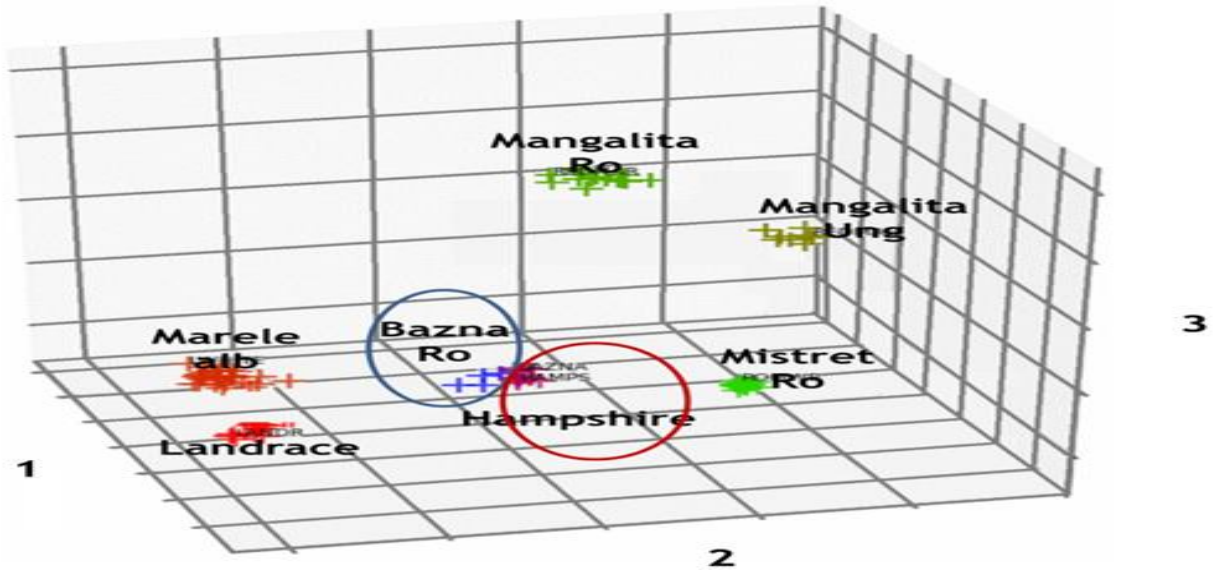


**Figure 2. 3D MDS diagram of the relationships between the studied swine breeds and the wild boar / Diagramă 3D MDS privind relațiile dintre rasele de porcine studiate și porcul mistreț**

The values of the Fst index, determined on the basis of the genotypes obtained at the loci analyzed, confirmed these results (table 4.). This index measures the degree of genetic differentiation (genetic distance) between breeds due to their genetic makeup.

The **Red Mangalitsa** breed population is genetically distant both from wild boar with a genetic differentiation index of 0.186, and from commercial breeds (table 4.).

Surprisingly, the **Bazna** breed shows a higher degree of differentiation compared to the **Mangalitsa** breed, although as was highlighted in the case of the mt DNA analysis, this breed contributed on the maternal line to the formation of the **Bazna** breed. This difference, however, is due to the polyracial origin of the **Bazna** breed. A significant genetic difference between the **Bazna** breed and the wild boar was also found.

**Table 4. Indices of genetic differentiation (Fst) between the studied swine breeds and wild boar calculated from SNP genotyping data / Indicii de diferențiere genetică (Fst) dintre rasele de porcine studiate și porcul mistreț, calculați pe baza datelor de genotipare SNP**

| | Red Mangalitsa (H) | Red Mangalitsa (Ro) | Bazna | Hampshire | Landrace | The great White | Wild boar (Ro) |
|---|---|---|---|---|---|---|---|
| **Red Mangalitsa (H)** | - | - | - | - | - | - | - |
| **Red Mangalitsa (Ro)** | 0,115 | - | - | - | - | - | - |
| **Bazna** | 0,259 | 0,234 | - | - | - | - | - |
| **Hampshire** | 0,279 | 0,259 | 0,329 | - | - | - | - |
| **Landrace** | 0,201 | 0,180 | 0,207 | 0,241 | - | - | - |
| **The great White** | 0,204 | 0,175 | 0,202 | 0,238 | 0,125 | - | - |
| **Wild boar (Ro)** | 0,185 | 0,186 | 0,243 | 0,267 | 0,196 | 0,198 | - |

## CONCLUSIONS

We could conclude that the **Red Mangalitsa** breed, raised in the mother core from RDSA Turda, presents a reduced genetic diversity at the level of mitochondrial DNA. This indicates a high degree of maternal inbreeding, which could affect the future genetic stability of this population. However, the population of the Romanian **Red Mangalitsa** shows at the autosomal level a certain degree of genetic differentiation (Fst=0.115) compared to the population of the **Red Mangalitsa** from Hungary.

The **Bazna** swine breed, raised in the maternal nucleus from RDSA Turda, shows a fairly high genetic diversity at the level of mitochondrial DNA, which confirms the existence of a large number of maternal lines in this population. This finding is consistent with the admixed (polyracial) origin of the **Bazna** breed. The results of the DNA analyzes highlight the fact that a proper management of this population can lead to its numerical increase, without affect the genetic stability of the **Bazna** breed.

The effective management of the domestic swine breeds **Red Mangalitsa** and **Bazna**, breeds with a vulnerable status, currently represents one of the most important challenges for the breeders' associations that lead breeding programs, as well as for the authorities in the field. The use of DNA markers in the classical breeding programs of the two breeds, currently based only on phenotypic selection, can contribute substantially to their more efficient management and to their genetic stabilization.

The advantages of using the information provided by DNA markers in classical breeding programs can be multiple, among which we mention: the precise establishment of the inbreeding coefficient within the pig populations of the two breeds, which allows a more efficient matching of matings between individuals little related; tracking with greater precision the transmission of characters from parents to offspring by knowing the real parentage of the offspring; fixing in the population some mutations associated with morpho-productive characters of interest; determination of genetic distances to other pig breeds based on the calculation of genetic differentiation indices (e.g. Fst).

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# THE PREVALENCE OF DIGESTIVE PARASITES OF PIGS IN SMALLHOLDERS FROM HÂRTIBACIU VALLEY, SIBIU COUNTY, ROMANIA

# PREVALENŢA PARAZIŢILOR DIGESTIVI LA PORCI ÎN GOSPODĂRII DE PE VALEA HÂRTIBACIULUI, JUDEŢUL SIBIU, ROMÂNIA

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***Abstract***

*Parasitic diseases cause significant economic losses in pigs, but are often overlooked because of the apparent lack of clinical signs. The current study aimed to identify the parasitic profile of swine raised in 14 smallholders from Hârtibaciu Valley, Sibiu. A number of 260 samples were collected, during winter and spring, from piglets, fatteners, and sows. The coproparasitological examination used usual methods. The number of eggs (EPG), oocysts (OPG), and cysts (CPG) were counted per gram of fecal matter.The examination revealed parasitic infections with B. coli, Eimeria spp., A. suum, T. suis, and Oesophagostomum spp. The prevalence (P) and average intensity (AI) of infections varied between farms, seasons and age group. The overall prevalence in all smallholders, according to the age category, was 60% for Eimeria spp., 22% - B. coli, 27% - Oesophagostomum spp., 29% - A. suum, and 8% - T. suis, in piglets. In fatteners, Eimeria spp. had a prevalence of 28%, B. coli - 49%, A. suum - 33%, T. suis - 8%, Oesophagostomum spp. – 29% and in sows Eimeria spp. - 23%, B. coli - 43%, A. suum - 27%, and Oesophagostomum spp. - 37%. Therefore, the present study revealed statistically significant differences between age groups, seasons, and smallholders for all diagnosed parasites. The presence of infections with B. coli, A. suum, and T. suis, parasites with zoonotic potential, justifies their supervision and control, in the vision of the "One health, one medicine" concept.*

***Keywords: digestive parasites, epidemiology, smallholders, pigs***

***Rezumat***

*Bolile parazitare provoacă pierderi economice semnificative la porci, dar sunt adesea trecute cu vederea din cauza lipsei aparente a semnelor clinice. Studiul actual a avut ca scop identificarea profilului parazitar al suinelor crescute in 14 gospodarii din Valea Hârtibaciu, Sibiu. Un număr de 260 de probe au fost colectate, în timpul iernii și primăverii, de la purcei, grăsuni și scroafe. Examenul coproparazitarologic a folosit metode uzuale. Numărul de ouă (OPG), oochisturi (OPG) și chisturi (CPG) au fost numărate per gram de materie fecală. Examinarea a relevat infecții parazitare cu B. coli, Eimeria spp., A. suum, T. suis și Oesophagostomum spp. Prevalența (P) și intensitatea medie (IM) a infecțiilor au variat între ferme, anotimpuri și grupa de vârstă. Prevalența totală la toate gospodăriile, în funcție de categoria de vârstă, a fost de 60% pentru Eimeria spp., 22% - B. coli, 27% - Oesophagostomum spp., 29% - A. suum și 8% - T. suis, la purcei. La grăsuni, Eimeria spp. a avut o prevalență de 28%, B. coli - 49%, A. suum - 33%, T. suis - 8%, Oesophagostomum spp. – 29% și la scroafe Eimeria spp. - 23%, B. coli - 43%, A. suum - 27%, și Oesophagostomum spp. - 37%. Prin urmare, studiul de față a evidențiat diferențe semnificative statistic între grupele de vârstă, anotimpuri și gospodării pentru toți paraziții diagnosticați. Prezența infecțiilor cu B. coli, A. suum și T. suis, paraziți cu potențial zoonotic, justifică supravegherea și controlul acestora, în viziunea conceptului "One health, one medicine".*

***Cuvinte cheie: paraziţi digestivi, epidemiologie, gospodării, suine***

## INTRODUCTION

In pig-rearing systems, parasitic diseases are responsible for substantial losses in both pig reproduction and productivity, which represents a significant obstacle to an efficient and profitable animal management (Kochanowski et al., 2017; Ózsvári et al., 2018). Parasitic infections can lead to intestinal malabsorption, compromised or delayed immunity following vaccination protocols, impaired fertility, increased susceptibility to bacterial and viral illnesses, as well as a decline in meat quality (Lai et al., 2011).

Factors such as age and sex of the animals, hygiene, management practices, and geographical location serve as risk factors that, in association with the digestive parasites of pigs, affect the welfare and health of the animals (Permin et al., 1999; Geresu et al., 2015; Roesel et al., 2017). Flooring, waste drainage facilities, humidity, and temperature provide optimal conditions for the propagation and survival of parasitic stages in feed and contaminated environments (Ali et al., 2018; Sharma et al., 2020). Animal overcrowding, the presence of rodents, access to non-potable water, open defecation, inadequate shelter, improper feeding, health status, and host immunity are other significant factors in the development of parasitic stages, creating optimal conditions for infection. This could be attributed to coprophagy in pigs, which makes them more susceptible to parasitic infections (Ali et al., 2018; Sharma et al., 2020).

Gastrointestinal parasitic diseases generally exhibit a relatively slow progression, and frequently, pigs do not show any noticeable symptoms (Băieş et al., 2021). Within animal populations, there are infected swine with a low number of parasites (intensity) and low pathogenicity, resulting in an unaffected clinical condition. However, these animals serve as carriers and eliminators of parasites, disseminating invasive elements into the external environment, thus contaminating the rest of the herd (Geresu et al., 2015; Roesel et al., 2017).

The most common mistakes in combating pig parasitoses include the lack of regular coproparasitological examinations, inappropriate administration and dosage of antiparasitic drugs, ineffective disinfection, or the incorrect choice of disinfectant agent (Selzer and Epe., 2021).

This study aimed to identify the parasitic profile of pigs from three age categories (piglets, fatteners, and sows) raised in smallholders in the Hârtibaciu Valley, Sibiu County.

## MATERIAL AND METHODS

This study aims to ascertain the prevalence and intensity of parasitic infections in pigs of various breeds, raised in a low-input system (smallholder) in the Hârtibaciu Valley area.

The Hârtibaciu Valley, also known as the ”Green Valley”, offers a unique landscape with abundant pastures, forests, and agricultural fields. Iacobeni is a commune located in Sibiu County, Transylvania, consisting of villages such as Iacobeni (the administrative center), Movile, Netuș, Noiștat, and Stejărișu. Positionated 70 km from the city of Sibiu, 12 km from Agnita, and 32 km from Sighișoara, the commune is characterized by hilly terrain, traversed by the Hârtibaciu River. The fertile lands, cultivated without the use of chemicals, establish the Green Valley as one of the most suitable places for agriculture, especially for organic farming.

Within the commune, there is an estimated population of around 700 pigs. The main swine breeds found among the local smallholders include Pietrain, Large White, Duroc, Landrace, Bazna, and their crossbreeds. Pigs are raised in either individual or collective pens. However, the practice of free-range pig rearing is becoming less common due to restrictions aimed at preventing domestic pigs from encountering wild boars and some others potentially spreading African swine fever (ASF).

Faecal samples were collected from 14 smallholders residing in the villages of Iacobeni, Netuș și Stejarișu. The shelters are subject to a daily cleaning routine, and some of them have outdoor areas. Additionally, a few smallholders have adopted a free-range pig-rearing system. The sampling procedure encompassed three distinct age groups (piglets, fatteners, and sows). The collection of faecal took place during two seasons: winter and spring, spanning the years 2021-2022. A total of 135 animals were sampled during the winter season, followed by 125 animals during the spring season. Specifically, in the winter, 35 faecal samples were procured from piglets, 50 from fatteners, and 50 from sows. In the subsequent spring season, 25 faecal samples were obtained from piglets, while 50 samples each were collected from fatteners and sows. Age-wise, the piglets ranged between 6 to 12 weeks, fatteners were between 4 and 8 months, and sows ranged from 2 to 5 years in age.

Faecal samples were individually collected from the rectum of the animals. The samples were examined macroscopically (color, consistency, and presence of any visible parasites). Subsequently, the sterile containers were labeled and stored at 2-8 °C until testing. Each faecal sample underwent an individual coproparasitological examination.

Throughout the study, the animals did not exhibit any clinical signs attributed to parasitic infections. Certain smallholders adhered to an established deworming protocol, while a limited number of animals were not dewormed. The deworming procedure was overseen by the local veterinarian upon the owners' s request. Sows were dewormed with injectable ivermectin (0.3 mg/kg body weight), fatteners received injectable levamisole solution (5 mg/ kg body weight) and piglets were orally administered toltrazuril (20 mg/ kg body weight).

The collected samples were processed and examined at the Department of Parasitology and Parasitic Diseases of the Faculty of Veterinary Medicine from Cluj-Napoca. The coproparasitological methods used for processing the faecal samples included flotation (Willis, McMaster), active sedimentation, modified Ziehl-Neelsen method, modified Blagg method, and the cultivation of strongyle eggs and coccidian oocysts. The number of eggs/oocysts/cysts (EPG/OPG/CPG) was counted per gram of faecal matter using established protocols (Mircean et al., 2011).

## RESULTS AND DISCUSSION

The coproprasitological examination revealed parasitic infections with *Balantioides coli, Eimeria* spp., *Ascaris suum, Oesophagostomum* spp., and *Trichuris suis* (Figure 1). The prevalence (P) and mean intensity (MI) of the infection varied according to the age and category of investigated pigs (Table 1 and Table 2). It is noteworthy that all samples subjected to active sedimentation, modified Ziehl-Neelsen, and modified Blagg methods, were negative. Furthermore, all identified oocysts belonged to the *Eimeria* genus, and all the strongyle larvae were attributed *Oesophagostomum genus***.**

Among piglets, in both seasons, the most prevalent parasite was *Eimeria* spp. (52%; 68%), followed in the winter season by *Oesophagostomum* spp. (34%), *A. suum* (17%), *T. suis* (12%), and *B. coli* (3%), and in the spring season *A. suum* (40%), *B. coli* (40%), *Oesophagostomum* spp. (20%), and *T. suis* (4%) (Tables 1, 2 and 3).

In the case of fatteners, the most prevalent parasite during both seasons was *B. coli* (44%; 54%). In the winter season, this was followed by *Oesophagostomum* spp. (34%), *Eimeria* spp. (28%), *A. suum* (24%), and *T. suis* (4%), and during the spring season, by *A. suum* (42%), *Eimeria* spp. (28%), *Oesophagostomum* spp. (24%), and *T. suis* (4%) (Tables 1, 2, and 3).

Among sows, the highest prevalence, during the winter season was observed for *A. suum* (38%), followed *by B. coli* (36%), *Oesophagostomum* spp. (26%), and *Eimeria* spp. (16%). In the spring season, *B. coli* (50%) exhibited the highest prevalence, followed by *Oesophagostomum* spp. (48%), *Eimeria* spp. (30%), and *A. suum* (16%) (Tables 1, 2, and 3).

In the current study, *Trichuris suis* was identified only in piglets and fatteners displaying a prevalence of 8% (Table 3). In Europe, the prevalence of this nematode ranged from 2.9 to 11.2% among fatteners in Poland and reached up to 14.3% in the Netherlands (Eijck et al., 2005; Popiołek et al., 2009; Kochanowski et al., 2017). Notably, the prevalence of *T. suis* infection in India was lower at 6.43%, similar to other reports from Ghana (4.6%) and Zimbabwe (4.2%) (Sharma et al., 2020).

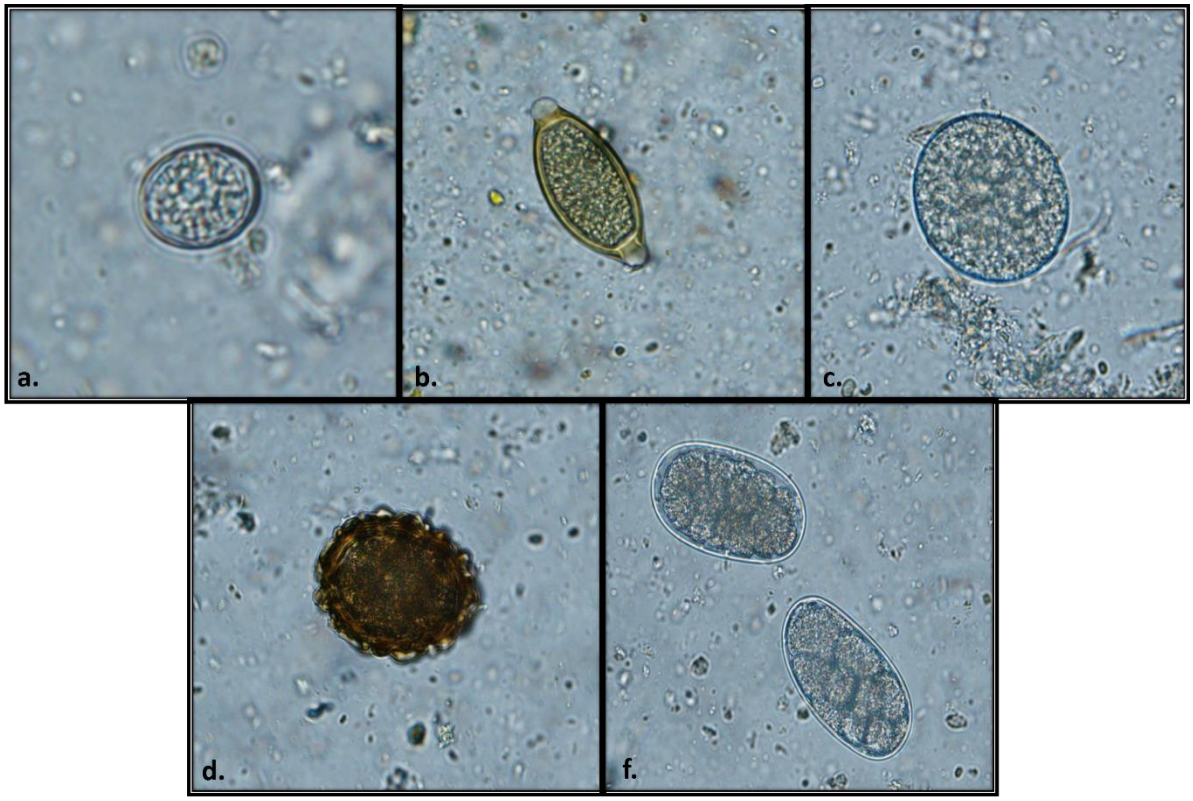


**Figure 1. The results of coproparasitological examination: a. *Eimeria* spp. oocyst; b. *T. suis* egg; c. *B. coli* cyst; d. *A. suum* egg; f. *Oesophagostomum* spp. egg (400x) / Rezultatele examenului coproparazitologic: a. oochist de *Eimeria* spp.; b. ou de *T. suis*; c. chist de *B. coli*; d. ou de *A. suum*; f. ou de Oe*sophagostomum* spp. (400x).**

**Table 1. The prevalence (P%) and mean intensity (MI) in the 3 age groups in the winter season / Prevalenţa (P%) şi intensitatea medie (IM) la cele 3 categorii de vârstă în sezonul de iarnă**

| | Piglets | | Fatteners | | Sows | |
|---|---|---|---|---|---|---|
| **Parasite** | P % | MI | P % | MI | P % | MI |
| *Eimeria* spp. | 52 | 881 | 28 | 1311 | 16 | 288 |
| *B. coli* | 4 | 400 | 44 | 173 | 36 | 160 |
| *A. suum* | 17 | 3508 | 24 | 667 | 38 | 727 |
| *Oesophagostomum* spp. | 34 | 246 | 34 | 433 | 26 | 131 |
| *T. suis* | 12 | 175 | 4 | 125 | - | - |

**Table 2. The prevalence (P%) and mean intensity (MI) in the 3 age groups in the spring season / Prevalenţa (P%) şi intensitatea medie (IM) la cele 3 categorii de vârstă în sezonul de primăvară**

| | Piglets | | Fatteners | | Sows | |
|---|---|---|---|---|---|---|
| **Parasite** | P % | MI | P % | MI | P % | MI |
| *Eimeria* spp. | 68 | 454 | 28 | 6519 | 30 | 600 |
| *B. coli* | 40 | 182 | 54 | 129 | 50 | 62 |
| *A. suum* | 40 | 1485 | 42 | 1384 | 16 | 994 |
| *Oesophagostomum* spp. | 20 | 140 | 24 | 480 | 48 | 958 |
| *T. suis* | 4 | 200 | 12 | 309 | - | - |

**Table 3. The prevalence (P%) and mean intensity (MI) in the 3 age groups in the both season / Prevalența (P%) şi intensitatea medie (IM) la cele 3 categorii de vârstă în ambele sezoane**

| | Piglets | | Fatteners | | Sows | |
|---|---|---|---|---|---|---|
| **Parasite** | P % | MI | P % | MI | P % | MI |
| *Eimeria* spp. | 60 | 667 | 28 | 3915 | 23 | 444 |
| *B. coli* | 22 | 291 | 49 | 151 | 43 | 111 |
| *A. suum* | 29 | 2496 | 33 | 1025 | 27 | 860 |
| *Esophagostomum* spp. | 27 | 193 | 29 | 456 | 37 | 544 |
| *T. suis* | 8 | 187 | 8 | 217 | - | - |

**Table 4. The overall prevalence (P%) and mean intensity (MI) / Prevalența (P%) şi intensitatea medie (IM) totală**

| | Total | |
|---|---|---|
| **Parasite** | P % | MI |
| *Eimeria* spp. | 37 | 1675 |
| *B. coli* | 38 | 184 |
| *A. suum* | 30 | 1460 |
| *Oesophagostomum* spp. | 31 | 398 |
| *T. suis* | 8 | 202 |

In the current study, *Ascaris suum* was identified in piglets, fatteners, and sows with a prevalence of 29%, 33%, and 27%, respectively (Table 3). Sharma et al. (2020) demonstrated that *A. suum* was the most prevalent digestive parasite in pigs (11.1%), within subtropical regions of India. In another study conducted on pigs of various ages, in Nordic countries, varying prevalences of *A. suum* were reported: 3.5% in Finland, 16% in Norway, and 15% in Sweden (Roepstorff et al., 1998). In South Africa, a study conducted in 2019 revealed a prevalence of 63.9% among fatteners (Nwafor et al., 2019). Several studies in China and Greece concluded that the prevalence of *A. suum* infection is lower in commercial farms (ranging from 0.9 to 16.5%), compared to free-range farms (Lin et al., 2013; Symeonidou et al., 2020).

The prevalence of *B. coli* infection in Italy was 36.66%, 40% in Bangladesh, and 22% in Malaysia, which concurs with our study's findings (38%), and contrary to the observations in Korea, where it was notably lower (4.5%) (Tan et al., 2014; Sharma et al., 2020). *B. coli* has been diagnosed in piglets from Bangladesh, Greece, and Germany, with prevalences of 28.6%, 2.6%, and 0.7%, respectively (Wieler et al., 2001; Dey et al., 2014; Symeonidou et al., 2020), while our study recorded a prevalence of 22% (Table 4).

In a study carried out in Azerbaijan involving pigs of different ages, a prevalence of *Eimeria* spp. ranging from 21.4% to 32.2% was identified (Ali et al., 2018). In contrast, Roepstorff et al. (1998) reported *Eimeria* spp. prevalence rates of 7% in Finland, 1% in Norway, and 8% in Sweden. Our study, however, found a higher prevalence of 37% (Table 4). Likewise, a study conducted in Poland on piglets revealed a coccidiosis prevalence of 31.4% (Kochanowski et al., 2017), while our study recorded a higher prevalence of 60% (Table 4). An investigation spanning African countries such as South Africa, Ethiopia, and Rwanda, recorded a prevalence of *Eimeria* spp. infection ranging from 5.6% to 88% across all age groups, with the highest prevalence in piglets, followed by fatteners and sows (Abdu and Gashaw, 2010; Nwafor et al., 2019; Tumusiime et al., 2020).

*Oesophagostomum* spp. was identified in all three age categories, with the highest prevalence in sows (37%), followed by fatteners (29%), and piglets (27%) (Table 3). Similar findings were observed in other countries such as Greece (sows-5.2%), Netherlands (piglets-12.5%, sows-37.5%), Poland (3.44-20.8%), South Africa (piglets-8%, sows-43.8%), and Ethiopia (6.75%) (Eijck et al., 2005; Abdu and Gashaw, 2010; Knecht et al., 2011; Nwafor et al., 2019; Symeonidou et al., 2020).

The presence of infections with potentially zoonotic parasites such as *B. coli*, *A. suum*, and *T. suis*, justifies their surveillance and control under the vision of the "One health, one medicine" concept. Nevertheless, further research is needed for a better understanding of the epidemiology of these infections in pigs raised in low-input, occasionally free-range systems, from our country.

## CONCLUSIONS

The present study was conducted during the winter and spring seasons of 2021-2022, and involved a total of 135 pigs from 14 smalholders of the local population, located in the villages of Iacobeni commune, in the Hartibaciu Valley area, Sibiu County. The animals were raised in low-input systems, and occasionally had access to the outdoor environment (free-range).

In piglets, the most prevalent parasite was *Eimeria* spp. (60%), followed by *A. suum*, *Oesophagostomum spp*., *B. coli*, and *T. suis*. Among fatteners, the highest prevalence was recorded for *B. coli* (49%), followed by *A. suum*, *Oesophagostomum* spp., *Eimeria* spp., and *T. suis*. In sows, the highest prevalence was for *B. coli* (43%) infection, followed by *Oesophagostomum* spp., *A. suum*, and *Eimeria* spp.

Despite the significant prevalence and intensity levels of parasitic infections, all pigs were clinically asymptomatic, and no correlation was observed between parasitic infections and clinical manifestation.

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# STUDY ON THE REPRODUCTIVE PERFORMANCE AND MILK PRODUCTION OF DAIRY BUFFALOES FROM THE CENTRAL AREA OF ROMANIA

# STUDIU PRIVIND PERFORMANȚELE DE REPRODUCȚIE ȘI ALE PRODUCȚIEI DE LAPTE LA BIVOLIȚELE DIN ZONA DE CENTRU A ROMÂNIEI

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***Abstract***

*Buffaloes raised in Romania have mixed productive abilities, milk-meat, the main ability is for milk production. In the center of the country, buffalo breeding has a long tradition of over a century. Buffalo milk has great nutritional value, lends itself very well to processing into specific products. On December 1, 2021, there were 18,328 buffaloes, of which 97% of the herds were in the center and northwest of Transylvania. In the center of the country, 5,388 heads are raised, in 592 farms of different sizes, which represents 29.40% of the total herd. The research carried out during six years, in ten farms, on a herd of 396 dairy buffaloes raised in the central area of Romania, revealed that the average interval between calvings is 438.04 days, with a high variability of the character in the population. This result is influenced by the average duration of the service period of 123.93 days, with V=89.06%, the average duration of gestation being relatively constant. The duration of lactation was influenced by the age of the animals and technological factors. The average amount of milk, fat and protein per total lactation, as well as the average content in fat and protein recorded different average values, being influenced by the rank of lactation. From the analysis of the milk production data recalculated at 270 days, it follows that there are no significant differences compared to the milk production per total lactation. The explanation lies in the fact that the total duration of lactation is not much longer than 270 days.*

***Key words: buffalo, milk production, farms, milking, fat, protein, reproduction***

***Rezumat***

*Bivolii crescuți în România au aptitudini productive mixte, de lapte-carne, aptitudinea principală este pentru producerea laptelui. În centrul țării creșterea bivolilor are o tradiție îndelungată, de peste un veac. Laptele de bivoliță are valoare nutritivă deosebită, se pretează foarte bine la procesare în produse specifice. La 01 decembrie 2021 existau 18328 bivoli, din care 97% din efective fiind în centrul şi nord-vestul Transilvaniei.. În centrul țării se cresc 5388 capete, în 592 ferme de diferite dimensiuni, ceea ce reprezintă 29,40% din efectivul total. Cercetările derulate pe parcursul a șase ani, în zece ferme, pe un efectiv de 396 bivolițe crescute în zona de centru a României, a relevat că intervalul mediu între fătări este de 438,04 zile, cu o variabilitate mare a caracterului în populație. Acest rezultat este influențat de durata medie a service-periodului de 123,93 zile, cu V=89,06%, durata medie a gestației fiind relativ constantă. Durata lactației a fost influențată de vârsta animalelor și de factorii tehnologici. Cantitatea medie de lapte, grăsime și proteină pe lactație totală, cât și conținutul mediu în grăsime și proteină a înregistrat valori medii diferite, fiind influențate de rangul lactației. Din analiza datelor producției de lapte recalculată la 270 zile, rezultă că nu sunt diferențe semnificative față de producția de lapte pe lactație totală. Explicația constă în faptul că durata totală a lactației nu este cu mult mai mare de 270 zile.*

***Cuvinte cheie: bivoli, producție de lapte, ferme, muls, grăsime, proteine, reproducție***

## INTRODUCTION

At the origin of the Romanian Buffalo is the wild Indian buffalo (*Bubalus arni*), but also the domestic one - the common buffalo (*Bubalus microceros*). From a genetic and geographical point of view, the Romanian buffalo is part of the river type, considered the common riparian buffalo. Regarding the period of entry of buffaloes into Romania, several assumptions are made. Information about buffaloes in Romania, since ancient times, is scarce. In Porumbacu (Sibiu county), 35 buffaloes and female buffaloes are recorded in the live inventory of the estate from 1632 (Mateș, 1974). Over time, since entering the country, the Romanian buffalo has been isolated from a reproductive point of view, through "geographical enclavization", determined by state administrative borders, acclimatizing in Transylvania where the climate is harsher and in the Danube Plain. Until 1990, the buffalo herds in Romania were maintained at an approximately constant level, they being considered a safe population, raised in Agricultural Production Cooperatives, State Agricultural Enterprises and households of the population. Thus, the animals were registered, infusion crosses were made with mixed-breed Murrah bulls imported from Bulgaria, the breed was approved in 1987, with the name Romanian Bubaline Breed, presenting distinct morphological and productive characteristics, for which the breeding program was also developed and the genealogical register was opened. The highest number of buffaloes in the country was in 1980 (228,000 buffaloes). After 1990, Romania's buffalo population decreased, as did most species of domestic animals, reaching 18,826 heads in 2014. The main causes of the numerical reduction of buffalo herds, after 1990, were multiple, of which we specify the massive slaughters, the abusive trade in animals, the retention of a small number of replacement female youth and the advanced age of the buffalo breeders in the rural area. Buffaloes raised in our country have mixed productive skills, milk-meat, the main skill is for milk production and it is spread in two large areas: Transylvania and the Danube Plain. In the center of the country, buffalo breeding has a long tradition of over a century. In the period 1899-1908, the average milk production of buffaloes in the area of Făgăraș, in the town of Sâmbăta was 1300 kg of milk/year. From the ranking of the best buffaloes, it can be seen that the total duration of lactation varied between 256 and 303 days, the amount of milk recorded values between 1398-1484 kg milk/lactation, with 7.28-11.21% fat (Rostafinski, 1912). On December 1, 2021, there were 18,328 buffalo heads in Romania, of which 14,060 were females for reproduction, and 4,268 youth heads (INS), 97% of the herd being in the center and north-west of Transylvania, mainly in the counties of Brasov, Sibiu, Cluj and Salaj. In the center of the country (Brașov and Sibiu counties) 5388 head are raised, in 592 farms of different sizes, which represents 29.40% of the total herd raised in the country (Genealogical Register of the Romanian Buffalo breed).

Considering the aspects specified above, the purpose of this work was to know thoroughly and realistically the parameters of reproduction and production of buffaloes in the central area of Romania.

## MATERIAL AND METHOD

The existence in the central area of Romania of some buffalo breeding farms with herds of more than 20 heads made the research in the present study possible. At the same time, the grouping of large herds of buffaloes in a small area also offered the opportunity for research to be carried out in all farms in a short period of time, eliminating the errors given by the general and special environmental conditions.

The research took place over six years, in ten farms, on a herd of 396 buffaloes. The study of the reproductive parameters was carried out by analyzing the data from the passports of the females, from the individual animal reports (SNIIA), the breeding and calving register of the farms and from the Genealogical Register of the **Romanian Buffalo** breed. The study of the parameters of milk production in buffaloes was carried out by performing the control of milk production at intervals between 38-46 days, method (B6), by recording the amount of milk (kg). The quality parameters of the milk (fat and

protein content) were determined using the portable milkoscanner. For this purpose, 1483 completed lactations were analyzed. The rank of lactations was from lactation 1 to lactation 13. Milk production, in the case of each lactation, was recalculated as equivalent to 270 days (ICAR, 2017). At the same time, the data from the breeding and calving register of the farms and from the Genealogical Register for the Romanian Buffalo breed were analyzed. The obtained data were statistically processed and interpreted. In order to determine the main statistical indicators (mean, mean error, standard deviation and variability) a simple variance analysis was used.

## RESULTS AND DISCUSSION

The analysis of reproductive rhythmicity in buffaloes takes into account the following indicators of interest, such as the gestation period (DG), the calving interval (CI) and the service period (SP). Calving interval is the interval in days between two successive calvings. The duration of the calving interval can be calculated according to the formula CI = SP+DG. For buffaloes, the service-period represents the interval from calving to the fertile mount and can be calculated according to the formula SP = dif-df, in which, dif is the interval from calving-insemination to the fertile mount, expressed in days and df is the calving date. Gestation duration represents the interval between fecund mounting/insemination and calving, expressed in days. The duration of gestation in buffaloes ranges between 300-330 days, with an average of 315 days.

From the analysis of reproduction parameters with implications on rhythmicity, in the farms studied, the values presented in table 1 resulted.

**Table 1. Reproduction indices for dairy buffaloes in the analyzed farms / Indici de reproducție a bivolilor de lapte în fermele analizate**

| Specification | Total farms | | |
|---|---|---|---|
| | n | $\overline{X} \pm S^2_{\overline{X}}$ | V% |
| **Gestation duration – DG (days)** | 396 | 314.10±0.22 | 1.50 |
| **Calving interval - CI (days)** | 396 | 438.04±6.30 | 30.88 |
| **Service period – SP (days)** | 396 | 123.93±6.30 | 89.06 |

An average calving interval on 396 buffaloes of 438.04±6.30 days is noted, with a high variability (V=30.88%), which indicates a heterogeneous population. This result is influenced by the average duration of the service period (123.93±6.30 days) and the very high variability (89.06%), the average duration of gestation being relatively constant (314.10±0.22 days), with a very small variability (1.50%). In order to have a more accurate picture of the duration of the interval between calvings and the service period calculated in the present study, we make the comparison with the average values determined in other buffaloes. Thus, in other studies (Vidu, 2008), on 686 buffaloes from other areas of the country, the average calving interval was 486.33 days and the service period was 171.33 days.

The study shows that the duration of the service period is influenced by the heat detection rate. For a very good reproduction activity of the buffaloes, it is necessary that the service period has a duration of up to 60 days.

The characterization of buffaloes in terms of milk production was carried out by studying three parameters, namely: the duration of lactation, the amount of milk milked, and the quality of the milk in terms of fat and protein content. The results of the milk production study are shown in table 2 (n = heads analyzed; ± = mean and standard error of the character; DL = duration of lactation; CL = average amount of milk in kg; CG = amount of fat in kg; CP = amount of protein; % G = fat content %; % P = protein content in %).

The research reveals that the average duration of lactations in buffaloes did not show significant differences between farms even from one rank to another, the variability of the character being below 10% (table 2, chart 1). The longest average duration of lactation was recorded in the 6th lactation, and

the lowest in the 8th - 13th lactations. An increase in the duration of lactation is observed from lactation I to lactation VI, followed by a decrease until lactation 13. The duration of lactation in buffaloes from the studied farms was influenced by the age of the animals as well as by technological factors.

The average amount of milk per total lactation in the buffaloes from the farms analyzed, recorded different average values, being influenced by the rank of lactation (table 2, chart 2). At the same lactation rank, the variability of the character is below 10%, the differences between the average amounts of milk per lactation between farms being insignificant.

**Table 2. Productive parameters for lactations 1 - 13, recorded in dairy buffaloes from 10 farms / Parametri productivi pentru lactațiile 1 - 13, înregistrați la bivolițele de lapte din 10 ferme**

| Specification | | | First lactation | | | Second lactation | | | Third lactation | | | Fourth lactation | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | n | X ± S²x | V | n | X ± S²x | V% | n | X ± S²x | V | n | X ± S²x | V |
| Centralizer 10 farms | Total milk production | DL | 225 | 275.68 ±1.03 | 5.58 | 201 | 280.81 ±1.24 | 6.27 | 211 | 288.86 ±1.42 | 7.12 | 172 | 294.17 ±1.81 | 8.07 |
| | | CL | 225 | 1615.11 ±9.68 | 8.99 | 201 | 1725.95 ±10.78 | 8.85 | 211 | 1837.18 ±11.99 | 9.48 | 172 | 1939.18 ±12.17 | 8.23 |
| | | CG | 225 | 120.28 ±0.70 | 8.79 | 201 | 128.79 ±0.81 | 8.90 | 211 | 137.49 ±0.91 | 9.64 | 172 | 145.55 ±0.93 | 8.37 |
| | | %G | 225 | 7.49 ±0.01 | 1.34 | 201 | 7.50 ±0.01 | 1.24 | 211 | 7.51 ±0.01 | 1.12 | 172 | 7.51 ±0.01 | 1.14 |
| | | CP | 225 | 67.97 ±0.40 | 8.72 | 201 | 72.22 ±0.44 | 8.56 | 211 | 77.12 ±0.52 | 9.83 | 172 | 81.55 ±0.53 | 8.56 |
| | | %P | 225 | 4.23 ±0.01 | 1.80 | 201 | 4.22 ±0.00 | 1.62 | 211 | 4.21 ±0.00 | 1.48 | 172 | 4.20 ±0.00 | 1.55 |
| | Milk production for 270 days | CL | 225 | 1578.42 ±7.26 | 6.90 | 201 | 1680.87 ±8.95 | 7.55 | 211 | 1763.42 ±9.53 | 7.85 | 172 | 1850.18 ±9.84 | 6.97 |
| | | CG | 225 | 118.19 ±0.55 | 7.01 | 201 | 125.96 ±0.68 | 7.66 | 211 | 132.37 ±0.73 | 8.00 | 172 | 138.97 ±0.76 | 7.14 |
| | | %G | 225 | 7.48 ±0.01 | 1.34 | 201 | 7.49 ±0.01 | 1.31 | 211 | 7.50 ±0.01 | 1.15 | 172 | 7.50 ±0.01 | 1.16 |
| | | CP | 225 | 66.80 ±0.30 | 6.66 | 201 | 70.71 ±0.36 | 7.23 | 211 | 74.32 ±0.42 | 8.18 | 172 | 77.90 ±0.43 | 7.31 |
| | | %P | 225 | 4.23 ±0.01 | 1.80 | 201 | 4.21 ±0.00 | 1.64 | 211 | 4.21 ±0.00 | 1.46 | 172 | 4.20 ±0.00 | 1.53 |
| | | | Lactation 5 | | | Lactation 6 | | | Lactation 7 | | | Lactations 8 - 13 | | |
| | Total milk production | DL | 130 | 299.46 ±2.19 | 8.35 | 112 | 295.29 ±2.11 | 7.56 | 90 | 279.23 ±1.62 | 5.50 | 342 | 272.41 ±0.83 | 5.62 |
| | | CL | 130 | 1953.58 ±14.53 | 8.48 | 112 | 2024.47 ±14.80 | 7.74 | 90 | 1722.94 ±19.41 | 10.69 | 342 | 1552.12 ±8.15 | 9.72 |
| | | CG | 130 | 146.15 ±1.13 | 8.84 | 112 | 151.97 ±1.12 | 7.80 | 90 | 128.82 ±1.49 | 10.94 | 342 | 116.55 ±0.60 | 9.58 |
| | | %G | 130 | 7.50 ±0.01 | 1.25 | 112 | 7.51 ±0.01 | 1.14 | 90 | 7.49 ±0.01 | 1.21 | 342 | 7.50 ±0.01 | 1.34 |
| | | CP | 130 | 82.04 ±0.66 | 9.22 | 112 | 85.26 ±0.63 | 7.85 | 90 | 72.63 ±0.86 | 11.20 | 342 | 65.88 ±0.32 | 8.98 |
| | | %P | 130 | 4.19 ±0.00 | 0.95 | 112 | 4.20 ±0.01 | 1.64 | 90 | 4.22 ±0.01 | 1.42 | 342 | 4.24 ±0.00 | 1.80 |
| | Milk production for 270 days | CL | 130 | 1841.35 ±11.21 | 6.94 | 112 | 1922.57 ±12.71 | 7.00 | 90 | 1678.88 ±16.18 | 9.14 | 342 | 1534.80 ±6.81 | 8.22 |
| | | CG | 130 | 137.84 ±0.88 | 7.26 | 112 | 144.44 ±0.95 | 6.96 | 90 | 125.95 ±1.26 | 9.50 | 342 | 115.38 ±0.50 | 8,10 |
| | | %G | 130 | 7.49 ±0.01 | 1.27 | 112 | 7.50 ±0.01 | 1.09 | 90 | 7.49 ±0.01 | 1.17 | 342 | 7.50 ±0.01 | 1.34 |
| | | CP | 130 | 77.35 ±0.51 | 7.45 | 112 | 80.96 ±0.55 | 7.20 | 90 | 71.04 ±0.73 | 9.72 | 342 | 65.22 ±0.26 | 7.40 |
| | | %P | 130 | 4.19 ±0.00 | 0.97 | 112 | 4.19 ±0.01 | 1.63 | 90 | 4.21 ±0.01 | 1.43 | 342 | 4.24 ±0.00 | 1.80 |

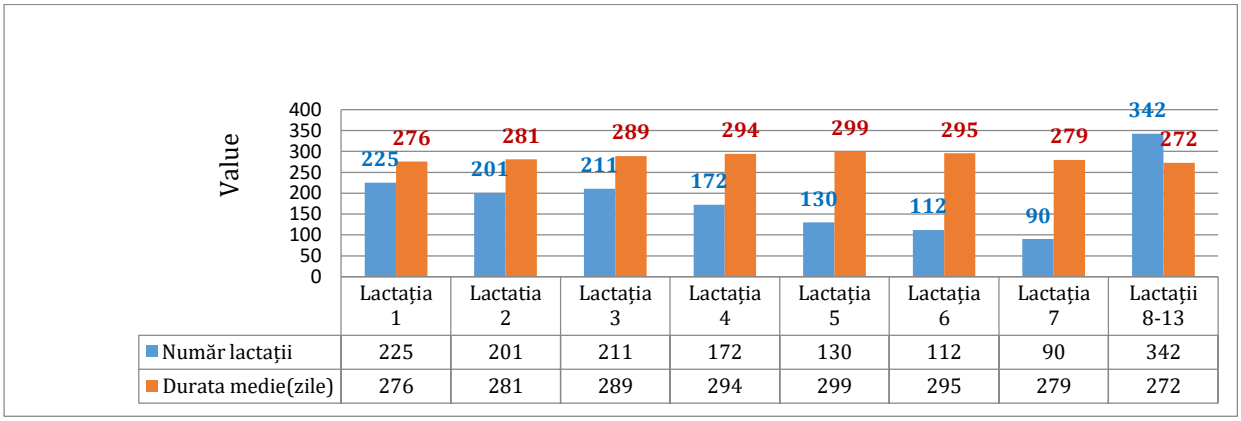
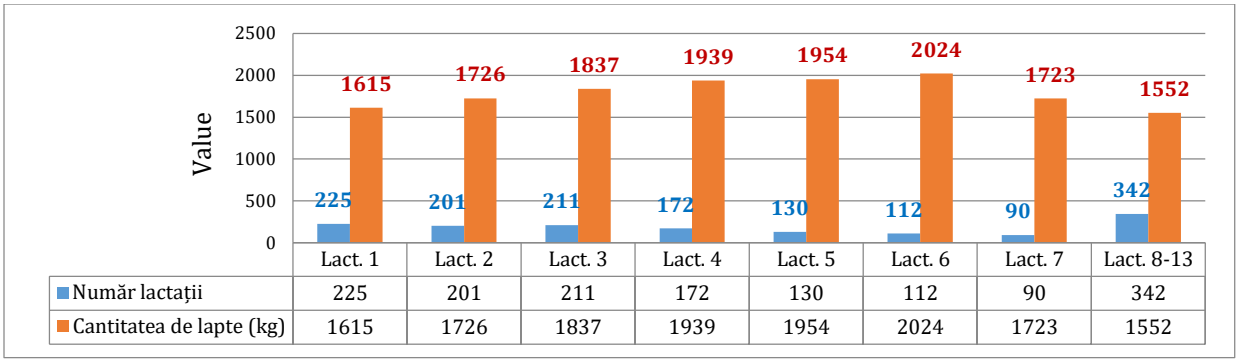


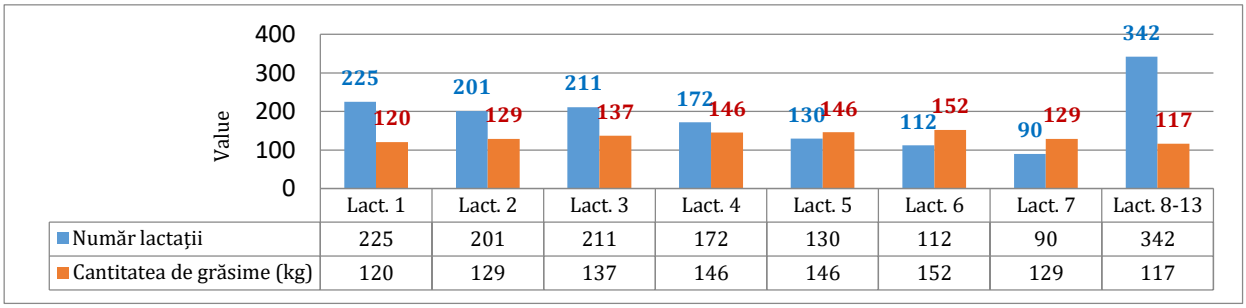

**Chart 1. Evolution of lactation duration in dairy buffaloes / Evoluția duratei lactației la bivolițele de lapte**




| | Lact. 1 | Lact. 2 | Lact. 3 | Lact. 4 | Lact. 5 | Lact. 6 | Lact. 7 | Lact. 8-13 |
|---|---|---|---|---|---|---|---|---|
| Număr lactații | 225 | 201 | 211 | 172 | 130 | 112 | 90 | 342 |
| Cantitatea de lapte (kg) | 1615 | 1726 | 1837 | 1939 | 1954 | 2024 | 1723 | 1552 |

**Chart 2. The average amount of milk per total lactation in dairy buffaloes from the analyzed farms / Cantitatea medie de lapte per lactație totală la bivolițele de lapte din fermele analizate**

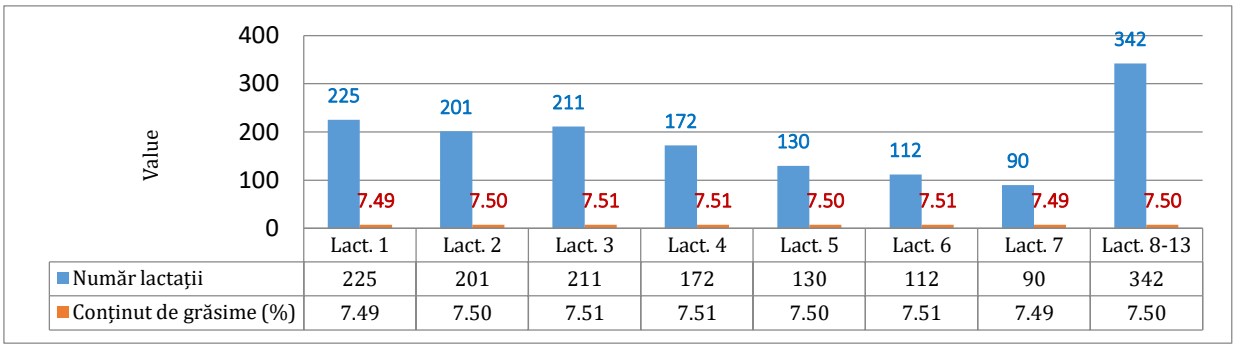
The average amount of fat per total lactation in buffaloes from the farms analyzed, recorded different average values, being influenced by the rank of lactation (table 2, chart 3).




| | Lact. 1 | Lact. 2 | Lact. 3 | Lact. 4 | Lact. 5 | Lact. 6 | Lact. 7 | Lact. 8-13 |
|---|---|---|---|---|---|---|---|---|
| Număr lactații | 225 | 201 | 211 | 172 | 130 | 112 | 90 | 342 |
| Cantitatea de grăsime (kg) | 120 | 129 | 137 | 146 | 146 | 152 | 129 | 117 |

**Chart 3. The average amount of total lactation fat in dairy buffaloes from the farms analyzed / Cantitatea medie de grăsime per lactație totală la bivolițele de lapte din fermele analizate**

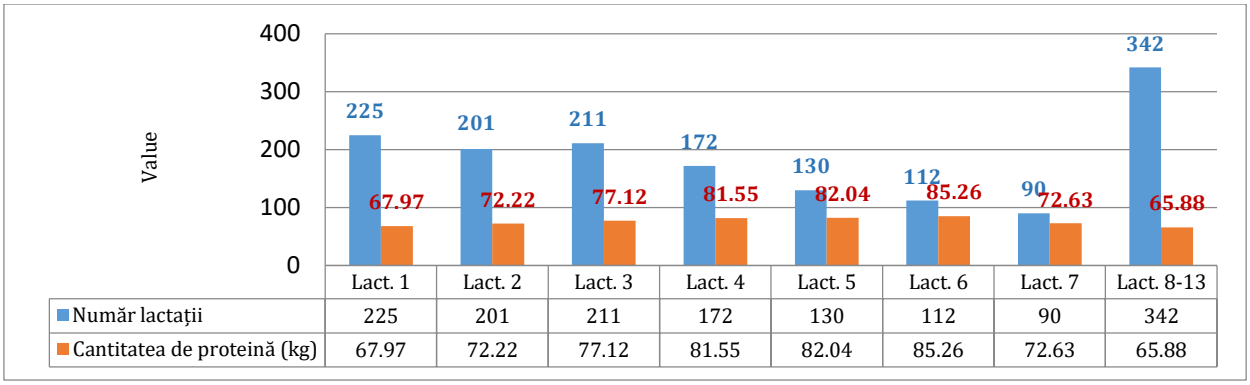
At the same lactation rank, the variability of the character is below 10%, the differences between the average amounts of milk per lactation between farms being insignificant. We specify the same conclusion for the fat content per total lactation (table 2, chart 4). At the same lactation rank, the variability of the character is below 10%, the differences between the average amounts of milk per lactation between farms being insignificant.




| | Lact. 1 | Lact. 2 | Lact. 3 | Lact. 4 | Lact. 5 | Lact. 6 | Lact. 7 | Lact. 8-13 |
|---|---|---|---|---|---|---|---|---|
| Număr lactații | 225 | 201 | 211 | 172 | 130 | 112 | 90 | 342 |
| Conținut de grăsime (%) | 7.49 | 7.50 | 7.51 | 7.51 | 7.50 | 7.51 | 7.49 | 7.50 |

**Chart 4. The average milk fat content per total lactation in dairy buffaloes from the analyzed farms / Conținutul mediu de grăsime din lapte pe lactație totală la bivolițele de lapte din fermele analizate**

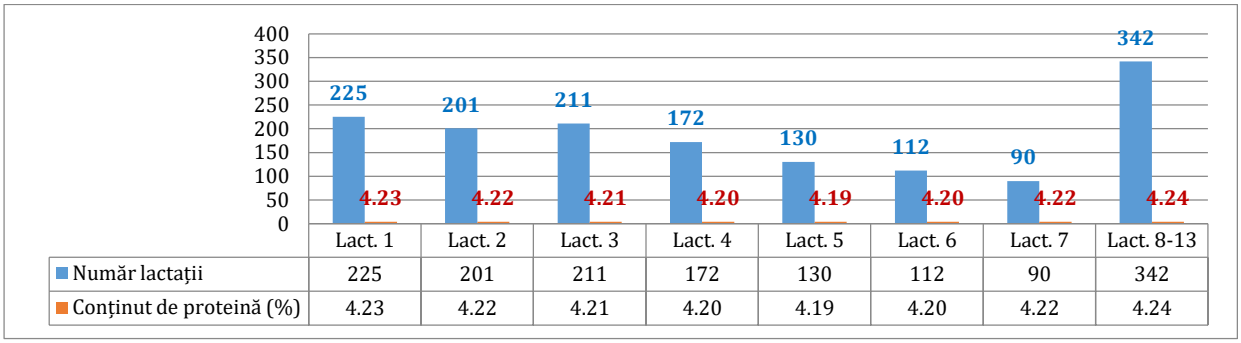
The average amount of protein per total lactation in buffaloes from the farms analyzed, recorded different average values, being influenced by the rank of lactation ( table 2, chart 5 ). At the same lactation rank, the variability of the character is below 10%, the differences between the average amounts of milk per lactation between farms being insignificant.




| | Lact. 1 | Lact. 2 | Lact. 3 | Lact. 4 | Lact. 5 | Lact. 6 | Lact. 7 | Lact. 8-13 |
|---|---|---|---|---|---|---|---|---|
| Număr lactații | 225 | 201 | 211 | 172 | 130 | 112 | 90 | 342 |
| Cantitatea de proteină (kg) | 67.97 | 72.22 | 77.12 | 81.55 | 82.04 | 85.26 | 72.63 | 65.88 |

**Chart 5. The average amount of protein per total lactation in dairy buffaloes from the analyzed farms / Cantitatea medie de proteine pe lactație totală la bivolițele de lapte din fermele analizate**

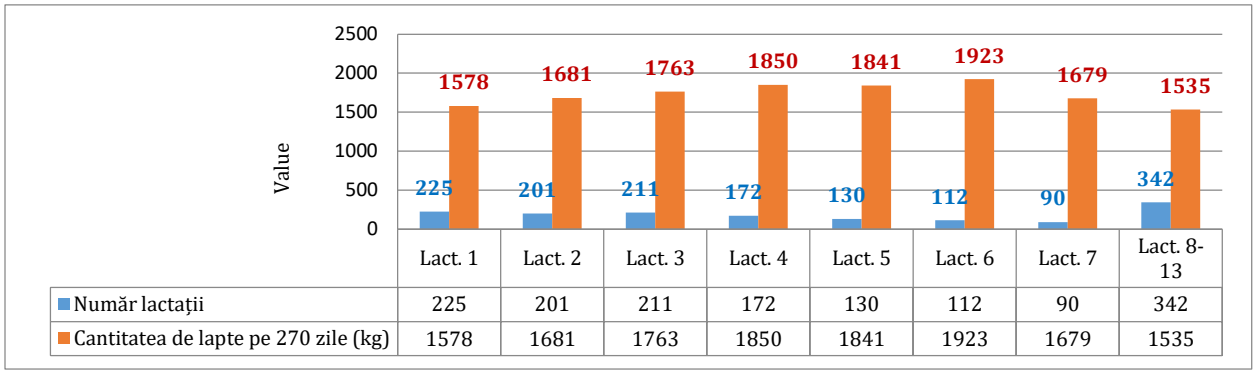
The results for the average protein content of milk per total lactation (table 2, chart 6). At the same lactation rank, the variability of the character is below 10%, the differences between the average amounts of milk per lactation between farms being insignificant.




| | Lact. 1 | Lact. 2 | Lact. 3 | Lact. 4 | Lact. 5 | Lact. 6 | Lact. 7 | Lact. 8-13 |
|---|---|---|---|---|---|---|---|---|
| Număr lactații | 225 | 201 | 211 | 172 | 130 | 112 | 90 | 342 |
| Conținut de proteină (%) | 4.23 | 4.22 | 4.21 | 4.20 | 4.19 | 4.20 | 4.22 | 4.24 |

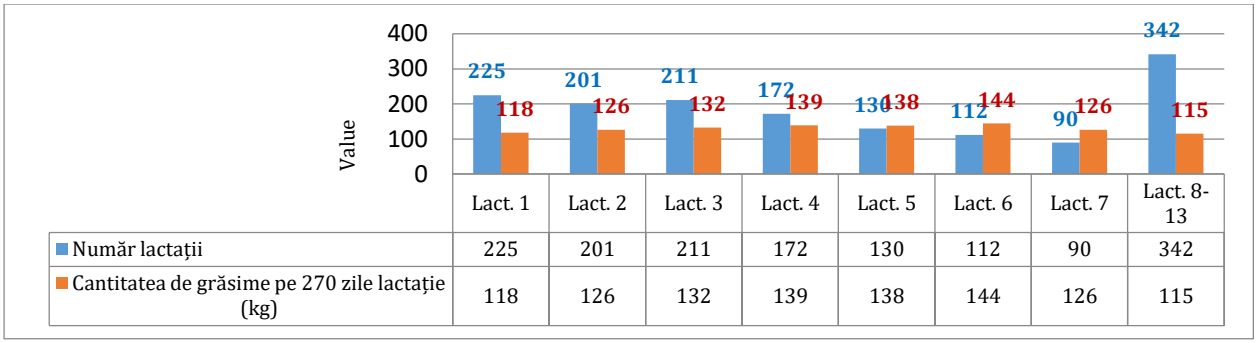
**Chart 6. The average milk protein content per total lactation in dairy buffaloes from the analyzed farms / Conținutul mediu de proteine din lapte pe lactație totală la bivolițele de lapte din fermele analizate**

From the analysis of milk production data on lactations 1-13, recalculated at 270 days (table 2, charts 7-11), it follows that there are no significant differences compared to milk production on total lactation. The explanation lies in the fact that the total duration of lactation is not much longer than 270 days, the highest average duration of lactation being recorded in the 6th lactation.



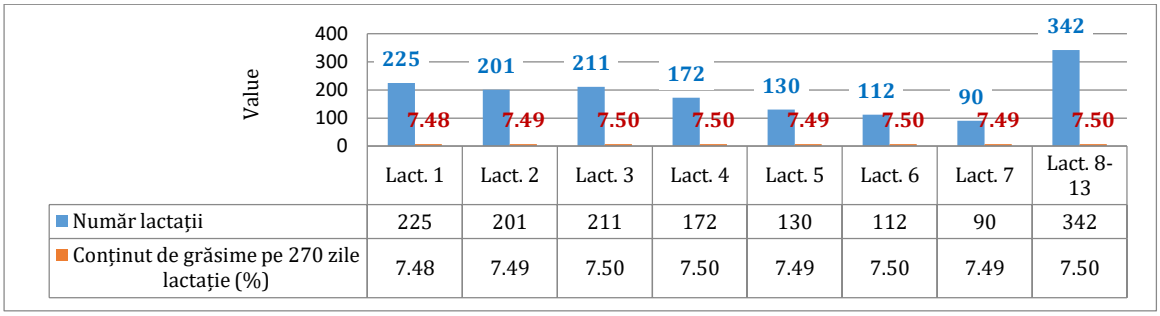

| | Lact. 1 | Lact. 2 | Lact. 3 | Lact. 4 | Lact. 5 | Lact. 6 | Lact. 7 | Lact. 8-13 |
|---|---|---|---|---|---|---|---|---|
| Număr lactații | 225 | 201 | 211 | 172 | 130 | 112 | 90 | 342 |
| Cantitatea de lapte pe 270 zile (kg) | 1578 | 1681 | 1763 | 1850 | 1841 | 1923 | 1679 | 1535 |

**Chart 7. The average amount of milk per 270 days lactation in dairy buffaloes from the farms analyzed / Cantitatea medie de lapte la 270 de zile de lactație la bivolițele din fermele analizate**



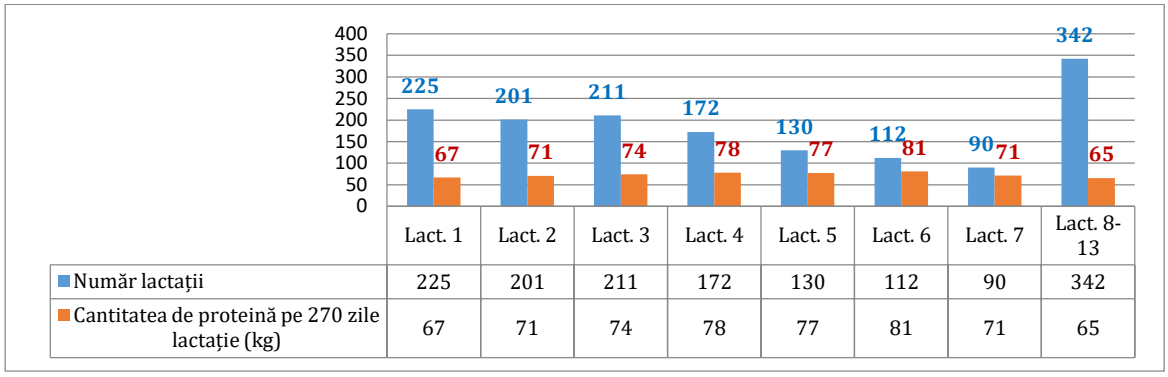

| | Lact. 1 | Lact. 2 | Lact. 3 | Lact. 4 | Lact. 5 | Lact. 6 | Lact. 7 | Lact. 8-13 |
|---|---|---|---|---|---|---|---|---|
| Număr lactații | 225 | 201 | 211 | 172 | 130 | 112 | 90 | 342 |
| Cantitatea de grăsime pe 270 zile lactație (kg) | 118 | 126 | 132 | 139 | 138 | 144 | 126 | 115 |

**Chart 8. The average amount of fat per 270 days of lactation in buffaloes from the analyzed farms / Cantitatea medie de grăsime la 270 de zile de lactație la bivolițele din fermele analizate**



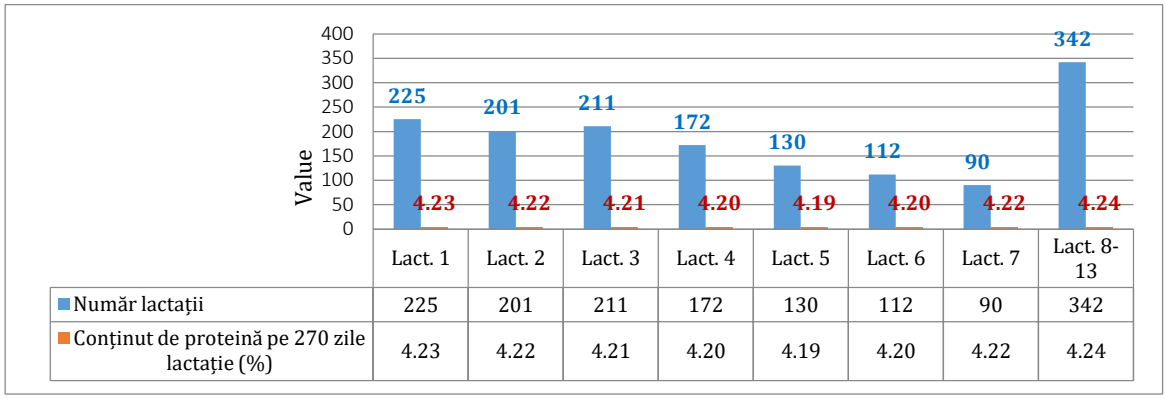

| | Lact. 1 | Lact. 2 | Lact. 3 | Lact. 4 | Lact. 5 | Lact. 6 | Lact. 7 | Lact. 8-13 |
|---|---|---|---|---|---|---|---|---|
| Număr lactații | 225 | 201 | 211 | 172 | 130 | 112 | 90 | 342 |
| Conținut de grăsime pe 270 zile lactație (%) | 7.48 | 7.49 | 7.50 | 7.50 | 7.49 | 7.50 | 7.49 | 7.50 |

**Chart 9. The average milk fat content per 270 days lactation in dairy buffaloes from the analyzed farms / Conținutul mediu de grăsime din lapte pe 270 de zile de lactație în lactatele de bivoli din fermele analizate**




| | Lact. 1 | Lact. 2 | Lact. 3 | Lact. 4 | Lact. 5 | Lact. 6 | Lact. 7 | Lact. 8-13 |
|---|---|---|---|---|---|---|---|---|
| Număr lactații | 225 | 201 | 211 | 172 | 130 | 112 | 90 | 342 |
| Cantitatea de proteină pe 270 zile lactație (kg) | 67 | 71 | 74 | 78 | 77 | 81 | 71 | 65 |

**Chart 10. The average amount of protein per 270 days lactation in dairy buffaloes from the farms analyzed / Cantitatea medie de proteine pe 270 de zile de lactație în lactatele de bivoli din fermele analizate**




| | Lact. 1 | Lact. 2 | Lact. 3 | Lact. 4 | Lact. 5 | Lact. 6 | Lact. 7 | Lact. 8-13 |
|---|---|---|---|---|---|---|---|---|
| Număr lactații | 225 | 201 | 211 | 172 | 130 | 112 | 90 | 342 |
| Conținut de proteină pe 270 zile lactație (%) | 4.23 | 4.22 | 4.21 | 4.20 | 4.19 | 4.20 | 4.22 | 4.24 |

**Chart 11. The average milk protein content per 270 days lactation in buffaloes from the analyzed farms / Conținutul mediu de proteine din lapte pe 270 de zile de lactație la bivolițele din fermele analizate**

## CONCLUSIONS

The average interval between calvings in 396 buffaloes dairy raised in the central area of Romania was 438.04±6.30 days, with a high character variability in the analyzed population (V=30.88%). This result is influenced by the average duration of the service period (123.93±6.30 days, with V=89.06%), the average duration of gestation being relatively constant (314.10±0.22 days), with a very low variability (1.50%). The research undertaken reveals that the average duration of lactations in buffaloes did not show significant differences between farms even from one rank to another, the variability of the character being below 10%. The longest average duration of lactation was recorded in the 6th lactation, and the lowest in the 8th-13th lactations. An increase in lactation duration is observed from lactation I to lactation VI, followed by a decrease until lactation XIII. The duration of lactation in the buffaloes from the studied farms was influenced by the age of the animals as well as by the technological factors. The average amount of milk per total lactation in buffaloes recorded different average values, being influenced by the rank of lactation. At the same lactation rank, the variability of the character is below 10%, the differences between the average amounts of milk per lactation between farms being insignificant. The average amount of fat per total lactation recorded different average values, being influenced by the rank of lactation. At the same lactation rank, the variability of the character is below 10%, the differences between the average amounts of milk per lactation between farms being insignificant. We specify the same conclusion for the content in fat and protein per total lactation. At the same lactation rank, the variability of the character is below 10%, the differences between the average amounts of milk per lactation between farms being insignificant. The results for the average fat and protein content of milk, at the same stage of lactation, have a variability of less than 10%, the differences between the average amounts of milk per lactation between farms being insignificant. From the analysis of milk production data on lactations 1-13, recalculated at 270 days, it follows that there are no significant differences compared to milk production on total lactation. The explanation lies in the fact that the total duration of lactation is not much longer than 270 days, the highest average duration of lactation being recorded in the 6th lactation.

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# STUDY ON BUFFALO BREEDING FOR MILK PRODUCTION IN THE CENTRAL REGION OF ROMANIA

# STUDIU PRIVIND CREȘTEREA BIVOLILOR PENTRU PRODUCȚIA DE LAPTE ÎN REGIUNEA CENTRALĂ A ROMÂNIEI

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***Abstract***

*Raising buffaloes for milk production is a tradition in many areas of the world, but also in Romania. Buffalo milk has great nutritional value, lends itself very well to processing into specific products. From a hygienic point of view, the number of somatic cells is lower than in taurines. Although the world population of buffalo showed a general upward trend, in Romania it decreased significantly in number during the same period. On December 1, 2021, there were 18,328 buffaloes, of which 14,060 females for reproduction, and 4,268 youth, 97% of the herd being in the center and northwest of Transylvania.. In the center of the country, 5,388 heads are raised, in 592 farms of different sizes, which represents 29.40% of the total workforce. In small farms, the focus should be on increasing individual milk production and increasing marketable production. In medium-sized farms, the specific recommended measures are to increase the herd of buffaloes, respectively from medium to large farms. For farms specialized in raising buffaloes cows, it is recommended to increase the individual milk production through selective reform and the retention of plus variants from the own pen, the application of optimized rations, artificial insemination, the use of MSC from bulls with superior breeding value for milk production, monitoring reproductive activity and increasing udder skills for mechanical milking.*

***Key words: buffaloes, milk production, farms, milking, feeding, breeding***

***Rezumat***

*Creșterea bivolilor pentru producția de lapte este o tradiție în multe zone ale lumii, dar și în România. Laptele de bivoliță are o valoare nutritivă mare și se pretează foarte bine procesării în produse specifice. Din punct de vedere igienic, numărul de celule somatice este mai scăzut decât la taurine. Deși populația mondială de bivoli a înregistrat o tendință generală ascendentă, în România aceasta a scăzut semnificativ ca număr în aceeași perioadă. La 1 decembrie 2021 erau 18.328 de bivoli, din care 14.060 femele pentru reproducție și 4.268 capete tineret, 97% din efective fiind localizate în centrul și nord-vestul Transilvaniei. În centrul țării sunt crescute 5.388 capete, în 592 ferme de diferite dimensiuni, ceea ce reprezintă 29,40% din totalul activității. În fermele mici, accentul ar trebui să fie pe creșterea producției individuale de lapte și pe creșterea producției comercializabile. În fermele de mărime medie, măsurile specifice recomandate sunt creșterea efectivului de bivoli, respectiv de la ferme medii spre mari. Pentru fermele specializate în creșterea bivolilor, se recomandă creșterea producției individuale de lapte prin reforma selectivă și reținerea plus variantelor din propriul efectiv, aplicarea de rații optimizate, inseminarea artificială, utilizarea MSC de la tauri cu valoare de reproducție superioară pentru producția de lapte, monitorizarea activității reproductive și creșterea abilităților ugerului pentru mulsul mecanic.*

***Cuvinte cheie: bivoli, producție de lapte, ferme, muls, hrănire, creștere***

## INTRODUCTION

Buffalo milk production is a tradition in many areas of the world such as the Caucasus, Asia, where sour milk, butter and yogurt from buffalo milk are very popular. In Italy, the buffalo milk industry is flourishing due to the popularity of mozzarella cheese. The large market for mozzarella cheese transforms buffalo farms into profitable enterprises and they can rely on modern exploitation techniques

and technologies in an organized manner. In South America, in countries such as Brazil and Argentina, buffaloes are raised for both milk and meat production, and more recently, buffalo milk and buffalo milk products have become very popular, and buffalo milk production has also reached countries that previously had no tradition in this production, such as Germany, Great Britain, and even the USA. Buffalo milk has a special nutritional value, being 58% richer in calcium than cow's milk, and the cholesterol content is 43% lower. It lends itself very well to processing into specific products (yoghurt, sour cream, Mozzarella, Camembert, Telemea cheeses). From a hygienic point of view, the number of somatic cells is lower, as a result of the lower risk of mastitis. In a study carried out in India, regarding buffalo milk compared to cow's milk, published in the Indian Dairy Industry magazine, the following was specified: from the point of view of cholesterol, its content in buffalo milk is 0.65 mg/g milk compared to cow's, which contains 3.14 mg/g milk. Much more buffalo milk is produced and marketed in India, and a number of its qualities make it more favorable for consumption. The analyzes carried out on buffalo milk showed that the value of the protein efficiency rate ( Protein Efficiency Ratio ) is 2.74 compared to that of cow's milk, which is 2.49. Thus, buffalo milk has approximately 11.42% more protein than cow milk. Buffalo milk is superior to cow's milk also in terms of mineral content, especially calcium, iron, potassium, which are approximately 92%, 37.7% and 118% higher than the content in cow's milk. The buffalo metabolizes carotene-rich products much better, which it completely metabolized into vitamin A, which will then pass into the milk, resulting in a greater amount of vitamin A in the buffalo's milk. Commercialization of buffalo milk is more viable than cow's milk in terms of manufactured products such as butter, a special product of buffalo milk, due to its low water content and high fat content. Very important is the low cholesterol content, which makes it much more popular on the market of healthy food products.

On the territory of Romania, buffaloes have been bred since the 4th-5th centuries, entering in two ways: from the Balkan Peninsula, being brought by the Turks and spread in the Romanian Plain and Dobrogea, and from the west, being brought by the Huns and spread by the Avars, in especially in the 9th and 12th centuries. The first written information was found in the town of Porumbarul de Jos and referred to the buffaloes from the Făgăraș region, and the first selection nucleus was formed in Șercaia (1870-1879). The chronicler Miron Costin reveals the importance given to buffaloes, especially in armed confrontations, when they were used to tow massive cannons, as a result of the impressive strength they developed. Although the total world population of buffalo showed a general upward trend, the population of buffalo in Romania decreased significantly in number during the same period. The strongest decrease occurred after 1990, being caused by several factors: the decrease in the importance of buffaloes in milk production (the demand for milk with a high fat percentage decreased); the decrease in the importance of buffaloes in labor production (mainly due to the degree of mechanization of work); the lack of support policies in the field of buffalo breeding; holsteinization of milk production.

On December 1, 2021, there were 18,328 buffalo heads in Romania, of which 14,060 were females for reproduction, and 4,268 youth heads (INS), 97% of the herd being in the center and north-west of Transylvania, mainly in the counties of Brasov, Sibiu, Cluj and Salaj. In the center of the country (Brașov and Sibiu counties) 5388 head are raised, in 592 farms of different sizes, which represents 29.40% of the total herd raised in the country (Genealogical Register of the Romanian Buffalo breed). Studies related to the economic efficiency of buffalo breeding, by type of farm, in Romania are insufficient. As a result, through this study, we aimed to analyze milk production, its destination, the feeding and milking technology used, as well as the way to ensure animal welfare, in farms of different sizes, from the most representative growing area in country, to formulate recommendations to increase milk production.

## MATERIAL AND METHOD

This scientific study was carried out in 48 buffalo farms, in 10 localities in Brașov and Sibiu counties. In order to make relevant comparisons between holdings, three groups of farms were created, namely the group of small farms (1-5 heads), medium farms (6-19 heads) and large farms (over 20 heads). Of the 48 farms analyzed, 50% were medium-sized farms, 29% large-sized farms and 21% small-sized farms or households.

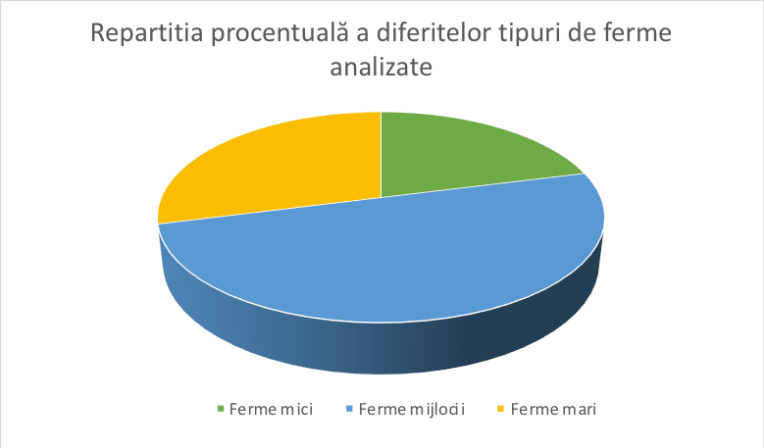


**Chart 1. Breakdown of farm types analyzed / Repartiția tipurilor de ferme analizate**

The working method applied was based on the application of questionnaires to all farmers participating in the study. The questions in the content of the questionnaire mainly related to the buffalo herd, broken down by age category, the amount of milk recorded daily and how the milk obtained on the farm is used. Thus, information was obtained regarding the consumption of buffalo milk at the farm level (own consumption and consumption for calves) and the amount of milk processed on the farm, delivered as raw material or as fresh milk and distributed through milk vending machines. Also, direct analyzes were made regarding the feeding technology used in the farm, the types of shelters and the way to ensure well-being at the farm level, as well as the applied milking technology. For the statistical evaluation of the indicators in the analyzed farms, the average differences between the results of the collected samples were expressed as a function of the standard deviation (SD) and were interpreted using the analysis of variance.

## RESULTS AND DISCUSSION

**Analysis of buffalo rearing in small-scale farms**. In this first group, of small farms, there are 5 analyzed households. The total number of buffaloes in these farms varies between 6 and 10 heads, because along with the category of milk buffaloes there are also young animals. The average number of buffaloes was 4.6 heads, with ranges between 3 and 5 heads. The average amount of milk obtained per day is 8.4 liters of milk, with variations between 7 and 10 liters (table 1).

**Table 1. Synthetic indicators in small farms / Indicatorii sintetici în fermele mici**

| Specification | Number of farms | $X \pm S_X$ | MAX | Min | S | V% |
|---|---|---|---|---|---|---|
| **Number buffaloes on exploitation** | 10 | $4.6 \pm 0.4$ | 5 | 3 | 0.89 | 10 |
| **Amount of milk per head of dairy buffalo per day** | 10 | $8.4 \pm 0.5$ | 10 | 7 | 1.14 | 13 |
| **The total amount of milk obtained on the farm per day** | 10 | $38 \pm 2.54$ | 45 | 30 | 5.7 | 15 |
| **Own consumption of milk in the farm or household** | 10 | $2 \pm 0.03$ | - | - | 0.2 | 3 |
| **Milk consumption for calves** | 10 | $18.8 \pm 5.9$ | 40 | 4 | 5.9 | 15 |
| **The amount of milk processed** | 10 | $22.5 \pm 0.8$ | 26 | 0 | 1.48 | 14 |

On average, in the analyzed farms, a quantity of 38 liters of buffalo milk is obtained. It is found that farmers do not consume large amounts of milk for their own food, but a significant amount is used to feed calves (18.8 kg). Regarding the category of milk intended for processing, we note that the milk is capitalized as such by selling it at vending machines located in cities. The selling price is 6 lei/liter (chart 2).

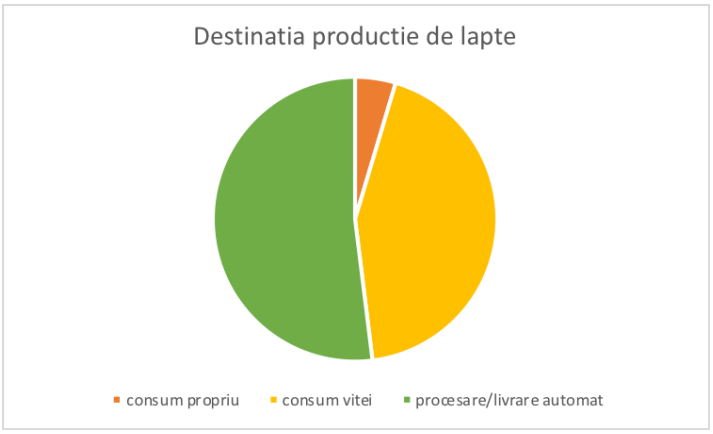


**Chart 2. Distribution of milk production in small farms / Distribuția producției de lapte în fermele mici**

From the analysis of the 10 holdings, a household was distinguished with 7 buffalo heads, respectively 3 lactating dairy buffaloes, which have an average daily production of 10 liters of milk, the amount of milk intended for the calves is very small, and the largest amount is delivered to vending machines sale of milk (chart 3).

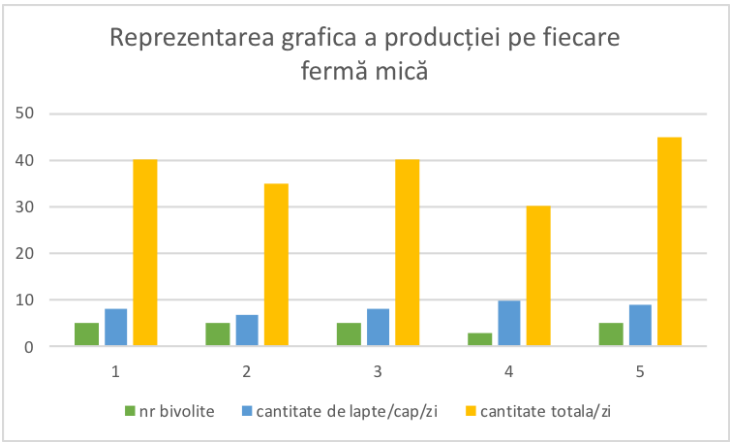


**Chart 3. Distribution of milk production on 5 analyzed farms / Distribuția producției de lapte în 5 ferme analizate**

In these small holdings, the work is done by family members. Buffalo breeding is carried on in a traditional system, the animals being kept in common stables with other animals (cows, sheep, pigs). The shelters have very limited interior arrangements, namely stands for rest and mangers for the administration of fodder during the winter period. Animals are kept in tethered system. As for feeding, it is carried out seasonally. During the summer season, the animals are kept on pasture and consume green mass ad libitum . During the winter, the buffaloes receive hay (8 kg), corn or beet silage (20 kg), roughage (4 kg) and, in some cases, concentrates (2 kg). The dairy buffaloes are milked manually, with the exception of one holding where the mobile milking group is used, equipped with a milking machine.

**Analysis of buffalo rearing in medium-sized farms**. Table 2 shows that 24 farms from Brașov and Sibiu counties were analyzed, with buffalo herds that varied between 50 and 20 total herds. The average number of dairy buffaloes per holding was 12.58, with variations between 24 and 9 heads.

**Table 2. Synthetic indicators in medium-sized farms / Indicatorii sintetici din fermele de dimensiune medie**

| Specification | Number of farms | $X \pm S_x$ | MAX | Min | S | V% |
|---|---|---|---|---|---|---|
| **Number buffaloes on exploitation** | 24 | 12.58 ± 1.26 | 24 | 9 | 4.37 | 30 |
| **Amount of milk per head of dairy buffalo per day** | 24 | 7.65 ± 0.26 | 10 | 6.5 | 0.91 | 12 |
| **The total amount of milk obtained on the farm per day** | 24 | 94.3 ± 7.92 | 168 | 75 | 2.7 | 20 |
| **Own consumption of milk in the farm or household** | 24 | 2 ± 0.01 | - | - | 0.2 | 2 |
| **Milk consumption _ for calves** | 24 | 43.91 ± 6.5 | 102 | 24 | 2.7 | 19 |
| **The amount of milk processed** | 24 | 53.18 ± 5.35 | 94 | 33 | 1.78 | 18 |

The total amount of milk in the farm is twice as much as in the small farms, but it is observed that the same trend of giving a significant amount of milk to the calves is maintained.

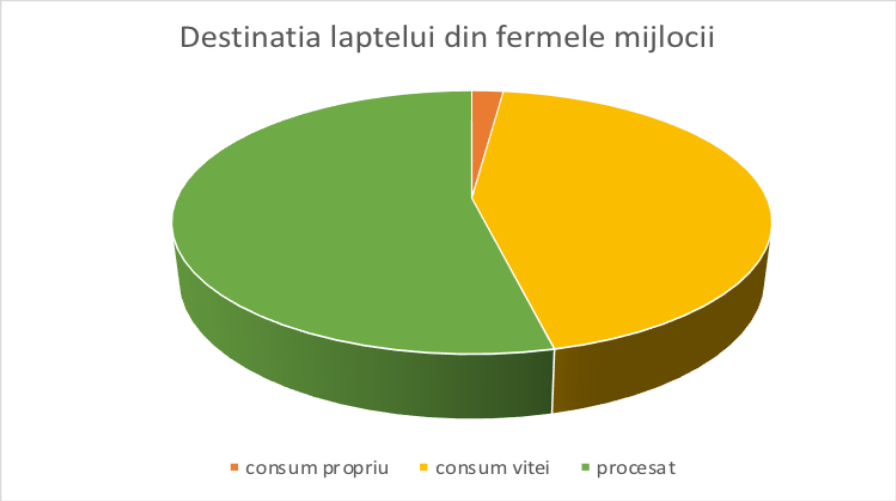


**Chart 4. Distribution of milk use at the average farm level / Destinația laptelui din fermele de dimensiune medie**

An interesting feature of medium-sized farms is that 85% of market milk is processed at the farm level. Milk processing at the farm level involves turning it into buffalo milk. The consumption to obtain one kilogram of Telemea cheese is between 3.5 kg of milk/kg of cheese and 4 kg of milk/kg of cheese. Regarding the selling price of cheese, it varies between 13 lei/kg and 20 lei/kg, which shows us a large variation depending on the ability to negotiate with customers. From chart 5, a great variability of the average milk production can be observed between the analyzed farms. A negative correlation is generally observed between the number of animals and the average amount of milk per head of dairy buffalo. As the number of buffaloes on the farm increases, the average amount of milk per dairy buffalo decreases, which is an indicator of the farmers concern for the quality of biological material.

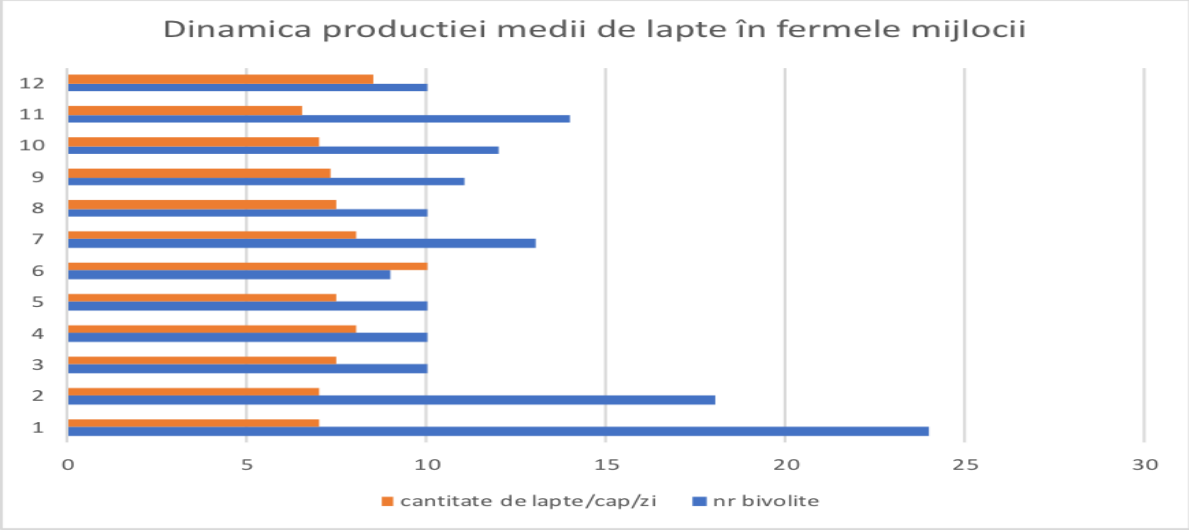


**Chart 5. Production indicators in medium-sized farms / Indicatorii pentru producție în fermele de dimensiune medie**

In medium-sized farms, dairy buffaloes are reared in the traditional system, with stable maintenance involved in more than 80% of cases. Buffaloes are milked mechanized in 35% of cases, and in the rest of the farms it is done manually. The feeding technology is seasonally differentiated, in the summer the animals stay on the pasture and the feed consists exclusively of green mass, and in the winter the feeding is done from the stock, with canned fodder.

**Analysis of dairy buffaloes growth in large-scale farms**. In this category we have grouped the farms that have a queen herd of more than 20 heads. 14 farms were analyzed, with herds of lactating buffaloes dairy between 20 and 200 heads. It can be seen from Table 3 that although the rearing system is intensive or semi-intensive, the average milk production is lower (6.01 liters of milk/dairy buffalo /day) compared to medium and small farms.

**Table 3. Synthetic indicators in large farms / Indicatorii sintetici în fermele mari**

| Specification | Number of farms | $X \pm S_x$ | MAX | Min | S | V% |
|---|---|---|---|---|---|---|
| **Number buffaloes on exploitation** | 14 | 63.85 ± 0.26 | 200 | 20 | 0.68 | 30 |
| **Amount of milk per head of dairy buffalo per day** | 14 | 6.01 ± 0.33 | 7,6 | 5 | 0.89 | 29 |
| **The total amount of milk obtained on the farm per day** | 14 | 386.85 ± 0.54 | 1200 | 108 | 20.9 | 25 |
| **Own consumption of milk in the farm or household** | 14 | 2 ± 0.02 | - | - | 0.31 | 3 |
| **Milk consumption _ for calves** | 14 | 113.16 ± 0.98 | 323 | 44 | 4.7 | 21 |
| **The amount of milk processed** | 14 | 332 ± 5.41 | 1200 | 58 | 4.22 | 22 |

In large farms, significant daily quantities of milk are obtained, respectively between 108 and 1200 liters. Milk intended for feeding calves takes up about 1/3 of the total production, which means that a much larger amount is used (chart 6).

As far as the way of capitalizing the milk is concerned, we found a much greater variability. For the safety of the distribution, several beneficiaries are kept, who purchase the milk at different prices. A significant part is delivered to the processing factory, with different prices, depending on the supplier, between 2.5 and 4 lei/liter. There are farmers who also deliver to milk vending machines at the price of 5-6 lei/liter, but they also retain a certain amount of milk on the farm, which they transform into Telemea cheese (3.5 liters/kg of cheese) and on which uses it at the price of 20 lei/kg.

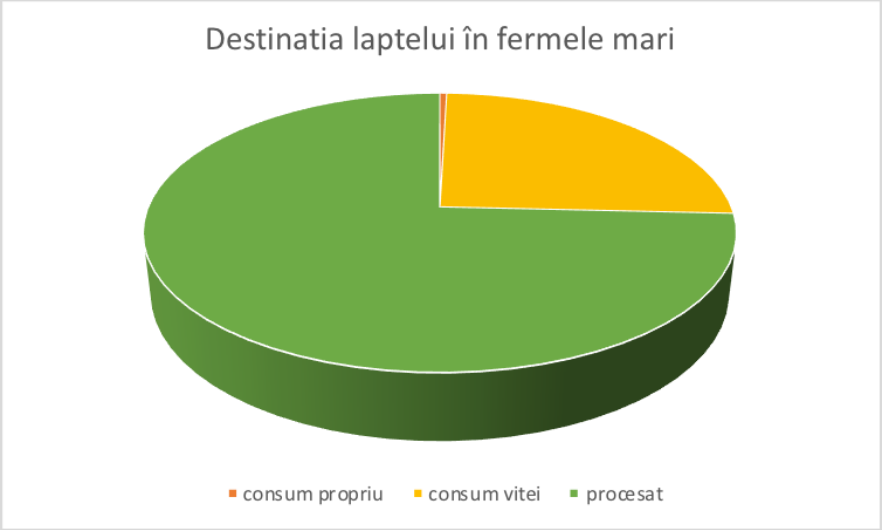


**Chart 6. Distribution of milk production in large farms / Distribuția producției de lapte în fermele mari**

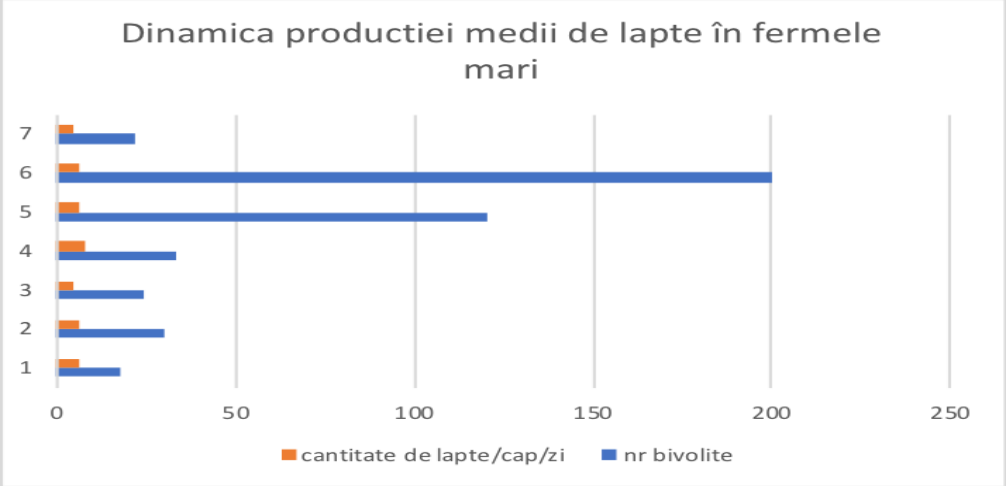


**Chart 7. Correlation between livestock and milk production per head of buffalo cow per day in large farms / Corelația dintre efectivul de animale și producția zilnică pe cap de bivoliță în fermele mari**

In large dairy buffaloes farms, a seasonally differentiated feeding technology is practiced, without concentrate supplements in the summer season. The maintenance of dairy buffaloes is carried on in large shelters, in tied or free housing. The applied milking technology is different, from manual milking in a farm of 22 milk dairy buffaloes, to milking in special rooms (barrel-type milking room). We have also identified mobile milking facilities (individual milking group) and pipe lines, with centralized milk transport in the milk cooling and storage tank.

## CONCLUSIONS

In small farms, the focus should be on increasing individual milk production and increasing marketable production (commodity production). The main recommended measures are the following: the gradual increase of the queen stock, respectively of the size of the farm from a small farm to a medium farm; judicious selection of biological material by increasing the percentage of selective reform; replacement of reformed animals with heifers from own breeding, with high biological value, but also by purchasing heifers with high genetic potential, from medium and large farms; avoiding inbreeding by introducing artificial insemination; the introduction of official performance control; reducing the

consumption of whole milk for feeding calves by introducing feeding schemes in which the share of milk substitute increases; separating calves from buffaloes immediately after calving, so that the maternal reflexes specific to buffaloes do not develop and they no longer yield milk without the presence of the calf; keeping buffaloes with a high milk fat content on the farm; the development of balanced fodder rations, based on the calculation of what is needed for the maintenance of vital functions and for production, so that the animals benefit from all the nutrients necessary for the expression of genetic potential; the construction or modernization of shelters for the maintenance of dairy buffaloes taking into account the principles of welfare, which do not contradict the traditional breeding system; the gradual transition from the tied maintenance of the dairy buffaloes to the free maintenance, by increasing the accommodation surface provided per animal; increasing the degree of luminosity (increasing the glazed surface) and air volume (realizing higher shelters) in the shelters, so that the well-being principles regarding the microclimate to be respected; the introduction of mechanical milking both in shelter and on pasture, in summer camps - individual milking groups up to the size of 20 heads.

In medium-sized farms, the specific recommended measures are the following: increasing the herd of dairy buffaloes, respectively the size of the farm from a medium-sized farm to a large farm; setting a productivity threshold for buffaloes and keeping only those with average daily production above this threshold on the farm; avoiding inbreeding by using artificial insemination, with semen from bulls with superior breeding value; the development of fodder rations differentiated by physiological states and production levels, as well as the introduction of concentrate supplements during the summer period, simultaneously with the careful supervision of green mass production; the application of periodic care measures and overseeding of pastures, so that there is sufficient and constant production of green mass throughout the grazing period; the application of rational grazing in the summer period and the creation of a sufficient fodder base for the winter period; the development of modern shelters, which allow the herd to increase, without affecting the welfare of the animals; training or continuous training of farmers so that they know all the opportunities for farm development and improvement of milk production; for the efficiency of milk production, it is recommended to consult the farmers within the association, so that the price of the products sold does not fluctuate so much (between 13 and 20 lei/kg of Telemea) and to ensure the profitability of all farmers.

For farms specialized in raising milk buffaloes, the following is recommended: increase individual milk production by practicing a selective reform of up to 30% per year and retaining the plus variants from the own herd; the application of optimized rations for each technological group, respectively productive level and physiological state; applying artificial insemination to the entire herd and using semen from bulls with superior breeding value for milk production; monitoring the reproductive activity, namely the interval between calvings and the age of the first calving, so as to increase the production of goods per productive life; increasing the skills of the udder for mechanical milking by monitoring the morphology and function of the udder, the milking speed and the degree of milk yield; disseminating genetic progress by selling heifers to medium and small farms; assuming the role of a regional hub to engage with small and medium farmers to disseminate genetic progress and scientific advice; concluding firm and advantageous contracts with the processors, so that the buffalo milk brings a real profit for the farm; close welfare monitoring for all categories of buffaloes on the farm.

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# STUDY ON LACTATION CURVE FOR MILK PRODUCTION INDICES IN MONTBELIARDE DAIRY COWS

# STUDIU ASUPRA CURBEI DE LACTAȚIE PENTRU INDICII PRODUCȚIEI DE LAPTE LA VACILE DIN RASA MONTBELIARDE

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***Abstract***

*The aim of this paper was to highlight the effect of parity on the shape of the lactation curve for milk yield and composition in **Montbeliarde** cows. Researches were carried out on 367 lactations obtained from 115 **Montbeliarde** dairy cows from Hunedoara County. Data was obtained using the standard method of the official milk recording system. Only those lactations were used that had at least 11 controls. Test-day data was modelled using Wood's incomplete gamma function. Lactations were divided into three classes according to the parity of the cow: primiparous, secundiparous and multiparous. Parity had a significant influence ($p<0.001$) on the milk production. Milk production of multiparous cows (third lactation or higher) was 16-18% higher than in primiparous cows and 10-14% higher than in secundiparous cows. There were no significant ($p>0.05$) differences for milk production between primiparous and secundiparous cows. The shape of the lactation curve was significantly influenced ($p<0.05$) by parity in **Montbeliarde** dairy cows for milk yield, fat percentage, protein percentage and lactose percentage. Generally, primiparous cows had flatter lactations curves for all indices and lower test-day values, compared to multiparous cows that had sharper lactation curve with higher test-day values.*

***Key words: dairy cows, lactation curve, milk production, Montbeliarde, parity***

***Rezumat***

*Scopul acestei lucrări a fost acela de a evidenția efectul ordinii lactației asupra formei curbei de lactație pentru cantitatea și compoziția laptelui la vacile din rasa **Montbeliarde**. Cercetările s-au efectuat pe 367 lactații obținute de la 115 vaci de lapte crescute în județul Hunedoara. Datele s-au obținut din controlul oficial al performanțelor. S-au utilizat doar informațiile de la acele lactații care au avut cel puțin 11 controale. Datele de producție din ziua de control au fost modelate folosind funcția gamma incompletă a lui Wood. Lactațiile au fost împărțite în raport cu ordinea lactației, astfel: primipare, secundipare și multipare. Producția de lapte a vacilor multipare (lactația a treia sau mai mare) a fost cu 16-18% mai mare ($p<0,001$) decât cea a vacilor primipare și cu 10-14% mai mare ($p<0,001$) decât cea a vacilor secundipare. Nu au existat diferențe semnificative ($p>0,05$) în ceea ce privește producția de lapte între vacile primipare și secundipare. La vacile din rasa **Montbeliarde**, forma curbei de lactație pentru cantitatea de lapte, precum și pentru procentul de grăsime, proteină și lactoză din lapte a fost influențată în mod semnificativ de ordinea lactației ($p<0,05$). În general, vacile primipare au avut curbe de lactație mai plate pentru toți indicii productivi, precum și valori mai mici în ziua de control, comparativ cu vacile multipare, care au avut curbe de lactație mai ascuțite cu valori mai mari în ziua de control.*

***Cuvinte cheie: vaci de lapte, curba de lactație, producția de lapte, Montbeliarde, ordinea lactației***

## INTRODUCTION

The lactation curve represents the evolution over time of the daily milk production during a lactation. The lactation curve allows the evaluation of important characteristics of milk production, such as the maximum daily yield, the time of reaching the maximum yield or the persistence of lactation.

These curves are also used to predict the total production of a cow or a farm when the quantities produced in a certain period are known (Matinez Lopez et al., 2019).

The lactation curve provides useful information to selection programs, as well as to the development of appropriate management decisions and setting up the production strategies at the farm level (Bouallegue and M'Hamdi, 2020). Several types of mathematical models are used to study lactation curves, each of them having advantages and disadvantages. They can be divided into empirical models, parametric models, exponential functions, linear fitting models, as well as other unclassified mathematical models.

Most studies on lactation curves have considered milk yield and described a standard curve in dairy cows, showing that peak milk yield occurs between the 4th and 8th week after calving, followed by a daily decrease in the amount of milk produced until the cow is weaned (Silvestre et al., 2009). The most common model used to describe the lactation curve is the model of Wood, 1967. Research carried out by Silvestre et al., 2009 showed that the milk production traits (milk yield, fat yield, protein yield, fat percentage, and protein percentage), as well as parity have an influence on the degree of confidence of the Wood model. Jingar et al., 2014 argue that the incomplete gamma function can be successfully used to model the lactation curve for hybrid cows with or without mastitis.

Somatic cell count (SCC) is an indicator of mastitis, but at the same time it is also influenced by other factors, such as: parity, breed and days in milk. SCC shows greater variability than the daily milk yield. The shape of the lactation curve for SCC varies markedly between dairy farms. The variability of lactation curves for milk yield and SCC between farms highlights the importance of addressing them at the farm level (Græsbøll et al., 2016).

## MATERIAL AND METHOD

In order to understand the milk production from a quantitative and qualitative views in the **Montbeliarde** breed, periodic milk production controls were carried on, determining the following quantitative and qualitative indices of milk production: the daily milk yield, the fat percentage, the protein percentage, and the lactose percentage. The milk fat, protein and lactose yields were used for the analysis of variance.

Three hundred sixty-seven lactations obtained from 115 **Montbeliarde** cows, originating from a dairy farm of a commercial company in Hunedoara County, were studied, on cows that completed a normal lactation until September 30, 2021.

The experimental data were analysed using the National Agency for Animal Husbandry (ANZ) technique. The analysis of the chemical composition of the milk and the measurement of the milk yield obtained were carried on with built-in lactometers, at the milking parlour on the farm. The results were automatically collected with the help of a computer in the designed software of the milking parlour.

Only those cows that completed a normal lactation with at least 11 controls during lactation were considered. The data from the measurements made on each control day were used in the calculations. Thus, a number of 8,094 milk samples were used. The data from each control day were divided into three classes according to the parity of the cows, namely: primiparous, secondiparous and multiparous cows (third lactation and beyond third lactation).

The data were processed statistically with the help of the STATISTICA software (Hill, T. & Lewicki, P., 2006). Means and indices of dispersion on normal lactation were calculated for each parity, and by using ANOVA/MANOVA analysis of variance, the effect of this factor on the following characteristics was determined: milk yield per control day, fat yield, protein yield and lactose yield.

Based on the averages obtained, the parameters of the lactation curves were calculated using the mathematical model first proposed by Wood, 1967, called the "incomplete gamma function", having the following equation:

$$y_n = an^b e^{-cn}$$

where:

- e natural logarithm base
- $y_n$ is the milk yield (fat percentage, protein percentage or lactose percentage) at the *n* moment from calving;
- a is the initial milk yield (fat, protein or lactose percentage), in the first day of lactation;
- b is the increase rate of milk yield (decrease rate of fat, protein or lactose percentage) up to the peak yield (nadir percentage);
- c is the decrease rate of milk yield (increase rate of fat, protein or lactose percentage) after the peak (nadir) value.

The incomplete gamma function model estimates peak milk yield to be equal to $y_{max} = a(b/c)be^{-b}$, production which is achieved at $n = b/c$ days from calving. The total milk yield can be estimated using the formula $Y = a\Sigma n^b e^{-cn}$, and the persistence (e.g. the length of time maximum production is maintained) can be estimated using the formula $S = c^{-(b+1)}$.

Initially, Wood used this model to highlight the effects of different factors (age, calving season, etc.) on the shape of the lactation curve. He demonstrated a number of properties of this function in subsequent studies (see Wood, 1969, 1970a, 1970b, 1972, 1974, 1976, 1977, 1980).

A peculiarity of this function is the fact that it can be solved by non-linear as well as linear methods. Solving the incomplete gamma function nonlinearly requires a larger volume of mathematical calculations. By logarithmic transformation of the initial function, a linear model is obtained that can be solved by multiple linear regression. The results obtained for the parameters of the lactation curve (a, b and c) are then very easily obtained by dilogarithm transformation. The linear function corresponding to the incomplete gamma function has the form:

$$ln(y_n) = ln(a) + b\ ln(n) - cn$$

where:

- ln natural logarithm;
- $y_n$ is the milk yield at moment n from calving;
- a is the initial milk yield;
- n days in milk;
- b is the increasing rate of milk yield up to peak;
- c is the decreasing rate of milk yield after the peak.

The advantage of using the linear form of the incomplete gamma function is the possibility of statistical processing of the obtained parameters.

Lactation curves were drawn, in relation to the parity, for the following traits: milk yield, fat percentage, protein percentage and lactose percentage.

## RESULTS AND DISCUSSION

### Influence of parity on milk yield and milk chemical components on normal lactation

One of the important factors influencing individual milk production is parity, which is generally correlated with cow age, with lifetime milk production varying from one lactation to another (Stanciu, 1999). The smallest milk yield is obtained in the first lactation, the production increases until the 4$^{th}$ lactation, after which it begins to decrease. The causes that determine the variation in the milk production from one lactation to another are multiple, the most important being the productive potential of the cow, the intensity of metabolism, the volume and structure of the udder, the capacity of the digestive system, the state of health, the body condition of the cow at calving, the regime of feeding before and after calving.

Maximum milk production is generally achieved in different lactations, in relation to the precocity of the breed and its degree of genetic improvement. From an economic point of view, it is important for cows to reach the maximum productive level at a young age and to maintain this productive level for as long as possible, and in first lactation to achieve a milk production as close as possible to the maximum production.

Table 1 shows the averages and indices of dispersion for the milk yield per normal lactation in Montbeliarde cows in relation to the parity.

The milk yield during the normal lactation increased with the parity, being on average 4974.49 kg in the first lactation, 5231.43 kg in the second lactation and 5788.73 kg in the third lactation.

The cows in lactation 1 had the lowest milk yield, the minimum value being 3325.1°kg of milk and the maximum value of 6608.9 kg of milk. Cows in lactation 2 had the minimum milk yield of 3288.6 kg, while the maximum yield was 7604 kg of milk. Also, cows in lactation 3+ achieved a minimum production of 2749.1 kg of milk and a maximum production of 12,239.1 kg of milk. The coefficient of variability for the amount of milk in cows in lactation 3+ had the highest value (24.43%) and the lowest coefficient of variability (13.84%) was obtained in cows in lactation 1.

**Table 1. Averages and dispersion indices for milk yield (kg) on normal lactation according to parity in Montbeliarde cows / Mediile și indicii dispersiei pentru cantitatea de lapte (kg) pe lactația normală în raport cu ordinea lactației la vacile de rasă Montbeliarde**

| Parity | n | Average ± SEM | SD | V% | Min | Max |
|---|---|---|---|---|---|---|
| First lactation | 76 | 4974.49±78.972 | 688.458 | 13.84 | 3325.1 | 6608.9 |
| Second lactation | 73 | 5231.43±93.095 | 795.403 | 15.20 | 3288.6 | 7604.0 |
| Third+ lactation | 218 | 5788.73±95.773 | 1414.065 | 24.43 | 2749.1 | 12239.1 |

The averages and indices of dispersion for fat yield per normal lactation in relation to parity in Montbeliarde cows are shown in Table 2. As the cows get older, the fat yield per normal lactation increases from 193.94 kg in primiparous cows to 204.87 kg in second-lactation cows and up to 229.4 kg in third-lactation cows.

From Table 2, it can be seen that the cows in lactation 1 have the minimum fat yield 116.1 kg, reaching the maximum fat yield of 282.4 kg. Also, cows in lactation 2 achieved the lowest fat yield at 110.4 kg and the maximum at 350.7 kg. Cows in lactation 3 and above had the lowest fat yield at 92.5 kg and the highest at 496.5 kg of fat. The coefficient of variability for the fat yield in cows was the highest in lactation 3 (28.26%) and the lowest was obtained in cows in lactation 2 (18.28%).

**Table 2 .Averages and dispersion indices for milk fat yield (kg) on normal lactation according to parity in Montbeliarde cows / Mediile și indicii dispersiei pentru cantitatea de grăsime (kg) pe lactația normală în raport cu ordinea lactației la vacile de rasă Montbeliarde**

| Parity | n | Average ± SEM | SD | V% | Min | Max |
|---|---|---|---|---|---|---|
| First lactation | 76 | 193.94±4.217 | 36.761 | 18.95 | 116.1 | 282.4 |
| Second lactation | 73 | 204.87±4.384 | 37.459 | 18.28 | 110.4 | 350.7 |
| Third+ lactation | 218 | 229.40±4.391 | 64.825 | 28.26 | 92.5 | 496.5 |

Table 3 shows the averages and indices of dispersion for the protein yield per normal lactation in relation to the parity in Montbeliarde cows. The protein yield produced during normal lactation increases with the age of the cows, the lowest value was recorded in secundiparous cows (176.17 kg), followed by primiparous cows (180.4 kg), and the highest value was obtained in cows in the third lactation (201.12 kg).

Lactation 1 cows had the lowest protein yield at 119.5 kg and the highest at 260.9 kg protein. Lactation 2 cows had the minimum yield of 102.9 kg protein, while the maximum yield was 288.1 kg protein. Also, lactation 3 cows achieved a minimum yield of 85.5 kg of protein and a maximum yield of 454.6 kg of protein. The coefficient of variability for the protein yield in cows in lactation 3+ had the highest value (27.51%) and the lowest coefficient of variability for the protein yield was obtained in cows in lactation 1 (15.74%).

**Table 3. Averages and dispersion indices for milk protein yield (kg) on normal lactation according to parity in Montbeliarde cows / Mediile și indicii dispersiei pentru cantitatea de proteină (kg) pe lactația normală în raport cu ordinea lactației la vacile de rasă Montbeliarde**

| Parity | n | Average ± SEM | SD | V% | Min | Max |
|---|---|---|---|---|---|---|
| First lactation | 76 | 180.40±3.258 | 28.401 | 15.74 | 119.5 | 260.9 |
| Second lactation | 73 | 176.17±3.655 | 31.225 | 17.72 | 102.9 | 288.1 |
| Third+ lactation | 218 | 201.12±3.748 | 55.335 | 27.51 | 85.5 | 454.6 |

Table 4 shows the averages and indices of dispersion for the lactose yield per normal lactation in relation to the parity in the **Montbeliarde** cows. Primiparous cows achieved the lowest lactose yield per lactation (246.87 kg), followed by secundiparous cows (260.12 kg), while the highest lactose yield was achieved by cows in the third+ lactation (287.47 kg).

**Table 4. Averages and dispersion indices for milk lactose yield (kg) on normal lactation according to parity in Montbeliarde cows / Mediile și indicii dispersiei pentru cantitatea de lactoză (kg) pe lactația normală în raport cu ordinea lactației la vacile de rasă Montbeliarde**

| Parity | n | Average ± SEM | SD | V% | Min | Max |
|---|---|---|---|---|---|---|
| First lactation | 76 | 246.87±4.961 | 43.25 | 17.51 | 150.5 | 356.1 |
| Second lactation | 73 | 260.12±4.815 | 41.14 | 15.81 | 171.8 | 432.7 |
| Third+ lactation | 218 | 287.47±5.037 | 74.36 | 25.86 | 132.2 | 631.3 |

Primiparous cows had the lowest lactose yield of 150.5 kg and the highest of 356.1 kg. Secundiparous cows had the lowest lactose yield of 171.8 kg, while the maximum yield was 432.7 kg. Also, cows in third+ lactation had the minimum yield at 132.2 kg lactose and the maximum yield at 631.3 kg lactose. The coefficient of variability for the lactose yield in cows at the third + lactation had the highest value (25.86%) while the lowest coefficient of variability (15.81%) for the lactose yield was obtained in cows in second lactation.

Table 5 shows the differences and statistical significance for the milk, fat, protein and lactose yields in relation to the parity in **Montbeliarde** cows. For the milk yield per normal lactation, very significant differences ($p<0.001$) were obtained between cows in the first lactation and those in the second lactation (-814.24 kg milk), as well as between cows in the second lactation and those in the third+ lactation (-557.3 kg milk).

**Table 5. Differences and statistical significance for milk production indices according to parity in Montbeliarde cows / Diferențele și semnificația statistică pentru indicii producției de lapte în raport cu ordinea lactației la vacile din rasa Montbeliarde**

| Parity | Milk yield (kg) | | Fat yield (kg) | | Protein yield (kg) | | Lactose yield (kg) | |
|---|---|---|---|---|---|---|---|---|
| | 3+ | 2 | 3+ | 2 | 3+ | 2 | 3+ | 2 |
| Lactation 1 | -814.24*** | -256.94$^{ns}$ | -35.46*** | -10.93$^{ns}$ | -20.72*** | 4.23$^{ns}$ | -40.6*** | -13.25$^{ns}$ |
| Lactation 2 | -557.30*** | - | -24.53*** | - | -24.95*** | | -27.35*** | - |

ns – $p>0.05$; * - $p<0.05$; ** - $p<0.01$; *** - $p<0.001$

For milk fat yield, there were very significant differences ($p<0.001$) between lactation 1 and lactation 3+ (-35.46 kg fat), as well as between lactation 2 and lactation 3+ (-24.53 kg fat).

Between lactation 1 and lactation 3+ there were very significant differences (-20.72 kg, $p<0.001$) for the milk protein yield. Also, a highly significant difference ($p<0.001$) was observed between lactation 2 and lactation 3+ (-24.95 kg protein).

For the milk lactose yield, very significant differences ($p<0.001$) were obtained between lactation 1 and lactation 3+ (-40.6 kg), as well as between lactation 2 and lactation 3+ (-27.35 kg).

There were no statistically significant differences ($p>0.05$) between milk production indices during normal lactation between primiparous and secundiparous cows.

## Evolution of the milk yield and milk chemical components during lactation according to parity

Age is generally correlated with parity, and milk production varies from one lactation to another. The lowest milk production occurs in the first lactation, then increases until the maximum level is

reached, after which it begins to decrease. The increase in milk production occurs until cows reach adult age, during which the cow's body development and the capacity of the digestive tract increase. The udder is not fully functional after the first calving, increasing its weight, capacity and improving its histological structure during the first 1-2 lactations. The greatest increase in volume and the most significant changes in structure occur between the first and the second lactations. The decrease in milk production in the last part of life is the result of the aging phenomenon, due to which the intensity of vital functions is gradually reduced, the capacity to consume and digest feed decreases and, above all, the capacity to restore the alveolar system during the breast rest period (Stanciu, 1999).

The analysis of variance demonstrated the existence of a very significant influence ($p<0.001$) of the parity on all the properties studied (Table 5).

The shape of the lactation curve for daily milk yield is shown in Figure 1. The parity had a highly significant influence ($p<0.001$) on the shape of the lactation curve for milk yield. Thus, primiparous cows (lactation 1) showed a flatter lactation curve, which started from an average level (14.6 kg) increased to 16.1 kg milk/day, after which the daily production decreased slowly until the end of lactation. Peak milk yield was reached, on average, 175 days after calving for cows in lactation 1. Tekerli et al., 2000, showed that in the first lactation, peak milk yield, as well as, over the entire lactation was lower, but lactation persistence was better. On the other side, the multiparous cows (3 and more than three lactations) presented a much sharper lactation curve. They started from a production of 14.43 kg of milk per day, reaching a maximum level of 20.1 kg of milk per day at only 82 days of lactation. Secundiparous cows showed a lactation curve similar to that of cows in lactation 3 and above, thus the minimum milk yield was 9.89 kg, reaching a maximum level of 17.9 kg at 115 days after calving.

The parity also influenced the milk fat percentage, which decreases from the first lactation to the fourth lactation by about 0.2-0.3%. After the fourth lactation, changes in fat percentage are non-significant (Figure 2). Significant differences ($p<0.05$) were found between the shapes of the lactation curves depending on the age of the cows. For cows in lactation 1 and lactation 3 and above, milk fat percentage was high at the beginning of lactation, after which it decreased to a nadir, only to increase again towards the end of lactation. Different is the shape of the lactation curve for cows in lactation 2 where the evolution of the percentage of fat started from 3.68% and gradually increased, almost linearly, until the end of lactation.

Similar to the lactation curve for milk yield, milk fat percentage in primiparous cows showed a flatter curve, starting from an initial fat percentage of 3.04%, reaching a minimum of 2.92% at 36 days after calving, and towards the end of lactation it rose to a threshold of approximately 3.17%.

Multiparous cows (3+ lactations) started from a higher initial level of 3.36% fat, reached a nadir of 3.26% in the first 32 days after calving, after which, towards the end of lactation, a fat percentage of 3.53% was obtained.

In secundiparous cows, the fat percentage in the milk towards the end of lactation increases abruptly, up to the value of 3.49%, with accelerate increase in the ascending phase until weaning.

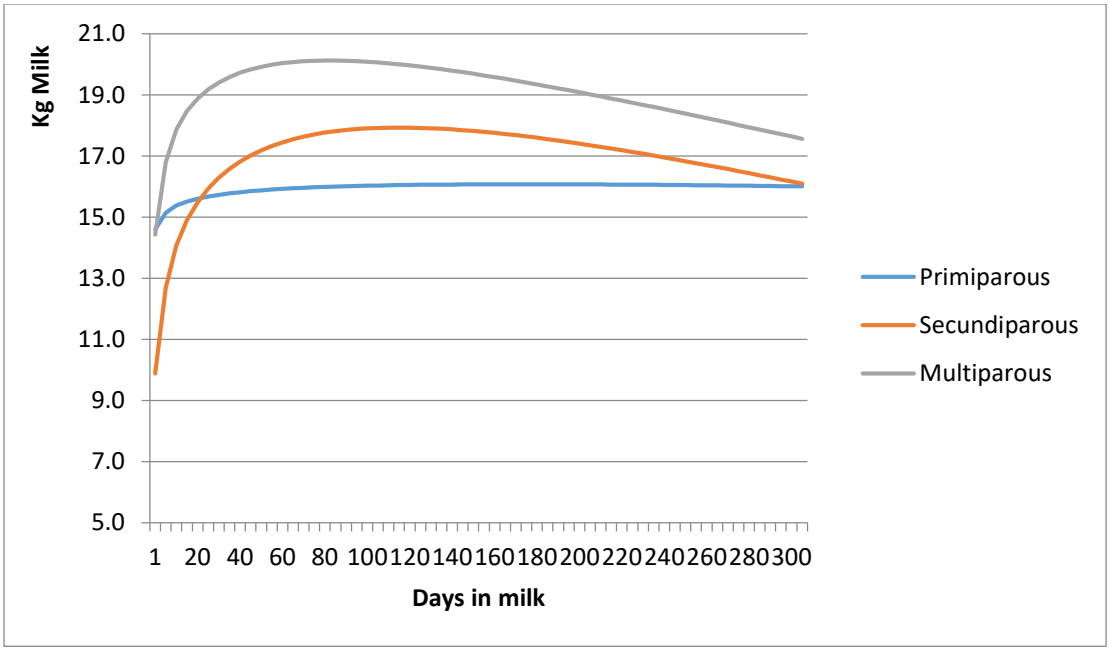


**Figure 1. Evolution of milk yield during normal lactation according to parity in Montbeliarde breed / Evoluția cantității de lapte pe lactația normală în funcție de ordinea lactației la rasa Montbeliarde**

The percentage of milk protein varied from one lactation to another, varying at the beginning of lactation between 3.01% in primiparous cows and 4.12% in cows in lactation 2 (Figure 3).

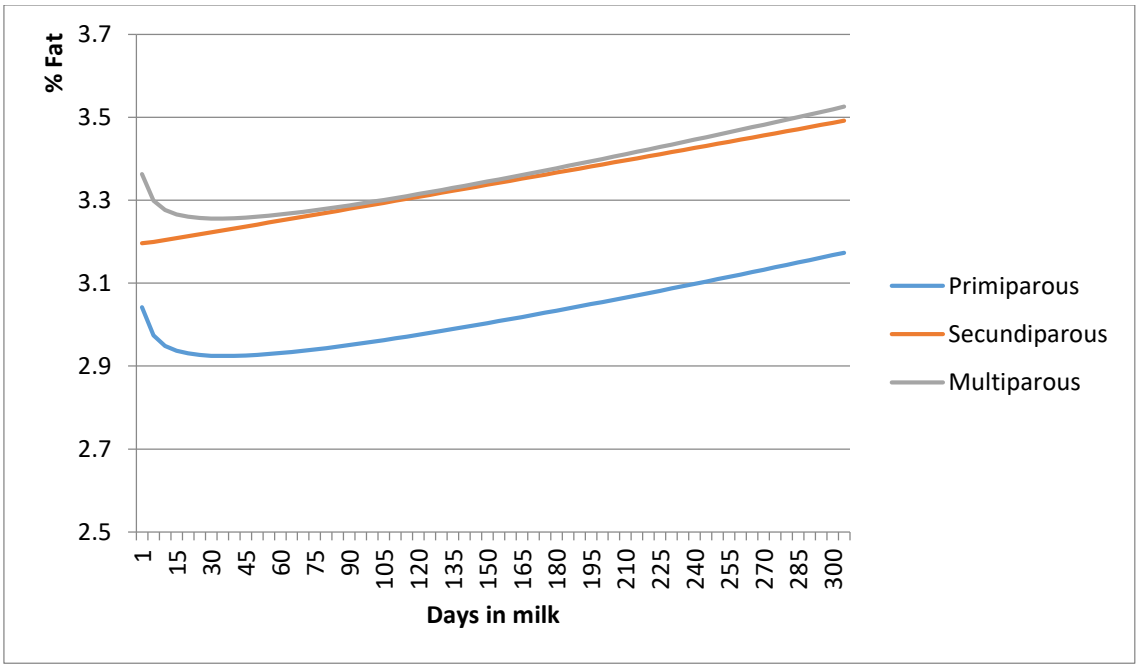


**Figure 2. Evolution of milk fat percentage during normal lactation according to parity in Montbeliarde breed / Evoluția procentului de grăsime din lapte pe lactația normală în funcție de ordinea lactației la rasa Montbeliarde**

As shape, primiparous cows had a flatter lactation curve, starting at an initial level of 3.01%, then reaching a nadir of 2.95% at 71 days postpartum, after the protein percentage increased slow until the end of lactation (2.98%). Secundiparous cows reached their minimum protein percentage of 2.76% at 224 days after calving, after which it increased very slowly, reaching 2.77% at the end of lactation.

Multiparous cows, with more than three lactations, showed a strong decrease in milk protein percentage, from the initial value of 3.92% reaching a nadir of 2.87% at 150 days after calving. After that, the milk protein percentage showed a more pronounced increase compared to secundiparous cows until weaning (2.94%).

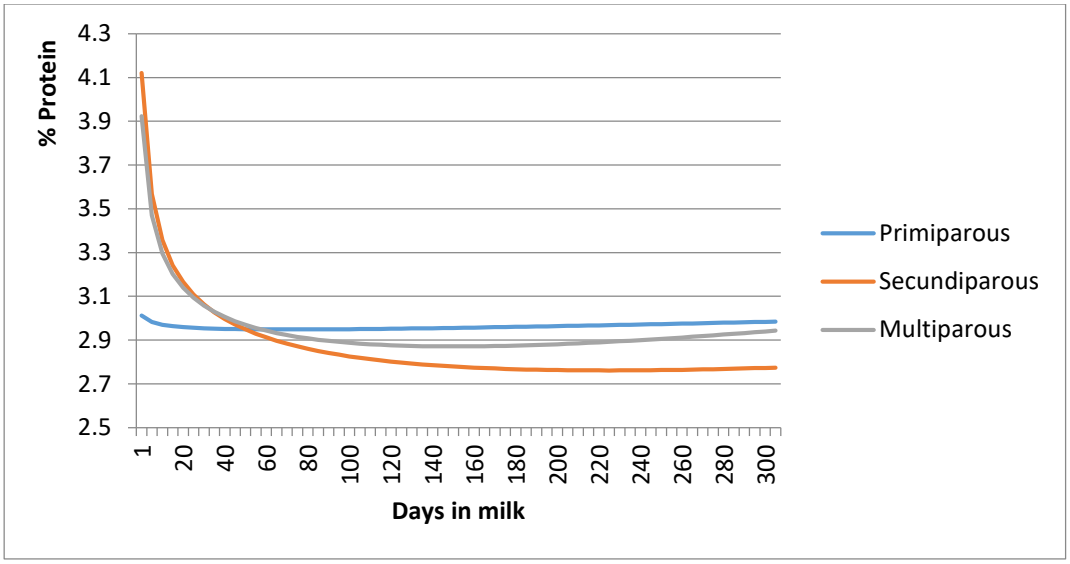


**Figure 3. Evolution of milk protein percentage during normal lactation according to parity in Montbeliarde breed / Evoluția procentului de proteină din lapte pe lactația normală în funcție de ordinea lactației la rasa Montbeliarde**

Parity had a significant effect on the shape of the lactation curve for milk lactose percentage ($p<0.001$, Figure 4).

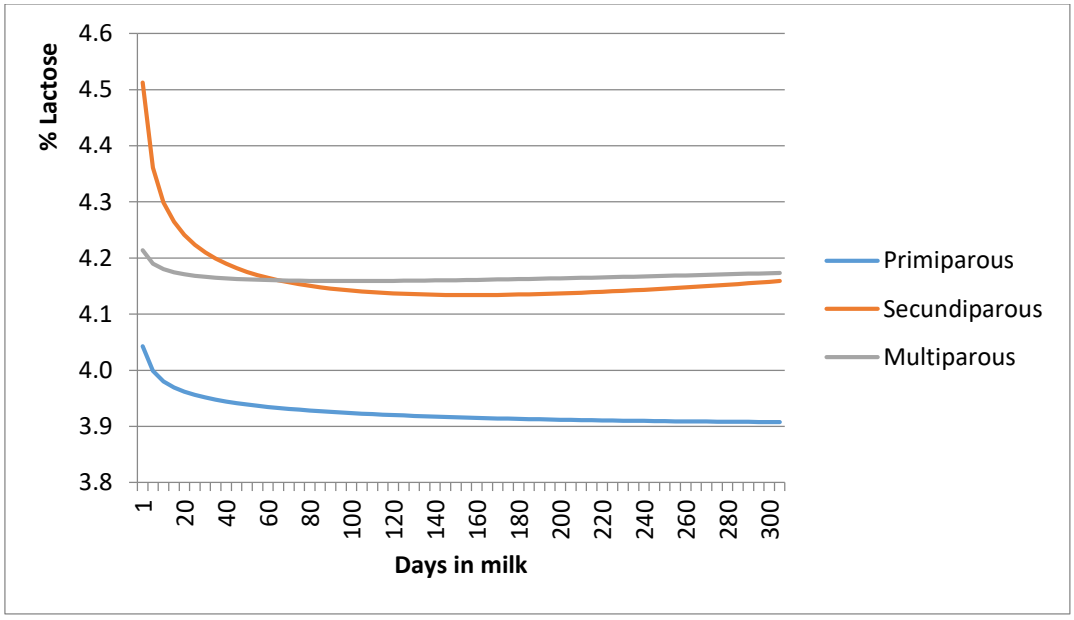


**Figure 4. Evolution of milk lactose percentage during normal lactation according to parity in Montbeliarde breed / Evoluția procentului de lactoză din lapte pe lactația normală în funcție de ordinea lactației la rasa Montbeliarde**

The lowest percentage of lactose in milk during lactation was recorded by cows in first lactation. In these cows, the milk lactose content decreased slightly from 4.04% at the beginning of lactation to 3.91% at weaning.

In the case of the other two groups of cows (secundiparous and multiparous) the lactation curve for the milk lactose percentage had an atypical shape, i.e., in both groups the lactose percentage decreased immediately after calving, reaching the nadir, after which growing slightly to weaning. Multiparous cows (3+ lactations) had a more flattened curve, where the percentage of lactose in milk started from a value of 4.21%, showing a slight decrease at the beginning of lactation, reaching a minimum of 4.16% at 99 days after calving, then remained close to this value until the end of lactation (4.17%).

Second lactation cows started at 4.51% lactose percentage, reached a nadir of 4.13% at 157 days after calving, and at weaning milk lactose percentage was 4.17%.

## CONCLUSIONS

Parity significantly influenced ($p<0.05$) both milk production in normal lactation and the shape of the lactation curve for milk yield as well as fat, protein and lactose percentage.

Multiparous cows, which completed three or more lactations produced, during a normal lactation, 16 to 18% more milk compared to primiparous cows and 10 to 14% more milk compared to secundiparous cows ($p<0.01$). Between primiparous and secundiparous cows there were no significant differences ($p>0.05$) for milk, fat, protein and lactose yields per normal lactation.

Parity had a highly significant influence ($p<0.001$) on the shape of the lactation curve for milk yield. Thus, primiparous cows (lactation 1) showed a flatter lactation curve. On the contrary, the multiparous cows (3 and more than three lactations) presented a much sharper lactation curve.

Significant differences ($p<0.05$) for milk fat percentage were recorded between cows in lactation 1 and cows in lactation 3 and above. The percentage of milk fat was high at the beginning of lactation, then decreased to reach a nadir, to increase again towards the end of lactation.

The milk protein percentage showed variations in relation to the age of the cows, being at the beginning of lactation between 3.01% in primiparous and 4.12% in secundiparous cows. Multiparous cows (3+ lactations) had the lowest content of milk protein, of 2.87% on the 150th day of lactation.

Parity had a highly significant effect ($p<0.001$) on the shape of the lactation curve for milk lactose percentage. The lowest level of lactose in milk during lactation was recorded by cows in lactation 1.

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# THE PRETABILITY OF THE TRANSYLVANIAN PINZGAU BREED FOR MEAT PRODUCTION

# PRETABILITATEA RASEI PINZGAU DE TRANSILVANIA PENTRU PRODUCȚIA DE CARNE

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***Abstract***

*The **Pinzgau** breed is a local breed formed in the environmental conditions of the mountainous area of Apuseni and Bucovina. A late breed, resistant to specific cattle diseases, it makes good use of natural meadows. Unfortunately due to excessive cross-breeding it is in danger of disappearing. The breed has a modest milk production, at the same time it has major udder defects, which is why it is not suitable for mechanical milking. Under these conditions, we studied the readiness of the breed for meat production using the "meat cow" technology, where the heifers are kept with the calves next to them, from calving to weaning, the period when the calves suck all the milk of the mother. Our studies showed that the young male achieves, up to the age of slaughter (181), an average body weight of 410 kg, a slaughter yield of 54-56% and an average daily gain of 700 g. From the point of view of the quality of the carcasses, assessed by the EUROP method, they have a superior quality, falling into the R, O+ category, with a level of good meat dressing of the body regions and a reduced meat dressing in fat* (2). *After analyzing the results obtained regarding the growth rate of the youth, the yield at slaughter and the quality of the carcasses, we can conclude that the **Pinzgau de Transilvania** breed lends itself to meat production using the "meat cow" technology with real chances of relaunching the breed in the years following.*

***Keywords: pretability, "meat cow", slaughter yield, growth rate, mechanical milking***

***Rezumat***

*Rasa **Pinzgau** este rasă locală formată în condițiile de mediu ale zonei montane din Apuseni și Bucovina, o rasă tardivă, rezistentă la bolile specifice bovinelor, valorifică foarte bine pajiștile naturale, din păcate datorită metisărilor excesive este în pericol de dispariție. Rasa are o producție modestă de lapte, totodată are defecte ale ugerului, motiv pentru care nu se pretează la mulsul mecanic. În aceste condiții, am studiat pretabilitatea rasei pentru producția de carne folosind tehnologia "vaca de carne", unde vacile fătate se întrețin cu viței lângă ele, de la fătare până la întărcare, perioadă în care viței sug toată cantitatea de lapte a mamei. Din studiile noastre a rezultat că tineretul mascul realizează până la vârsta de sacrificare (18 l), o greutate corporală medie de 410 kg, un randament la sacrificare de 54-56% și un spor mediu zilnic de 700 g. Din punct de vedere a calității carcaselor, apreciate prin metoda EUROP, acestea au o calitate superioară, încadrându-se în categoria R, O+, cu un nivel de îmbrăcare bună în carne a regiunilor corporale și o îmbracare redusa a cărnii în grăsime (2). După analiza rezultatelor obținute în privința ritmului de creștere a tineretului, a randamentului la sacrificare și a calității carcaselor, putem concluziona că rasa **Pinzgau de Transilvania** se pretează pentru producția de carne folosind tehnologia "vaca de carne", cu șanse reale de relansare a rasei în anii următori.*

***Cuvinte cheie: pretabilitate, "vaca de carne", randament la sacrificare, ritm de creștere, muls mecanic***

## INTRODUCTION

**Transylvanian Pinzgau** cattles have a conformation characteristic of mixed breeds with milk-meat, meat-milk production skills. Phenotypically they have a color specific to the breeding breed: dark red-chestnut, with a white stripe that starts from the withers towards the rear extremities of the trunk,

goes down on the inguinal region, up to the head of the chest, forming a ring of the same color on the calves and arms (Florian P., 2007).

The breed is well adapted to the current growing areas, body development is variable depending on the area of spread, respectively the specific climatic conditions.

The breed is resistant to environmental conditions, later and with lower production qualities in comparison with the domestic improved breeds, **Baltata Romaneasca** and **Bruna de Maramures**.

The interest in the meat from the **Transylvanian Pinzgau** breed is justified by the very good quality and by the fact that the increase in growth is consistent with the effectiveness of the breed in exploiting the meadows of the mountain areas (Ujica V., 2005).

From a qualitative point of view, the meat of this breed is among the best in the world. The fine fiber, the juicy red meat, with a light covering of fat, is remarkable (Bures D., 2006).

The breed is late compared to the other improved mixed breeds in the country, namely **Baltata Romaneasca** and **Bruna de Maramures**. At the same time it has major udder defects with asymmetrical quarters, long and thick nipples and modest milk production, 6-7 l per day.

Under these conditions, the **Pinzgau** breed does not lend itself to mechanical milking, and due to the small production, the expenses of manual milking are not justified.

The research aimed to study the readiness of the breed for meat production, using the 'meat cow' technology. In parallel we studied the quality of the meat after slaughter. With the help of the EUROP system we assessed the conformation of the carcasses and the degree of their coating with fat.

Starting from these premises, we set out to bring, through our studies, a substantial contribution to the knowledge of meat quality parameters in order to make better use of it.

Knowledge, the application of improved exploitation technologies, maintenance and feeding are the main factors in obtaining good results in the production process. Deviations from these desired or compromise solutions lead to a decrease in economic efficiency (Vaida Ghe., 2010).

**Picture 1. Young Transylvanian Pinzgau bull / Tăuraș rasa Pinzgau de Transilvania**

## MATERIAL AND METHODS

Considering the modest milk production of the breed, we have left the suckling calves with their mothers until their instant weaning. On average the decrease in milk production occured at 7 months of lactation, when the calves were transferred to collective stalls for youth. Cattle were fed with the varieties available on the farm, respectively: hay, corn silage, concentrates and protein supplements.

We prepared appropriate feed rations and recorded the evolution of body weight up to the age of slaughter. Control slaughters were performed and we collected meat samples for physico-chemical analysis. The organoleptic properties of the meat were assessed and finally the carcasses were assessed using the EUROP assessment system.

The obtained data were systematized, processed and interpreted by specific statistical methods specific for such studies.

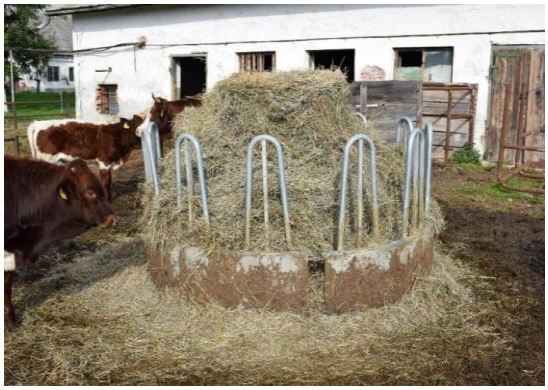
In order to carry out this research project, we studied the male youth of the **Transylvainian Pinzgau**, which form the core of the genetic conservation of the breed which, due to excessive cross-breeding, is threatened by extinction.

The number of animals that make up the population of **Pinzgau** was established in 2015-2016 by the purchase of adult cows, pregnant heifers, and female youth, totalling 25 individuals.

This herd of animals was identified and purchased from 10 different locations in the Bucovina area. Currently the total herd consists of 40 individuals, of which 22 are adult cows in different physiological states.

We mention that the population of **Pinzgau** from the station`s farm is very heterogeneous, especially in terms of body development, milk production and even the coat color. In this **Pinzgau** herd we also have several individuals of the '**Dorna Cow**', whose basic color is black with the same white line on the upper-lower part of the body (Draganescu C., 2006).

Mother cows and their calves, which were the object of the study were kept together during the calving-weaning period in a shelter with free housing.



**Picture 2. Feeding installment for hay / Tipul de hrănitoare pentru fân**

## RESULTS AND DISCUSSIONS

The **Transylvanian Pinzgau** breed has a rustic conformation. It is well adapted to the environmental conditions in which it was bred, namely the mountainous area. The breed is very resistant to specific cattle diseases. These cattle make good use of the meadows in the submontane and mountainous areas, with a very diverse and valuable floristic composition.

The milk production of the breed is very modest: on average, one cow produces 6-7 l per day. The duration of lactation - compared to other mixed breeds in the country - is of 210 days, compared to 305 days.

Unfortunately, major udder defects, such as asymmetrical quarters and thick and long nipples are characteristic for the breed. For these reasons, lactating cows are not suited for mechanical milking.

In these conditions, we resorted to the 'meat cow' exploitation technology, in which pregnant cows, including after calving, are kept together, in free stables, in simple shelters with a paddock for movement. Calves stay with their mothers and suckle the entire amount of milk produced. Calves stay with their mothers until weaning, which occurs on average at 210 days (7 l) when milk production deplets completely. Then the calves are weaned and transferred to collective stalls for youth.

The basic feed of lactating cows consists of good quality hay (15 kg/day) during the stable period, whereas in the summer they can be taken out to pasture. In addition to this diet, vitamin and mineral

supplements are also provided. It should be noted that the shelters comprise stalls for calving cows that stay in them for 5-6 days, after which they are allowed access to the exercise paddocks.

**Body development of youth**

According to the *'meat cow'* technology, newborn calves stay with their mothers until the average age of 7 months, when the mother's milk production deplets completely. At this point the calves are weaned, after which they will be fed with the existing assortments in the farm or household.

In order to study the growth rate of the youth, a series of measurements were taken for body weight, waist, rump height, chest circumference, chest depth and oblique chest length. These measurements were performed at regular time intervals: at birth, at 6 months, 12 months, respectively at 18 months.

After obtaining the data from the measurements, these were processed and statistically interpreted by calculating y, sy and V% (Czister L., 2008).

**Table 1. Physical development of youth / Dezvoltarea corporală a tineretului**

| Specification | Birth | 6 months | 12 months | 18 months |
|---|---|---|---|---|
| **Body weight (kg)** | 40.00 | 186.42 | 325.00 | 410.00 |
| **Waist (cm)** | 78.08 | 101.28 | 115.37 | 125.60 |
| **Height at the rump (cm)** | 82.83 | 107.71 | 122.50 | 131.80 |
| **Chest circumference (cm)** | 76.75 | 128.71 | 159.25 | 171.80 |
| **Chest depth (cm)** | 28.08 | 44.28 | 56.25 | 62.60 |
| **Length oblica trunk (cm)** | 65.33 | 96.71 | 121.87 | 135.00 |

According to the data presented in Table no. 1, calves are born with an average body weight of 40.10 kg and reach 410 kg at the age of 18 months.

At birth, the calf's waist measures 78.08 cm. Waist size reaches 125.6 cm at the age of 18 months. Height at the rump increases from 82.83 cm at birth to 131.80 cm at 18 months.

Chest circumference at birth is 76.75 cm, reaching 171.00 cm at the age of 18 months. The depth of the chest is on average 28.08 cm at birth and measures 62.60 cm at the age of 18 months.

The oblique length of the trunk measures 65.33 cm at birth, reaching 135 cm at the age of 18 months.

The data in Table no. 2 summarizes the intensity of growth of body weight and dimensions in the period birth - 18 months of age. At the age of 6 months the measurements represent 46.25-81.70%, whereas at 12 months 80.64-93.12% of the parameters achieved at 18 months.

**Table 2. Growth coefficient of body sizes (%) / Coeficientul de creștere a dimensiunilor corporale (%)**

| Age in months | Body regions | | | | | |
|---|---|---|---|---|---|---|
| | Weight % | Height % | Rump height % | Chest circumference % | Chest depth % | Oblique length of the trunk % |
| **Birth** | 11.26 | 62.16 | 62.80 | 44.88 | 44.35 | 48.39 |
| **6** | 46.25 | 80.64 | 81.70 | 75.25 | 70.70 | 71.63 |
| **12** | 80.64 | 91.85 | 92.50 | 93.12 | 89.85 | 90.27 |
| **18** | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |

**The chemical composition of meat**

Immediately after the animals' slaughter and during storage, a number of biochemical transformations and a series of other processes occur in the meat, which, if carried out properly, positively affect the nutritional value and the organoleptic characteristics.

On the other hand, incorrect storage conditions lead to its degradation, worsening the quality of the meat.

In the meat industry, all the compounds of this raw material constitute, by its chemical composition, a favorable environment for the development of microorganisms.

Along with the evaluation of the carcasses of the slaughtered youth, we collected samples to determine the meat's chemical composition. The results are presented in table no. 3.

**Table 3. Chemical composition of meat / Compoziția chimică a cărnii**

| Specification | Value % |
|---|---|
| Dry matter at 105°C | 31.58 |
| Fat content | 6.02 |
| Protein content | 20.28 |
| Water retention capacity | 5.60 |
| Bound water | 21.01 |
| Ash content | 1.29 |
| Thickness of muscle fibre | 52 |
| Meat color | 0.38 |
| pH | 5.50 |

From the analysis of the data presented in the table, the values are in accordance to those quoted in literature: the dry substance content is of 31.50% (25-30%), the fat content of 6.02% (4.6%), the protein content of 20.2% (18-22%), while the water retention capacity has values of 5.0-5.6%, and the binding water of 21.0%.

Regarding the thickness of the muscle fiber of the meat, we found that it is 52 u, and the color of the meat expressed in Lange units has a value of 0.380, pink to red, specific to young age.

The pH of beef varies depending on its freshness (V. Ujica, 2005), with values ranging between 5-7. As the pH value indicates the time since slaughter and the storage conditions, the pH of 5.5 shows that the collected meat samples were fresh.

**The organoleptic properties of meat**

This group of properties belongs to the character that confers the palatability, flavor and attractiveness of the meat. The most important qualities in this group are: tenderness, juiciness, aroma and taste, marbling and color of the meat.

The organoleptic examination of the meat is done in bright spaces, without foreign smells, with a temperature range of 16-20 °C.

**Tenderness** is the basic criterion in the appreciation of meat (Temisan, 1990), it is defined as the '*easiness*' with which the meat is fragmented into small pieces by mastication.

**Juiciness** is given by the amount of juice in the meat and the water-protein ratio. It is reflected in two effects: the impression of moisture that appears during the first part of mastication, following the release of juice under the pressure applied by the teeth, and the permanent preservation of the sensation of juiciness, produced of muscle fat components that stimulate saliva secretion.

Following the consumption (chewing) of meat from the young male **Transylvanian Pinzgau**, we can conclude that this is tender and juicy.

**The taste and aroma of the meat** is another important characteristic that favors the secretion of saliva and gastric juice, indirectly participating in the process of digesting the substances from the meat.

The taste and aroma are specific to each species and depend on the sulfur and ammonia content of the meat. If these two substances are found in higher concentrations, the meat has a less pleasant taste and smell (Ujica - 2007).

After tasting, the prepared meat has a pleasant taste and aroma, highly appreciated by consumers.

The second group of properties that attract consumers are marbling, meat and fat color.

**Marbling:** this characteristic means the visible proportion of fat in the muscle tissue. In EU countries, meat with an average degree of marbling is preferred, i.e. an intramuscular fat content of 2.5-4.0%, a situation in which the meat is tender and juicy. In our study, the fat content was average (2-3%).

**The color of the meat** is one of the basic characteristics that attracts meat consumers. There is a correlation between color and quality.

For bulls, the pale pink color is preferred for veal, intense red for young bulls and dark red in adult animals. In our case the meat of young bulls had a pink to red color.

Fat color: the fat of bulls is generally of a yellowish-white color. The dark color of the fat is associated with the age or lack of freshness of the meat and with the conditions of meat storage.

In our experiment, the fat had a white - slightly yellowish color characteristic to young males.

**Quantitative assessment of meat production**

At the end of the research project, we performed control slaughters on a number of 5 bulls at the age of 18 months and an average weight of 410 kg. The slaughter was carried out at an authorized slaughterhouse, specialized for the slaughter of bulls.

The results obtained after the slaughter are presented in Table no. 4.

**Table 4. Results after slaughter / Rezultatele în urma sacrificării**

| Specification | | MU | Value |
|---|---|---|---|
| **Live weight** | | kg | 410 |
| **Hot case weight** | | kg | 213 |
| **Cold case weight** | | kg | 208 |
| **Slaugthering yield** | | % | 54 - 56 |
| **Age at slaughter** | | days | 540 |
| **Moss eye sourface** | **Costal interval 8-9** | $cm^2$ | 53 |
| | **Costal interval 11-12** | $cm^2$ | 79 |

Analyzing the data in the table, it shows that at the age of slaughter (18 l) the young males had an average live weight of 410 kg, the weight of the hot carcass being of 213 kg and after cooling 208 kg. The slaughtering yield was of 54 - 56%.

Various studies (C. Velea, 1999) have reported a salughtering yield varying between 44-58%, depending on the breed, sex, category and state of maintenance of the animals.

The slaughtering yield and the surface of the muscle eye in the slaughtered youth reveals the readiness of the breed for meat production. Even if breed that reaches maturity later than the **Baltata Romaneasca** or **Bruna de Maramures** breeds, we must mention that these animals were not fed in a fattening regime, but only to maintain their breeding condition.

In order to evaluate the carcasses after slaughter, they were cut. The data collected are presented in the table below.

**Table 5. The structure of the casings / Structura carcaselor**

| Specification | MU | Value | % |
|---|---|---|---|
| **Meat** | **kg** | **155** | **74.7** |
| **Fat** | **kg** | **6.2** | **4.0** |
| **Renal sebum** | **kg** | **3.2** | **2.1** |
| **Bones** | **kg** | **27.0** | **17.8** |
| **Flesh to bone ratio** | | **155:27 = 5.7:1** | |
| **Case category** | | **A** | |
| **Conformation** | | **R; O** | |
| **Fat** | | **2-3** | |

We note that the literature cites the following values of the carcass structure (C. Velea, 1999): meat 70-78%, fat 4.9%, bones 16-18%. Thus our results fall within the quoted limits.

**The use of the EUROP method in assessing the quality of** carcasses

For the assessment of meat production from a qualitative point of view, we resorted to the method imposed by the EC for the assessment of carcasses and their classification by authorized classifiers. (Movileanu G., 2009).

To assess the meat production of the youth, we slaughtered 5 young individuals, with an average age of 18 months.

The classification of cattle carcasses according to the EUROP method refers to 5 categories of animals. The slaughtered youth fall into category A- carcasses of young, uncastrated males up to 2 years old. Carcass classification is based on 2 criteria: conformation and the degree of fat coverage:

- for conformation, 5 classes have been provided, expressed by the letters E, U, R, O, P, ranging from superior to medium

- for the degree of fat coverage, 5 classes have been established , expressed numerically by 1,2,3,4,5, from skinny to maximum fat coverage. Each class refers to the conformation and the degree of fat coverage, which can be divided into 3 subclasses (-, =, +).

In the case of the youth from the "*meat cow*" experiment, the carcasses fall into conformation classes R and O+, with a good conformation and muscle development, well-rounded calf, thick back, fairly well-developed scapula.

The degree of fat coverage is situated between 2 and 3. Category 2 means a light coverage with fat, with the meat being visible everywhere. Category 3 means that the meat - with the exception of the thigh and back - is almost completely covered with fat, small deposits of fat in the chest cavity.

The figures below show aspects regarding: case, semicasing and youth meat in section.

**Picture 3. Youth meat in the section / Carne de tineret în secțiune**

## CONCLUSIONS

Following the completion of the research project on the suitability of the **Transylvanian Pinzgau** breed for meat production we drew the following important conclusions:

The breed is well adapted to the environmental conditions of the mountainous area where it was formed. It resists to specific cattle diseases and, importantly, it makes good use of the meadows, either in the form of green grass or hay.

Compared to the group of mixed breeds in the country, it reaches maturity later: the calves first come into heat at the age of 22-23 months (considered the optimal age), with a body weight of 350 kg. The age of the first calving of the female youth is 31-35 months.

Body development is small compared to the **Baltata Romaneasca** breed. The weight of **Pinzgau** cows is on average 400-450 kg, with a waist of 127 cm, compared to the 550-600 kg weight and waist of 132 cm on average of the **Baltata Romaneasca** breed.

In addition to its mixed, rustic breed qualities of milk-meat-traction production, **Pinzgau** cows have major udder defects, asymmetrical quarters, long and thick teats, and for these reasons they are not suitable for mechanical milking.

The milk production of the cows is modest (1700 l/lactation) when compared to the **Baltata Romaneasca** cows (5500-6500 l/lactation) or to the **Bruna de Maramures** breed.

Considering the defects of the udder and the small production of milk per milked cow (of 6-7 l per day), we studied the suitability of the breed for meat production by applying the *'meat cow'* technology, which involves keeping cows together with their calves, which suckle the entire amount of milk until weaning (210 days), when the mammary gland stops secreting milk.

Calves left with their mothers during the calving-weaning period achieved a higher growth rate, with 80-100 g/day, compared to the calves whose mothers were kept in the classic system, whereby 5-6 days after calving the calves are fed with milk substitutes.

By using the *'meat cow'* technology, labor costs are eliminated for the manual or drum milking or for the purchase of the milking installation.

The maintenance of **Pinzgau** cows in the *'meat cow'* system, compared to the conventional system can bring a benefit to the farmers through the better values of the reproduction indices: calving interval reduced by 8 days, average number of inseminations/gestation 1.64 compared to 2.05, service period shorter by 5 days and the incidence of abortions lower, 0.17% compared to 1.44%.

The costs of setting up shelters for the 'meat cow' technology are greatly reduced, the consumption of milk substitutes is completely eliminated and the need for medicinal products is greatly reduced.

The ease of calving, the vigor of the calves at birth, the low percentage of ante- and post-partum diseases, and the sudden natural weaning of the calves do not affect at all the health status of the mammary gland in cows kept in the *'meat cow'* system.

After tasting and eating the meat from **Pinzgau** bulls, it was found to be tender, juicy, with a pleasant taste and aroma that were highly appreciated by consumers.

The yield at slaughter of 54 - 56%, the surface of the muscle eye of 53 $cm^2$, respectively of 79 $cm^2$ and the average body weight at slaughter (500 days) of 410 kg, reveals the suitability of the breed for meat production. This is true even if this breed reaches maturity later than the **Baltata Romaneasca** and **Bruna de Maramures** breeds, especially when considering that these animals were not fed in a fattening regime, but just to keep their breeding condition.

To assess the quality of the carcasses, we used the EUROP method, which showed that, after the slaughtering of the youth under study, the carcasses fell into the R and O+ classes, and presented a good conformation, as well as muscle development, well-rounded calf, thick back, and a well developed scapula.

The fat covering of the carcasses fell between grades 2-3, which means a light covering of the meat with fat, a commercial aspect much appreciated by buyers. The result of the evaluation of carcasses using the EUROP method is another argument that reflects the suitability of the Pinzgau breed for meat production using the *'meat cow*' breeding technology.

In conclusion, we can state that the keeping, breeding and exploitation of Pinzgau breed animals in the *'meat cow'* system is a viable option for cattle breeders in Romania, which can lead to the relaunch of meat production and the preservation of the genetic background of this breed, threatened by extinction.

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# NUTRITIVE VALUE, USE AND IMPORTANCE OF MULBERRY LEAF FOR THE SILKWORM *BOMBYX MORI L.*

# VALOAREA NUTRITIVĂ, UTILIZAREA ȘI IMPORTANȚA FRUNZEI DE DUD PENTRU VIERMII DE MĂTASE *Bombyx mori L.*

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***Abstract***

*If the silkworm is the main interest point of sericulture, the mulberry is the beginning point since it produces leaves, the only feed supply for silkworm species Bombyx mori L. Due to the production of silk fibre, on the one hand, and the secondary products produced by the sector (pupae with specific by-products, respectively cakes, oils etc.), on the other hand, the breeding of silkworms is of great economic importance. The mulberry, specifically the quality of the mulberry leaves, is one of the most important factors in determining silkworm growth, production, and health status. The mulberry (Morus sp) is widely distributed in various climate zones, from tropical to temperate areas. The mulberry leaves have an excellent nutritional composition (proteins and amino acids, lipids and fatty acids, carbohydrates, vitamins and minerals, flavonoids etc.). Some volatile compounds contribute to the mulberry leaf's attractiveness, and one of the silkworm antennae receptors reacts significantly to cis-jasmone, which is thought to be the most alluring volatile component, even though it is naturally found in only trace amounts in the mulberry leaf. The digestive system and enzymatic equipment of the larva associated with the fermentative substances contained by mulberry leaves enable digestion and absorption of nutritive substances from the leaves, which are then converted into nutrients required for growth within the proper ranges and the production for obtaining a high-quality of silk fibres and valuable nutritional by- products. Given the crucial importance of the mulberries, new measures are necessary to grow and promote Bombyx mori silkworms, especially in rural regions.*

***Keywords:*** **mulberry, leaves, nutrients, silkworms, metabolism, applications**

***Rezumat***

*Dacă viermele de mătase reprezintă punctul central de interes în sericicultură, dudul, prin producția de frunze, reprezintă punctul de plecare, ca unica sursă de hrană pentru viermii de mătase din specia Bombyx mori L. Creșterea viermilor de mătase are o importanță economică deosebită dată, pe de o parte, de producția de fibră de mătase, iar pe de altă parte de produsele secundare generate de sector (pupe cu subproduse specifice, respectiv turte, uleiuri etc). Creșterea, producția și starea de sănătate a viermilor de mătase, sunt aspecte care depind de o serie de factori, dintre care primordial este dudul, și anume calitatea frunzei de dud. Dudul (Morus sp.) este dispersat extensiv în diverse suprafețe climatice, de la zone tropicale la temperate. Frunza are o compoziție excelentă în substanțe nutritive (proteine și aminoacizi, lipide și acizi grași, carbohidrați, vitamine și minerale, flavonoizi etc.). Atractivitatea spre frunza de dud este dată de o serie de compuși volatili, iar unul din receptorii din antene, răspunde puternic la cis-jasmone, considerat compusul volatil cel mai puternic atractant, deși este prezent natural în cantități mici în frunza de dud. Sistemul digestiv al larvei și echipamentul enzimatic specific, asociat cu fermenții din frunza de dud, permit digestia și absorbția substanțelor nutritive din frunză, ce sunt convertite mai departe în nutrienți necesari pentru creșterea în parametrii corespunzători și obținerea unor fibre de mătase de calitate și a unor sub-produse valoroase nutrițional. Dată fiind importanța crucială a dudului pentru sector, se impun noi măsuri de extindere a suprafețelor cultivate cu dud și de promovare a creșterii viermilor de mătase, cu precădere în zone rurale.*

***Cuvinte cheie: dud, frunza, nutrienți, vierme de mătase, metabolism, aplicații***

## INTRODUCTION

Sericulture is a complex science that combines art and science to obtain silk fibre (as the main product) and secondary by-products (e.g., pupae with their waste cakes and oils), with multiple applications in various economic sectors (Jasmine and Mandal, 2014; Oduor et al., 2021). The economic value of sericulture depends on several ways of valorising products and by-products in the industry sector (Sharma et al., 2022).

The starting point of sericulture is the mulberry tree which belongs to the *Morus* genus and *Moraceae* family and has a wide geographic distribution in tropical and temperate regions. *Moraceae* is a family of plants from more than 64 species, subspecies, and a minimum of 100 varieties identified. The *Morus* word comes from Latinus *"mora*" and means, most probably, *delay* due to the slow development of its buds.

*M. alba* is the dominant species (Jan et al., 2021). The leaves are very important from the ecological point of view and as feed for silkworms being the single diet for *Bombyx mori (B. mori).* The pupae are directed principally to obtain silk production.

Greeks and Romans have cultivated mulberry since ancient times. Since 220 D.H., Emperor Elagabal was wearing a silk coat. The clergy members in England have worn silk robes since the 1500s. Mulberry and silk industry play a major role in Virginia (Tang et al., 2016; www.wikipedia.com).

Mulberry (*Morus sp*.) is a fast-growing plant adapted to temperate, tropical, and subtropical climates. In Romania, sericulture first developed in Transylvania and Banat in the 14$^{th}$ century (1348). In the 18$^{th}$ century, the Turks introduced sericulture practices in Moldova and Muntenian. For supporting sericulture, 60000 mulberry saplings were distributed for free in Moldova in 1845, for being cultivated in the South of the region. In Banat, mulberry is regarded as a symbol. In the 18$^{th}$ century, the Habsburgs introduced mulberry to feed silkworms. Sericulture developed in this area, such as Banat becoming the first silk exporter (Doliș, 2008). Leaves were considered the single feed for silkworm *Bombyx mori* L. (Sanchez, 2000; Thaipitakwonga et al., 2018).

Due to the essential oils, flavonoids, terpenes, and flavonoids used by silkworms for digesting processes, along with the nutritional content of the leaves, there may be an unpleasant odour. Palatability issues can be due to this odour (Finke, 2002; www.feedpedia.com). The ability to obtain high-quality cocoons depends on how effectively the nutritional components of the mulberry leaf are used. Pupae represent approximately 60% of the dry cocoon (Tassoni et al., 2022).

## 1. NUTRITIONAL VALUE OF MULBERRY LEAVES

The studies show that mulberry leaves contain toxic substances and have higher palatability than other vegetable leaves (Srivastava et al., 2006). The composition depends on variety, age, harvest time, and environmental conditions. The primary nutrients from the mulberry tree are proteins, carbohydrates, vitamins, sterols, and minerals (Raghuvanshi et al., 2019; Alipanah et al., 2020). Such as Srivastava et al., (2006) mentioned, the powder of mulberry leaves has a content in water ranging between 5.11–7.24%, crude protein (CP) between 15.31–30.91%, ash 14.59–17.24%, neutral detergent fibre 27.60–36.66%, fat 2.09–4.93%, carbohydrates 9.70–29.64%, and energy between 113–224 kcal/100 g.

Generally, the CP composition is comparable to that of most legumes. Several authors (Sanchez, 2000; Raghuvanshi et al., 2019) determined a CP level ranging from 15–28%, while Sanchez-Salcedo et al. (2017) found a level of 13.4–19.4%, Iqbal et al. (2012) a level of 18.41–24.63%, and Adeduntan and Oyerinde (2010) reported a level of 21.24–21.66%.

Compared with other plants, the fibre level is lower, although it depends on many factors such as season, age, etc. Calcium concentration is about 0.14–0.24%, and phosphorus is 1.8–2.4% (Sanchez, 2000). In 2000, Liu et al. specified that the leaves have a higher nutritional value in spring than in autumn.

Furthermore, Srivastava et al. (2006) highlighted that mulberry leaves contain important quantities of beta-carotene (14.688 mg/100 g) *vs* other plant leaves consumed usually (spinach, amaranth, fenugreek). Furthermore, iron and zinc, acid ascorbic and antioxidants are contained in mulberry leaves.

Two new flavonoids were also identified in leaves (Wang et al., 2009). Phytochemical components isolated from leaves and fruit extract from mulberry *Morus rubra* were reported by Sharma (2010). Recently, the antioxidant potential of extracts from different parts of mulberry plants leaves, roots, and fruits have been investigated by many authors (Andallu and Varadacharyulu, 2002; Arfan, 2012).

As perennial plants, the mulberry tree is characterised by a deep root system, high biomass production and remarkable adaptability. The mulberry leaf has a strong absorption capacity that is believed to be helpful in controlling air pollution. It has demonstrated an encouraging result for stabilizing the adverse effects of heavy metals in various polluted soils (Ghosh et al., 2017).

The attractivity of silkworms for mulberry leaves intake can be explained by 20 volatile compounds. From a neutral fraction of essential oil of old mulberry leaves, Ɓ-Ɣ hexanol and α-Ɓ hexenal compounds attract the larvae. Twenty olfactive receptors are active in the antennae of silkworm larvae. From these receptors, one named *cis-jasmone* is considered the most strong and most attractive volatile, although it is in small quantities in leaves (0.3–300 ng). When the olfactive receptor is triggered to become active, insects move to the odour source. Citral also attracts silkworms when administrated within 30-300 ng, but not in small amounts. Linalol, hexenal, hexyl acetate and acetate 2-hexenyl presented an attractive activity at 30 ng, or 3 ng (Tanaka et al., 2009).

## 2. PROCESSES OF DIGESTION AND ABSORPTION IN SILKWORMS

The digestion and absorption of nutritional compounds from mulberry leaves present particularities due to the specificity of the digestive system described below.

It is essential to the fact that throughout the life cycle of the silkworm, only in the larval period does the silkworm consume feed based exclusively on mulberry leaves. In this phase, silkworms use nutritive compounds for growing and development and accumulate reserves for successive periods of growth.

### 2.1. The digestive tract of the silkworm

In the silkworm's body structure, such as seen by the larva's dissection, immediately under the skin at the median line of the dorsal surface of the larva, there is a dorsal vessel that surrounds many muscles and fat. Along the inside of the body is the digestive tube in the form of a straight tube with the trachea on both sides, and its dorsal side is the sericigenic gland. Beneath these organs are muscles.

Three main parts characterize the larva: the foregut (stomodeum), where feed is fragmented and stored; the middle intestine (mesenteric), where take place the digestion and absorption processes and the posterior intestine (proctodaeum), where waste is collected and decomposed substances are excreted or reabsorbed.

The foregut has the oral cavity, oral cavity, pharynx and oesophagus. At the end of the foregut, there is a cardiac valve that retain for a while, shredded leaves, to avoid regurgitation. The middle intestine (stomach) is the most significant part of the digestive tube. Goblet cells (intraepithelial, unicellular, scattered mucin secretors in simple epithelia) secrete the digestive fluid, and the cylindrical cell absorbs the digesta. The foregut and hindgut have a chitinous lining, and the peritrophic membrane in the midgut protects the epithelium from damage caused by feed particles. The posterior intestine has three components, the small intestine, cecum and rectum; a pyloric valve placed at the end of the small intestine protects and regulates the passage of feed particles from the middle to the posterior intestine. At the posterior segment of the digestive tube, the water is absorbed (a part re-enters the circuit), and the

undigested substances are eliminated. The rectal muscles exert mechanical pressure on the excreta (https://hbmahesh.weebly.com; Doliș, 2008).

## 2.2. Enzymatic equipment of silkworm

The enzyme involved in the digestion process is secreted in saliva and the middle intestine, the last having a vital role in digestion and absorption. The intestinal microorganisms can facilitate digestion. In other words, digestion consists of converting insoluble substances into soluble ones, while the impermeable substances become permeable. The most intense digestion process is in the middle intestine, where enzymes are secreted.

The digestive juice includes salivary and gastric juice. Saliva is a solution weakly alkaline and contains amylase. The gastric juice is strongly alkaline, pH of 9.2–10.3. The main enzymes contained in it is tyrosine (protease enzyme), lipase (the enzyme that acts on lipids), amylase, maltase and glycogenase (enzyme for decomposition of carbohydrates), oxidase, peroxidase, catalase, tyrosinase (enzymes oxidative). Amylase breaks down starch into destrins and maltose.

## 2.3. Digestion and absorption of silkworm

### 2.3.1. *Digestion*

The digestive system of the larva is well developed, while at pupae and moth are vestigial and non-functional.

Larva uses the digestive system to digest and absorb nutrients and other substances from consumed leaves. Macromolecules and other complex substances, such as proteins, polysaccharides, fats, nucleus acids etc., are taken over from mulberry leaves and subjected to a metabolic process. These substances must be beaked down by catabolic reactions in the small molecules, such as amino acids, fatty acids, and simple sugars, for passing into tissues and are used by own body cells for energy, growth, reproduction and, on the other hand, as substances required for body tissue synthesis. This process is named digestion (https://drive.google.com/file/d/1_H4Nps4-pkOX_K31Ni1QGgKy4BscnBiq/view).

The silkworms have considerable variation in the digestion, absorption and conversion capacity of the nutritive substance into body substances, depending on leaves quantity, climate variations, and feed (Rahmathulla and Suresh, 2012).

It is generally accepted that nutritive parameters are correlated with feed intake and silk production. The conversion of mulberry leaves into silk is highly correlated with the digestion capacity of the silkworm. A more accurate economic indicator for producing cocoons is the effectiveness of the conversion of mulberry leaves intake in silk or the conversion rate of active substances from leaves (Rahmathulla and Suresh, 2012). Only 620.70 kg of 2472.80 kg mulberry leaves produced/ha cultivated with mulberry plants are digested by silkworms and converted to 211.20 kg silk (Priyadharshini et al., 2017).

At *Bombyx mori* silkworm the haemolymph composition consists of 24–30 mg/mL protein and more than 1500 mg/mL lipids, while pupae have a weight that ranges between 705–885 mg (Mahmoud, 2017).

### *2.3.2. Absorption*

The absorption of nutritional substances implies the passages from the digestive tube into the blood directly or indirectly through the lymph. Among the leaves' components, certain elements, such as glucose, soluble salts in the water etc., are absorbed directly by epithelial-specific cells in the middle intestine. Still, proteins and other macromolecules of large size can't penetrate through the cell wall. In this regard, by the decomposition into simple particles must be easily assimilable.

There are differences between the protein in mulberry leave and the protein from the silkworm body. The specific protein of mulberry leaves is broken down by an enzyme in amino acids and peptones

and then is absorbed in the middle epithelial intestine and transported to organs through haemolymph. Into organs, the amino acids and peptones are re-synthesised in animal protein, a process named assimilation. The fat is re-synthesised in fatty acids and glycerol. Behind this synthesis, the fat can be synthesized from carbohydrates. An important part of mulberry leaf carbohydrate is synthesised in fat into the larva body.

## 3. EFFECTIVENESS OF FEED UTILISATION

Appropriate feeding is a critical indicator of the economic value of silkworm development and a factor in determining the superiority of specific breeds or hybrids' productive characteristics (growth, development, and silk production (Rahmathulla et al., 2005). The efficacity of digestion implies a superior conversion of nutrients from silkworm into silk.

The conversion efficacity of ingesta (ECI) represents an important economic indicator of the assessment of cocoon production. The digestion and absorption process depend on hybrid/breed, the environmental factors, sex and quantity of feed.

Several methods to improve the rate of conversion in silk were highlighted by many authors (Benchamin et al., 1984; Chandrakala et al., 1999; Rahmathulla et al., 2005). The methods focus on respecting specific environmental conditions are the, growing the variety/ hybrids with superior characteristics, appropriate mulberry variety, diets according to qualitative and quantitative requirements, and respect for specific growing procedures. A high feed intake in larval phase 5 positively influences body weight and silk production. Females consume more feed than males, and monovoltine breeds ingest and digest a higher quantity of feed. The digestibility of leaves is higher in monovoltine species, although in polyvoltine breeds, diet digestibility is significantly higher (Tzenov et al., 1999).

A positive correlation is found between leaves intake and the protein level in silk fibre. The leaves' intake influences as well the reproduction activity and quantity of biomass accumulated. The newly created hybrids valorise the feed better compared to the traditional one.

In the larvae phase, digestibility remains more or less constant from the first day up to the middle of the growing phase; after that, a decline is registered up to the spinning process. The efficacity with which the feed is digested is converted to body mass decreases with age (Rahmathulla et al., 2005). The quantity of dry matter consumed and digested, ECI, the final weight of the larva and the weight of the cocoon increase significantly, being correlated with water quantity from leaves. A less water content extends larval phases.

The mean of parameters relies on ingesta and digesta of silkworm feed with three varieties of mulberry Kenmochi (*Morus bombycis Kodiz*), black mulberry (*Morus nigra* L.), and native mulberry (*Morus alba* L.) on the entire larval period, and they were presented by Alipanah et al., (2020), respectively 34% DM, leaves ingested 5.16 g, excreta 2.20 g, leaves digested 2.42 g, digestibility 44.2%.

Figure 1 shows the biological cycle of silkworm growing, production and health, and potential industry application.

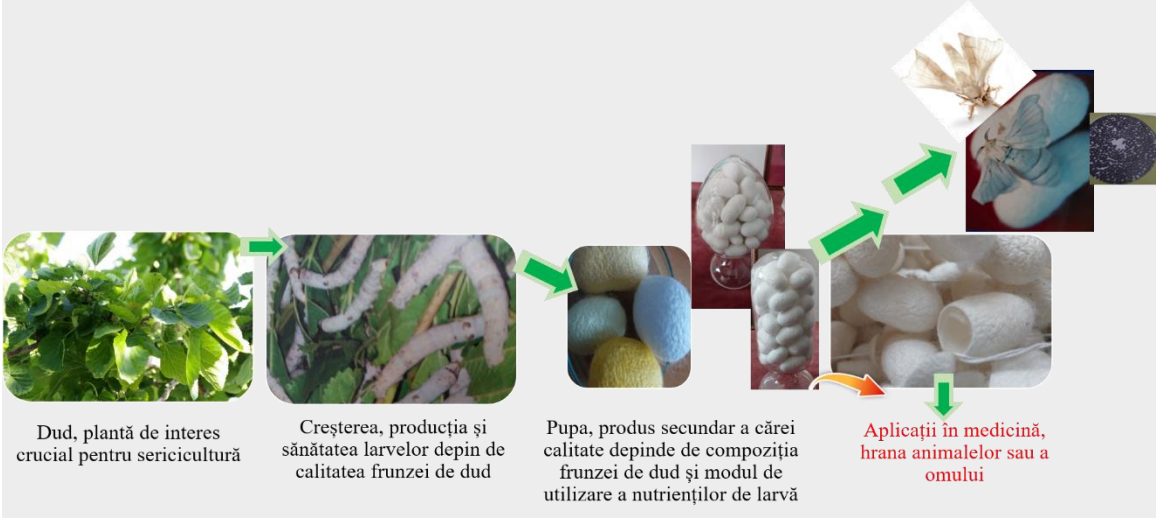


**Figure 1. Biological cycle, nutritional quality and applications of the silkworm growth / Ciclul biologic, calitate nutrițională și aplicații ale creșterii viermilor de mătase**

## 4. IMPORTANCE OF MULBERRY CULTIVATION

All those described above highlight mulberry leaves' primary role in the silkworm diet. Of crucial importance is that leaves are stocked with all the nutrients the larva needs for body functions (growing, reproduction, silk production). In addition, the positive involved for health through the conversion of nutrients in compounds beneficial to the insect's health is also important. From an economic point of view, silkworms also have an important role. However, this species cannot survive without mulberry plants.

The valuable components from leaves and fruits make these plants a suitable functional food for human health besides the primary nutritional value (Kadam, 2019).

Practically, the protein contained in mulberry leaves is transformed into silk that includes 2 types of protein (fibroin and sericin). This means that the silk produced in this way can be used commercially for silk-coats. It is estimated that silk obtained from silkworms greatly contributes to the global production of silk (approx. 90%) and is a very attractive economic activity, especially for the rural population, significantly contributing to several social groups. Behind these aspects, many nutritional and medicinal benefits are demonstrated by the mulberry plant. Thus, mulberry leaves are used for numerous other things besides feeding silkworms. The leaves and fruits are protein and vitamins-rich and were exploited long ago as feed to animals in many countries. Due to its high nutritional value and savoury, the mulberry fruit can be considered a valuable food.

Mulberry bark and wood are also useful for making paper and sporting goods. The evaluation of several medicinally important pharmacological compounds present in the mulberry plant has recently opened new avenues of medical science research. Furthermore, studies demonstrate antioxidant effects, antivirals, anti-inflammation, hypolipidemic, anti-hyperglycaemic, neuroprotective activities (Pan and Lou, 2008), anti-HIV, anti-hypotensive and cytotoxic of different mulberry species (Du et al., 2003). Furthermore, fruits, roots and leaves extract can be used in cosmetics, dermatological skin care products, gels, and many others due to a greater capacity for capturing free radicals.

The mulberry is very important from the economic point of view and for the environment, taking into consideration many aspects of its role as feed for animals, phytomedicine, and cleaning up contaminated soils and air etc. (Ghosh et al., 2017). Large quantities of wastes from the sericulture industry (such as parts of mulberry plants and excrement of silkworms), nutritive-rich, are generated during the growth period of silkworms fed with mulberry leaves. It is estimated that 15 tonnes of waste

(including crop and agricultural waste) are generated annually from one hectare of the mulberry farm. Identifying ways to utilize these wastes properly can open up new opportunities for income generation for sericulture farmers. However, the surface cultivated with mulberry trees recorded a significant decline in Europe, including Romania, and immediate measures must be taken to revitalize and expand cultivated areas. In this way, the Government Rules (H.G. no. 695/2022) on the management and administration of the Research Station for Sericulture Baneasa-Bucharest for the further development and restoration of the value of the sericulture sector is very important.

## CONCLUSION

Besides the aspects that rely on the economic importance of growing silkworms due to silk production and valuable by-products, mulberry leaves are a central point of interest. The mulberry (genus *Morus*, family *Moraceae*) is widely dispersed in various climatic areas, from tropical to temperate zones. Mulberry leaves have become very important for sericulture, especially since they are the only feed source for the species *B. mori* L. silkworms.

The nutritional value of mulberry leaves, the protein and amino acids, lipids and fatty acids, carbohydrates, vitamins and minerals, flavonoids etc., are adequate for growth and good health status. We can mention that mulberry has economic and environmental importance, considering many aspects of their role in various sectors, such as animal feed, phytomedicine, and cleaning up the soil of pollutant substances and the atmosphere. Large quantities of sericulture wastes, such as leftover plants and excreta of silkworms rich-nutritive are generated from this industry. Identifying correct ways of waste valorisation opens new opportunities for generating income for farmers practising sericulture. In particular, during the early stages of growth, the silkworm's digestive tract possesses properties that enable it to consume the leaf and absorb nutrients in a relatively high proportion. The silkworm's enzymatic equipment and the fermentation process in mulberry leaves considerably aid in the nutritional principles' ability to be digested.

New initiatives are needed to increase the mulberry cultivation areas and encourage the development of silkworms, particularly in rural areas.

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# STUDY ON THE *APINOSEM FORTE* EFFECTIVENESS IN RABBITS COCCIDIOSIS

# STUDIU PRIVIND EFICACITATEA PRODUSULUI *APINOSEM FORTE* ÎN COCCIDIOZA IEPURILOR

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***Abstract***

*Coccidiosis is a parasitic disease frequently encountered in rabbit farms, affecting especially young animals. Influenced by the factors of non-specific aggression, this parasitosis can cause, depending on the location, digestive disorders, weight loss, and hepatomegaly. In large farms, chemoprophylaxis provides protection for rabbits. In households, once diagnosed, a specific treatment is instituted. The veterinarian must evaluate the degree of toxicity, the cost, and the method of administration, but also the risk of blood absorption (in the case of hepatic localization) when it must choose the therapy. Sulfaveridin, Toltrazuril, Diclazuril, Sulfachloropyrazine, Robenidin, and Salinomycin are just a few of the anticoccidials whose effectiveness has been demonstrated, although currently natural products, natural extracts from plants, fungi, and microorganisms, Aloe vera and the unconventional use of liquid paraffin are becoming remarkable in the treatment of coccidiosis in rabbits. The present therapeutic study aimed to test the efficacy of Apinosem forte, a product obtained from natural plant extracts, in treating an experimental group consisting of 80 rabbits naturally infested with Eimeria spp. Coprological, necropsy, and histopathological examinations were performed; the therapeutic protocol was applied and the results were interpreted from a statistical point of view. We recommend Apinosem forte, administered 3 ml/5 l water, in the prevention and combating of coccidiosis in rabbits. It is the first report on the therapeutic results of Apinosem forte used in the prevention and control of this parasitosis in rabbits.*

***Keywords: rabbits, Eimeria spp., Apinosem forte, therapeutic efficacy***

***Rezumat***

*Coccidiozele sunt afecțiuni parazitare întâlnite frecvent în crescătoriile de iepuri, afectând mai ales animalele tinere. Influențate de factorii de agresiune nespecifică, aceste parazitoze pot determina, în funcție de localizare, tulburări digestive, scăderea în greutate, hepatomegalie. În marile crescătorii, chimioprofilaxia asigură protecția iepurilor. În gospodăriile populației, odată diagnosticate, se instituie un tratament specific. În alegerea anticoccidicului, medicul veterinar trebuie să evalueze gradul de toxicitate, costul și modul de administrare, dar și riscul absorbției sangvine (în cazul localizării hepatice). Sulfaveridin, Toltrazuril, Diclazuril, Sulfacloropirazina, Robenidin, Salinomicin sunt doar câteva dintre anticoccidicele a căror eficacitate a fost demonstrată, deși, în prezent, produsele naturiste, extractele naturale din plante, ciuperci și microorganisme, Aloe vera și utilizarea neconvențională a parafinelor lichide devin candidate remarcabile în tratamentul coccidiozei iepurilor. Studiul terapeutic prezent a urmărit testarea eficacității Apinosem forte, un produs obținut din extracte naturale din plante, în tratamentul unui lot experimental alcătuit din 80 de iepuri infestați natural cu Eimeria spp. Au fost efectuate examene coproparazitologice, necropsice, histopatologice; s-a aplicat protocolul terapeutic și s-au interpretat statistic rezultatele obținute, care recomandă Apinosem forte, administrat 3 ml/5 l apă, în prevenirea și combaterea coccidiozei iepurilor. Este prima raportare privind rezultatele terapeutice ale Apinosem forte utilizat în prevenirea și combaterea acestei parazitoze la iepuri.*

***Cuvinte cheie: iepuri, Eimeria spp., Apinosem forte, eficacitate terapeutică***

## INTRODUCTION

The most notable disease of rabbits, coccidiosis, causes massive economic losses, high mortality, and morbidity and is caused by fifteen species of *Eimeria* of varying pathogenicity. Rabbit coccidiosis is a ubiquitous infection caused by obligate intracellular protozoa belonging to the genus *Eimeria* and is considered a major cause of significant morbidity and mortality [3, 8, 35]. Three forms of coccidiosis are known: intestinal coccidiosis, in which the protozoan invades the epithelial cells of various regions of the intestines, leading to moderate to severe damage, depending on the virulence of the species, hepatic coccidiosis, the site of predilection for the species *E. stiedae* and otorhinopharyngeal localization [3, 8].

The occurrence of coccidiosis in rabbits is aggravated by poor hygiene and high animal densities that allow the spread of the parasite. Oochists have a remarkable ability to survive in the environment, which makes parasitological control difficult [8, 35].

Currently, the attention of researchers is directed toward different natural strategies regarding the treatment and prevention of parasitic infestations. Studies show that proper hygiene combined with strict biosecurity and effective therapy plays a significant role in preventing and spreading these conditions [19].

Despite their success in the poultry industry, vaccination attempts with live or attenuated vaccines have had unsatisfactory results in rabbits. In the rabbit, coccidiosis control should address not to only prevention of disease and excess mortality, but also mild and subclinical infections, as even minor intestinal lesions can interfere with growth and feed conversion and thus profitability, state Bhat et al., 2010 [5]. Therapy of rabbit eimeria focuses on the products Amprolium and Toltrazuril [18]. The development of varying degrees of tolerance to coccidiostats drugs in feed and the side effects inherent to ionophore coccidiostats prompt producers to explore other control measures [27]. This is of particular relevance because, with the exception of some alternative rearing systems, most systems depend on pharmacological control of coccidiosis [12, 24].

According to the bibliographic studies, we note the therapeutic efficacy of various medicinal substances used to control this parasitosis. The therapeutic evaluation was based on the estimation of production data, body weight, body weight gain, feed consumption, feed conversion rate, performance index, and reduction of oocyst production indicating anticoccidial activity [11]. In the choice of the anticoccidial, when we talk about hepatic coccidiosis, the veterinarian must evaluate the degree of toxicity, the cost, and the way of administration, but, in particular, the risk of blood absorption [8].

Starting from the herbal food supplement (patent no. RO130489-B1) intended to prevent and combat microsporidiosis (*Nosema* spp.) which contributes to the massive loss of bee colonies in Europe and all over the world, we arrived at the product *Apinosem forte*, extract natural from plants, which proved to be effective in treating this parasitosis. Administration of the product to bees infested with *Nosema* spp. or in the prevention of the disease is not followed by the presence of residues in the products of the hive, thus fulfilling one of the most important European requirements, regarding human health [8].

Although synthetic anticoccidials remain the main agents of therapy, natural products, natural extracts from plants, fungi, and microorganisms, Aloe vera and the unconventional use of liquid paraffin are becoming outstanding candidates in the treatment of rabbit coccidiosis [1,10].

In this context, the aim of the study was to assess the therapeutic efficacy of the product *Apinosem forte*, a natural extract from plants, in the treatment of coccidiosis in rabbits.

## MATERIALS AND METHODS

### *Animals taken in the study*

The study was carried out over a period of one year, on a number of 80 rabbits naturally infested with *Eimeria* spp. During the study, the rabbits were hospitalized in the Clinical Veterinary Hospital of

the Faculty of Veterinary Medicine Timișoara. They were raised in metal cages under the same microclimate conditions. The rabbits were aged between 2-6 months. They were given water at will. No other antiparasitic treatment was administered to them.

***Coproparasitological examination***

Fecal samples were examined in the laboratory of the Parasitology and Parasitic Diseases discipline of the Timișoara Faculty of Veterinary Medicine. The coprological methods used for the identification and quantification of *Eimeria* spp. oocysts were the flotation method, respectively the Mc Master method [9]. Coprological methods were applied throughout the treatment to estimate EPGs.

***Necropsy examination***

It was performed in accordance with the necropsy technique [9].

***Histopathological examination***

In the succumbed individuals, we performed a histopathological examination from the liver sections parasitized with *Eimeria stidae*, respectively from the intestinal sections, using the usual work techniques [20, 22, 38].

***The therapeutic protocol***

In the present study, we administered the product *Apinosem Forte* – a product recommended for the prevention and combating of bee nosemosis and obtained through the collaboration between ULS Timișoara and the company Dulcofruct Cirast Focșani (Table 1; figure 1) [20].

**Table 1: *APINOSEM FORTE* data sheet / Fișa tehnică *APINOSEM FORTE***

| | | | | |
|---|---|---|---|---|
| **Product** | **APINOSEM FORTE** | | | |
| **Origin** | Romania | | | |
| **The description product** | **Apinosem Forte – Stimulant adjuvant for preventing and combating nosemosis** | | | |
| **Ingredients** | Essential plant oils, plant maceration, and infusion, apple cider vinegar, garlic tincture | | | |
| **Properties organoleptic** | **appearance** | **Color** | **Smell** | **Taste** |
| | Liquid | Brown | plants | Specific |
| **Administration** | **Syrup**:-2 ml/1kg syrup.<br>200ml syrup with APINOSEM FORTE/hive<br>**Cake**:4 ml/kg cake<br>500g with APINOSEM FORTE/hive<br><br>**RECOMMENDATION**: In the case of prevention, two administrations in the spring and two administrations at the beginning of autumn are recommended (in total 4 administrations per year). The interval between administrations is 10 days. In the case of the visible presence of the disease, the preventive administration is not sufficient and it is recommended 4-5 administrations with a break of 4 days between administrations. | | | |
| **Use** | This product is used in the prophylactic treatment of *Nosema apis* and *Nosema ceranae* parasites | | | |
| **PACKING** | Bottles 50 ml, 200 ml | | | |
| **Storage and validity** | **STORAGE CONDITIONS**: at a temperature between -15ºC and + 35ºC<br>**TERMS OF VALIDITY**: 12 months from the date of manufacture written on the packaging | | | |
| **Author/Producer** | **USAMVB Timisoara/SC CIRAST SRL-ROMANIA** | | | |


**Figure 1.** ***Apinosem Forte / Apinosem Forte***

The rabbits were divided into three groups of 20 individuals each.
Batch I – treated with *Apinosem Forte* – 2 ml/5l water
Lot II – treated with *Apinosem Forte* – 3 ml/5l water
Lot III – treated with *Apinosem Forte* – 4 ml/5l water
Lot IV – infected and untreated
The product was administered daily, in drinking water, for 30 days.

***Statistical interpretation***

To determine the effectiveness of the treatment, the results were statistically interpreted by applying the Kruskal Wallis and Mann-Whitney tests, to determine the significant differences between the three infected and treated groups, respectively the infected and untreated group 4 (control group). Descriptive statistics established the differences between the characteristics followed throughout the experiment: degree of the infestation, color of feces, clinical signs, EPG value, and water consumption [44].

## RESULTS AND DISCUSSION

All infested rabbits in groups 1, 2, 3, and the control group showed clinical signs: restlessness, depression, dry fur, loss of appetite, weight loss, bloody watery diarrhea, and dehydration.

*Eimeria* spp. oocysts were identified in the flotation examination (figure 2) and we determined in the Mc Master examination, a parasite load between 1500 – 58,000 EPG.

The results of the necropsy examination revealed the presence of intestinal lesions: thickening of the intestinal wall, small ulcerations, hemorrhages, desquamation of the intestinal mucosa, as well as liver lesions: hepatomegaly, the presence of numerous gray-whitish nodules.

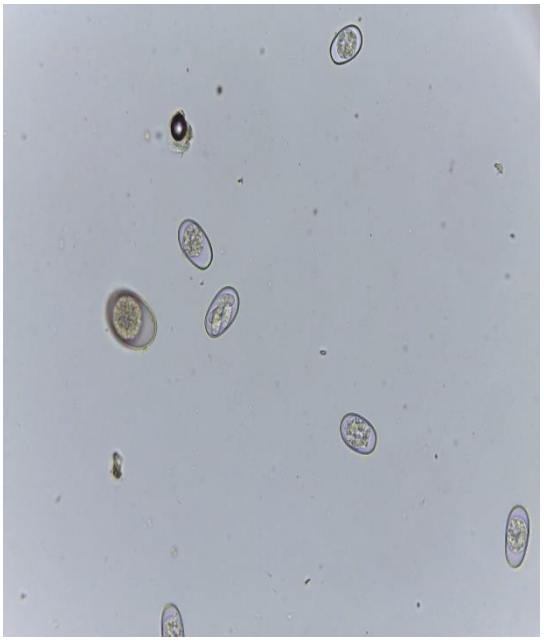

**Figure 2. *Eimeria* spp. – oocysts / *Eimeria* spp. – oochiști**

The histopathological examination revealed hepatic coccidiosis caused by *Eimeria stiedae*. The presence of extensive biliary hyperplasia with numerous intralesional coccygeal elements was found. The bile ducts were dilated and lined with hyperplastic columnar epithelial cells. Numerous developmental stages of the protozoa, ranging in size from 25-50 microns in diameter, were present within the epithelial cells. The bile ducts were surrounded by large amounts of fibrous connective tissue with lymphohistiocytic inflammatory infiltrates (figure 3).

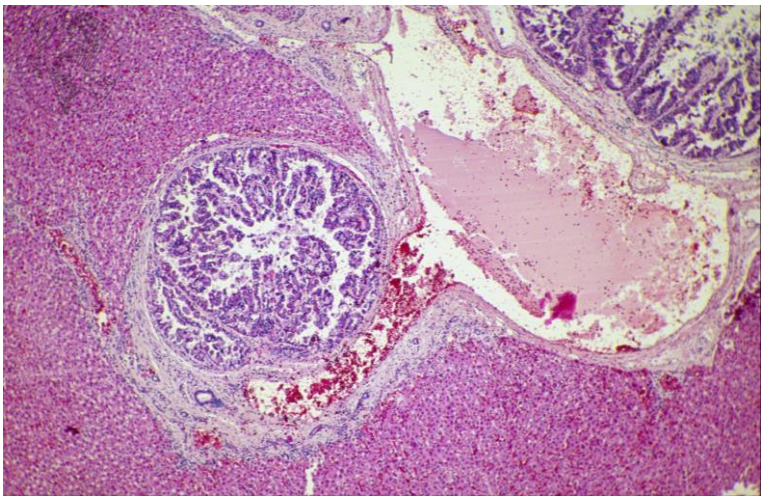

**Figure 3. HP aspect on the liver - epithelial hyperplasia – *Eimeria stiedae* (H&E, 40x) / Secțiune HP în ficat - hiperplazie epitelială – *Eimeria stiedae* (H&E, 40x)**

In the intestines infested with *Eimeria* spp., different stages of development of the protozoan involved in the etiology were highlighted. HP examination also revealed severe infiltration with inflammatory cells, mainly lymphocytes (figure 4, a-f).

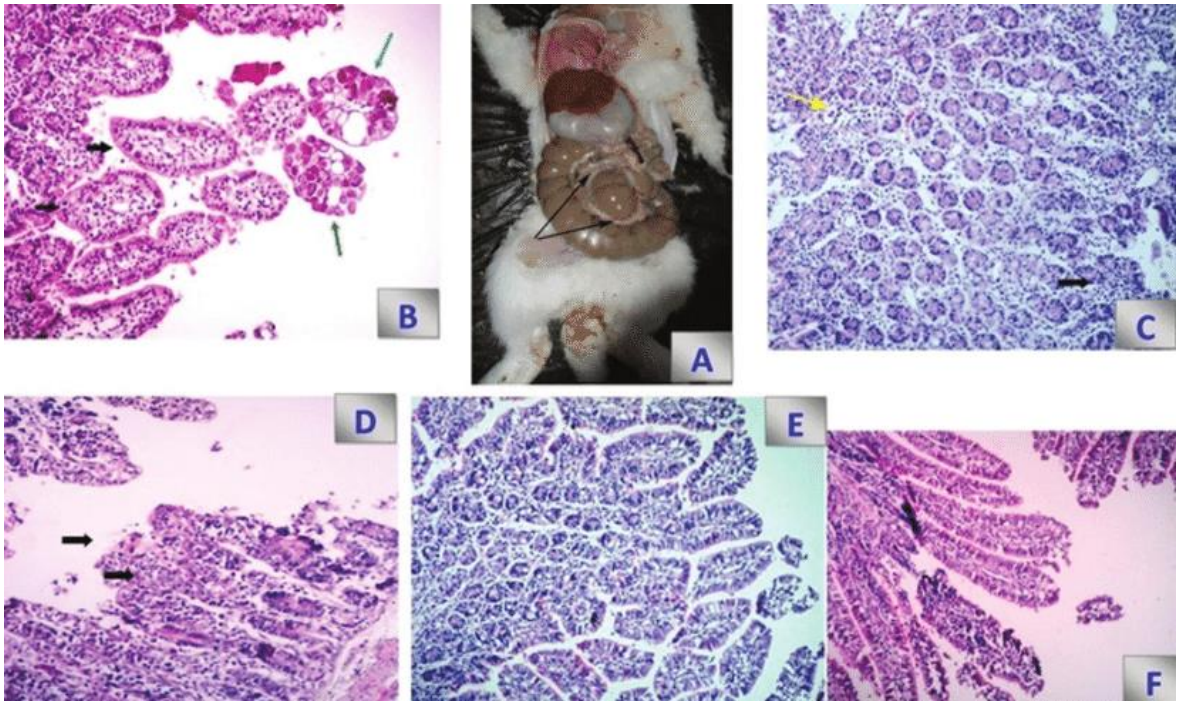


**Figure 4. HP aspects on the *Eimeria* spp. infested intestines A. congestion B. cell necrosis, *Eimeria* spp. presence C, D, E, F. epithelial desquamation, lymphocytes infiltrated (H&E × 40) / Secțiune HP în intestin parazitat cu *Eimeria* spp. A. congestie B. necroza celulelor, prezente stadii de dezvoltare ale *Eimeria* spp. C, D, E, F. descuamare epitelială, infiltrate limfocitare (H&E × 40)**

During the treatment, rabbits from the three infected and treated groups (L1, L2, L3) showed an improvement in health status, remission of clinical signs, and a significant ($P < 0.05$) reduction of EPG in feces in comparison with the infected and untreated group (L4). In the control group, the clinical signs did not remit, the EPG did not decrease, and 5 individuals succumbed 1 week after the start of experiment.

The results of the descriptive statistics are presented in table 2.

**Table 2. Kruskal Wallis results / Rezultatele testului Kruskal Wallis**

| **Statistics test** [a,b] | | | | | |
|---|---|---|---|---|---|
| | Infestation | Fecal color | Clinical signs | OPG | Water consumption |
| Chi-Square | 18,869 | 7,047 | 25,143 | 23,692 | 9,455 |
| df | 2 | 2 | 2 | 2 | 2 |
| Asymp. Sig. | 0,000 | 0.029 | 0,000 | 0,000 | 0.009 |
| a. Kruskal Wallis Test | | | | | |
| b. Grouping Variable: Batches | | | | | |

Analyzing the data obtained comparing the control group and the group that was administered ***Apinosem forte* 2ml/5l water**, we can notice significant differences in the case of infestation (U=30.00, p=0.000<0.05), fecal color (U=37.50.00, p= 0.000<0.05), clinical signs (U=22.5, p=0.000<0.05) and water consumption (U=72.00, p=0.000<0.05) (table 3).

**Table 3. *Apinosem forte 2 ml* - Mann-Whitney test / Testul Mann-Whitney - *Apinosem forte 2ml***

| | Infestation | Fecal color | Clinical signs | OPG | Water consumption |
|---|---|---|---|---|---|
| Mann-Whitney | 30,000 | 102,000 | 37,500 | 22,500 | 72,000 |
| | 150,000 | 222,000 | 157,500 | 142,500 | 192,000 |
| | -3,994 | -.493 | -3,808 | -4,318 | -2,282 |
| | .000 | .622 | .000 | .000 | .022 |
| | .000b | .683b | .001b | .000b | .098b |

Between the control group and the group that was administered ***Apinosem forte* 3ml/5l water**, significant differences were evident in all the studied manifestations (table 4), the corresponding probabilities being all lower than 0.05.

**Table 4.** ***Apinosem forte 3ml*** **- Mann-Whitney test / Testul Mann-Whitney -** ***Apinosem forte 3ml***

| | Infestation | Fecal color | Clinical signs | OPG | Water consumption |
|---|---|---|---|---|---|
| Mann-Whitney | 30,000 | 63,500 | 37,500 | 30,000 | 44,500 |
| | 150,000 | 183,500 | 157,500 | 150,000 | 164,500 |
| | -3,994 | -2,239 | -3,808 | -3,985 | -3,348 |
| | .000 | .025 | .000 | .000 | .001 |
| | .000b | .041b | .001b | .000b | .004b |

Analyzing the results after the administration of the treatment in the case of the ***Apinosem forte* 2ml** group vs the ***Apinosem forte* 4ml group**, significant differences were obtained only in the case of the fecal color variable (U=63.5, p=0.011<0.05) (table 5).

**Table 5.** ***Apinosem forte 4ml*** **- Mann-Whitney test / Testul Mann-Whitney -** ***Apinosem forte* 4ml**

| | Infestation | Fecal color | Clinical signs | OPG | Water consumption |
|---|---|---|---|---|---|
| Mann-Whitney | 107,000 | 63,500 | 112,500 | 105,000 | 109,500 |
| | 227,000 | 183,500 | 232,500 | 225,000 | 229,500 |
| | -.243 | -2,543 | .000 | -.482 | -.134 |
| | .808 | .011 | 1,000 | .630 | .893 |
| | .838b | .041b | 1,000b | .775b | .902b |

The results of the study reveal that the product *Apinosem forte*, administered 3 ml/5 l of water, showed the highest efficacy on *Eimeria* spp. oocysts.

From the perspective of clinical diagnosis, the results of the present study are consistent with those reported by other authors who identify in infected rabbits, typical clinical signs: watery diarrhea with streaks of blood, lack of appetite, dehydration, bloated abdomen, drowsiness, weight loss, hepatomegaly [2, 5, 16, 24, 25, 28].

Histopathological investigations reveal the diagnosis of chronic proliferative cholangitis marked with coccygeal intraepithelial elements and liver atrophy. Bibliographic histopathological studies confirm the appearance of the lesions identified in the present study: hypertrophic liver, dilated bile ducts, white nodules of variable size and creamy liquid content, on the liver, damage to the epithelium of the bile ducts with extensive hyperplasia of the epithelium, the presence of developmental stages of the protozoan in the epithelial cells of bile ducts, cytoplasmic vacuolations in hepatocytes [3, 4, 22, 40].

Therapeutic studies demonstrate the superior efficacy of the curative use in coccidiosis of rabbits of diclazuril and sulfachloropyrazine, substances established in the control of coccidiosis in birds, in favor of amprolium which proves to be ineffective [7, 13, 15, 17, 18, 23, 27, 30, 34, 36, 41, 42, 43].

A study by Peeters et al. [24, 29] demonstrated the efficacy of narasin in the treatment of mixed intestinal and hepatic coccidiosis. Robenidin and salinomycin have been widely used in Europe with varying efficacy against hepatic coccidiosis [26, 33]. Similarly, studies have reported varying efficacies following prophylactic and curative use of sulfonamides against coccidiosis [14, 37, 39]. The prophylactic and therapeutic use of toltrazuril has shown good results in the countries where it is used [11, 31, 37, 39].

In India, amprol, bifuran, and sulfur-based drugs have been widely used for the prevention of rabbit coccidiosis [5]. The prophylactic and curative use of diclazuril against rabbit coccidiosis has shown impressive results in Italy, France, and Spain [26, 41].

A study in Kenya looked at the curative (therapeutic) efficacy of sulfachloropyrazine, amprolium hydrochloride, and trimethoprim versus diclazuril which has never been used in Kenya, but has been shown to be effective [30, 41, 43].

Currently, there are no specific anticoccidials for rabbits in Kenya, and farmers use the anticoccidials used in the treatment of avian eimeriosis, not knowing the specific dose and efficacy for rabbits [6, 30, 32].

Studies on the effectiveness of the prevention and treatment of coccidiosis with natural extracts from garlic, pomegranate, and sweet wormwood plead for the introduction of these natural products in the control of protozoa [1, 20].

In the present study, we chose the product *Apinosem forte* – a natural extract from plants and garlic, used with great success in preventing and combating microsporidiosis produced by *Nosema* spp. [19, 21].

## CONCLUSIONS AND RECOMMENDATIONS

The diagnosis of coccidiosis was confirmed following the coproparasitological, necropsy, and histopathological examinations.

Reduction to the disappearance of oocysts of *Eimeria* spp., the easy way of administration (in water), the remission of clinical signs, the return to the normal consistency of feces represent the advantages of the product *Apinosem forte*, administered 3 ml/5 l water, which we recommend in the prevention and combating of coccidiosis in rabbits.

It is the first report on the therapeutic efficacy of the product *Apinosem forte* used in the parasitological control of coccidiosis in rabbits.

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# RESEARCH ON THE EFFICIENCY OF THE ARTIFICIAL INSEMINATION WITH FRESHLY DILUTED AND FROZEN SEMEN IN EWES DURING NATURAL ESTROUS

# CERCETĂRI PRIVIND EFICIENȚA INSEMINĂRILOR ARTIFICIALE CU MATERIAL SEMINAL PROASPĂT DILUAT ȘI CONGELAT LA OI ÎN ESTRU NATURAL

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***Abstract***

*In the context of the development and expansion of the sheep farming system, the main concern of sheep breeders has become the achivement of controlled reproduction and the practice of artificial insemination with semen from breeder rams. The study is carried out in a farm of 550* ***Merino de Palas*** *sheep in Constanța county, the owner aiming to improve the quality of the wool fiber. After about 3 weeks from the beginning of the mounting campaign, when the ewes in estrous were detected twice a day, in the morning and in the evening, the ewes came out to the mounting in a group due to the male effect. This was the moment when we started the double artificial insemination campaign, approximately 10 hours apart, on natural estrous, with semen from 2* ***Australian Merino*** *rams - UK import, aged 5 and 6 years. Thus, 308 ewes were artificially inseminated, respectively 226 ewes with freshly diluted semen and 82 ewes with frozen semen. After these 14 days, the two males were used for the natural mounting of 64 ewes in natural estrous, during the next 14 days, these being the control group. After the artificial insemination, a calving rate of 64.86 - 67.95% was obtained when inseminating with freshly diluted semen, 40.24% when inseminating with frozen semen and 72 - 76.92% after natural mounting of ewes from the control group.*

***Key words: ewes, artificial insemination, semen***

***Rezumat***

*În contextul dezvoltării și extinderii sistemului de exploatare a ovinelor, principala preocupare a crescătorilor de oi a devenit realizarea reproducției dirijate și aplicarea inseminărilor artificiale cu material seminal de la berbeci amelioratori. Studiul este realizat într-o fermă de 550 oi* ***Merinos de Palas*** *din județul Constanța, proprietarul urmărind ameliorarea calității fibrei de lână. După aproximativ 3 săptămâni de la debutul campaniei de montă, când s-a realizat depistarea oilor în estru de două ori pe zi, dimineața și seara, femelele au ieșit la montă în mod grupat datorită efectului mascul. Acesta a fost momentul în care am demarat campania de inseminare artificială dublă, la interval de aproximativ 10 ore, pe estru natural, cu material seminal de la 2 berbeci* ***Merinos Australian*** *– import Marea Britanie, în vârstă de 5 și 8 ani. Astfel au fost inseminate artificial 308 oi, respectiv 226 oi cu material seminal proaspăt diluat și 82 oi cu material seminal congelat. După aceste 14 zile, cei doi masculi au fost utilizați la monta naturală a 64 oi în estru natural, pe parcursul următoarelor 14 zile, acestea constituind lotul martor. În urma inseminării artificiale s-au obținut o rată a fătării de 64,86 - 67,95% la inseminarea cu material seminal proaspăt diluat, 40,24% la inseminarea cu material seminal congelat și 72 – 76,92% în urma montei naturale a oilor din lotul martor.*

***Cuvinte cheie: oi, inseminare artificială, material seminal***

## INTRODUCTION

The present study was carried out as a result of the need to perform artificial insemination with semen from breeding rams. By applying the selection and improvement criteria within the Genealogical Registers, the ranking and sorting of the purebred rams that are at the top of the hierarchy for production in the desired selection direction was achieved. These rams from the top of the pyramid, respectively from the elite farms, are distributed to the selection and breeding farms but also to the production farms to improve the livestock productions. The number of males that reach the production farms is relatively small, the purchase being quite expensive. Thus, many sheep breeders have the tendency to excessively and irrationally use these rams in the natural mounting that leads to their physical and/or reproductive exhaustion. Another risk is the use for mounting without taking into account parentage, reaching a high degree of inbreeding. The solution to this situation is the exchange of rams between breeders or, easier to achieve, resorting to artificial insemination with semen from breeding rams from other farms or from elite farms. Under these conditions, it is necessary to check the quality of the semen from rams in terms of mobility and viability (Druart X. et al., 2009, Baril G. et al., 1993, Fatet A. et al., 2008) and establish its suitability for collecting, dilution and artificial insemination. Artificial insemination represents the most efficient reproductive biotechnology that has become a common practice for the sheep breeders in Europe. According to the website idele.fr, in 2017, in France, more than 800,000 inseminations were carried out in dairy sheep by means of nine artificial insemination centers, creating a herd that remains approximately constant for over 20 years. This trend has been maintained until now when there is a network of semen collection and preservation centers and frozen semen insemination centers, achieving a good diffusibility in time and space of the valuable genetic material.

In our country, sheep reproductive biotechnologies have been developed within the Research and Development Institute for Sheep and Goat Breeding Palas, Constanța and the Research and Development Stations for Sheep and Goat Breeding. Research in the field of reproduction has been oriented in the direction of increasing productivity and management of controled reproduction, through themes imposed by the needs of sheep breeders. All results have as their main purpose the dissemination of information, new or upgraded techniques and technologies in the production activity of the sheep sector.

## MATERIAL AND METHOD

The present study was carried out on a farm of 550 **Merino de Palas** ewes, 17 **Merino de Palas** rams and 2 **Australian Merino** rams, exploited mainly for wool production. The farmer, engaged in wool sales contracts, is interested in the quality of the wool fiber, aiming to obtain specimens with a fineness below 20 microns. In this sense, he purchased 2 years ago, from Great Britain, the two **Australian Merino** rams, aged 5 years (hereinafter referred to as ram A) and 6 years old (hereinafter referred to as ram B), with a fiber fineness of wool of 17.5 microns and 13.91 microns respectively. After the first year of use at mounting and registration of calvings, we were asked to check the quality of the semen and advise him on how to carry out the mounting to maximize the use of the 2 imported males. Following the analysis of the semen and taking into account the age of the rams, we proceeded to collect and cryopreserve the ejaculates with a volume of over 1 ml and with a motility of 85-95% from the 6-year-old ram (ram B), making a stock of 150 medium sequins (0.5 ml).

In July 2022, the controlled breeding season began, by introducing test rams among the ewes, in the morning and in the evening, separating the females that show clinical signs of estrous and the natural mounting. After about 3 weeks from the beginning of the mounting season, when the ewes in estrous were detected twice a day, the ewes came out to mounting in a group due to the male effect, at which time we applied the artificial insemination protocol.

For 14 days, ewes in estrous were detected with rams provided with an apron, twice a day, in the morning (5.30 am) and in the evening (7 pm). Females detected in estrous were artificially inseminated 10-12 hours after detection, with the repetition of insemination at approximately 10 hours.

The collection regime of the 2 **Australian Merino** rams was 3 consecutive days followed by a rest day for recovery, collecting 2-3 ejaculates/day, depending on the number of females detected in estrous and the volume of the ejaculate at the first insemination. In the situation where the number of females was higher and the number of doses of freshly diluted semen was not sufficient, frozen semen from ram B was used for both inseminations (table 1).

**Table 1. The situation of artificial insemination in Merino de Palas sheep, in natural estrous, during the 14 days period / Situația inseminărilor artificiale la oile Merinos de Palas, în estru natural, în perioada de 14 zile**

| Experiment day | No. Of ewes detected in estrous | Total number of ewes artificially inseminated with freshly diluted semen | Number of ewes artificially inseminated with freshly diluted semen ram A | Number of ewes artificially inseminated with freshly diluted semen ram B | Number of ewes artificially inseminated with frozen semen ram B |
|---|---|---|---|---|---|
| 1 | 20 | 20 | 14 | 6 | 0 |
| 2 | 26 | 26 | 19 | 7 | 0 |
| 3 | 18 | 14 | 14 | Ram recovery break | 4 |
| 4 | 26 | 10 | Ram recovery break | 10 | 16 |
| 5 | 23 | 21 | 14 | 7 | 2 |
| 6 | 21 | 19 | 10 | 9 | 2 |
| 7 | 18 | 10 | 10 | Ram recovery break | 8 |
| 8 | 24 | 8 | Ram recovery break | 8 | 16 |
| 9 | 23 | 22 | 16 | 6 | 1 |
| 10 | 21 | 18 | 11 | 7 | 3 |
| 11 | 24 | 13 | 13 | Ram recovery break | 11 |
| 12 | 19 | 9 | Ram recovery break | 9 | 10 |
| 13 | 24 | 18 | 12 | 6 | 6 |
| 14 | 21 | 18 | 15 | 3 | 3 |
| **Total** | **308** | **226** | **148** | **78** | **82** |

**The protocol for obtaining diluted raw semen**.

Semen collection is done with the artificial vagina using a dummy ewe in estrous, contained in a special stand. To perform the collection, the artificial vagina must meet the three basic conditions to achieve ejaculation: the lubrication of the vaginal shirt with neutral ointment, the temperature of 38-39$^0$ C, and the pressure by blowing air at the level of the cannula of the artificial vagina.

Immediately after collecting the ejaculate, the volume is visually assessed by reading the graduations of the collecting cup, obtaining between 0.9 and 2.4 ml. The consistency and color of the semen can be evaluated through the transparency of the collecting cup, which varies from white-slightly yellowish (normal color) to white-brown (Zamfirescu et al., 2004; Zamfirescu et al., 2011).

To determine the degree of dilution, the Accucell analyzer was used - produced by IMV Technologies (photo 1a). After calibrating the device and entering the volume of the ejaculate and the concentration of spermatozoa/ml target for the insemination dose (200x10$^6$ spermatozoa/0.25ml), the sample of 10 microliters of semen is introduced and the photometer will indicate the volume of diluent to be added and the number of doses obtained. To achieve the dilution, we use the OviXcell diluent, produced by IMV Technologies (photo 1b). Until the moment of insemination, the glass with the diluted semen is kept on the water bath so that the temperature gradually decreases and to be valid until the moment of artificial insemination.

**The cryopreservation protocol of frozen semen**.

In order to cryopreserve the semen, we proceeded to the collection with an artificial vagina, the reading of the volume and the qualitative analysis directly on the graduated glass, respectively the motility and viability by the colorimetric method. The semen was subjected to three phases of dilution: the initial dilution in a ratio of 1:1 with TRIS diluent containing 20% egg yolk. The operation is done on

a water bath at $30^0$ C, followed by gradual cooling to $+4^0$ C, the intermediate dilution with the same diluent, at room temperature, the amount being calculated according to the sperm concentration. The next stage is the equilibration of the diluted sperm at $+5^0$ C for 3-4 hours, followed by the final dilution with diluent II (saline diluent with 20% egg yolk and 8% glycerin); the amount used of diluent II is equal to that of diluent I and is added in one or two batches. This final dilution is done a few minutes before aspiration of the sperm into medium sequins with a capacity of 0.5 ml or fine sequins of 0.25 ml. The sequins are sealed with polyvinyl powder, which gels in contact with the aqueous medium. Freezing is done in two phases: pre-freezing in liquid nitrogen vapor at a temperature of $-120^0$ C for different times depending on the form of freezing (fine sequins are pre-frozen for 2 minutes at 8 cm and 8 minutes at 2 cm above the level of liquid nitrogen and medium sequins pre-freeze 4 cm above the level of liquid nitrogen for 8-10 minutes) and final freezing by immersing the sequins in liquid nitrogen (at $-196^0$ C). The control of the freezing is done 24 hours after freezing, by checking the viability of the spermatozoa from each ejaculate of several sequins, storing in the cryobank only the ejaculates which after freezingthawing have a viability of over 40%.


1a – ACCUCELL


1b - OviXcell

**Figure 1. The equipment and the used preservation medium / Echipamentul și mediul de conservare utilizat**

The artificial insemination of the ewes was carried out on natural estrous, following the detection of estrous with test rams with an apron. The insemination was carried out with 0.25 ml of diluted raw semen, with an average concentration of 200 x $10^6$ spermatozoa/dose. For artificial insemination, the vaginal speculum with its own light was used, with the help of which the place of sperm deposition was visualized, respectively at the level of the involte flora, and when the opening of the cervix allowed, the insemination pipette penetrated along the path of the cervix, at 0.5-1 cm. The second insemination was made with semen from the same ram.

After the completion of the 14 days of insemination, the study continued for another 14 days in which the natural mounting of 64 more ewes with the two rams was carried out. The ewes detected in estrous were distributed to the 2 rams, these managed to achieve up to 4 mountings/day – Ram A and a maximum of 3 mountings/day – ram B, as can be seen in table 2. The difference of the ewes detected in estrous during the respective days were mounted with **Merinos de Palas** rams, a situation we did not include in the present study.

**Table 2. Distribution of natural mounts in Palas Merino ewes with 2 Australian Merino rams / Distribuția montelor naturale la oile Merinos de Palas cu 2 berbeci Merinos australian**

| Specification | Number of naturally mounted ewes | | | | | | | | | | | | | | Total mounted ewes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Day 8 | Day 9 | Day 10 | Day 11 | Day 12 | Day 13 | Day 14 | |
| Ram A | 3 | 3 | 4 | 3 | 4 | 2 | 3 | 2 | 1 | 2 | 3 | 3 | 4 | 2 | 39 |
| Ram B | 3 | 3 | 1 | 2 | 1 | 2 | 1 | 2 | 2 | 1 | 2 | 2 | 2 | 1 | 25 |
| Total mounted ewes | 6 | 6 | 5 | 5 | 5 | 4 | 4 | 4 | 3 | 3 | 5 | 5 | 6 | 3 | 64 |

## RESULTS AND DISCUSSION

The flock of artificially inseminated ewes with the two forms of semen presentation, but also the naturally mounted one were supervised and subjected to oestrous detection with test rams, at intervals of 15-19 days after insemination/mounting to determine the non-pregnant females, respectively the rate of no return. At an interval of 50 days after insemination/mounting, the ultrasound examination was performed with the mobile ultrasound machine WED 3000, with a multifrequency probe, set to 3.5Hz to confirm the gestational state. Females that showed estrous in the next estrous cycle or were not confirmed pregnant were subsequently mounted naturally with rams according to the pair matching chart, not being included in the calculation of the reproductive indices of the present study.

Table 3 shows the reproductive indices obtained during this study and graphic representation 1 illustrates the comparative analysis of each reproductive parameter in the five variants of the study: artificial insemination with raw diluted semen from the two rams and with frozen semen from ram B, as well as the natural mounting with the two rams.

In this study, we did not take into account the age of the ewes and the previous reproductive status that could, in addition to the individual factor, influence the results and the correlations between the monitored indicators.

**Table 3. Recorded breeding rates of Palas Merino ewes artificially inseminated/naturally mounted to Australian Merino rams during natural estrous / Ratele de reproducție înregistrate de oile Palas Merino inseminare artificial/montate în mod natural cu berbeci Merinos australieni în timpul estului natural**

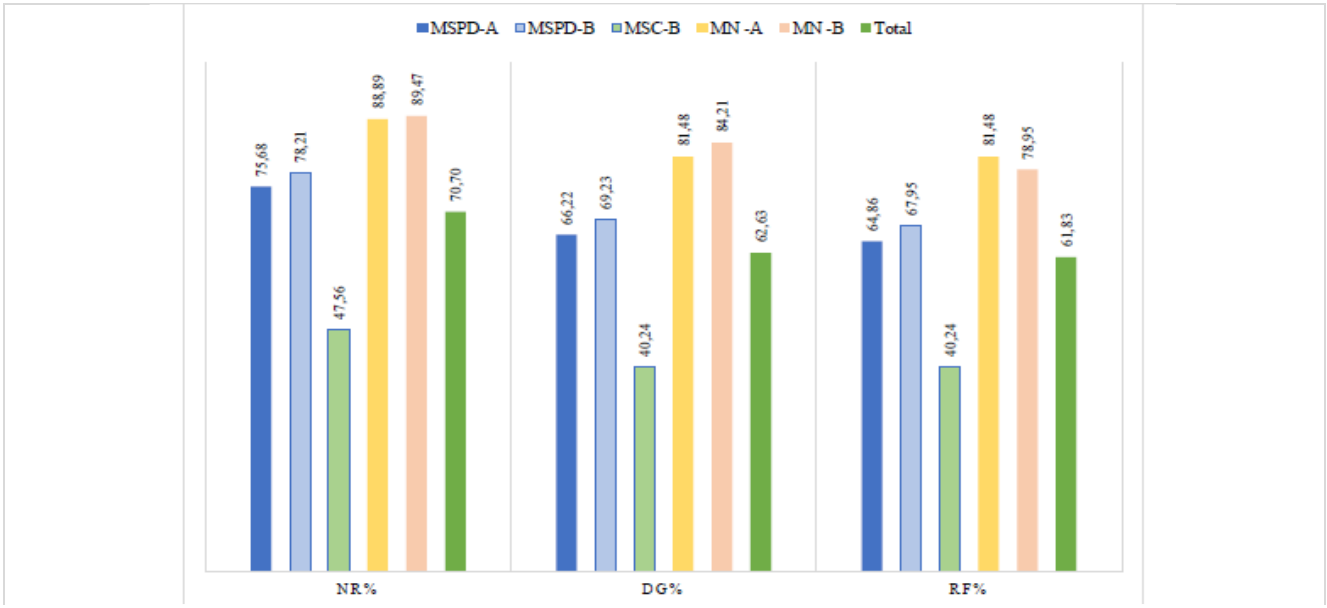
| No. crt. | No of ewes | Specification IA / MN | No. Of ewes returned to estrous | NR% | No of ewes pregnant. | DG% | No. of calves | RF% | No of lambs | P% |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 148 | MSPD-A | 112 | 75.68 | 98 | 66.22 | 96 | 64.86 | 114 | 118.75 |
| 2 | 78 | MSPD-B | 61 | 78.21 | 54 | 69.23 | 53 | 67.95 | 64 | 120.75 |
| 3 | 82 | MSC-B | 39 | 47.56 | 33 | 40.24 | 33 | 40.24 | 40 | 121.21 |
| 4 | 39 | MN -A | 32 | 82.05 | 30 | 76.92 | 30 | 76.92 | 37 | 123.33 |
| 5 | 25 | MN -B | 19 | 76.00 | 18 | 72.00 | 18 | 72.00 | 21 | 116.67 |
| Total | 372 | | 263 | 70,70 | 233 | 62,63 | 230 | 61,83 | 276 | 120,00 |

AI – artificial insemination; MN – natural mount; MSPD-A – freshly diluted semen - collected from ram A; MSPD-B – freshly diluted semen - collected from ram B; MSC-B – frozen semen - collected from ram B; MN -A – natural mount with ram A; MN -B – natural mount with ram B; NR %- – non-return rate; DG% – gestational diagnosis; RF% – calving rate; P – prolificacy

The difference between the rate of non-returns established at 15-19 days after artificial insemination/mounting and the ultrasound confirmation of the gestational state is generated by two situations: embryonic death without visible clinical signs due to the small gestational age or the females were not pregnant but did not manifest the specific signs of estrous (heats erased) or presented a short or longer estrous cycle not being captured in the monitored interval. In all five situations, the percentage of pregnant ewes is lower at the ultrasound diagnosis compared to the non-return rate. The best values are

observed in naturally mounted ewes (81.48-84.21%), followed by those artificially inseminated with fresh semen diluted (66.22-69.23%) and the lowest values are obtained following artificial insemination with frozen semen. The same situation occurs in the case of calvings, when the results of insemination with frozen semen register the lowest value (RF=40.24%). The results of this study are inferior to previous research when we performed artificial insemination on experimental lots, selected according to strict criteria, without differences between the animals in the batch, with previous normal cyclic reproduction and which were inseminated in the second estrous, therefore knowing the reproductive status at the time of insemination , the fecundity being 82% by using refrigerated semen (Nadolu et al., 2019). Superior results are recorded when artificial insemination is performed in ewes with synchronized and hormonally induced estrous, respectively 78% after artificial insemination with fresh diluted semen, but by using frozen semen the fertility was only 43% (Langford G.A et al., 1979 ).

Both artificial insemination and natural mounting being carried out during natural estrous, the prolificacy is within the specific limits of the breed (116.67 - 123.33%) not being influenced by hormones.

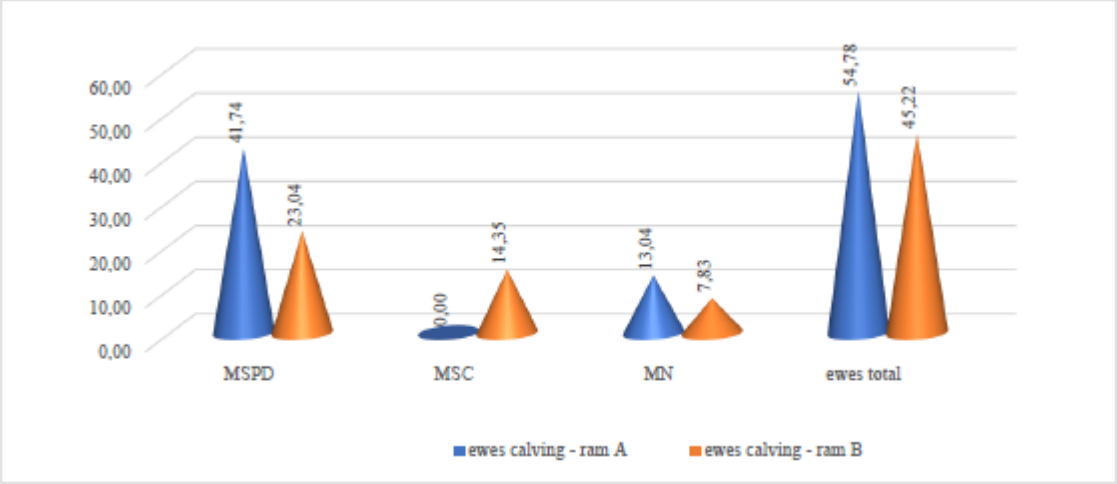



MSPD-A – freshly diluted semen - collected from ram A; MSPD-B – freshly diluted semen - collected from ram B; MSC-B – frozen semen - collected from ram B; MN -A – natural mount with ram A; MN -B – natural mount with ram B; NR %- – non-return rate; DG% – gestational diagnosis; RF% – calving rate

**Figure 2. The reproductive indices variation in Palas Merino ewes artificially inseminated/naturally mounted with Australian Merino rams during natural estrous / Variația indicilor de reproducție la oile Merinos de Palas inseminate artificial / montate în mod natural cu berbeci Merinos australian în timpul estului natural**

From the data analysis and graphical representation, the differences between the two males are observed. In natural mounting, ram B aged 6 years but with superior productive performance manages to mount fewer females (n=19) during the same time interval compared to ram A that mounts 32 ewes. Regarding artificial insemination, it is observed that the results obtained by using freshly diluted semen from ram B are better than from ram A (DG= 69.33% vs 66.22% and RF= 67.95% vs 64 .86%). These results can be explained based of the data observed at freezing. The semen of ram B, although it recorded smaller volumes, the suitability for freezing was good, and only the processed ejaculates that after freezing - thawing had a viability higher than 40% were kept in the cryobank.

During the 4 weeks study 187 ewes were inseminated/ mounted with ram A and 185 ewes with ram B, out of a total of 372 ewes.




MSPD – artificial insemination with freshly diluted semen; MSC – frozen semen; MN – natural mount

**Figure 3. The efficiency of artificial insemination use in Merino de Palas ewes during natural oestrous / Eficiența utilizării inseminării artificiale la oile Merinos de Palas în timpul estrului natural**

From figure 3 it can be seen that ram B, due to its age, could not be used for natural breeding or semen collection with the same intensity as ram A but the quality of the cryopreserved semen was good that the final percentage of pregnant ewes with this ram increased by 14.35%.

## CONCLUSION

The use of reproductive biotechnologies, i.e. semen collection, semen processing and dilution, cryopreservation and artificial insemination, increases the main reproductive indices and thus the productivity of sheep farming.

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# THE WEANING STRESS EFFECT ON CALF BEHAVIOUR AND PERFORMANCES IN ROMANIAN SPOTTED CALVES

# EFECTELE STRESULUI DE ÎNȚĂRCARE ASUPRA COMPORTAMENTULUI ȘI PERFORMANȚELOR VIȚEILOR DE RASĂ BĂLȚATĂ ROMÂNEASCĂ

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***Abstract***

*The aim of the study was to analyses the effects of weaning method on calf welfare based on body weight post weaning and behavior patterns. The study aimed to set the most appropriate weaning method in order to ensure the calves welfare. Study was carried out on 120* ***Romanian Spotted*** *calves weaned at 60 days of age (60 calves) either abruptly or with a 14-day pre-weaning period (60 calves). Comparisons between the weaning methods for body weight and feeding behavior were performed using one-way ANOVA assay with categorical factor "weaning method". A positive effect of the gradual weaning was observed on calves' body weight post calving. The abrupt weaning induced an 8.4 % loss in calves body weight within the first 10 days after weaning compared to 4.1 % recorded in gradual weaning (P≤0.001). Gradually weaned calves allocated 174.11±6.31 min. / day for feeding divided into 9.2±0.61 meals compared to 104.26±9.71 min. / day feeding and 5.22±0.34 meals (P≤0.001) recorded in abruptly weaned calves. Performing gradual weaning decreases loss of calves' body weight also increasing the feeding behavior patterns.*

***Keywords****:* ***behavior, calves welfare, Romanian Spotted, weaning method***

***Rezumat***

*Studiul urmărește efectele metodei de înțărcare asupra condiției de bunăstare a vițeilor, evaluată în baza dinamicii greutății corporale post înțărcare și a manifestărilor comportamentale specifice categoriei de vârstă. Scopul acestei analize a fost stabilirea unei metode de înțărcare fiabile în încercările de a asigura vițeilor o bunăstare deplină. Observațiile s-au desfășurat pe un lot de 120 de viței de rasă* ***Bălțată Românească****, înțărcați la vârsta de 60 de zile, fie prin înțărcare bruscă (60 viței) fie prin asigurarea unei perioade de preîncărcare de 14 zile (60 viței). Comparațiile în ceea ce privește efectele metodelor de înțărcare asupra greutății corporale și a comportamentului s-au efectuat în baza protocolului ANOVA în care a fost inclus factorul de influență "metoda de înțărcare". Un efect pozitiv semnificativ asupra dinamicii greutății corporale post înțărcare a fost asociat metodei graduale. Înțărcarea bruscă a vițeilor a indus o pierdere în greutate de 8,4% în primele 10 zile după sistarea dietei lactate comparativ cu un regres de doar 4,1% asociat înțărcării treptate. Vițeii care au beneficiat de perioada de pre înțărcare au alocat consumului de furaje un interval mediu zilnic de 174,11±6,31 min., interval împărțit în 9,2±0,61 tainuri zilnice, comparativ cu cei înțărcați brusc, care au prezenta o durată redusă de consum 104,26±9,71 min. asociat unui număr de doar 5,22±0,34 tainuri (P≤0.001). Rezultatele obținute pledează evident în favoarea implementării înțărcării treptate a vițeilor, pierderile în greutate fiind semnificativ reduse în paralel cu accentuarea manifestărilor specifice comportamentului de ingestie a furajelor.*

***Cuvinte cheie****:* ***Bălțată Românească, bunăstare viței, comportament, metodă de înțărcare***

## INTRODUCTION

The weaning of calves is necessary in order to improve the farm's efficiency, reproduction activity of the dams and not at least to increase the quantity of milk available to humans (Meyers et al., 1999). Due to the weaning method several issues may occur both in dams or calves. The weaning constitutes as a second major stress factor after birth. Intensity and duration of the calves' response depend on many influential factors such as weaning method, age at weaning, housing or feeding program. The calves body weight decreases and behavioral patterns suggest a negative emotional state being essential elements in assessment of animal welfare condition (Sweeney et al., 2010). Generally, the weaning represents the total independence of offspring from their dam and ability to survive on its own. Despite of its evidence, the endpoint of natural weaning is largely unknown and it is reported to vary surveys. Knowledge of weaning effects implies understanding of calves responses (body weight dynamics, behavior patterns and health status) according to "*honest signals of needs*" (Miller K., 2011). The negative effects of dams are intensely studied in beef breeds due to the natural nursing witch exert a negative impact on somatic development and subsequent performances (Borderas et al., 2009). In dairy or dual purpose breeds, the studies aim on the one hand the economic efficiency of farms according to the milk quantity and on the other hand, the efficiency in calves rearing assessment on daily gain and its costs. Economy is common practice to early weaning allowing use of vegetable forage in order to reduce costs. The intensive rearing systems require and imposes a technological efficiency in weaning, early and abrupt weaning being most often chosen. The effects of abrupt weaning are obvious in both calves' body weight dynamic and behavioral patterns. Moreover, undesirable behavioral patterns occur (frequent agonistic rounds, prolonged exploring behavior, reduced meals frequency). The institutional requirements as well as consumer concerns regarding animal welfare claim intensive and varied researches about the impact of applied technology on animal condition. The aim of the current research was to assess the effects of weaning method on calves' body weight dynamic and behavioral response.

## MATERIAL AND METHOD

The study was carried out on livestock farm from the Research and Development Station for Bovine Arad, Romania (location: 46° 10' 36" N, 21° 18' 4" E, 107 m altitude, 582 mm annual average rainfall 21°C / -1°C average of temperature corresponding seasons summer / winter). In the current study, 63 Romanian Brown calves (41 females and 22 males) were randomly included. Calves were from eutocia, singles (n = 59) or twins calving's (n = 4 calves). Calves were separated from their dams within the first hour after birth and moved either individually (1.85 x 1.85 m) in straw-bedded pens, in the maternity until 14 days of age. Up to 90 days of age, the calves were housed outside the maternity barn in group pens (6 heads / box) up to weaning (except 3 calves housed individually due to several health issues).

The calves were fed with milk replacer (10 % of body weight) 6-8 kg / day (95 % DM, 22 % CP and 18 % Fat), alfalfa hay (84 % DM, 13 % CP) and concentrates (88 % DM, 17% CP, and 2.5 % Fat) ad libitum. The calves had constant visual and auditory contact with congeners from neighbouring pens, both in maternity and in external facility. The weaning was abruptly for 30 calves and gradual for another 33 calves, with 10 days pre-weaning period, 20 between day 90 and 100. After weaning, the calves were moved in the common paddock (n=63) where the feeding was allowed throughout 24 –h period with vegetal fodder and concentrate mixture. For all calves we recorded the body weight at weaning day and within the first 10 day after. Also, feeding behavior patterns were recorded. Behavior patterns were recorded for 24 hours in the weaning day (day 90 or day 100 for calves weaned gradually) using 4 cameras (Sony HDR-CX24OE). The recorded data were analyzed by The Observer XT specialized software. Data were expressed as means ± standard errors. Comparisons between calves were carried out

using the one-way ANOVA protocol, with categorical factor being considered "*weaning method*". All the statistical inferences were carried out using Statistica® software v. 13 (Hill and Lewicki, 2007). Decisions about the acceptance or rejection of statistical hypothesis have been made at the 0.05 level of significance. This research was mainly observational and non-invasive. Ethical considerations of this study were evaluated according to the European Union's Directive for animal experimentation (Directive 2010/63/EU) and were approved by the Scientific Council at the Research and Development Station for Bovine Arad (No. 50/29.10.2015).

## RESULTS AND DISCUSSION

The weaning method exerts obvious effects on calves' body weight dynamics in the post weaning period. Pre-weaning feeding programs play an important role in both calves´ body weight and distress. Early feeding of forage allows a proper development of the rumen. Increasing the forage dietary can lead to a decrease level of stress by reducing the calves' milk requirements. Previous surveys have shown that calves that drink milk as much as they wish were healthier, recorded fewer visits to the vets, produced more milk as adults and shown an increased daily gain compared to calves nursed in conventional milk feeding program.

Although pre-weaning average daily gain is reduced, the differences fade post weaning, the calf's body weight being comparable (Gorgulu M., 2012). Higher milk amounts are desirable from an economic point of view in order to ensure an increased average daily gain (Diaz et al., 2001). However, the milk allowance and duration of the pre-weaning period influence the calves' body weight dynamics. The results obtained in the current research show decreased tendency of calves' body weight post weaning in both abrupt and gradual weaning methods, the intensity of its being different.

On the weaning day, no significant differences ($P>0.05$) were recorded between the studied calves (98.3±2.24 vs. 97.72±0.38 kg). The post weaning weights dynamics meets a different tendency, according to weaning method. The abrupt weaning calves recorded a decrease in body weight (98.3±2.24 vs. 92.16±0.59 kg, $P\leq0.004$) representing 6.6 % compared to gradual weaning calves recording a 3.73 % loss (97.72±0.38 vs. 94.2±0.26 kg, $P>0.074$). The length of the pre-weaning period exerts positive effects on calves´ body weight dynamics.

In this respect, the 10 days of pre-weaning period allocated in the current study proved to be sufficient in order to record a lower loss in body weight for gradual compared to abrupt weaning calves, similar results have been reported in previous research also by Sweeney et al., (2010). The calves' body weight dynamics proved to be strongly influenced by preweaning feeding strategy.

Intensified milk feeding programs providing higher milk amounts are able to determine the calves' growth dynamics in pre and post weaning time. Calves included in intensified milk feeding programs providing more milk amounts are able to increase their nutrient intake leading to a greater body weight compared to calves associated with conventional program characterized by restricted amounts of milk. (Appleby M., 2001; Jasper and Weary, 2002). This strategy allows an increased daily gain reach up to 1.2 kg / day. Comparatively, the conventional milk feeding program used in our study allows an average daily gain up to 0.65 Kg / day in pre-weaning follow by a loss of weight range between 3.6-6.2 % post weaning according to weaning method for both gradual and abrupt method respectively. Previous surveys regarding the influence of higher milk amounts in calves weight gain suggest a favorable impact on pre-weaning up to 100 g / day.

Also, the intensified milk feeding programs allow a growth tendency of calves' biometrics measurements in both, pre and post weaning. In dairy farms, intensified milk feeding programs involve increased costs. There are studies that proved these supplementary costs will be amortized during the first lactation (Gonyou and Stookey, 1987). The greater body weights as a result of pre-weaning intensified milk feeding programs could be maintained in post weaning period (Jasper and Weary, 2002).

According to conventional milk feeding program used in our study, the results recorded a significant body weight loss (P≤0.004) in abrupt compared to gradual weaning method. Slightly decrease of body weight was recorded for gradual weaning calves, according to time spent for it and meals frequency.

**Table 1. Means (±standard error) for calves behaviour patterns according to weaning method / Mediile (±eroarea standard) manifestărilor comportamentale ale viţeilor în raport cu metoda de înţărcare**

| The weaning method | Parameters | Feeding behavior (min.) |
|---|---|---|
| **Abrupt weaning (60 calves)** | Meals frequency | 5,7±0,37[a] |
| | Meal average duration | 16,19±1,36[a] |
| | Daily meal duration | 92,34±2,74[a] |
| **Gradual weaning (60 calves)** | Meals frequency | 9,2±6,31[b] |
| | Meal average duration | 18,92±1,4[b] |
| | Daily meal duration | 174,11±6,31[b] |

a,b Columns mean with different superscripts differ significantly at p≤0.05

Feed consumption is strongly influenced by technological factors. Particular importance is exercised by the calves' age of providing forage in ratio, forage quality and the amount of milk available in preweaning period. The confinement system exerts an important role, the common rearing being an incentive for calves feeding behavior. In our study, calves were reared in pairs up to 14 days of age, in common pens (6 head / pen) up to weaning and in the common paddock (30 heads / paddock) post weaning. The concentrate forage was administered since 4th day of age and water was freely available. A conventional milk feeding program with restricted milk allowance (10 % of body weight) was used. The vegetal diet structure was maintained in post weaning compared to the pre-weaning period in order to stimulate the consumption, the calves do not change their preferences. According to a conventional milk feeding program which provides restricted milk allowance, the current study recorded significant differences (P≤0.001) regarding meals frequency in gradually (8.1±0.31 meals / day) and abruptly weaned calves (6.3±0.17 meals / day).

Gradually weaned calves show an increase meals frequency (P≤0.001) in the post weaning period compared to abruptly weaned calves. Gradually weaned calves show an increased meals frequency comparable to meals frequency in calves feeding with higher milk amounts reported in previous surveys. Myers et al., 1999; Bach, 2012). Conventional milk feeding program in the pre-weaning period leads to an increased forage consumption and prolonged daily meal time. The effect was maintained also in the post weaning period. Nevertheless, prolonged meals do not indicate a high feed consumption. In intensified milk feeding programs and in abruptly weaned calves, previous studies found a negative correlation between meals time and forage intake. The current study found an average meal time of 14.9±0.3 min. / meal for abruptly weaned calves compared to a significant increased (P≤0.036) average meal time of 18.7±0.84 min. / meal in gradually weaned calves. These results were found by Miller et al., 2011) in a previous study (15.4 min. / meal). Conventional milk feeding program used in our study allowed a total daily meal range between 93.87±6.11 min. / day for abruptly and 151.47±4.28 min. / day for gradually weaned calves. Reducing of the milk amount administered further ceasing of it leading to an upward trend of forage feeding daily time in calves with range between 93.87±6.11 min. / day and 151.47±4.28 min. / day (P≤0.001) in abrupt and gradual weaning. These outcomes are similar to previous results reported by Overvest et al., 2016 (87-155 min. / day at weaning) with higher values in post weaning period (over 180 min. / day).

## CONCLUSIONS

The weaning method has been shown to exert a significant influence in calves body weight dynamics, inducing a lose weight particularly in abruptly weaned calves. The current study recorded a weight lose up to 6.2 % in abruptly compared to 3.6 % in gradually weaned calves. The daily meals

frequency associated to abruptly weaned calves was lower (6.3 meals / day) compared to the higher frequency (8.1 meals / day) recorded for the gradually weaned calves. Also, total meals daily time have been reduced in abrupt weaning (93.87 min. /day) compared to 151.47 min. / day for spent by gradually weaned calves. The current findings still remain contradictory comparing to others previous studies, requiring further researches which include the specific parameters.

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# A RISK-BASED „ONE HEALTH" PERSPECTIVE APPROACH - THE FUTURE OF DAIRY FARMS MANAGEMENT

# VIITORUL MANAGEMENTULUI FERMELOR DE VACI PENTRU LAPTE: ABORDAREA CONCEPTULUI „ O SINGURĂ SĂNĂTATE" PRIN ANALIZA DE RISC

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***Abstract***

*All food businesses, including food primary producers, are accountable on a legal level for ensuring the quality of their products. Primary production is the first crucial stage in the food chain, where food safety and traceability must be guaranteed. The maintenance of high-quality standards for milk and dairy products continues to be a challenge for farmers and manufacturers, who in turn ask the scientific community to provide them with appropriate tools for hazard identification and risk management. This overview describes the novelties of an integrated risk-based system that relies on the HACCP principle, corroborated by the incorporation of technological innovation, and the alerts from the multidisciplinary interactions of environment, animal health, and food safety SMART key indices of the "One Health" profile concept. This represents the future benchmark for primary dairy production. Here, farm characterization, agriculture practices (with particular attention to main crops, management of pesticides and fertilizers, and degree of groundwater pollution), animal nutrition (with particular attention to feeding quality, safety, and origin), animal health and welfare (anti-microbial and anti-parasitic drugs usage and management, udder health, etc.), milking techniques, milking parlour hygiene, and their consistent risk analyze are the main inputs used to develop a custom-focused grid of instruments for the top platforms in order to track a panel of analytical variables, unique to each dairy production farm. The overcoming benefit is that the above resumed integrated method of dairy production takes into account the effect that products entering and exiting the dairy farm have on the health condition of the larger network and ensures that food safety begins on the farm and does not negatively affect the environment or downstream consumers.*

***Keywords: one health, hazard analysis and critical control point, risk assessment, risk management;***

***Rezumat***

*Toți operatorii din sectorul producției agro-alimentare sunt responsabili din punct de vedere juridic de calitatea și inocuitatea produselor lor. Producția primară este prima verigă a lanțului alimentar unde este necesară garantarea siguranței alimentului și trasabilitatea produsului. Menținerea standardelor de calitate a laptelui și / sau produselor lactate reprezintă o provocare continuă pentru fermieri, dar și producători, care, la rândul lor solicită comunității științifice furnizarea de instrumente eficiente în identificarea pericolelor și gestionarea riscurilor asociate. Lucrarea de față descrie rezumativ noutățile unui sistem integrat, bazat pe evaluarea riscurilor directe dar și a celor asociate conform principiilor HACCP (Sistem de analiză a riscurilor și determinare a punctelor critice de control), coroborat cu integrarea inovării tehnologice exprimate prin alertele din interacțiunile multidisciplinare dintre mediu, sănătatea animală și siguranța alimentului, indicatori SMART ai conceptului „O Singură Sănătate". Acest concept reprezintă viitorul în definirea criteriilor de referință pentru producția primară de produse lactate. Aici, caracterizarea fermelor, practicile agricole, nutriția animalelor, sănătatea și bunăstarea lor, tehnologia mulsului, igiena sălii de muls și analiza consecventă a riscurilor potențiale la nivelul fiecărei etape din matricea proceselor sunt principalele elemente de intrare utilizate pentru a dezvolta un panel personalizat de instrumente pentru platformele de top în vederea exprimării printr-o serie de variabile analitice unice pentru fiecare fermă de vaci de lapte. Avantajul principal al acestei metode integrate la nivel industrial ia în*

*considerare efectul tuturor intrărilor dar și ieșirilor din ferma de vaci pentru lapte asupra stării de sănătate a rețelei de consumatori, fără efecte negative asupra mediului.*

***Cuvinte cheie: O Singură Sănătate, HACCP (Sistemul de analiză a riscurilor și determinare a punctelor critice de control), analiza de risc;***

## INTRODUCTION

The European White Book is a comprehensive guide that highlights the utmost importance of ensuring food safety in the food industry. It emphasizes that all food operators, from primary producers to manufacturers and distributors, have a significant ethical, scientific, and legal responsibility to ensure that their products are safe for consumption. This responsibility cannot be taken lightly, and it is essential that all parties involved take the necessary steps to guarantee that their products are of the highest quality and meet all safety standards. Any negligence or oversight in this regard can have severe consequences, not only for the consumers but also for the reputation of all operators from the food chain, including primary processors, like dairy farms [8].

Additionally, there is a growing awareness of issues relating to animal health and welfare. This is exemplified by past occurrences such as the BSE epidemic, the dioxin problems, and the *Salmonella* survey that affected various species in recent years. However, it is important to recognize that in recent years, there has been a growing emphasis on the interconnectedness of humans, animals, and the environment. This concept, known as *One Health*, has led to an increased understanding of the importance of prevention and safety measures in primary livestock production. It is important to understand that the issues we face today cannot be solved by a single solution. Instead, a comprehensive and all-encompassing approach is required to navigate these challenges successfully. This is where the One Health/One Medicine philosophy comes into play. This approach recognizes the interconnectedness between animal, human, and ecosystem health and seeks to improve them all simultaneously. By adopting a holistic mindset, we can create sustainable solutions that not only address the immediate problems but also ensure long-term health and well-being for all [14].

In order to ensure the well-being of dairy cattle and the safety of the products they provide, it is essential to prioritize access to clean water, safe food, and proper hygiene. These key components are integral to maintaining the health of dairy herds and preventing the spread of disease *(Fig. 1*).

Also, the One Health approach to dairy production medicine and management considers the interconnectedness of human, animal, and environmental health. By implementing effective farm management systems, such as *Dairy Dynamic Management*, and practicing the prudent use of antimicrobial agents, we can address these critical issues and promote the overall health and sustainability of the dairy industry. It is important to consider the entire system of dairy production, from animal welfare to environmental impact, in order to ensure a safe and responsible food supply. By prioritizing these key components and adopting a holistic approach, we can support the health and well-being of both dairy cattle and the communities they serve.

As we continue to learn more about the complex interactions between these various elements, it becomes evident that a comprehensive approach to food safety is essential, with prevention and safety measures at the level of primary production taking precedence, implying the following areas of approach: critical control points in the production of dairy products, prevalence of antibiotic-resistant mastitis pathogens, foodborne pathogens from farm to table, prudent use of antibiotics, residues of antibiotics in milk, dairy dynamic management and with big importance alternative treatments to antibiotics (*Fig. 1*).

To achieve this goal, food business operators [FBOs] often rely on a comprehensive and science-based approach to food safety known as the Hazard Analysis and Critical Control Point (HACCP) system or commonly named risk analysis. This system is designed to identify potential hazards and risks associated with food production and distribution and to establish specific control measures to mitigate

these risks, which implies that the farmer's agricultural practices align with animal welfare standards in addition to following the guidelines for food safety [2, 7].

The implementation of the Hazard Analysis and Critical Control Points (HACCP) methodology in the context of environmental changes and animal health management in dairy farms is a sensible and rational decision that encompasses seven guiding principles and twelve developmental steps. The 7 principles that have been established are primarily aimed at facilitating effective risk assessment, risk management, and the implementation of specific documentation and verification procedures. Additionally, they emphasize the importance of maintaining accurate and up-to-date documentation, as well as implementing rigorous verification procedures to ensure that all processes and procedures are functioning as intended [2, 7, 12].

The implementation of these management techniques in the dairy industry serves to reinforce the notion that worldwide dairy producers are committed to supplying milk that is not only safe and nutritious but also yields numerous health benefits. It is crucial that these practices are adopted in developing countries as well, as they have the potential to vastly improve the overall nutrition, health, and well-being of their populations. By prioritizing the implementation of these techniques, we can ensure that people across the globe have access to the same high-quality dairy products that we enjoy in the European Union.

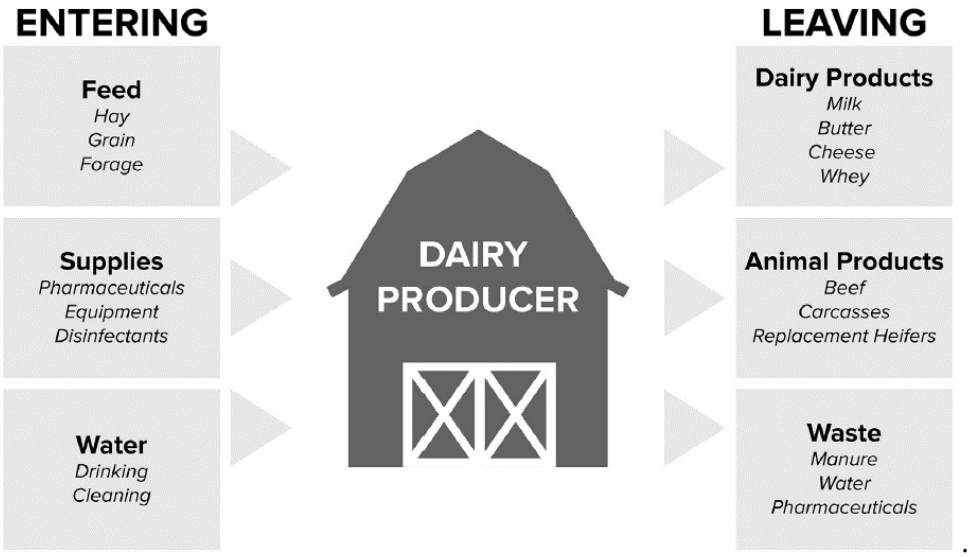


**Figure 1. The visual translation of the "One Health" concept by centralizing the active participatory factors within a milk cow farm / Translatarea vizuală a conceptului "O Singură Sănătate" prin centralizarea factorilor participativi activi în cadrul unei ferme de vaci pentru lapte [8, 12, 16]**

Further, in this paper, we will explore „*an example*" how the implementation of HACCP principles can effectively control potential public health threats on dairy farms that are accessible to the general public, named „***multifunctional farms***". It will be described a clear example of a HACCP principles risk analysis for the biological hazard "***cuddling of the animals***" by the visitors. Multifunctional farms serve multiple purposes simultaneously. They may include commercial dairy farms that produce milk and sometimes cheese, but also offer the general public one or more additional services, such as camping or hosting groups of children. These farms provide educational opportunities, training, information, a venue for social gatherings, recreational amenities, animal "therapy," the opportunity to taste fresh animal products, and — for young children — "animal snuggling" [14].

Multipurpose farms actively encourage the equitable participation and access of children, adolescents, and adults through hands-on learning opportunities in a variety of social, recreational, educational, and professional endeavors. These initiatives are centered on agriculture, enabling

individuals to better their own lives and the environment. The necessity for such farms has grown over the past few decades, not the least of which is the widening knowledge gap between professional agriculture and urban residents. From the perspective of public health and safety, it is important to think about how HACCP may be applied to these kind of farms. It is important to mention that there is a specific "*hygiene code for city farms*" in various nations [13].

## 2. UTILIZING HACCP PRINCIPLES TO ENSURE PUBLIC HEALTH SAFETY ON OPEN DAIRY FARMS

### 2.1 Potential activities and services of city farms

City farms and commercial dairy farms that are accessible to the general public can offer a wide range of activities and services. Whatever activities and services are precisely offered depends primarily on the requirements and traditions of the host community. A type of list of these initiatives and services implies:

- food growing and food production in community gardens, with instructions;
- animal handling, animal husbandry and animal welfare-related activities;
- conservation and nature preservation-related activities (farm camping);
- young children's activities, including animal cuddling;
- young children city farm clubs (play schemes; excursions);
- senior citizen city farm clubs (including grandparents with grandchildren);
- visits and programs for particular groups (e.g. mentally disabled persons);
- venues for arts and crafts workshops (classes; demonstrations; practicals)
- visits of school classes; activities for instructing school children;
- summer holiday camp for children;
- a social meeting place in the format of a „farm café";
- venue for seasonal festivals, special events, or celebrations;
- evening recreation, conservation projects, and training programs;
- venue and support for local self-support groups;
- the basis for volunteering and for learning new skills;
- production of (raw) food products of animal origin.

In terms of public health, animal health, and animal welfare, farms with animals are required to protect both their visitors and the animals from potential risks. This includes bodily risks (trauma) brought on by an aggressive animal or unexpected behavior. In that regard, they are also required to abide with the General Food Law (EC regulation 178/2002). The hygiene directives (EU 852/853/854-2004) must also be followed by these farms when they offer visitors the ability to create food items on-site [14, 17].

### 2.2 Dairy farms open to the general public: context

The number of dairy farms has significantly decreased in many European countries and is likely to continue to decline in the upcoming years for the reasons outlined below. The choice to abandon farming is frequently influenced by urbanization and shrinking economic margins between farm income and production costs. In addition, many young people no longer have the desire to take over the farm. To boost herd productivity per man and per hectare, remaining farmers frequently expand their herds and adopt new technology. By using services or products targeted at residents as a method to create a „*better*" living in the future, hazards can occasionally be turned into opportunities [9].

Along with economic advantages, the growth of welcoming visitors to farms is frequently advantageous for a better public perception of the agricultural industry. Today's general populace knows very little about agricultural production. Only a small percentage of people have a history with farming, connections to farmers, or a sense of affinity with the place where the food they purchase originated. Strict hygiene regulations and the upscaling of farms are to blame for this. As a result, the public's

perspective is largely shaped by incidents involving animal health and food safety, as well as, the media's portrayal of those events [18].

The public perception of bioindustry and modern farm management, such as dairy farms with robotic milking or farms where cows are housed indoors all year round, might be harmed by negative press from animal welfare organizations. To sustain its right to exist, the agricultural sector, and especially more so in nations with dense populations, needs the compassion and understanding of the general public. By allowing people to learn more about the industry, farms that are accessible to the general public can help with this [4].

Direct animal interaction is one of the many created goods and services that include bed and breakfast, traditional or organic food production, camping, activities, and sports. The target audience for this service may include children, adults with physical or mental disabilities, or those looking to renter the workforce. In addition to the benefits of making farms accessible to the public, hazards may also exist [14].

This section uses the on-farm service of "***animal hugging***" as an illustration of how the HACCP principles be used to control public health hazards on dairy farms that are open to the public. By using HACCP, businesses can give customers more assurance regarding the calibre of their goods and services. Such an application's main goal is to protect users from potential dangers. Moreover, examples of and discussions about the potential (advisory) role of veterinarians in helping farmers apply HACCP will be provided [1].

**2.2.1 The HACCP team (step 1 of the 12 developmental steps in HACCP)**

The interdisciplinary HACCP team must be formed` before HACCP principles are used, as was described by *Codex Alimentarius*. The HACCP team will be tiny if there are just one or two individuals managing the farm, which is typical on most dairy farms. It is possible that in a small HACCP team, not all expertise is instantly available since distinct skills, particular knowledge, and experience suited to the product or process are needed for the formulation of a HACCP plan. It is advised in such circumstances to enlist external assistance for particular areas [5].

In areas like hygiene, public health (zoonoses), animal health and welfare, or animal handling practice, veterinarians with training in quality risk management and veterinary public health can be very helpful. So, the farmer, an employee, and a veterinarian can make up the HACCP team. As necessary, specialists can be added to the HACCP team to provide guidance in specific areas, such as caring for certain target visitor groups or building appropriate housing for animals used for cuddling [10].

**2.2.2 Description of the provided service and the target group (step 2 and 3 of the 12 developmental steps in HACCP)**

Once the HACCP team has been put together, it is necessary to provide a detailed description of the targeted service, as well as, the target group itself. The phrase "***animal snuggling***" which is used as an example in this paper, can be presented in a variety of ways. Either directly or through a fence, people can interact with animals. Also, the service can be targeted at various demographics and have a variety of goals. Contact with animals not only makes kids happy but also helps the sick, addicted to drugs or alcohol, overworked, and disabled individuals by instilling in them a sense of duty, self-worth, or positive sensations. The target group's mental and physical health may necessitate higher safety standards for the service.

Before choosing animals for animal cuddling, the service must be precisely defined. Some animal species or humans will be better suited for animal cuddling than others, depending on the service's objectives and its intended audience. Animal characteristics like age, body size, and natural behaviour must be considered, but unpredictable animal and possibly human behavior must also be considered when designing the selection and Quality Risk Management Program [17].

### 2.2.3 Development of flow diagrams of the production process (step 4 and 5 of the 12 developmental steps of HACCP)

Where appropriate, interactions between the various steps of a production process are also shown in a flow diagram together with their logical and chronological order. Several production procedures must be completed before the intended product, "*raw milk*," is shipped to the industry. These phases can be shown in the general flow diagram used to convey an overview description of the process steps. Detailed diagrams or charts can be used to work out secondary, detailed procedures like feeding various animal species, utilizing land, and treating animals while being fed. In addition to the previously mentioned basic flow diagrams for the raw milk production process, flow diagrams for services targeted at visitors must be produced in order to apply HACCP principles on dairy farms that are accessible to the public. *Fig. 2.* shows an illustration of a particular flow diagram of the animal cuddling procedure. When dairy farming and pet-holding procedures clash, complexity might result. To visualize interactions and points of contact between humans and animals, a (full) specification of the flow diagram must be produced depending on the farming areas where the service of "*animal cuddling*" is offered. For instance, animal hugging can be provided during calving, cow feeding, snuggling dry cows, feeding calves, and calves' grouping [16, 19].

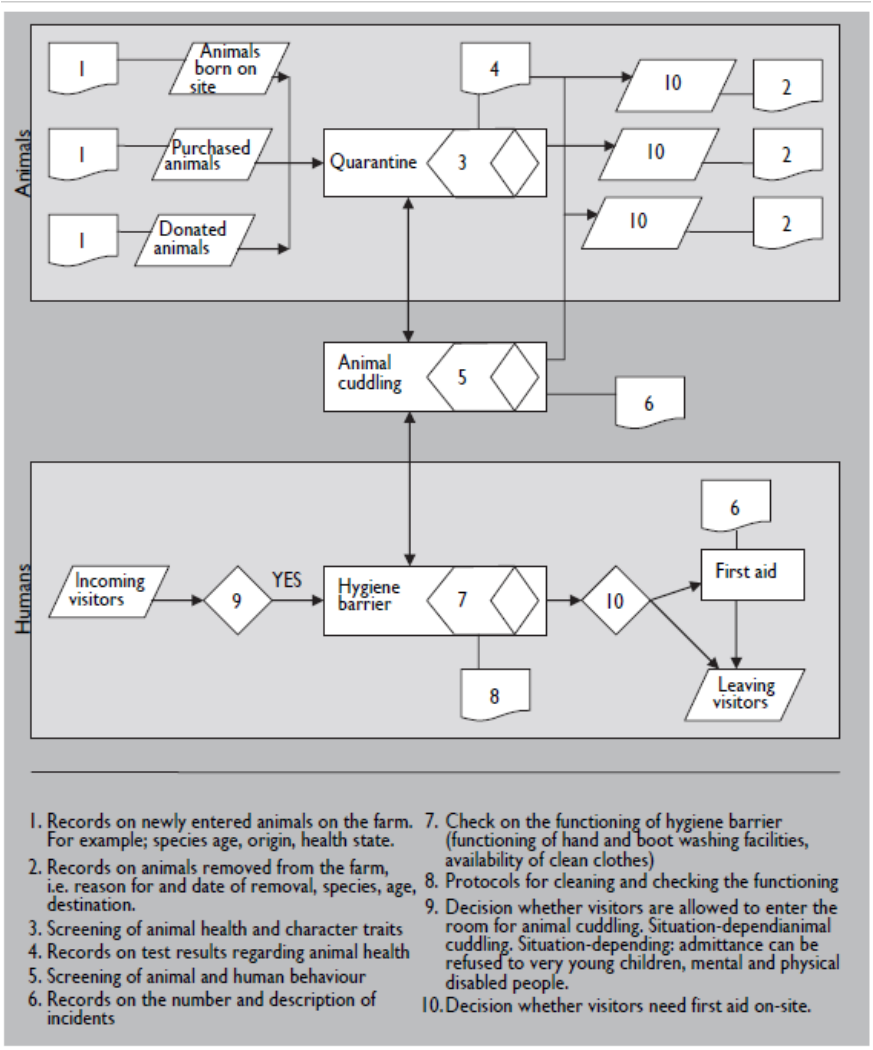


**Figure 2. General flow diagram showing the phases of the interactive process between mean with animals by the "animal cuddling"/ Diagrama generală de flux a etapelor procesului de interacțiune a omului cu animalele prin "îmbrățișarea lor" [18, 19]**

Before the HACCP team can audit the procedure to confirm that each step in the diagram is an accurate reflection of the actual scenario, the general flow diagrams discussed above must be modified to the unique circumstances of a single farm. The flow diagrams will raise awareness among team members and other farm visitors and will enable additional discussion about hazards and risks within the HACCP team.

Physical risks have a close connection to both animal and human behaviour. Individuals may sustain injuries as a result of animals biting, kicking, or scratching them, as well as, from poorly maintained buildings, fences, or machinery [21].

**2.2.4 Hazard analysis (step 6 – principle 1 in the 12 developmental steps of HACCP)**

Microbiological, chemical, and physical hazards and risks are the three most common categories of hazards. On dairy farms, unique managerial risks and hazards are identified. Keeping in mind the "*animal cuddling*" service, the risks can be separated into two categories: risks that affect the quality and quantity standards of the targeted product of animal origin and risks that affect the safety of the public's activities (zoonoses and injuries). In addition to having an impact on the amount and quality of raw milk, for instance, risks to animal welfare and the health of the production system might affect consumers that will receive products and services. Different risks and dangers must be evaluated depending on the product or service [1].

Also, microbiological dangers such as zoonotic bacteria, viruses, and parasites may be present on animal skin and in an area contaminated by animal faces (e.g., manure). According to the animals chosen for snuggling, their health status, and regional and national variations in the incidence of zoonotic pathogens, the precise results of the (microbial) hazard analysis will vary on each farm [6].

In case of Europe, pathogenic *Enterobacteriaceae*, such as *Salmonella spp., Campylobacter jejuni, E. coli O157,* and *Yersinia enterocolitica*, are zoonotic bacteria that can be common without symptoms. In addition, zoonoses such as dermatophytosis, zoonotic scabies, infectious ecthyma, giardiasis, and cryptosporidiosis should be considered, especially for elderly and young people, as well as, those with impaired immune systems. We must not ignore the possibility that brucellosis or tuberculosis still provide a risk to humans in some nations where they have not yet been completely eradicated.

People may come into touch with chemical risks on some public farms, such as cleaning supplies, pesticides, herbicides, and veterinary medications, when these supplies are, for example, left lying around the farm. Also, when animals are treated, such as with pour-on treatments of veterinary antiparasitic medications, chemicals can be discovered on their hair coats.

**2.2.5 Risk Assessment (step 6 of the 12 developmental steps of HACCP)**

The HACCP team will talk about the risks and determine which risk factors are prevalent on the farm throughout the risk assessment process. The team must decide the importance and priority of each hazard to be handled during the procedure. Risk assessment can be done using locally available veterinary epidemiological data or using an approach called adaptive conjoint analysis which gathers, validates, and ranks expert opinions on a given topic (such as the applicability of specific risk factors for disease). The third way to assign a weight to hazards is to qualitatively evaluate both their likelihood of happening and their potential effects should they do so. To the best of their knowledge and experience, the members of the HACCP team undertake this weighing. Focusing on the risks associated with "*animal hugging*," which are outlined in Table 1, other risk factors can be identified [20].

Visitors who have direct contact with animals and those who are present in an environment that is contaminated with animal waste (such as manure) have significant risk factors in connection to the threat posed by "*animals harboring zoonotic diseases*". Also, many visitors to farms that are open to the public, such as young children and pregnant women, tend to be in a more weakened condition of health, making them more susceptible to skin lesions than other visitors (*Table 1*).

**Table 1. Risk factors associated to biological hazard concerning the pathogenic zoonoses transmission / Factori de risc asociați hazardului biologic privind transmiterea zoonozelor patogene [19]**

| |
|---|
| Direct contact of humans with animals |
| A stay in an environment which is contaminated with animal waste (manure; scabs) |
| Lesions of the human skin |
| Fragile state of health of visitors (young, old, pregnant, immuno-deficient people) |

Besides zoonotic disease infections, other significant risks include head injuries from animal contact, animal bites, scratches, and animal kicking. Unpredictable animal behavior and visitor target group behavior can both be risk factors for these dangers (*Table 2*). Also, persons who have little expertise in handling animals and little knowledge of typical and abnormal animal behavior are more in danger than, say, well-trained animal keepers [20].

**Table 2. Example of some specific risk factors for a certain hazard / Exemplu a unor factori de risc specifici unui anumit hazard [19]**

| |
|---|
| Direct contact of humans with animals |
| Contact with animals through a fence |
| Unpredictable behaviour of animals (e.g. mother behaviour; male behaviour) |
| Unpredictable/undesirable behaviour of visitors due to e.g. ignorance, health or mental state |
| Limited knowledge of visitors about animal behaviour |

To enable control, the targets for each POPA and each CCP standard or tolerance must be established. The circumstances and objectives of each dairy farm must be taken into consideration while setting targets. For the majority of CCPs and POPAs shown in *Tables 3* and *4*, stated objectives may be set with a zero-tolerance policy, such as prohibiting visitors from entering certain farm areas. Objectives for the POPA's "*Exposure of visitors to animal waste is as low as achievable*" standard can be established in reference to bacterial plate agar counts, which are common in the food business. For instance, allowing no more than a particular amount of colony forming units per $cm^2$ for aerobic growth and having zero tolerance for *Enterobacteriaceae* on the plate agar obtained from a dinner table. Furthermore, it suggests that a sanitation strategy needs to be implemented.

**Table 3. CCP and POCA associated to microbiological hazards at the zoonotic carries animals / PCC și PA asociate hazardelor microbiologice la animalele purtătoare de patogeni zoonotici**

| | |
|---|---|
| No contact between visitors and animals is allowed | CCP |
| Animals are free from selected zoonotic pathogens | CCP or POPA[1] |
| Exposure of visitors to animal waste materials is as low as possible | POPA |
| Isolation of sick animals | CCP[2] |

Besides from zoonotic disease infections, other significant risks include head injuries from animal contact, animal bites, scratches, and animal kicking. Unpredictable animal behavior and visitor target group behavior can both be risk factors for these dangers. Also, persons who have little expertise in handling animals and little knowledge of typical and abnormal animal behavior are more in danger than, say, well-trained animal keepers.

**Table 4. CCP and POCA associated with physical hazards as a result of physical aggression against animals during contact with them (bitten, hit) / PCC și PA asociate hazardelor de natură fizică ca rezultat a agresării fizice a animalelor în timpul contactului cu acestea (mușcat, lovit) [19]**

| | |
|---|---|
| No contact between humans and animals is allowed | CCP |
| No direct contact of humans with 'dangerous' animals is allowed | CCP |
| Avoiding situations which provoke dangerous behaviour of animals | POPA |
| Sufficient information for visitors about natural and abnormal animal behaviour | POPA |

**2.2.6 Critical Control Points, CCP, and Points of Particular Attention, POPA (step 7– principle 2 – of the 12 developmental steps of HACCP**

A critical control point (CCP) is a step, series of steps, or procedure in the production process that can be measured or observed, that is connected to the risk of concern, at which process control can be applied, where control is necessary to prevent or eliminate a safety risk, and where related corrective measures must ensure the complete restoration of control once it has been lost. A control point is referred to be a Point of Particular Attention, or POPA, when it is not a CCP but is nonetheless thought to be extremely relevant despite not meeting the CCP criteria.

**2.2.6.1 CCP's and POPA's in relation to the hazard: 'animals carrying zoonotic pathogens'**

To effectively avoid zoonotic pathogen infections and animal-caused illnesses, people should be prohibited from coming into direct contact with animals and from entering cowsheds or pastures (CCP). When the primary objective of the service known as "animal hugging" is described as having direct touch with animals, implementing this control point will not be desirable. Nonetheless, in circumstances where infections are known to be common, the establishment of such a control point for specific populations, such as very young children or immune-compromised individuals, may be advised. Furthermore, when animals are recognized as zoonotic infection carriers, this control point must be put into practice. This issue can be controlled with the use of adequate fencing and supervision. More control points (POPA) are required when direct human interaction with animals is permitted.

It is preferable for animals that have been exposed to people to have a priori negative tests for specific zoonotic infections. Only zoonotic infections that can be reliably and quickly tested, such as by visual examination of clinical indications or through on-site and laboratory tests, can have critical control points established. Sadly, there aren't many zoonotic infections for which there are fast, dependable, and inexpensive diagnostic. In addition to this issue, it is impossible to rule out the reintroduction of zoonotic infections on many farms where animals and tourists come and go. To minimize hazards to the public's health, it is crucial in these open systems to optimize the herd's health state. It will be beneficial to have a stringent policy (good farming practice guidelines) regarding the acquisition and addition of additional animals to the herd. Also, it's important to limit (as much as possible) visitors' exposure to animal feces to lessen the likelihood that they'll contract zoonotic diseases.

Several levels of hygiene can be the focus of this point of special attention. For instance, by cleaning the animal snuggling area of animal droppings at least once every two hours or by cleaning the hair coats of animals before exposing them to guests [19].

**2.2.6.2 CCP's and POPA's** in relation to the hazard: 'trauma as a result of being hit, kicked, bitten or scratched by an animal'

Some animal species and individual animals can be classified as being dangerous in nature depending on the exact farm scenario, target service, and target group.

Male animals that are typically utilized for animal snuggling, such as bulls and stallions must be excluded. Some creatures can also be categorized as harmful. For instance, due to their more forceful or unpredictable behavior, animals in estrus may act less desirably. Yet, a two or four-month-old free-ranging calf can also be classified as dangerous due to its relatively large size and forceful (inquisitive) behavior when it is around young children. CCP can be characterized as the prohibition of exposing

visitors to harmful animals. Even animals that are normally trustworthy can act dangerously under certain circumstances, such as when they feel threatened. In order to prevent situations that cause animals to behave dangerously, supervision and a proper environment (i.e. housing) are required (POPA). In addition, in order to avoid potentially dangerous circumstances, employees and volunteers must possess or acquire appropriate knowledge of natural animal behavior. Posters with instructions could be put up throughout the property to let guests know what is expected of them and avoid potentially harmful situations. When visitors must take an exam to gauge their level of understanding, this area of focus could turn into a crucial control point. Targets for adequate knowledge could be set in such customized control programs [19, 20].

**2.2.7 Targets associated to CCP's and POPA's (step 8 – principle 3 – of the 12 developmental steps of HACCP)**

To enable control, objectives must be established for each POPA and each CCP standard/tolerance. KPI's must be determined based on the conditions and objectives of each dairy farm. The majority of CCPs and POPAs shown in Tables 3 and 4 can have their stated targets set on a zero-tolerance basis, such as prohibiting guests from entering certain portions of the farm's grounds. Targets can be set in relation to bacterial plate agar counts, which are utilized in the food business, for the POPA's "*Exposure of visitors to animal waste is as low as practicable*" KPI. For example, a zero-tolerance for *Enterobacteriaceae* on the plate agar taken from a dining table and a restriction on the maximum number of colony-forming units per cm2 for aerobic growth. It also suggests that a sanitation strategy has to be implemented [21].

**2.2.8 Establishment of a monitoring system for CCP's and POPA's (step 9 – principle 4 – of the 12 steps of HACCP)**

It is necessary to design a particular monitoring system for all CCP or POPA combined.

Regular tests on, say the presence of zoonotic infections will be required in order to optimize and sustain the health state of a herd. Depending on the pathogen, this may be accomplished through a visual assessment of the clinical symptoms or with laboratory testing following a sample of blood, urine, or feces to look for pathogen carriers or shedders.

The majority of sanitary procedures to reduce visitor exposure to animal waste as much as possible (POPA) and to prevent harmful circumstances (POPA) can be observed visually. Visitors should be watched, for instance, to ensure that they are dressing appropriately and wearing appropriate footwear, and cleaning their hands.

Visual inspection is a practical way to assess the cleanliness of the environment.

Agar bacterial count or contact plates should ideally be used to regularly inspect cleaning methods. Monitoring lists can be created for the CCP and POPA examples shown in *Tables 3* and *4*. The following information will be included on these lists: the CCP or POPA of concern; their standard/tolerance or target values; the location in the production process where it must be monitored; the frequency of the monitoring; the method of monitoring (for example, visual; testing after sampling); the responsible person to do it; and the action to be taken once monitoring has revealed loss of control [17].

**2.2.9 Establishment of intervention methods & corrective action plans (step 10 – principle 5 – of the 12 developmental steps of HACCP)**

The critical limit or target of a process stage can be identified using monitoring data. To ensure that the CCP or POPA has been brought under control once more or at the very least that its influence has been lessened, corrective measures must be implemented [3].

**2.2.10 Corrective actions in relation to the microbiological hazard: 'animals carrying zoonotic pathogens'**

Visitors must not be exposed to any animals that are suspected of being carriers of zoonotic illnesses until the monitoring data from the daily visual health inspection or routine sample shows signs

of zoonotic diseases. Entrance must be temporarily denied when a visual assessment reveals an inadequate degree of hygiene. The level of hygiene can be raised by changing the cleaning procedure or frequency or by redesigning cowsheds and stables to be more sanitary.

Corrective measures must be performed when visitors do not follow the farm's prescribed regulations. These measures may include warnings, bringing attention to the restrictions, or even denying entry. Examples of prescription regulations include requiring visitors to wear appropriate clothing while around animals and washing their hands following contact with them. Further visitor behavior prescription criteria might be introduced based on the animal species present [12].

**2.2.11 Corrective actions in relation to the physical hazard: 'trauma as a result of being hit, kicked, bitten or scratched by an animal'**

As was indicated in the last example, when people act irresponsibly, such as when potentially deadly animal behavior is ignored, corrective actions must be performed. Animal-related injuries to humans require prompt first aid or specialized medical attention. To be prepared for this task, farm workers must receive frequent first aid instruction and training. If an animal consistently causes problems or displays undesirable behavior, it must be prevented from snuggling or even replaced by another animal, or, if necessary, another species of animal [11].

**2.2.12 Establishment of verification procedures and record keeping (step 10 and 12 – principles 6 and 7 – of the 12 developmental steps of HACCP)**

Verification processes must be created in order to ascertain whether the HACCP-like program is operating properly. Preferably, the person in charge of carrying out the monitoring and remedial actions should not execute the verification procedures. A local veterinarian with the necessary expertise can complete this task, other competent outside parties can.

The HACCP-like program and its records, deviations, and product dispositions must all be examined as part of the verification process, and it must be confirmed that CCPs and POPAs are maintained under control. Where practical, validation operations have to involve procedures that verify the effectiveness of each component of the HACCP-like program. It is probable that external verification through auditing will soon need to be adopted in addition to these internal validity checks. The latter might ultimately result in certification for these types of dairy farms.

When a HACCP-like program is used, the results of the hazard identification and risk evaluations, as well as the determination of CCPs, POPAs, and their critical limits or objectives, must be documented. Additionally, written procedures and documentation of CCP and POPA monitoring activities (frequency, methods, results, responsible person), as well as, the associated corrective measures for improvement (CCP or POPA, date, area, type of measures taken, effects), are crucial for helping the farmer confirm that the HACCP-like program is operating in accordance with the goals. Furthermore, short- and long-term evaluations can be carried out using the stored papers.

The Good Dairy Farming Codes of Practice and their precise operating instructions on the farm are another area of documentation in such Quality Risk Management programs [13].

## CONCLUSIONS

Dairy farms that are open to the public must deal with specific hazards relating to the activities carried out by lay persons visiting the farm in addition to risks associated with the production of milk. A risk management program similar to HACCP can be used to control safety risks for visitors entering the farm. The HACCP concept focuses on hazard detection, risk analysis, and risk management. As a result, it might be very appropriate for such use.

This paper demonstrates that a HACCP-like program may be created and implemented for dairy farms that are open to the public, in order to manage the microbiological, chemical, and physical risks to the public's health. The HACCP-like program can be used to demonstrate to third parties that safety

hazards associated with farm products and on-farm services are kept as under control as possible in case of complaints in addition to catastrophe prevention. It can also be utilized as a marketing tool (including potential official accreditation).

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# RESEARCH ON THE FATTENING APTITUDES OF CROSSBREDS OBTAINED BY CROSSING TSIGAI SHEEP WITH FRENCH MEAT BREED RAMS

# CERCETĂRI PRIVIND APTITUDINILE PRODUCTIVE ALE METIȘILOR OBȚINUȚI PRIN ÎNCRUCIȘAREA OILOR ȚIGAIE CU BERBECI DIN RASE DE CARNE DIN FRANȚA

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***Abstract***

*Crossing of local sheep breeds with specialized breeds for meat production in order to obtain crossbreeds with superior meat characters is a rapid method of increasing quantitative and qualitative meat production. The main aim of our research was to study the productive abilities for meat production of crossbreeds obtained by crossing **Tsigai** breed from the rust variety with rams of French meat breeds, namely **Vendeen, Blanche du Massif Central** and **Berrichon du Cher**. The experiences took place within the biobase of the RDSSGB Secuieni Bacau research station in the period 2018-2020. The results obtained show that the heterosis has a higher manifestation on the productive performance in the F1 crossbred lambs (**Berrichon du Cher** x **Tsigai**) with very significant differences being found (P<0.001) in the average body weight of all ages compared to the maternal breed, followed of F1 crossbreds (**Vendeen** x **Tsigai**) with highly significant differences (P<0.001) in the average body weight at birth, 100 days and at 6 months and with significant differences (P<0.05) at the age of 60 days. As a result, the **Berrichon du Cher** and **Vendeen** breeds have a better degree of genetic combinability with the local **Tsigai** breed, the resulting F1 crossbreeds having, in the case of intensive fattening, clearly superior performances to the local breed, being able to be used by farmers at any age after weaning. The F1 crossbreds (**Blanche du Massif Central** x **Tsigai**) can be used by farmers only after the age of 100 days, the weight gain up to this age being much lower than the other two crossbred variants.*

***Key words: meat production, sheep, crossbreeding, fattening, crossbreeds, meat breeds***

***Rezumat***

*Încrucișarea raselor locale de ovine cu rase specializate pentru producția de carne în vederea obținerii de metiși cu caractere superioare pentru carne este o metodă rapidă de creștere a producției cantitative și calitative de carne. Scopul principal al cercetărilor noastre a fost de a studia aptitudinile productive pentru producția de carne a metișilor obținuți prin încrucișarea oilor **Țigaie din varietatea ruginie** cu berbeci din rase de carne din Franța, respectiv **Vendeen, Blanche du Massif Central** și **Berrichon du Cher**. Experiențele s-au derulat în cadrul biobazei stațiunii de cercetare SCDCOC Secuieni Bacău în perioada 2018-2020. Rezultatele obținute arată că heterozizul are o manifestare mai ridicată asupra performanței productive la mieii metiși F1 (**Berrichon du Cher** x **Țigaie**) constatându-se diferențe foarte semnificative, (P<0.001) la greutatea corporală medie de la toate vârstele față de rasa maternă, urmați de metișii F1 (**Vendeen** x **Țigaie**) cu diferențe foarte semnificative (P<0.001) la greutatea corporală medie de la naștere, 100 zile și la 6 luni și cu diferențe semnificative (P<0.05) la vârsta de 60 de zile. Ca urmare, rasele **Berrichon du Cher** și **Vendeen** dispun de un grad mai bun al combinabilității genetice cu rasa locală **Țigaie ruginie**, metișii F1 rezultați având în cazul îngrășării în sistem intensiv performanțe net superioare rasei locale, putând fi valorificați de către fermieri la orice vârstă după înțărcare. Metișii F1 (**Blanche du Massif Central** x **Țigaie**) pot fi valorificați de fermieri doar după vârsta de 100 zile, sporul de greutate până la această vârstă fiind mult inferior celorlalte două variante de metiși.*

***Cuvinte cheie: producție de carne, ovine, încrucișare, îngrășare, metiși, rase de carne***

## INTRODUCTION

Currently, on an international level, research in the field of sheep breeding is directed towards the creation of mixed types with high precocity and prolificacy, with a strong capacity for adaptability and high combinability with an increased attention to the improvement of reproductive aspects (Pop et al., 1983; Tafta, 2010). Sheep breeding for meat production has a priority role worldwide (Taftă et al., 1997; Pascal, 2013). The interest in the quantitative and qualitative increase in meat production determined the creation of specialized breeds for this production, the development of methods for the production of lambs for meat and high-performance technologies for growing and fattening young sheep.

Breeding for meat production of local sheep breeds is an extremely important field of activity. The socio-economic changes combined with the requirements and regulations of the European Union regarding the exploitation of meat production and the changes in the genetic structure of the sheep herds highlight the need to carry out and develop some activities regarding the quantitative and qualitative improvement of meat production in local sheep breeds.

The improvement for meat production aims to improve some morphoproductive characteristics such as: body mass, growth speed, reduction of feed consumption, increase of prolificacy, improvement of slaughter yields, meat-bone ratio, tissue composition of carcasses (Mochnacs et al., 1978; Taftă, 1983; Maier and Domnicar, 1989; Pascal, 2015).

Crossing local breeds with breeds specialized for meat production in order to obtain crossbreeds with superior meat characters is a rapid method of increasing production and carcass quality using the advantages of the phenomenon of heterosis (Sandu, 1993). This method of increasing meat production and improving the carcasses intended for delivery has begun to make its way timidly among sheep breeders in Romania.

In our country, the sheep flock is predominantly made up of breeds with a high degree of rusticity and very heterogeneous, with mixed productions. The increased interest in sheepmeat and animals with a high growth rate to give a higher quality carcass has created an opportune environment for research into crossbreeding to increase meat production. Recently, increased attention has been paid to this branch of sheep breeding, the strategy of the national authorities being to encourage the establishment and development of fattening farms and the opening of new markets for sheep meat, Romania has the potential to deliver quality young sheep for all tastes, here referring both to the requirements of the market in Europe and to the requirements of the market in Arab countries that prefer carcasses from rustic sheep breeds.

The main aim of our research was to analyze the productive abilities for meat production of crossbreds obtained by crossing **Tsigai** ewes from rusty variety (Tsi) with rams of meat breeds from France, namely **Vendeen** (V), **Blanche du Massif Central** (BMC) and **Berrichon du Cher** (BC).

## MATERIAL AND METHOD

The experiences took place within the biobase of the RDSSGB Secuieni Bacau in the period 2018-2020. In the research, the direct crossing method was used between breeding rams for meat production originating in France and autochthonous **Tsigai** sheep of the rust variety. The goal was to obtain F1 mestizos with superior qualities to the native breed in meat production.

In order to carry out the research in this direction, the products of the pure breed **Tsigai** and the F1 crossbreeds were monitored, being 4 batches of lambs, which were distributed after weaning for fattening in an intensive system. To evaluate the body development of the products obtained, weighings were carried out at birth, at 60 days, at 100 days and at 6 months.

**Animal feeding and accommodation**

All batches of lambs received the same treatment throughout the experiments. Thus, during the nursing period, the lambs benefited from mother's milk and permanent access to combined starter feed (18% crude protein), alfalfa hay of the best quality, water and mineral blocks for licking. After weaning, lambs were fed ad libitum fully granulated fattening lamb feed containing 16.8% crude protein, alfalfa hay and water. Mineral blocks for licking were also administered to each batch throughout the fattening period. The way of valorization of feed was followed by weighing administered feed and unconsumed leftovers, thus tracking the evolution of feed consumption during the growth and fattening periods and over the entire experimental period.

The intensive fattening system adopted consisted in the maintenance of the young sheep in collective boxes with access to the paddock, the watering being provided with constant level watering cans, with a watering front of 2 linear m, the feeding with concentrates was carried on in high feeders to eliminate wastage and facilitated ingestion, and hay was administered in gridded feeders with a feed front of 3 linear meters on each side, ensuring the required feed front per head of animal.

The health status of the animals that were the subject of the research was constantly monitored during the experiments, performing all the necessary diagnostic, treatment, deworming and vaccination actions.

**Monitoring body growth and development**

To evaluate the body development of the obtained products, body measurements were performed at birth, at the age of 60 days, at 100 days and at the end of the fattening period, namely at the age of 6 months. At the ages mentioned above, the body weight of the lambs was also determined using the animal scale (accurate to four decimal places) for the best possible accuracy.

In order to ascertain the gain through the effect of heterosis and the combinability of the breeds involved in the crossbreeding program, the performance of the meat production of the crossbreeds was compared with the performance of the pure breed batch **Tsigai**, rust variety, subjected to fattening under the same environmental conditions as the crossbreeds.

Body measurements of length and height were taken using a zoometer, widths with a compass, and perimeters with a tape measure. Later, the data obtained from body measurements were used to calculate the main body indices that give us information on body size, compactness and skeleton.

**Assessing the state of fattening in live animal and in the slaughtered animal**

Fattening status was assessed on the live animal by palpation of body regions with rich muscle mass and manipulative points, as well as by free assessment using the point method. For a correct and conclusive evaluation of the quality of the carcasses, 5 lambs from each batch were slaughtered. The evaluation of the slaughtered animal was done by determining the body mass of the live animal and the carcass resulting from the slaughter in order to be able to calculate the "*slaughter yield*" which is expressed in percentages and is calculated as the ratio between the mass of the carcass and that of the live animal multiplied by 100, and the "*commercial yield*" when added to the weight of the carcase and organs. The measurements performed on the slaughtered animal were used to calculate the main indices of the carcasses, for a better picture of their quality.

**Statistical data analysis**

The data recorded following the observations as well as the determinations made were processed using modern statistical processing methods. Microsoft Office Excel 2016 was used to calculate all statistical parameters (mean, standard deviation, coefficient of variation and standard error of the mean) and the t-test (Student) to determine the significance of the difference between the mean values of the characteristics analyzed. Differences were considered statistically significant at $P<0.05$ and indicated by specific signs of differentiation. The results obtained for all the characteristics analyzed were then presented in the form of tables and graphs, and their interpretation was made by comparing them with data from the specialized literature.

## RESULTS AND DISCUSSION

As a result of the crosses, the first generation of hybrids was obtained between the **Tsgaie** females of the rust variety and the three imported breeds, which were evaluated regarding the performance related to meat production (body growth and development, feed consumption, carcass quality, etc.), in order to determine the effect of heterosis for meat production.

The obtained data indicate a tendency to manifest the positive type of heterosis for the performance related to meat production in the three mixed populations. This statement is supported by the fact that the three groups of mestizos had a better body development compared to the maternal race for all characteristics analyzed (Table 1).

**Table 1. Heterosis effect for body weight determined at specific ages /**
**Efectul de heterozis pentru greutatea corporală determinată la anumite vârste**

| Batch | n | Age | UM | The weight corporal in F1 mestizos | The weight corporeal to Tsigai sheep rust variety | Differences (heterozis) | |
|---|---|---|---|---|---|---|---|
| | | | | | | Real | $\frac{\overline{X}_{F_1} - \overline{X}_P}{\overline{X}_P}$ x 100 |
| F1 (**Vendeen** x **Tsigai**) | 39 | birth | kg | 5.05 | 3.97 | 1.08 | 27.20 |
| | 30 | 60 days | kg | 16.30 | 14.44 | 1.86 | 12.88 |
| | 25 | 100 days | kg | 28.75 | 23.99 | 4.76 | 19.84 |
| | 25 | 6 months | kg | 44.60 | 31.84 | 12.76 | 40.08 |
| F1 (**Blanche du Massif Central** x **Tsigai**) | 30 | birth | kg | 4.26 | 3.97 | 0.29 | 7.30 |
| | 30 | 60 days | kg | 15.61 | 14.44 | 1.17 | 8.10 |
| | 27 | 100 days | kg | 25.87 | 23.99 | 1.88 | 7.84 |
| | 25 | 6 months | kg | 42.51 | 31.84 | 10.67 | 33.51 |
| F1 (**Berrichon du Cher** x **Tsigai**) | 20 | birth | kg | 5.20 | 3.97 | 1.23 | 30.98 |
| | 20 | 60 days | kg | 18.51 | 14.44 | 4.07 | 28.19 |
| | 16 | 100 days | kg | 30.02 | 23.99 | 6.03 | 25.14 |
| | 15 | 6 months | kg | 43.35 | 31.84 | 11.51 | 36.15 |

Following the analysis of the evolution of body mass, from birth to 6 months in the breeds studied, in the offspring resulting from the purebred **Tsigai** and in the hybrids **Blanc du Massif Central** x **Tsigai**, **Berrichon du Cher** x **Tsigai** and **Vendeen** x **Tsigai**, it appears that:

- the lowest weight at parturition was recorded in the purebred progeny (**Tsigai**) with an average of 3.97 kg, while in the hybrid progeny the first place was the progeny from **Berrichon du Cher** x **Tsigai** with an average of 5.20 kg at parturition, closely followed (5.05 kg) by the **Vendeen** x **Tsigai** crossbreds progeny;

- at 6 months the lowest weight was recorded in the pure offspring (**Tsigai**) with an average of 31.84 kg, while in the hybrid progeny the first place was the offspring from **Vendeen** x **Tsigai** with an average of 44.60 kg, followed by **Berrichon du Cher** x **Tsigai** crossbreeds with an average of 43.35 kg.

The data obtained for calving weight are close to those obtained by Borzan et al. (2017), respectively an average of 3.60 kg for the **Tsigai rust variety**, which falls within the breed standard, and in the hybrid progeny, the first place was the F1 crossbreeds (**Blanche du Massif Central** x **Tsigai**) with an average of 5, 19 followed by F1 crossbreds (**Berrichon du Cher** x **Tsigai**) with an average of 4.50 kg.

Regarding the average daily gain achieved from weaning (60 days) to the age of 100 days, the first place is the progeny from the **BC** x **Tsigai** breeds with 287 g, followed by the progeny obtained from the **Vendeen** x **Tsigai** crossbreeds with an average daily gain of 277 g, the **BMC** x **Tsigai** crossbreeds with an average daily gain of 239 g and respectively the pure offspring from the **Tsigai** breed with an average daily gain of 214 g/day (Table 2).

**Table 2. The average daily gain recorded during periods of growth (kg) / Sporul mediu zinic înregistrat pe perioade de creștere (kg)**

| Batch | Nr. crt. | Specification | n | $\bar{X}$ | $\pm s\bar{x}$ | s | V% | Min. | Max. |
|---|---|---|---|---|---|---|---|---|---|
| **Tsi** | 1. | Average daily gain 0-30 days | 30 | 0.190 | 0.011 | 0.062 | 30.339 | 0.093 | 0.350 |
| | 2. | Average daily gain 30-60 days | 30 | 0.194 | 0.016 | 0.086 | 23.301 | 0.101 | 0.370 |
| | 3. | Average daily gain 60-100 days | 30 | 0.214 | 0.009 | 0.049 | 22.840 | 0.105 | 0.321 |
| **V** x **Tsi** | 1. | Average daily gain 0-30 days | 30 | 0.229 | 0.009 | 0.050 | 21.806 | 0.126 | 0.359 |
| | 2. | Average daily gain 30-60 days | 30 | 0.253 | 0.020 | 0.104 | 41.162 | 0.149 | 0.601 |
| | 3. | Average daily gain 60-100 days | 25 | 0.277 | 0.010 | 0.052 | 18.868 | 0.201 | 0.389 |
| **BMC** x **Tsi** | 1. | Average daily gain 0-30 days | 30 | 0.198 | 0.008 | 0.046 | 23.031 | 0.116 | 0.285 |
| | 2. | Average daily gain 30-60 days | 30 | 0.210 | 0.008 | 0.046 | 21.670 | 0.138 | 0.302 |
| | 3. | Average daily gain 60-100 days | 25 | 0.239 | 0.012 | 0.061 | 25.442 | 0.157 | 0.410 |
| **BC** x **Tsi** | 1. | Average daily gain 0-30 days | 20 | 0.214 | 0.008 | 0.040 | 20.205 | 0.128 | 0.361 |
| | 2. | Average daily gain 30-60 days | 20 | 0.229 | 0.019 | 0.114 | 39.062 | 0.151 | 0.402 |
| | 3. | Average daily gain 60-100 days | 16 | 0.287 | 0.009 | 0.048 | 16.866 | 0.201 | 0.419 |

The values obtained in the present study are superior to those obtained by Borzan et al. (2017), who found that for the average daily gain, the **BMC** x **Tsigai** crossbreeds are in first place, with an average daily gain of 240 g, while for the pure descent from the **Tsigai** breed, the average daily gain recorded was only 154 g /day. For the **Tsigai** breed, the SMZ value is higher compared to that reported in the specialized literature, namely 152 g/day (Pascal et al., 2013).

Regarding the effect due to heterosis, it is found that after testing the differences between the resulting crossbreeds and the local breed (Student test application), it has a higher manifestation on the productive performance of the F1 products resulting from the crossing of the females of the **Tsigai** breed with rams from the **Berrichon du Cher** breed (highly significant differences in mean body weight at all ages - Table 5), followed by F1 crossbreeds obtained from crosses with rams belonging to the Vendeen breed (highly significant differences in mean body weight at birth, 100 days and at 6 months and significant differences at 60 days -Table 3).

**Table 3. Differences between average body weight in F1 crossbreds (Vendeen x Tsigai) and Tsigai breed rusty variety (kg) / Diferențe între greutatea corporală medie la metișii F1 (Vendeen x Țigaie) și rasa Țigaie varietatea ruginie (kg)**

| Specification | F1 (Vendeen x Tsigai) | Tsigai | Real difference | Differences (%) | The meaning of the differences |
|---|---|---|---|---|---|
| Birth weight | 5.05 | 3.97 | 1.08 | 27.20 | *** |
| Weight at 60 days | 16.30 | 14.44 | 1.86 | 12.88 | * |
| Weight at 100 days | 28.75 | 23.99 | 4.76 | 19.84 | *** |
| Weight at 6 months | 44.60 | 31.84 | 12.76 | 40.08 | *** |

NS – insignificant differences (P>0.05); * significant differences (P<0.05); ** distinctly significant differences (P<0.01); *** very significant differences (P<0.001).

**Table 4. Differences between average body weight in F1 crossbreds (Blanche du Massif Central x Tsigai) and Tsigai breed rusty variety (kg) / Diferențe între greutatea corporală medie la metișii F1 (Blanche du Massif Central x Țigaie) și rasa Țigaie varietatea ruginie (kg)**

| Specification | F1 (Blanche du Massif Central x Tsgai) | Tsigai | Real difference | Differences (%) | The meaning of the differences |
|---|---|---|---|---|---|
| Birth weight | 4.26 | 3.97 | 0.29 | 7.30 | NS |
| Weight at 60 days | 15.61 | 14.44 | 1.17 | 8.10 | NS |
| Weight at 100 days | 25.87 | 23.99 | 1.88 | 7.84 | NS |
| Weight at 6 months | 42.51 | 31.84 | 10.67 | 33.51 | *** |

NS – insignificant differences (P>0.05); * significant differences (P<0.05); ** distinctly significant differences (P<0.01); *** very significant differences (P<0.001).

**Table 5. Differences between average body weight in F1 crossbreds (Berrichon du Cher x Tsigai) and Tsigai breed rusty variety (kg) / Diferențe între greutatea corporală medie la metișii F1 (Berrichon du Cher x Țigaie) și rasa Țigaie varietatea ruginie (kg)**

| Specification | F1 (Berrichon du Cher x Tsigai) | Tsigai | Real difference | Differences (%) | The meaning of the differences |
|---|---|---|---|---|---|
| Birth weight | 5.20 | 3.97 | 1.23 | 30.98 | *** |
| Weight at 60 days | 18.51 | 14.44 | 4.07 | 28.19 | *** |
| Weight at 100 days | 30.02 | 23.99 | 6.03 | 25.14 | *** |
| Weight at 6 months | 43.35 | 31.84 | 11.51 | 36.15 | *** |

NS – insignificant differences (P>0.05); * significant differences (P<0.05); ** distinctly significant differences (P<0.01); *** very significant differences (P<0.001).

Regarding the F1 crossbreds (**BMC** x **Tsgaie)**, it was found that the differences are insignificant in the average body weight achieved at birth, at 60 days and at 100 days, and the differences become very significant only at the age of 6 months, registering a better weight and implicitly a higher average daily increase in the period 3-6 months (Table 4).

In accordance with these results, it can be stated that the **Berrichon du Cher** and **Vendeen** breeds have a better degree of genetic combinability with the local **Tsigai** breed, rust variery, the resulting F1 crossbreds having, in the case of intensive fattening, clearly superior performances to the local breed, being able to be exploited by to farmers at any age after weaning. The F1 half-breeds obtained with the **Blanche du Massif Central** breed can only be used after the age of 100 days, the weight gains up to this age being much lower than the other two half-breed variants.

The results obtained confirm those reported by Mochnacs et al. (1978), which shows that in F1 crossbreds (**BC** x **Tsigai**) growth is improved by 4.97%, specific consumption is reduced by 9.08% and yield at slaughter increases by 4.73%, compared to the maternal breed. Maier and Domnicar (1989), in the crossbreeding of **Tsigai** sheep with **Romney-Marsh** rams, found that the F1 crossbreds achieved a genetic gain of 3.50 kg in terms of body weight compared to the **Tsigai** breed.

The calculated specific consumption was on average 6.3 UN/kg gain in the **Tsigai rust variety** group, 5.9 UN/kg gain in F1 crossbreds (**BC** x **Tsi**), 5.6 UN/kg gain in crossbreds (**BMC** x **Tsi**) and 5.5 UN/kg gain in crossbreds (**V** x **Tsi**). The lowest consumption was therefore recorded by the mixed-breeds (V x Tsi), which is 12.7% lower compared to the whole-registered **Tsigai** lambs. In the **Tsigai** breed, the consumption is similar to that reported in the specialized literature, namely 6.2 UN/kg weight gain (Ionescu et al., 1985).

**Table 6. Specific consumption / Consumul specific**

| Specificare | Tsigai | Breed F1 (V x Tsi) | Breed F1 (BMC x Tsi) | Breed F1 (BC x Tsi) |
|---|---|---|---|---|
| Specific consumption (UN/kg gain) | 6.3 | 5.5 | 5.6 | 5.9 |
| Differences (%) compared to Tsigai | - | 12.7 | 11.1 | 6.3 |

According to the findings, it can be said that, when it is desired to obtain larger amounts of meat, one of the methods can be the crossing with parents from the three breeds because in the expression of body development in the mestizo generation, the number of loci involved increases due to the differences between breeds in terms of relevant allele frequencies at these loci. The greater the difference in gene frequency, the greater the effect due to heterosis (Bonnes et al., 1991; Bodin et al., 1999).

Assessment of fattening status on the live animal consisted of palpation of regions with rich muscle masses and manipulative points, as well as free assessment using the point method. Following the evaluation of a number of 5 heads from each lot, it was found that in the animals from the **Tsigai** lot, there were 4 animals graded 2 (poor) and one animal graded 3 (good). Regarding the **V** crossbreds (Figure 2), four of them were rated four very good) and one was good. **BC** crossbreds were graded 4 and **BMC** crossbreds (Figure 1) received grades 4 and 3. Thus, it can be seen that the best fattening condition was the crossbreds (**BC** x **Tsi**).

**Figure 1. BMC x Tsigai crossbred male at 6 months of age / Metis mascul BMC x Țigaie la vârsta de 6 luni**

**Figure 2. Vendeen x Tsigai crossbred male at 6 months of age / Mascul metis Vendeen x Țigaie la vârsta de 6 luni**

a b c d

**Figure 3. Carcases from the 4 lots / Carcase provenite de la cele 4 loturi**
a -**Tsi**; b -**V** x **Tsi**; c - **BC** x **Tsi**; d - **BMC** x **Tsi**

Lambs slaughtered at 60 days of age (weaning) had live weights from 16 kg in **Tsigai** to 25 kg in **BMC** with a commercial yield of 52% in **Tsigai**, 54% in crossbreds (**V** x **Tsi**), 55% in crossbreds (**BC** x **Tsi**) and 57% in crossbreds (**BMC** x **Ti**). Slaughter yield without head, without organs and without gastrointestinal contents was 43% in **Tsigai**, 44% in **V** x **Tsigai** crossbreds, 44% in **BC** x **Tsigai** crossbreds and 47% in **BMC** crossbreds (Table 7, Figure 3, Figure 4).

From the analysis of these data, it can be seen that the best yield at slaughter was obtained by the half-breeds (**BMC** x **Tsigai**). For the **Tsigai** breed, the yield value at slaughter is similar to that reported in the specialized literature (Pascal et al., 2007).

**Table 7. Slaughter yield and commercial yield / Randamentul la sacrificare și randamentul comercial**

| Specification | Tsigai | Breed F1 (V x Tsi) | Breed F1 (BMC x Tsi) | Breed F1 (BC x Tsi) |
|---|---|---|---|---|
| Slaughter yield (%) | 43 | 44 | 47 | 44 |
| Commercial yield (%) | 52 | 54 | 57 | 55 |

Compared to the data found in the specialized literature, it is found that the values obtained for the slaughter yield are lower (Borzan et al., 2017), a fact that can be explained by the fact that the slaughter age in the case of our project was only 2 months, compared to the 4 months encountered in the cited experience. Also, Borzan et al. (2017), found that the best yield at slaughter was achieved by the mixed breeds (**BC** x **Tsigai**) being 50.6%, followed by (**BMC** x **Tsigai**) with 50% and the mixed breeds (**V** x **Tsigai**) with 48, 8%.

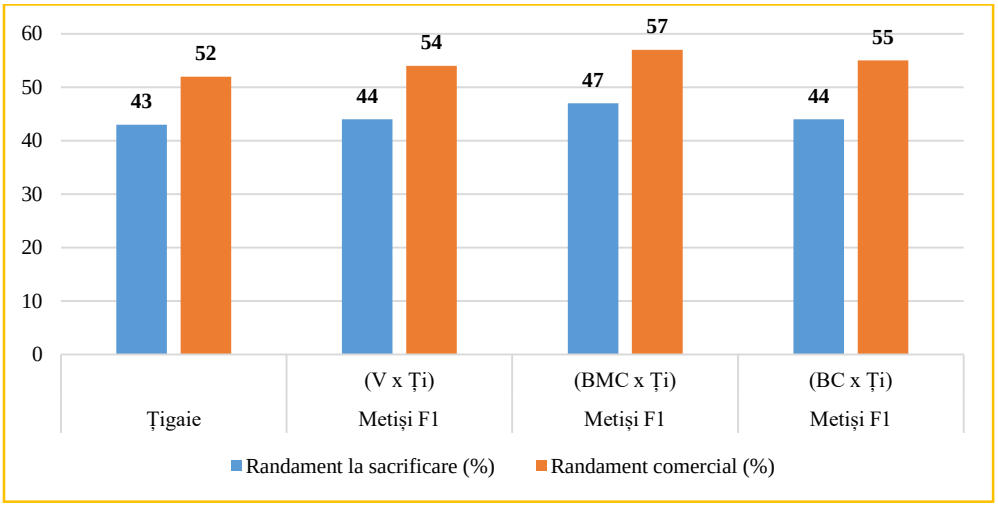


**Figure 4. Slaughter yield and commercial yield / Randamentul la sacrificare și randamentul comercial**

Following the average values of the carcase indices, it can be seen that the best carcase format has the half-breeds (**V** x **Tsi**) followed by the half-breeds (**BMC** x **Tsi**). The jigo format shows more obvious muscle masses in crossbreds (**BMC** x **Tsi**) followed by crossbreds (**BMC** x **Tsi**). The compactness of the carcass recorded the highest values in the **Tsigai** group, and the homogeneity of the carcass is the best in crossbreds with **BMC** being 100% as well as the best development of the jig represented by the index of jig development (Table 8).

**Table 8. Average values of the main carcass indices calculated for the 4 batches / Valorile medii ale principalilor indici ai carcaselor calculați pentru cele 4 loturi**

| Nr. crt. | Lot | Carcass format index | Pulp format index | The compactness of the carcass index | The width of the carcass index | The development of the pulp index | Thorax depth index |
|---|---|---|---|---|---|---|---|
| 1 | **BMC** x **Tsi** | 36.84 | 72.41 | 112.28 | 100.00 | 120.00 | 36.84 |
| 2 | **V** x **Tsi** | 37.25 | 70.37 | 119.60 | 110.52 | 112.5 | 39.21 |
| 3 | **Tsi** | 34.78 | 64.00 | 121.73 | 106.25 | 109.09 | 41.30 |
| 4 | **BC** x **Tsi** | 36.25 | 69.30 | 121.00 | 109.72 | 114.5 | 40.51 |

Following the heterosis effect for the main carcass indices calculated we observe a slightly negative heterosis effect for the carcass compactness index and the chest depth index. On the other hand, a positive heterosis can be observed for the size and development indices of the pulp, a portion of the carcass of increased importance in the capitalization of the carcass (Table 9).

**Table 9. Calculation of the heterosis effect for the main carcass indices / Calcularea efectului de heterozis pentru principalii indici ai carcaselor**

| Lot | n | Indices | UM | Medium values half-breeds F1 | Average values Tsigai rust variety | Difference (heterosis) | |
|---|---|---|---|---|---|---|---|
| | | | | | | Real | $\frac{\overline{X}_{F_1} - \overline{X}_P}{\overline{X}_P}$ x 100 |
| **V** x **Tsi** | 10 | carcass format | % | 37.25 | 34.78 | 2.47 | 7.10 |
| | | pulp format | % | 70.37 | 64.00 | 6.37 | 9.95 |
| | | compactness of the carcass | % | 119.60 | 121.73 | -2.13 | -1.75 |
| | | the width of the carcass | % | 110.52 | 106.25 | 4.27 | 4.02 |
| | | the development of the pulp | % | 112.50 | 109.09 | 3.41 | 3.13 |
| | | thorax depth | % | 39.21 | 41.30 | -2.09 | -5.06 |
| **BMC** x **Tsi** | 10 | carcass format | % | 36.84 | 34.78 | 2.06 | 5.92 |
| | | pulp format | % | 72.41 | 64.00 | 8.41 | 13.14 |
| | | compactness of the carcass | % | 112.28 | 121.73 | -9.45 | -7.76 |
| | | the width of the carcass | % | 100.00 | 106.25 | -6.25 | -5.88 |
| | | the development of the pulp | % | 120.00 | 109.09 | 10.91 | 10.00 |
| | | thorax depth | % | 36.84 | 41.30 | -4.46 | -10.80 |
| **BC** x **Tsi** | 10 | carcass format | % | 36.25 | 34.78 | 1.47 | 4.23 |
| | | pulp format | % | 69.30 | 64.00 | 5.3 | 8.28 |
| | | compactness of the carcass | % | 121.00 | 121.73 | -0.73 | -0.60 |
| | | the width of the carcass | % | 109.72 | 106.25 | 3.47 | 3.27 |
| | | the development of the pulp | % | 114.50 | 109.09 | 5.41 | 4.96 |
| | | thorax depth | % | 40.51 | 41.30 | -0.79 | -1.91 |

Based on these data, it can be said that the application of crosses represents a faster and more efficient solution for obtaining populations of a new type in which an improvement of characters can be found that require a long time for improvement through pure breed selection and the application of breeding programs breeding based on selection.

## CONCLUSIONS

1. The **Berrichon du Cher and Vendeen** breeds have a better degree of genetic combinability with the local **Tsigai** from rusty variety, the resulting F1 crossbreds having clearly superior performance to the local breed in case of intensive fattening, being able to be used by farmers at any age after weaning.

2. The use of first-generation crosses of **Tsigai** ewes with **Berrichon du Cher** rams lead to an increase in meat production on the farm by 25.1 to 36.2% depending on the age at which the delivery is made and to obtaining clearly superior carcasses to obtained from the **Tsigai** breed.

3. The use of first-generation crosses of **Tsigai** sheep with **Vendeen** rams leads to an increase in meat production on the farm by 13 to 40% depending on the age at which delivery is made and to obtaining carcasses superior to those obtained from the **Tsigai** breed.

4. The F1 crossbreds obtained with the **Blanche du Massif Central** breed can be used by farmers only after the age of 100 days, the weight gains up to this age being much lower than the other two variants of hybrids.

5. Industrial crosses of first-generation remain an effective and fast solution for increasing meat production in farms that own herds of sheep from the **Tsigai** breed, the rust variety, using the benefits of

the heterosis effect, the farmer's additional investment being only the purchase of rams from the meat breeds.

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# THE *IN VIVO* AND *IN VITRO* INFLUENCE OF SOME VEGETABLE EXTRACTS ON THE IMMUNOLOGICAL PROFILE IN CHICKENS

# INFLUENŢA *IN VIVO* ŞI IN VITRO A UNOR EXTRACTE VEGETALE ASUPRA PROFILULUI IMUNOLOGIC LA PUII DE GĂINĂ

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***Abstract***

*Plant extracts are defined by their bioactive properties, thus providing the scientific basis for their use in various pathologies. The investigations were carried out on 28, 47-day-old broiler chickens (**Rock** x **Cornish**), divided into 4 equal groups treated orally for 7 days with: I –alcoholic extract of Calendula officinalis, 0.5 ml; II - Echinacea angustifolia, 0.5 ml; III - 70% alcohol, 0.5 ml; IV – untreated control. On days 0 and 7, the birds were antigenically stimulated with 0.5 ml of a 5% suspension of sheep red blood cells in saline, administered sc. Blood taken from all groups on days 0, 7 and 14 was processed by leukocyte counts (Natt-Herrick), evaluation of phagocytic activity (incorporation of carbon particles) and blast transformation test. Treatment with alcoholic extracts of C. officinalis and E. angustifolia did not significantly influence the total number of leukocytes during the experiment. The plant extracts used induced a stimulation of phagocytic activity for both extracts (Echinacea angustifolia: OD 0.157 ± 0.07 and Calendula officinalis: 0.152 ± 0.056 OD). Of the two plant extracts, only the Echinacea angustifolia extract (54.86% ± 7.76%) showed stimulating effects in the group treated in vivo with the same extract. Thus, plant taxonomy defined the immunological efficacy of the tested extract in chickens.*

***Key words: plant extracts, Calendula officinalis, Echinacea angustifolia, chickens, immunity, phagocytosis, blast transformation***

***Rezumat***

*Extractele vegetale sunt definite de proprietăţile lor bioactive, oferind astfel bazele ştiinţifice pentru utilizarea lor în diferite patologii. Investigaţiile au fost efectuate pe 28 de pui broiler de 47 de zile (**Rock** x **Cornish**), divizaţi în 4 loturi egale tratate per os timp de 7 zile cu: I- extract alcoolic de Calendula officinalis, 0,5 ml; II –extract alcoholic de Echinaceea angustifolia, 0,5 ml; III –alcool 70º, 0,5 ml; IV netratat. În zilele de 0 şi 7 păsările au fost stimulate antigenic cu 0,5 ml suspensie de hematii de oaie 5 % în ser fiziologic, administrată sc. Sângele prelevat de la toate loturile în zilele 0, 7 şi 14 a fost prelucrat prin numărarea leucocitelor (Natt-Herrick), evaluarea activităţii fagocitare (înglobarea particulelor de carbon) şi testul de transformare blastică. Tratamentul cu extracte alcoolice de C. officinalis şi E. angustifolia nu a influenţat semnificativ numărul total de leucocite pe parcursul experimentului. Extractele vegetale folosite au indus o stimulare a activităţii fagocitare pentru ambele extracte (Echinaceea angustifolia: DO 0,157 ± 0,07 şi Calendula officinalis: 0,152 ± 0,056 OD). Dintre cele două extracte vegetale doar cel de Echinaceea angustifolia (54,86 % ± 7,76 %) dovedeşte efecte stimulatoare la lotul tratat in vivo cu acelaşi extract. Astfel, taxonomia plantei defineşete activitatea imunologică a acesteia la puii de găină.*

***Cuvinte cheie: extracte vegetale, Calendula officinalis, Echinacea angustifolia, pui de găină, imunitate, fagocitoză, transformare blastică***

## INTRODUCTION

In recent years, the study of plant extracts has gained new momentum due to the favorable results obtained in the therapy of some conditions in which even synthetic active substances have proven less effective (Roitt et al., 1989, Dhama et al., 2018, Qassadi et al. col., 2023). Phytochemicals show promise in supporting and maintaining health. There are numerous structural compounds in plant extracts such as polyphenols, terpenoids, phenolic acids, essential oils, lectins, polypeptides and alkaloids. Due to the presence of these substances, some of which are secondary metabolites, plant extracts are characterized with different biological properties, thus providing the scientific basis for their use in different pathologies (Qassadi et al., 2023). By associating plant extracts with the preparations used in classical therapeutic protocols, it is possible to intervene in increasing the body's non-specific defense capacity through the complex immune stimulation effect it exerts. The problem is all the more urgent in veterinary medicine where, by using such extracts, various disturbances of the immune status induced by stress states can be corrected. These conditions are increasingly common in the conditions of an increase - increasingly artificial exploitation of birds and animals, associated with a diet that is not always correct and balanced (Zarnea 1980, Vior et al., 1982, Nawaz et al., 2021). Numerous extracts from different plants are currently used to increase the natural resistance of organisms to various infections or in their effective treatment. Moreover, some extracts are used for the partial restoration of the functional capacity of the immune system in subjects with congenital immune depression. We should also not neglect the anti-inflammatory effects that some plant extracts can exert and due to which the extracts can acquire a wide use of interest in therapeutics (Arstila et al., 1994, Benencia et al., 1995, Bernd et al., 1995, Bussing et al., 1997, Fernandez-Puntero et al., 1997). A less researched but promising field of study on the use of extracts is the discovery of new adjuvants for vaccines or the improvement of existing ones, a phenomenon considered essential for the development of modern vaccines. Thus the use of plants and extracts obtained from them, although known for millennia, is far from being a closed subject. In this context, the purpose of the study was to evaluate the potential of some plant extracts on the immunological profile of chickens. The interest that today is medicine and biochemistry gives to this field is constantly growing and results in the introduction into practice of new preparations, which acquire an increasing importance among modern prophylactic and therapeutic means.

## MATERIAL AND METHOD

The investigations were done on 28 broiler chickens (**Rock** x **Cornish**), males and females, aged 47 days. The chickens came from the private farm. During the period before the experiment, the birds were kept in bunk batteries, 6 individuals/battery. The feeding was carried out in the first two weeks with combined feed prepared according to the 21/1 recipe, and later with type 21/2 feed. The average weight of the chickens at the beginning of the experiment was 1.5 kg. Later, the chickens were introduced into a breeding system in batteries on 3 levels, two individuals/box. During the entire period of the experiment, the birds were fed ad libitum, with feed obtained according to the 21/2 recipe, and the watering was done drop by drop, in the waterers in the box. No immunoprophylactic actions were performed during the experiment. The chicks were distributed in 4 groupes of 7 birds each. The groups were treated with plant extracts as follows: in group I, alcoholic extract of *Calendula officinalis* was administered for 7 days, in the amount of 0.5 ml, per bone; in group II the alcoholic extract of *Echinacea angustifolia*, in the amount of 0.5 ml/bone; in group III (control group) alcohol 70º, in the same quantity and by the same route of administration. Lot IV represented the absolute untreated control (environmental control and handling stress). Day 0 was considered the start day of the experiment. Blood samples collections were performed on days 0, 7 and 14. On days 0 and 7 the chickens were inoculated subcutaneously with a suspension of sheep red blood cells (SH, freshly prepared in the laboratory) 5%

in saline, administered in an amount of 0.5 ml. The samples were evaluated by the leukocyte counting technique, the evaluation of the phagocytic activity and the blast transformation test. The Natt-Herrick Tic® test (BioAnalytic) was used to determine the total number of leukocytes. To determine non-specific cellular reactivity, blood samples were collected by jugular vein puncture. To the blood samples, 3 µl of China ink was added. From the samples thus prepared, 100 µl were taken and transferred to 2 ml physiological serum; the mixture represented the control for the optical density conferred by the presence of plasma proteins and non-phagocytosed mucus. After homogenization by repeated overturning, the samples were incubated at 37ºC and to establish the optimal reading time, 100 µl of the blood and swab mixture were taken at 15 and 30 minutes. Each of these quantities was placed in 2 ml physiological serum. Finally, the tubes with a mixture of physiological serum, blood and tissue were centrifuged for 5 minutes at 800 revolutions/minute. The optical density of the supernatants was read in Sumal PE 2 at a wavelength of 535 nm in 96-well plates. Readings for controls were made against saline.

The values of phagocytosis were considered the ones read, expressed in units of optical density with physiological serum as standard. In parallel, the effects of the administered extracts on the reactivity of phagocytosis *in vitro* were investigated. The working variants assumed the same operations and in addition the addition of 3 microliters of extract at the initial moment. The extracts used were those of *Echinacea angustifolia* and *Calendula officinalis*, and the control, 70° alcohol.

The blast transformation test was used to determine specific cellular reactivity. For this purpose, the blood taken was diluted with RPMI 1640 medium (Sigma-Aldrich) supplemented with 5% fetal calf serum (Gibco) and antibiotics (penicillin 1,000 IU/ml; streptomycin 1,000 µg/ml). The obtained suspension was distributed in sterile plates with 96 wells, 200 µl/well. The experimental variants were: control (untreated), PHA M1 1 µl/well, alcohol 1.5 µl/well and alcoholic extracts of *Calendula, Arnica, Symphytum, Echinacea angustifolia, Echinacea purpurea* each 1.5 µl/well. The response to red blood cell lysate was also tested, the birds being inoculated with sheep red blood cells. Optical density of samples was performed at 610 nm wavelength in 96-well plates.

Initially, an average supernatant sample was made for each variant, homogenizing 12.5 µl from the two wells in a single tube. 12.5 µl of this mixture was added to 0.5 ml ortho-toluidine reagent, and after homogenization, the test tubes were kept in a water bath at 100ºC for 8 minutes. Each series of samples was identically worked with the glucose standard (with a concentration of 100 mg %) and initial medium.

The concentration of glucose in the samples to be researched was found starting from their optical density and the optical density of the glucose standard according to the formula: Cp = Ep/Es x 100, in which: Cp = sample concentration in mg glucose %; Ep = sample extinction and Es = standard extinction. Considering the residual concentrations of glucose after stopping cell growth and the initial concentration of the medium, it is possible to calculate the blasting index expressed as a percentage for both control and stimulated cultures. The reference value is the integral medium. The blasting indices induced by PHA and the various extracts were calculated using the formula: IS = glucose from the medium - glucose sample / glucose from the medium x 100; where, IS is the spontaneous blasting index. All the results obtained through these tests were processed statistically to obtain the mean values, standard deviations and variance using the usual formulas of statistical calculation. The significance of the differences was assessed using the t-student test.

## RESULTS AND DISCUSSION

Following the evolution of the total number of leukocytes in group I treated with *Calendula* extract, it is found that at the first collection the parameter varies between 11,500-19,500/mm3, with an average of 16,571,429±2,636,736 (standard deviation, SD). At the second collection, the number of leukocytes showed variable values between 17,500 - 39,000/mm$^3$, the average recorded was 29,785

±8,459.99 (SD). At the third collection, the leukocyte values were between 19,000 and 44,500/mm$^3$, the average being 26,785.71 ±8,557.92 (SD) (Fig. 1). In group III treated with *Echinacea* extract, the total number of leukocytes varied from 18,000 to 39,000/mm$^3$ with a mean of 27,333,333 ±7,167,054 (SD). Later, an increase in the variation is observed from 17,000 to 47,000/mm$^3$, the average having the value of 28,357,143 and the standard deviation of 10,391,139, while at the third collection the values obtained varied between 18,500 and 37,500/mm$^3$, the average being of 28,500, and the SD was ± 6,331.14 (Fig. 1).

In group III, treated with alcohol, at the first harvest the minimum value was 27,500, and the maximum was 51,000/mm$^3$, with an average of 37,428.57 and a SD of 8,974.169; at the second harvest, a decrease in variation is observed from 22,000 to 49,000/mm$^3$, with an average of 30,357.14 and a standard deviation of 9,432.7. The values of the third collection were between 24,000 and 45,000/mm$^3$, with a mean of 33,857.14 and SD of 6,878.192 (Fig. 1).

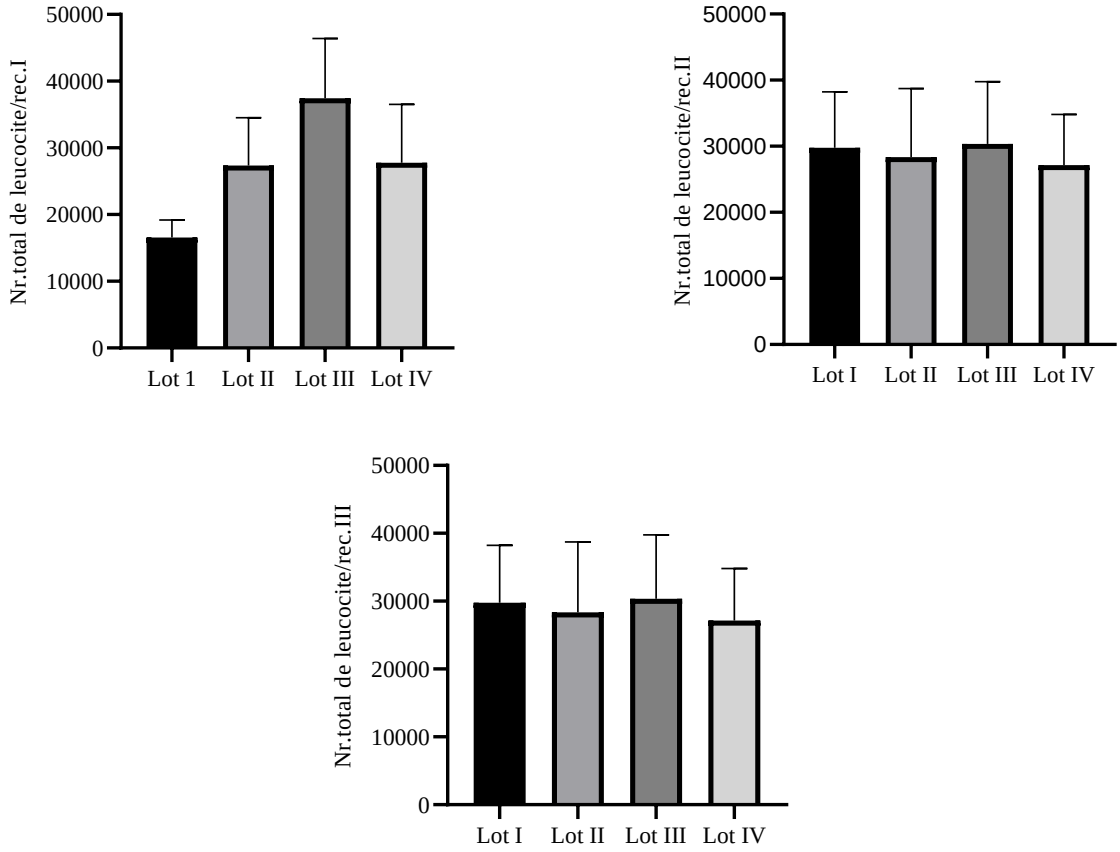


**Figure 1. Total leukocyte count at the three sampling / Numărul total de leucocite la cele trei probe**

In the control group, the total number of leukocytes at the first collection varied between 16,500 and 42,000/mm$^3$, with an average of 27,785.71 and a SD of 8,717.11. At the second harvest, an increase in individual values was observed, varying from 21,000 to 43,000/mm$^3$ with an average of 27,142.86 and a SD of 7,685.019. At the last harvest (III), the minimum value was 24,500, and the maximum value was 41,000/mm$^3$, with an average of 35,857.14 and a standard deviation of 5,406.125 (Fig. 1). Leukocytes represent some of the first effectors of defense (Czarnetzki et al., 1990). Their intervention can be destructive, with the aggressor microorganisms being incorporated, degraded and then eliminated, or constructive, when the aggressor microorganisms are incorporated, degraded and their constituents are

expressed on the cell surface, the antigenic information is transmitted to the immunocompetent cells and a response translated by the appearance of antibodies is elaborated (Rodenberg et al., 1994).

Establishing the total number of leukocytes can be a valuable indicator in estimating the efficiency of non-specific cellular protection. Phagocytosis represents one of the first intervention protection mechanisms, with a special significance especially in the prevention of bacteriosis and viruses. In this function, they are active, together with polymorphonuclear granulocytes and mononuclear cells of the macrophage type, whether they are in circulation or fixed in tissues. In the case of our study in the phagocytosis test of the control sample at harvest I, at T15 (minutes) a minimum value between 0.052 - 0.713 was found with a mean of 0.4247 ±0.172 (SD). The phagocytic activity of samples treated with alcohol at the same time have values that varied between 0.036 and 0.606, with a mean of 0.439 and a SD of 0.170. In the samples treated with *Echinacea* extract, at 15 minutes, the phagocytic activity values were between 0.032 and 0.796 with an average of 0.512 and a standard deviation of 0.187. Regarding the results of the second harvest, in the case of the control sample, at T15, the lowest value is 0.062 and the highest value is 0.197, the mean being 0.10 ± 0.04 (SD). In samples treated with alcohol, the lowest value was 0.14, and the highest was 0.291 with an average of 0.18 and a standard deviation of 0.07. Regarding the samples treated with Calendula extract, at T15, the minimum value is 0.067 and the maximum is 0.326 with an average of 0.17. In the case of samples treated with *Echinacea angustifolia*, the maximum value is 0.201, and the minimum is 0.067, with an average of 0.13 and a standard deviation of 0.045. At harvest II, it can be seen in the case of the control sample, at T15, that the lowest value is 0.21, and the highest is 0.378, with an average of 0.28.

In the case of samples treated with 70° alcohol, the values obtained at T 15 are 0.063 the minimum value and 0.366 the maximum value. Samples treated with *Calendula* show a fluctuation of phagocytic activity between 0.25 and 0.566 with an average value of 0.24 and a standard deviation of 0.11. In the case of samples treated with *Echinacea angustifolia* extract, the phagocytic activity is variable, with values between 0.154 and 0.304, with an average of 0.22 and a standard deviation of 0.051.

At T 30, at the first harvest, in the case of the control sample, the minimum value is 0.049, and the maximum value is 0.745 with an average of 0.36 and a standard deviation of 0.16.

The phagocytic activity of the samples treated with alcohol (also at 30 minutes) shows values that vary between 0.036 - 0.606 with an average of 0.43±0.17 (SD). In the samples treated with *Echinacea* extract, at the same time, the phagocytic activity values were on average 0.46±0.18. At collection 2, in the case of the control sample, at T 30, the minimum value was 0.03, and the maximum was 0.158, with an average of 0.07±0.039 (SD). In samples treated with alcohol, at 30 minutes, the minimum value was 0.05, and the maximum was 0.187, with an average of 0.11 and a standard deviation of 0.12. Samples treated with *Calendula* the minimum value was 0.053, and the maximum was 0.341 with an average of 0.18 and a standard deviation of 0.08. In the case of samples treated with *Echinacea angustifolia* extract, the minimum value is 0.063 and the maximum is 0.201, with an average of 0.135 ±0.045 (SD).

At harvest III in the case of the control sample, at T 30, the lowest value was 0.097, and the highest was 0.362 with an average of 0.21 ±0.06 (SD).

In the case of samples treated with 70° alcohol, the values obtained at T 30 were 0.049 the minimum value and 0.373 the maximum value with an average of 0.23 ±0.08 (SD). Samples treated with *Calendula* show a fluctuation of phagocytic activity between 0.04 and 0.231 with a mean value of 0.15 ±0.05 (SD). In the case of samples treated with *Echinacea angustifolia*, the phagocytic activity is variable with values between 0.028 and 0.215, with an average value of 0.157 ±0.06 (SD).

Numerous methods have been devised for the evaluation of phagocytic activity. Thus, the phagocytic capacity of whole blood *in vitro* against different live or inactivated bacterial suspensions was tested. Other methods of investigating phagocytic activity assume the association of this function *in vivo*. Thus, the animal is injected intravenously with the supernatant of a suspension of carbon particles (Chinese ink), centrifuged at 6,000 revolutions/minute for 15 - 20 minutes, in an amount evaluated according to

the species. Before and at different intervals after inoculation, blood samples are collected, the optical density of the plasma is evaluated spectrophotometrically and a correlation is established between this and the phagocytic activity of the body. This method assesses the body's phagocytic activity, summing up the activity of both mobile and fixed phagocytes. The acute antimicrobial anti-inflammatory response is the result of complement activation, translated by polymorphonuclear leukocyte chemotaxis and increased vascular permeability (Giurgea, 1994, Ostrycharz et al., 2022). Phagocytosis, i.e. intracellular killing by hydrolytic enzymes, represents one of the most primitive protection mechanisms, which in higher animals are performed by specialized cells: microphages and macrophages (Giurgea, 1994).

Microphage activity can be monitored *in vitro* by the carbon particle incorporation assay. This test also gives the possibility of following the effects of some extracts or other compounds, directly, in the presence of ink particles.

The data obtained initially, in the untreated birds, suggest a pronounced phagocytic activity in the untreated variant and stationary phagocytic activity, more intense at the beginning of incubation and slower afterwards, in the case of the alcohol-treated variant. The treatment with the *Echinacea* extract, although it does not induce phagocytosis as intense as that of the control group, thus acting Following the one-week treatment with the two extracts and the inoculation, the intensification of the phagocytic activity of the untreated control variant is observed. Alcohol treatment reduces phagocytic activity compared to the control, while *Calendula* extracts ensure an increase in phagocytosis initially and then a stationary evolution. *Echinacea* induces a more intense stimulation than *Calendula*, but the effects compared to those of the alcohol control are inhibitory.

At the end of the experiment, although the antigenic stimulation was repeated, the phagocytosis values decrease both in the control group and in the variants treated with alcohol, except for the plant extracts. Both extracts determine in the final moment, the stimulation of phagocytosis, compared to the control and the group treated with alcohol.

Regarding the stimulation indices in the case of the control variant, a fluctuation of the stimulation indices was observed between 49.06% and 62.79% with an average of 55.71%±5.42(SD). Phytohemagglutinin acts as a more intense stimulant, thus the stimulation indices are higher than in the case of the control variant from 54.41% to 75.81% with an average of 60.32% ±7.13 (SD).

The alcoholic extracts used induced variable stimulation indices, ranging between 11.16% in the case of the application of the *Arnica* extract and 82.32% in the case of the application of the *Calendula* extract. The highest average stimulation index was recorded in the case of cultures treated with alcohol (56.57%) and the lowest in the case of treatment with *Arnica* (18.43%). Red blood cell lysate determined an intermediate response. In group II, in the case of the control variant, the stimulation index indicated values between 26.27%-61.39%, with an average of 45.98 ±11.97 (SD).

Phytohemagglutinin showed values of the stimulation index close to those of the control sample, with values between 28.13% and 61.62%, with an average of 50.13% ±10.74 (SD).

The alcoholic extracts used induced variable stimulation indices between 14.18% in the case of the application of the *Arnic*a extract and 68.37% in the case of the application of the *Echinacea angustifolia* extract. The lowest average stimulation index was recorded in the case of crops treated with *Arnica montana* (20.23%), and the highest in the case of the treatment with *Echinacea purpurea*. Red blood cell lysate determined an intermediate response (49.14%). When analyzing the results of the first harvest in groupes 3 and 4 in the case of the control variant, values between 38.13% and 66.97% were observed, with an average of 50.26% ±9.78% (SD).

In the case of phytohemagglutinin, the stimulation index values were between 49.06% and 66.51% with an average of 58.97% ±5.92% (SD).

The alcoholic extracts used induced variable stimulation indices, ranging between 13.72% in the case of *Arnica* extract application and 68.37% in the case of alcohol application (extract control). The lowest average stimulation index is registered in the case of cultures treated with *Arnica* extract (26.91

%), and the highest in the case of alcohol treatment (57.17 %). Red blood cell lysate determined an intermediate response (54.14%).

In group 4, in the case of the control variant, there was a fluctuation of the stimulation indices between 53.25% and 88.13%, with an average of 72.49% ±17.69% (SD).

Phytohemagglutinin acts as a more intense stimulant, thus the stimulation indices were slightly higher than in the case of the control variant from 51.86% to 91.86%, with an average of 74.55% ± 17.10%.as an inhibitor, allows sensing the phagocytic dynamics. In the case of alcoholic extracts, the minimum value of the stimulation index was 9.30% in the case of the use of the *Arnica* extract, and the maximum value of 91.86% in the case of the use of the *Echinacea purpureea* extract with an average of 46.24%. At harvest 2 in the case of the control variant, in group I, a fluctuation of the stimulation indices was observed between 36.02% and 54.03%, with an average of 41.87% ± 8.87 (SD).

The alcoholic extracts used induced variable stimulation indices between -4.02% in the case of the use of the *Arnica* extract (so a negative action) and 51.86% in the case of the use of the *Calendula* extract with the highest average value of 36.37% in the case of using *Echinacea angustifolia* extract and the lowest of 10.24% in the case of *Arnica* extract. Red blood cell lysate determined an intermediate response.

In group II, by analyzing the results in the case of the control variant, a fluctuation of the stimulation indices was observed between 30.12% and 49.37%, with an average of 40.81% ±8.14 (SD). Phytohemagglutinin acted as a more intense stimulant, so the stimulation indices were higher than in the case of the control variant (25.15% - 56.20%). The alcoholic extracts used showed a maximum value in the case of using the alcohol extract (50.30%), while the *Arnica* extract had a negative effect (-15.53%). The average values of the stimulation index were 7.44% in the case of the use of the *Arnica* extract and 33.36% in the case of the use of the alcohol extract, while the red blood cell lysate has a response close to that of the alcohol extract, respectively 33 .71%.

In the case of the control variant in group I at the second harvest, the stimulation index indicates values that vary from 23.59% to 43.47%, with an average of 36.59% ±7.58 (SD).

Phytohemagglutinin indicated a more intense stimulatory potential. In the case of alcoholic extracts, the stimulation indices showed values ranging from -10.56% in the case of the *Arnica* extract (so a negative action), to 59.31% in the case of the alcohol extract. The highest average stimulation index was recorded in the case of cultures treated with *Echinacea purpureea* extract (42.18%), and the lowest (16.98%) in the case of treatment with *Arnica montana*. Red blood cell lysate causes an intermediate response (42.05%). In group IV, in the case of the control sample, the stimulation index indicates values between 24.5% and 57.76%, with an average of 39.97% ±12.88% (SD). In the case of phytohemagglutinin, the values obtained were between 21.42% and 60.24%, with an average of 42.10% ±14.36% (SD).

Among the alcohol extracts used, the highest value of the stimulation index was obtained in the case of the use of the alcohol extract of 57.45%, while the extract of *Symphytum officinale* has a strong negative action (-13.04%). The highest stimulation index is recorded in the case of cultures treated with alcohol extract (36.77%), and the lowest in the case of treatment with *Arnica montana* (14.10%). Red blood cell lysate causes an intermediate response. Analyzing the results after the third harvest in the case of group I, the control sample showed a fluctuation of stimulation indices between 29.81% and 69.25% with an average value of 52.48% ±8.21(SD).

The alcoholic extracts used had values of the stimulation index between 10.86% in the case of the *Arnica* extract and 64.59% in the case of the use of the *Calendula* extract. The stimulation index had the highest value in the case of treatment with *Calendula* extract (56.65%) and the lowest in the case of treatment with *Arnica* (22.13%). Red blood cell lysate causes an intermediate response (54.65%). In group II, in the case of the control sample, the stimulation index had the maximum value of 65.83% and the minimum value of 30.74%, with an average of 50.13% ±13.76 (SD).

Phytohemagglutinin acted as a less intense stimulator, so the stimulation indices were lower than in the control variant, from 31.98% to 63.97%. In the case of the use of plant extracts, the stimulation index had the maximum value in the case of the use of the *Echinacea angustiolia* extract (67.39%), and the minimum value in the case of the use of the *Arnica* extract (22.04%).

In group III at the third harvest in the case of the control variant, a fluctuation of the stimulation indices was observed between 40.68% and 76.70% with an average of 58.11% ±12.13% (SD).

Phytohemagglutinin acts as a more intense stimulant, so the stimulation indices are higher than in the case of the control variant, from 51.55% to 78.88%, with an average of 63.66% ±10.80% (SD).

The highest value of the stimulation index was obtained in the case of the use of the alcohol extract (74.84 %) and the lowest value in the case of the use of the *Arnica* extract (24.04 %). In group IV, the control variant showed a fluctuation of stimulation indices between 51.24 and 80.12% with an average of 60.86% ±9.28 (SD).

Phytohemagglutinin induced stimulation index values between 55.89 and 74.84% with a mean of 63.26% ±6.44 (SD).

The alcoholic extracts used induced variable stimulation indices, ranging between 18.32% in the case of the use of the *Arnica montana* extract and 84.78% in the case of the use of the *Calendula* extract. The highest average stimulation index was recorded in the case of cultures treated with alcohol (62.73%), and the lowest in the case of treatment with *Arnica montana.*

The study of the immune system in birds is particularly based on the knowledge gained by investigating immunological effectors and mechanisms in chickens. The cell cultures made from bird blood mainly focus on the blasting of lymphocytes, separated on special media, by concentration gradient centrifugation. Thus, the roles of T lymphocytes can be highlighted, the most important of which is to recognize self and non-self determinants (Giurgea, 1997). The blasting activity of lymphocytes is influenced by environmental factors, including the compounds included in the culture medium. The test of blast transformation *in vitro* allows the evaluation of the efficiency and the comparison of the effectiveness of such compounds. Estimating the values obtained from the blast transformation test, it is found that at the first harvest, the spontaneous blasting indices are heterogeneous, even though the birds were not treated. This fact is due to the inhomogeneity of the birds in the groupes. This discrepancy can also be found in the case of PHA-induced stimulation.

In our study, among the extracts used *in vitro*, the *Echinacea purpurea* extract proved to be the most effective, followed by the *Echinacea angustifolia* extract and the *Symphytum* extract.

Despite all the antigenic stimulation, at the second harvest, the spontaneous blasting indices decrease, still being maximum in the groupes treated with extracts. The differences are not statistically significant. The best response to phytohemagglutinin is given by the group treated with alcohol. Among the plant extracts, *Echinacea angustifolia* extract has the best answer. The response to red blood cell lysate is the most intense in the same group.

At the last harvest, spontaneous blasting indices increase more in groupes not treated with extracts than in treated ones. The response to phytohemagglutinin is similar. They increase the responses to plant extracts in all groups, however, the maximum ones are found in the control group, whose cells were treated *in vitro* with the extract of *Echinacea purpurea*. Comparing these results with those of alcohol stimulation, the differences argue for inhibition.

Lymphocytes maintained in contact with other types of blood cells or isolated in the culture medium, have the ability to multiply under appropriate conditions, spontaneously or under the influence of various non-specific (PHA M, PHA P) or specific mitogenic substances (bovine enzootic leukosis virus) . The evaluation of the degree of blastization by different methods: the incorporation of titrated thymidine or ethidium bromide, the dosage of residual glucose from the culture medium, allows the calculation of the stimulation index, providing the image of the reactive capacity of the T or B subpopulations in the case of an antigenic stimulation in vivo.

The blastic transformation of the cells *in vitro* is due to the stimulation of the cellular metabolism as a result of the reaction between the antigen and the lymphocyte membrane structures.

Following the coupling of the antigen with the receptors on the surface of specifically sensitized lymphocytes, the cell membrane is activated triggering the transmission of a signal to the nucleus, which determines the initiation of DNA synthesis. As a result, there is an increase in the size of the cell, the nucleus becoming larger, with loose chromatin and basophilic cytoplasm.

## CONCLUSIONS

The treatment with alcoholic extracts of *Calendula officinalis* and *Echinacea angustifolia* does not significantly influence the total number of leukocytes during the experiment, so that the final values (16,571.43 ± 2,636.74 respectively 27,333.33 ± 7,167.05) are close to the initial ones (26,785, 71 ± 8,557.93 and 28,500 ± 6,331.14, respectively). A slight decrease is observed in the case of the group treated with *Calendula officinalis* extract. Spontaneous phagocytic activity is diminished towards the end of the experiment, in the absence of antigenic stimulation (0.218 ± 0.060 OD). The alcohol-treated variant exhibits similar activity (0.230 ± 0.082 OD). The plant extracts used initially act inhibitory so that later, the *in vivo* treatment, combined with the *in vitro* one, induces stimulation in the case of both (*Echinacea angustifolia:* OD 0.157 ± 0.07 and *Calendula officinalis:* 0.152 ± 0.056 (OD). Spontaneous blasting indices are lower in the groups treated with extracts (group I: 52.48 ± 13.19 %, group II: 50.13 ± 13.177 %), compared to the one treated with alcohol (58.11 ± 12, 13 %) and untreated (60.87 % ± 9.29 %). Treatment with *Calendula* and *Echinacea* plant extracts reduces the mitogenic blast index (57.41 % ± 8.21 % and 53.15 % ± 11.16 %). Among the plant extracts, only that of *Echinacea angustifolia* (54.86% ± 7.76%) shows stimulating effects in the group treated *in vivo* with the same extract.

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# THE ROLE OF LABORATORY DIAGNOSIS IN THE CONTROL OF THE SILKWORM DISEASES, THE CURRENT STATE, PROSPECTS AND THE IMPACT ON SERICICULTURE

# ROLUL DIAGNOSTICULUI DE LABORATOR ÎN CONTROLUL BOLILOR CONTAGIOASE LA VIERMII DE MĂTASE, STAREA ACTUALĂ, PERSPECTIVE ȘI IMPACTUL ASUPRA SERICICULTURII

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***Abstract***

*With a tradition of thousands of years in the world and several hundred years in our country, sericulture, a branch of animal husbandry whose object is the growth and multiplication of silkworms in order to obtain cocoons intended for processing, is almost becoming a rarity in many areas of the globe. It has a long history in Romania, starting from the 19th century, when it developed steadily until 1990, after which there was a dramatic decline in this field so that currently silk is only processed at a traditional level. The silkworm is an insect with complete metamorphosis (egg, larva, nymph, adult) and, at the same time, a biological model, and the best-known species of silkworms, whose cocoons can be used for silk production, are:* ***Bombyx mori*** *breed (90% considered the most valuable, the only one held in the heritage, which feed on mulberry leaves),* ***Anthereae perny*** *breed (oak silkworm) and* ***Philosamia cynthia ricini*** *breed (Eri silkworm). After the dissolution of S.C. Sericarom SA, by H.G. no. 695/2022, the Baneasa Bucharest Sericulture Research Station was established, which currently operates under the Ministry of Agriculture and Rural Development (MADR) and under the scientific coordination of the "Gheorghe Ionescu-Şişeşti" Academy of Agricultural and Forestry Sciences (AAFS), having as its main object the research-development activity and the conservation of the sericulture genetic pool. The maintenance of the sericulture genetic pool (84* ***Bombyx mori*** *breeds, lines and hybrids) involves in addition to compliance with technical conditions and the performance of sanitary-veterinary examinations (direct microscopy and laboratory diagnostic methods for bacterial, internal parasitic, mycotic and viral diseases, according to the specific methodology. Thus, at the end of the active silkworm season (2022), morphological and microscopic investigations of the preserved silkworm genetic pool were carried out in the Laboratory of Silkworm Genetics, Breeding and Pathology of RSSBB in order to exclude the risk of the presence of pathogenic germs in the generations of silkworms from the next silkworm season (2023). The results of the laboratory examinations were negative for the samples taken in the analysis, a fact that allowed obtaining reproductive material free of diseases, of the best quality. The biological material free from infectious-contagious diseases can be utilized without restrictions by the main target beneficiaries (sericulture farmers, private silkworm breeders who obtain raw silk for economic purposes), who can be advised through specialist consultancy for the prevention and control of silkworm diseases, that can endanger their health and influence the quality production of silkworms cocoons.*

***Keywords: diagnosis, silkworms, contagious diseases***

***Rezumat***

*Cu o tradiție de mii de ani în lume și de câteva sute de ani la noi în țară, sericicultura, ramură a zootehniei care are ca obiect creșterea și înmulțirea viermilor de mătase, în vederea obținerii gogoșilor destinate prelucrării, aproape devine o raritate în multe zone ale globului. Ea are o istorie îndelungată în România, începând cu secolul al XIX-lea, când s-a dezvoltat constant până în 1990, după care s-a produs declinul dramatic al acestui domeniu, astfel că în prezent, mătasea se prelucrează doar la nivel tradițional. Viermele de mătase este o insectă cu metamorfoză completă (ou, larvă, nimfă, adult) și, totodată, un model biologic, iar cele mai cunoscute specii de viermi de mătase, sunt:* ***Bombyx mori*** *(90% considerată cea mai valoroasă, singura deținută în patrimoniu, care se hrănește cu frunze de dud),* ***Anthereae perny***

*(viermele de mătase al stejarului) și **Philosamia cynthia ricini** (viermele de mătase al ricinului). După desființarea Sericarom SA, prin H.G. nr. 695/2022 a luat ființă Stațiunea de Cercetări Sericicole Băneasa București (SCSBB) care funcționează în subordinea Ministerului Agriculturii și Dezvoltării Rurale (MADR) și în coordonarea științifică a Academiei de Științe Agricole și Silvice „Gheorghe Ionescu-Șișești" (ASAS), având ca scop principal activitatea de cercetare-dezvoltare și conservarea fondului genetic sericicol. Menținerea fondului genetic sericicol (84 rase, linii și hibrizi de **Bombyx mori**) implică, pe lângă respectarea unor condiții tehnice și efectuarea de examene sanitar-veterinare (microscopie directă și metode de diagnostic de laborator pentru boli bacteriene, parazitare interne, micotice și virale), conform metodologiei specifice. Astfel, în cadrul Laboratorului de Genetică, Ameliorare și Patologia Viermilor de Mătase al SCSBB au fost efectuate, la sfârșitul sezonului activ sericicol (2022), investigații morfologice și microscopice ale fondului genetic sericicol aflat în conservare, pentru a se exclude riscul de prezență a germenilor patogeni la generațiile de viermi de mătase din sezonul sericicol următor (2023). Rezultatele examenelor de laborator au fost negative pentru probele luate în analiză, fapt care a permis obținerea de material de reproducție liber de boli, de cea mai bună calitate. Materialul biologic indemn de boli infecto-contagioase poate fi valorificat fără restricții către principalii beneficiari țintă (fermieri sericicoli, crescători particulari de viermi de mătase, care obțin mătase brută în scop economic), ce pot fi consiliați prin consultanță de specialitate pentru prevenirea și combaterea bolilor la viermii de mătase, boli care pot periclita sănătatea acestora și pot influența calitatea producției de gogoși de mătase.*

***Cuvinte cheie**: **diagnostic, viermi de mătase, boli contagioase***

## INTRODUCTION

Sericulture is the science that deals with the study of the biology and technology of reproduction, growth and improvement of all sericulture species that produce cocoons in order to obtain natural silk. On the international level, the products obtained from silk represent one of the most expensive textile materials, also being used for non-textile purposes (silk protein used for medical use, cosmetics, and biomaterials). In many countries of the world, the sericulture sector is being developed (India, China, Japan, Thailand, France, Poland, Brazil, Cuba, Uganda, Kenya, Ethiopia, etc.), that tend to move to another performance (automation and digitization) (Yashaswini et al., 2020; Vankadara, 2021; Nalavadi et al., 2021).

Sericulture has a long history in Romania starting from the 19$^{th}$ century, when it developed steadily until 1990, after which the dramatic decline of this field occurred (Pau and Brailoiu-Tanase, 2006), so that currently silk is processed only at a traditional level. The Research Station for Sericulture Baneasa-Bucharest (SCSBB) currently operates under MADR and under the scientific coordination of ASAS (H.G. no. 695/25.05.2022), having as its main activity the research-development and conservation of the sericulture genetic fund (HG no. 695/2022; Law no. 319/2003; Law no. 72/2011; Law no. 45/2009).

Recently, in 2022, in Romania, the 26$^{th}$ edition of the Congress of the International Sericulture Commission (ISC) "*The New Concepts in Sericulture*" dedicated to sericulture specialists was organized at USAMV-Cluj, for the first time in Romania. The representatives of ISC member countries and prominent guests from the field of sericulture and the silk industry, from more than 15 countries, including India, China, Japan, Thailand, France, Romania, Poland, Brazil, Cuba, Uganda, Kenya or Ethiopia, participated.

Romania is a founding member of the International Sericulture Commission since 1959 and obtained from the ISC, in 2016, the Certificate of Recognition through which the Global Center of Excellence for Advanced Research in Sericulture and Promotion of Silk Production was created, based at USAMV Cluj-Napoca.

Sericulture is most developed in China, but also in Japan, India, Korea. In Europe, this field had the same trend as in Romania (*www.fzb.usamvcluj.ro*). There are also important points of work in Italy and Bulgaria.

The biological cycle of silkworms has 4 stages of metamorphosis: egg, larva, nymph (chrysalis) and adult (moth) (Bura et. al., 1995). Figure 1 presents the biological cycle of silkworms (www.wikipedia).

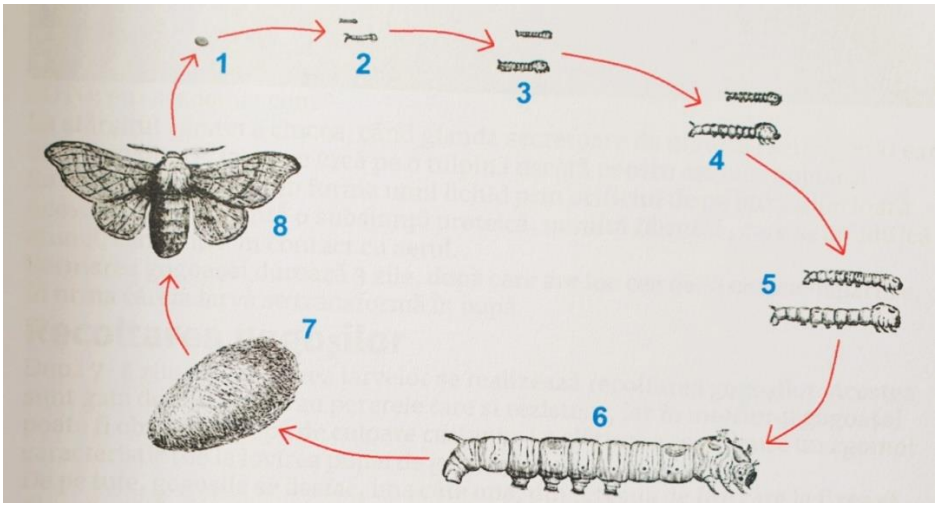


**Figure 1. The biological cycle of silkworms: eggs, larve, nynph and adult / Ciclul biologic al viermilor de mătase: 1. Stadiul de ou; 2-6. Stadiile de larvă; 7. Stadiul de gogoașă; 8. Stadiul de fluture (www.wikipedia.com)**

The silkworms (including the eggs and moth) are susceptible to numerous diseases (viral, parasitic, fungal and bacterial ones), with different routes of transovarian, oral, cutaneous transmission (Doliș, 2008), and currently there is no breed with totally resistance against them (Matei, 2002). Silkworm diseases are divided into two categories: infectious (viruses, bacteria, fungi, protozoa) and non-infectious (attacks by various arthropods, chemicals used in agriculture). The main factor in the production of the disease is the pathogen, without which no infectious disease can occur (Pau, 2000; Pau et al., 2006). Its development can be inhibited by good environmental conditions, and the physiological state of the host, (silkworm). All these elements have a special role in the occurrence of silkworm diseases; therefore, their prevention is of great practical importance. For most of the silkworm diseases, infection is achieved orally, along with the administration of feed (Shilpi and Praban, 2020). Therefore, avoiding contamination of the mulberry leaf, as a food source, represents an important step in the prevention of diseases in silkworms (Kumari et al., 2001; Matei, 2002).

The causes of diseases can be biological (pathogenic microorganisms, parasitic and non-parasitic insects), physical (mechanical injuries and physical irritations), chemical (chemicals used in agriculture), nutritional (food quality, over or under-nutrition), environmental (inadequate quality of food, temperature, humidity, ventilation, unfavorable lighting) or constitutional (differences due to genetic formation, developmental and sexual stage), which ultimately decrease vigor and reduce resistance.

The silkworm, a host for the development of various microbes, shows resistance thresholds in the prevention of diseases through the skin, digestive tract, intestinal juice, blood cells and the immune system (Pau et al., 2006).

Laboratory diagnosis plays a crucial role in ensuring the health and productivity of silkworms, and any disease can have a significant impact on silk production (Dolis, 2008). Laboratory diagnosis can help confirm disease and identify diseases early, allowing prompt treatment and prevention of further spread of diseases. Some of the common diseases that affect silkworms can cause symptoms such as loss of appetite, lethargy and abnormal behavior.

Laboratory diagnosis can help identify the specific pathogen causing the disease, guide the choice of appropriate treatment and management strategies (disinfection of necessity). In addition to diagnosing disease, laboratory analysis can help monitor the health and development of silkworms by assessing the

nutritional content of their diet, monitoring the quality of the mulberry leaves they feed on, and examining the genetic traits of different silkworm species.

In general, laboratory diagnosis is essential to ensure the health, productivity of silkworms, and by early identification and treatment of diseases, sericulture farmers can ensure a healthy and sustainable silk production process (impact on population productivity) (Kang and Guo, 2011).

In this context, the aim of the work was to evaluate the health status of the silkworm gene pool and highlight the importance of laboratory diagnosis in the control of contagious diseases in silkworms.

## MATERIAL AND METHODS

Health monitoring through morphoclinical and laboratory examinations on moth of **Bombyx mori** was carried out in order to prevent and control diseases that may later develop in silkworms. In the Laboratory of Silkworm Genetics, Breeding and Pathology of RSSBB, at the end of the 2022 active silkworm season, investigations were carried out on the biological material of silkworms of the **B. mori** species, respectively on the 84 existing breeds, lines and hybrids that constitute the genetic background, maintained in conservation.

Monitoring sheets were drawn up that included the following: breed/line or hybrid, female characteristics, clutch morphology, results of direct microscopic examination and for pathogens, number of clutch cells removed and admitted for preservation.

As an example, we present below the methodology used to analyze an indigenous race, chosen randomly, with a total number of 140 samples, of which 70 female butterfly samples and 70 stem cells were analyzed (Figure 2 and Figure 3).

**Figure 2. Femele moth of *B. mori* laying eggs / Femela de *B. mori* în timpul depunerii pontei (RSSBB)**

**Figure 3. Paper cell specially need for eggs laying / Celulă din hârtie pergament specială utilizată pentru depunerea pontei (RSSBB)**

The morphoclinical examination sought the elimination of clutches with a reduced number of eggs, unfertilized eggs, unevenly arranged eggs or conglomerates.

The microscopic examination (LEICA microscope, 400x and 100x resolution) was performed based on the regulations of the International Office of Epizootics (OIE, 2016), adapted for samples of female *B. mori* moth (www.idsa.ro). To detect endoparasitises, the presence and number of spores of *Nosema* sp. was determined, to detect mycotic agents, the presence of fungal agents was determined, and to detect viral diseases, the presence of polyhedral crystals was determined.

Briefly, the working technique consists in triturating the female butterflies by placing them individually in special 18-compartment trays/mortars that are attached to the triturating machine, adding

distilled water and triturating until a thick liquid is obtained. With the help of a rod, a drop of the liquid in each mortar is deposited on a glass slide which is covered with a slide, and then subjected to microscopic examination. If pathogenic agents are observed on microscopic examination, the respective cell with the pontine is removed and destroyed by burning. If no pathogens are observed after examining about 10 microscopic fields for each sample, it follows that the female was healthy, so the eggs she laid in the cell are also healthy. After microscopic examination, the stem cells from healthy females were kept under appropriate conditions in the cold room (2.5-5ºC) for preservation.

## RESULTS AND DISCUSSION

The analysis of the monitoring sheets regarding the morphoclinical examination of the moth showed the lack of even slight changes in the external appearance, finding they had developed properly, did not present physical integumentary injuries (intact integument, no changes in the integrity of the wings (wrinkling, malformations or shrinking of the wings) and legs (excrescences, anomalies), without skin color changes, lack of body fragility (dehydration), absence of smell (flacherie) and diarrheal excrement (nosemosis) (Figure 4 and 5). No typical symptoms of flacherie, grasserie or nosemosis were identified in *B. mori* moth.

The analysis of the monitoring sheets of the cells with the laying eggs related to each *B. mori* moth presented from a morphological point of view the concentric arrangement of the eggs, without identifying a scattered arrangement of them (nosemosis).

**Figure 4. Clinically healthy *B. mori* butterflies/ Fluturi de *B. mori* clinic sănătoși (RSSBB)**


**Figure 5. Concentrically arranged B. mori eggs from clinically healthy females / Ouă de *B. mori* cu dispunere concentrică provenită de la femele clinic sănătoase (RSSBB)**

Viral diseases of silkworms are produced by *Borrelina bombicys* with 2-6 microns polyhedra sizes, having triangular, hexagonal, tetragonal shape (Nuclear Polyhedra/NPV); or icosahedral, hexagonal, sometimes tetragonal or triangular polyhedra shape between 1 and 10 microns (Cytoplasmic Polyhedra/CPV) (Figure 6), as well as with globular viral particles of an yet undetermined structure (Flacherie infectious/FV) (Rahul et. al., 2019; Chopade et al., 2021).

Endoparasitic diseases (pebrina/nosemosis) are caused by the protozoan *Nosema bombycis* (Figure 7), with three stages of evolution, respectively spores, planont and meront (Chopade et. al., 2021).

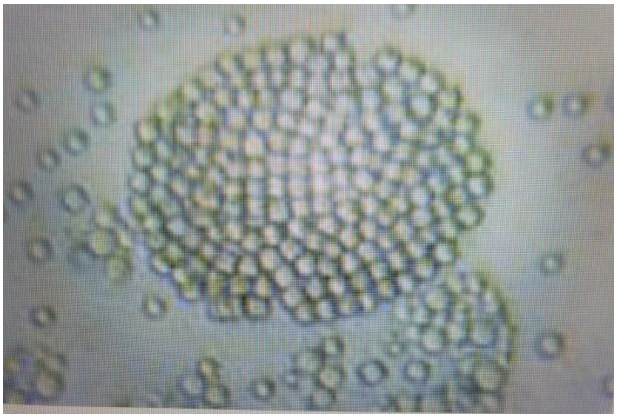

**Figure 6. The morphological aspect of the cytoplasmic polyhedra virus / Aspectul morfologic al virusului poliedriei citoplasmatice (Liu, 2017)**

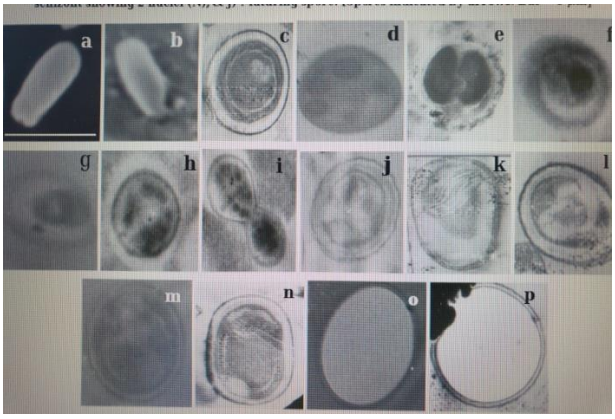


**Figure 7. Mature spore of *Nosema sp.* and intermediate forms / Spor matur de *Nosema* sp. și forme intermediare (b-p) (Chakrabarty et al., 2012)**

Mycotic diseases are caused by *Beauveria bassian*a (www.ansvsa.ro), a fungus that shows three stages in evolution: conidia, vegetative mycelium and aerial mycelium. The conidia have an oval, globular shape, visible as a white powder that penetrates the integument of the larva after germination (white muscardina) (Figure 8 and 9).

In aspergillosis, the pathogen is represented by fungi of the genus *Aspergillus sp*. (Chopade et al., 2021).

**Figure 8. The morphological aspect of the colony of *Beauveria bassiana* / Aspectul morfologic al coloniei de *Beauveria bassiana* (Shanmugam and Seethapathy, 2017)**

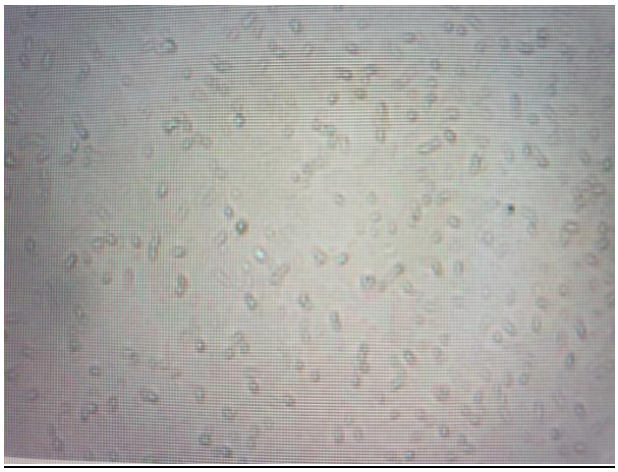

**Figure 9. Conidia of *Beauveria bassiana* / Conidia de *Beauveria bassiana* (Shanmugam and Seethapathy, 2017)**

Bacterial diseases are caused by bacteria of the genus *Bacillus*, with Gram positive bacilli measuring 1-1.5 x 3 microns, (Bacterial Septicemia) and bacteria of the genus *Streptococcus* sp. round diplococci or in the form of chains, Gram positive, 0.7-0.9 µ in size, (Infectious Anemia) (Chopade et al., 2021) (Figure 10 and 11).

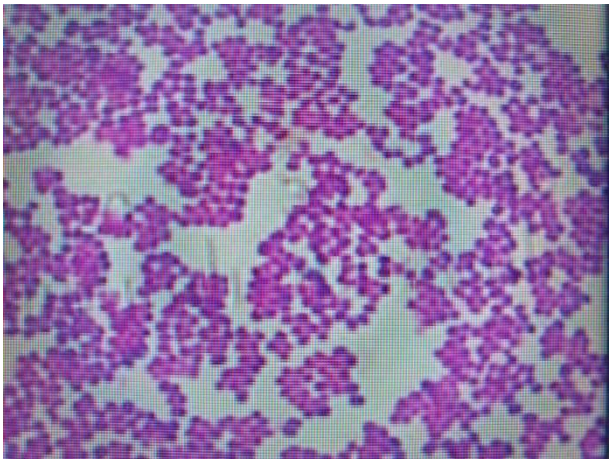

**Figure 10. Morphological appearance of diplococci and streptococci / Aspectul morfologic al diplococilor și streptococilor (Rahul et al., 2019)**

**Figure 11. The morphological aspect of the bacteria *Bacillus sp.* / Aspectul morfologic al bacteriilor *Bacillus* sp. (Liu, 2017)**

In the case of the example taken in the study, the microscopic examination of the analyzed samples (female moth of *B. mori*), no pathogens were identified: elementary particles of *Borrellina bombycis* in the form of polyhedral crystals (flacheria), chains of streptococci and staphylococci (infectious anemia), spores of internal parasitosis (nosemosis/pebrina), nor conidia, sterigma, conidiophores of mycoses (muscardina, aspergillosis).

For experimental purposes, during the inactiv season of March 2023, a eggs laying cell with 560 eggs (examined morphoclinically and microscopically) from a randomly selected breed was used in the Laboratory of Silkworm Genetics, Breeding and Pathology from RSSBB, preserved at 2.5ºC (diapause), with the aim of of verifying biological parameters of the laying eggs and hatching of the breed (% hatching and % viability). For the incubation of the eggs, the method of gradually raising the temperature and a relative humidity of 75-85% was used.

**Table 1. Percent hatching and viability during the first 3 days of the experiment / Procentul de ecloziune și viabilitate în primele 3 zile de experiment**

| Hatching *B. mori* | No. hatched larvae / % hatching 1 day | No. hatched larvae / % hatching 2 days | No. hatched larvae / % hatching 3 days | No. unhatched eggs/ % hatching | Hatching percentage / Survival rate after 3 days |
|---|---|---|---|---|---|
| 560 eggs | 106 larve (18,93 %) | 408 larve (72,86 %) | 21 larve (3,75 %) | 25 ouă / 4,46 % | 95,54 % (100 %) |

From the analysis of table 1, it can be seen that out of the total of 560 eggs that constituted the brood of a cell, the hatching percentage of the eggs in the breed in the experiment was 95.54%, distributed as follows: on the first day, 106 larvae hatched (18.93 %) (Figure 11 and 12), 2nd day 408 larvae (72.86%) and 3rd day 25 larvae (4.46%). During the experimental period (3 days after hatching), a 100% survival rate was observed for the larvae, which then entered sleep period 1 (moult 1) (Figure 13 and 14).

The high larval survival rate indicated that the preservation conditions of the sericicolic genetic material were adequate, and the eggs used in the experiment were able to withstand diapause stress and resume normal development.

**Figure 11. Hatched eggs of *B. mori*, dormant season 2023/ Ouă eclozionate de *B. mori*, sezon inactiv 2023 (RSSBB)**

**Figure 12. *B. mori* larvae hatched on the first day, dormant season 2023 / Larve de *B. mori* eclozionate în prima zi, sezon inactiv 2023 (RSSBB)**

**Figure 13. *B. mori* larvae hatched for 2 days, inactive season 2023 / Larve de *B. mori* eclozionate de 2 zile, sezon inactiv 2023 (RSSBB)**

**Figure 14. B. mori larvae, age 4 days, during sleep 1 (moult 1) / Larve de *B. mori,* vârsta 4 zile, în timpul somnului 1 (năpârlirea 1) (RSSBB)**

## CONCLUSION AND RECOMMENDATION

The maintenance of the sericulture genetic pool involves sanitary-veterinary examinations (morphoclinical, direct microscopy), as well as laboratory diagnostic methods for silkworm diseases (bacterial, internal parasitic, fungal and viral), according to the specific methodology, adapted to the conditions of the Laboratory of Silkworm Genetics, Breeding and Pathology of RSSBB.

Health monitoring through morphoclinical and laboratory examinations on samples of *B. mori* moth, was carried out in order to prevent and control diseases that may later develop in silkworms.

Health monitoring through morphoclinical and laboratory examinations on samples of *B. mori* female mouth was carried out in order to prevent and control diseases that may later develop in silkworms, our study did not identify pathogens in the females taken in the study, and the eggs that deposited in the cell were healthy, resisting the stress of diapause, with the resumption of normal development.

The results of the laboratory examinations were negative for the samples taken in the analysis, a fact that allowed obtaining disease-free breeding material of the best quality.

Ensuring optimal microclimate conditions and prophylactic disinfection are mandatory conditions for ensuring the well-being and health of silkworms, in order to obtain and preserve disease-free silkworm biological material.

## TRENDS AND PERSPECTIVES

- One of the main challenges in sericulture is the prevalence of diseases and infections affecting silkworms that can cause significant economic losses to sericulture farmers. To address these challenges, RSSBB's sericulture pathology researchers focus on developing new diagnostic tools and techniques (using DNA sequencing, advanced molecular biology techniques - PCR (Bebitha et al., 2020), identification of genes and markers associated with different diseases, understanding the mechanisms of pathogenesis (identification of key virulence factors and their interactions with silkworm host cells), investigating host-pathogen interactions (investigating the immune system, hemolymph and fat body), developing new strategies for disease management and investigating the role of environmental factors (temperature and humidity) on silkworm health.
- Another trend that could outline the future of pathology in sericulture is the development of new treatments and management strategies to control the spread of diseases (the use of probiotics and other biological agents to improve silkworm health).

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# THE EXTENT OF SYSTEMIC IMMUNOLOGICAL CHANGES DEPEND ON HIPERSENSITIVITY LEVEL IN BOVINE TESTED FOR TUBERCULOSIS

# AMPLITUDINEA MODIFICĂRILOR IMUNOLOGICE SISTEMICE DEPINDE DE NIVELUL DE HIPERSENSIBILITATE LA BOVINE TESTATE PENTRU TUBERCULOZĂ

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***Abstract***

*Tuberculosis (TB), a multi-host zoonotic disease, still represents a great concern for One Health in many countries worldwide. The research, carried out on bovine from a TB positive farm (n=47, divided in three groups: positive, uncertain and negative by the skin test), where an incomplet stamping out programme was implemented, aimed at monitoring the in vitro changes of systemic immunity in relation to the skin test results. Blood was sampled twice, before and at reading of the skin test, and subjected to carbon particle inclusion and blast transformation tests to evaluate cell mediated responses to mitogens, vegetal extracts and bovine/avian PPD. Circulating immune complexes (CIC) and immune globulins (Ig) were quantified by PEG 4.2% and ZnSO424%o preciptation methods, respectively. The results indicated the lowest values for phagocyatosis in the positive animals, while the blast transformation was most stimulated towards avian and bovine PPD (93.51±24.26% and 91.21±18.04%, respectively). An increased humoral immune activity was also recorded in the positive group, which showed the highest CIC (0.281 ± 0.017ODU) and Ig (0.182 ± 0.004 ODU) values. A positive, statistically relevant (p<0.001) correlation between the phagocytic capacity, CIC, Ig and the cutaneous reactivity was poited out. All tests indicated extensive changes in innate and adaptive immunity, suggesting their use, although not golden standard could be beneficial in surveillance of TB immunity in positive herds.*

***Key words: tuberculosis, skin test, bovine, phagocytosis, blast transformation, immune complexes, total immune globulins***

***Rezumat***

*Tuberculoza (TB), o boală zoonotică, continuă să reprezintă un pericol major în cadrul One Health în numeroase țări din întreaga lume. Cercetarea, efectuată pe bovine dintr-o fermă TB pozitivă (n=47, grup: pozitiv, neconcludent și negativ la testul cutanat), în care s-a implementat un program de eradicare incomplet, a urmărit monitorizarea modificărilor in vitro ale imunității sistemice în relație cu rezultatele testului intradermic. Sângele a fost prelevat de două ori, înainte și la citirea testului cutanat și prelucrat prin teste de înglobare a particulelor de carbon și de transformare blastică (TTB) pentru a evalua răspunsurile mediate celular la mitogeni, extracte vegetale și tuberculină bovină/aviară (PPD). Complexele imune circulante (CIC) și imunoglobulinele (Ig) au fost cuantificate prin metodele de precipitare cu PEG 4,2% și respectiv ZnSO424%o. Cele mai scăzute valori pentru fagocitoză s-au observat la animalele pozitive, în timp ce TTB a fost stimulată către ambele tuberculine (93,51±24,26% și 91,21±18,04%). O activitate imună umorală crescută a fost observată la grupul pozitiv, cu cele mai mari valori CIC (0,281 ± 0,017ODU) și Ig (0,182 ± 0,004 ODU). S-a evidențiat o corelație pozitivă, relevantă statistic (p<0,001) între capacitatea fagocitară, CIC, Ig și reactivitatea cutanată. Toate testele au indicat modificări extinse ale imunității înnăscute și adaptive, sugerând că utilizarea lor ar putea fi benefică în supravegherea imunității la TB în efectivele pozitive.*

***Cuvinte cheie: tuberculoză, intradermoreacție, bovine, fagocitoză, transformare blastică, complexe imune, imunoglobuline totale***

## INTRODUCTION

*Mycobacterium bovis*, one of the much-studied members of the *M. tuberculosis* complex, causes bovine tuberculosis, a zoonotic disease affecting cattle worldwide, induced by contact with or consumption of contaminated animal products (Cousins, 2001). Although relatively drastic measures are first implemented on farms, the disease continues to cause production losses when insufficiently controlled (Ballarini et al., 1971, Dungworth, 1992). WOAH includes tuberculosis on the list of diseases common to several animal species, as a disease considered to be of socio-economic or public health importance in countries and of importance to international trade in animals and animal products (WOAH, 2022). Consequently, most developed countries have engaged in campaigns to eradicate *M. bovis* from the cattle population or at least to try to control the spread of the infection. The success of these eradication and control programs has sometimes questionable.

*Mycobacterium bovis*, having multiple hosts, is found in both domestic and wild animals, and this range of hosts can complicate attempts to control or eradicate the disease in cattle. Thus, bovids, leporids and felids are susceptible to *M. bovis* but relatively resistant to *M. tuberculosis*. Wild ungulates are generally susceptible to *M. bovis*, with few reports of *M. tuberculosis* being detected in them. The susceptibility of pigs (Thoen, 2006) and dogs to *M. bovis* and *M. tuberculosis* is approximately equal. In the case of human patients, currently *M. bovis* causes a relatively small number of clinically manifest cases among those reported (LoBue, 2006). However, *M. bovis* remains a pathogen of significant importance in wild and domestic animals around the globe, with some areas of the world map being white islands, where the availability of information on the disease is absent or vague (LoBue et al., 2010).

The genus *Mycobacterium* belongs to the category of microorganisms transmitted orally or by respiratory route, but which survive intracellularly, and which in the absence of specific serum prevents the attachment of lysosomes to phagosomes. Even in the presence of specific antibodies, when the enzyme content overflows into the phagosome, *Mycobacterium* shows resistance to this attack. It should be mentioned that the partial destruction of some antigens by macrophages allows the expression of some fragments which are then presented to the cells of the immune system inducing the specific response (Muscoplast et al., 1975, Neill and Pollock, 1994). The presence in the infected body of agents from the genus *Mycobacterium*, induces a particular state of reactivity, not transmissible through the serum of sick animals (Moraru, 1984, Olsen et al., 2010). There are studies that support the pathogenicity of the *M. tuberculosis* complex being a multifactorial phenomenon. However, in cases where the host response cannot destroy the bacillus due to immune-compromising conditions resulting in low CD4+ T-cell counts, such as immune suppression due to chemotherapy, stress or HIV, reactivation can occur, resulting in the release of bacilli and transmission of infection (Brosch et al., 2002).

Phagocytosis represents one of the first intervention protection mechanisms, with particular significance, especially in the prevention of bacterioses (Răducănescu and Bica-Popii, 1986). The role of neutrophils in tuberculosis infection remains less studied compared to that of CD4+ T lymphocytes and macrophages. Thus, changes in the transcriptional profile of *M. tuberculosis* following phagocytosis by neutrophils and how these changes differ from those caused by phagocytosis by macrophages remain unknown (Kondratieva et al., 2022, Queval et al., 2017).

In this function, polymorphonuclear granulocytes are active, together with mononuclear cells of the macrophage type (Maphasa et al., 2021), whether they are in circulation or fixed in tissues (Saragea, 1987). Numerous methods have been designed for the evaluation of phagocytic activity. Testing can be done using whole blood samples against different live or inactivated bacterial suspensions, *in vitro*, or by assessing the activity *in vivo* (Roitt et al., 1989, Thoen, 1994). The *in vitro* carbon particle inclusion test has wide applicability and is based on the principle that under appropriate temperature conditions, the phagocytic activity of heparinized blood continues (Kumar et al., 2019). The suspension of carbon particles added in a certain proportion is incorporated by micro- and macrophages over time. Following

the clarification of the supernatant the phagocytic activity can be evaluated by spectrophotometry (Roitt, 1991, Rothel et al., 1992).

The dominant immune response in tuberculosis is cellular (Roitt, 1991, Thorns and Morris, 1983, Wood et al., 1992). The *in vitro* blast transformation of circulating leukocytes is due to the stimulation of cellular metabolism as a result of the reaction between antigen and lymphocyte membrane structures (Saragea, 1987, Gindeh et al., 2020). The response of circulating T lymphocytes as well as that at the site of infection are considered important (Sable et al., 2005).

Lymphocytes maintained in contact with other types of blood cells or isolated in a culture medium, have the ability to multiply under appropriate conditions, spontaneously or under the influence of various antigens that infect the host organism. Following the coupling of the antigen with the receptors on the surface of specifically sensitized lymphocytes, the cell membrane is activated triggering the transmission of a signal to the nucleus, which determines the initiation of DNA synthesis (Roitt et al., 1989). As a result, there is an increase in the size of the cell, the nucleus becoming larger, with loose chromatin and basophilic cytoplasm (Moraru, 1984). More advanced methods, with a pronounced objectivity in expressing the results, are the labeling of cultures with tritiated thymidine (Muscoplast et al., 1975; Rothel et al., 1992) or the dosing of glucose consumption as a metabolic indicator, by automated reading of the results.

In tuberculosis infection, humoral effectors do not represent elements of the hypersensitivity and resistance mechanism, however, they are endowed with a special specificity for antigens (Muscoplast et al., 1975, Cole et al., 1998), even allowing antigen detection (Răducănescu and Bica- Popii, 1986). Serological diagnosis can be made difficult by the presence of non-specific autoantibodies, which can react with tissue antigens and cross-react with *M. tuberculosis* (Roitt et al., 1989, Saragea, 1987, Gindeh et al., 2020). In the case of the humoral immune response, the role of immunoglobulins is a major one. The activity of gamma-globulins depends on: their molecular structure, their concentration in the serum and the reactive capacity of the body (Moraru, 1984, Stites and Terr, 1991). The application of the zinc sulfate precipitation test for the dosage of total immuneglobulins provides a completion of the immunological picture in affected animals, even without identifying the classes of immunoglobulins involved. The test is based on the fact that at pH 7.4 the electric charge and the colloidal stability of gammaglobulins is lower than that of serum albumins, thus the test using Serb solution can be performed (Roitt, 1991).

Immune complexes are continuously formed in the case of coupling between Ag and Abs and they are efficiently removed by the reticulo-endothelial system. In some cases, the formation of immune complexes leads to hypersensitivity reactions. The formation of immune complexes occurs in different cases: long-term infections, autoimmunopathies, for example in the lungs, following repeated inhalation of antigens of a different nature; their presence indicates either an effective way of removing the antigen, or, especially in the case of their chronic presence in the body, the ineffectiveness to remove antigens (Rothel et al., 1992). The structure of the immune complexes is dependent on the nature of the antigen and the antibody involved in their formation, the antibodies involved usually being of the IgG type. The dosage of circulating immune complexes provides a valuable element in defining the immunological profile (Lyashchenko et al., 2017). Several methods have been developed for the assessment of these complexes. One of these methods, polyethylene glycol - precipitation (Thorns and Morris, 1983, Thoen et al., 2009), was also used in the present experiment.

The research sought to establish some correlations between skin allergic reactivity and the variation in the concentration of total gammaglobulins, circulating immune complexes, phagocytic function, *in vitro* leukocyte blastogenic capacity in cattle from a unit with notified tuberculosis that was subjected to erradication by extraction.

## MATERIALS AND METHODS

The research was carried out on 47 animals divided into 3 groups (positive, uncertain, negative) based on the results obtained by the comparative skin test (TCS), performing two blood sampling on the technological flow: before intradermal injection of PPD (17 animals) and when reading the tuberculin test (30 animals). Blood samples have partially served the diagnosis of serological surveillance for enzootic bovine leukosis and brucellosis. Blood was collected on heparin (50IU/ml) for cellular tests and on procoagulant gel to obtain blood serum. The samples taken for cell tests were processed within a maximum of 4 hours after sampling and the sera were stored at -20ºC until processing. All assays were performed in duplicate.

**a. Quantification of total immunoglobulins**

In the wells of a 96-well plate, 3.3 µl serum were added to 196.7 µl Serb reagent. After shaking with the electric stirrer, the samples were incubated at room temperature for 30 minutes, then the optical densities were read on Sumal PE2 (Karl Zeiss, Jena) at a wavelength of 475 nm, against a blank sample represented by the reagent. The results were expressed in optical density units (ODU). Lipemic and hemolyzed sera were not tested.

**b. Quantification of circulating immune complexes (CIC)**

An amount of 193.4 µl of 4.2% polyethylene glycol solution in borate buffer was added to 6.6 µl of whole serum, using 96-well plates as a support for the reaction. After an incubation period of 60 minutes at room temperature, the optical densities of the mixtures were read spectrophotometrically on a Sumal PE2 apparatus (Karl Zeiss, Jena) at a wavelength of 450 nm, directly in the working plates, compared to the borate buffer +control research serum, tested under the same conditions. The levels of circulating immune complexes were calculated by performing the difference between the optical densities of the samples precipitated with PEG 4.2% and the corresponding samples treated with borate buffer. The obtained values were expressed in optical density units (ODU).

**c. Carbon particle inclusion assay to assess phagocytic activity**

To each sample 6 µl of China ink supernatant (centrifuged at 6,000 rpm for 20 minutes) were added. 150 µl of blood and india ink mixture were transferred into 2 ml of saline at 0, 30 and 75 minutes of incubation at 37ºC. Three variants were tested in parallel: control, with bovine tuberculin (12 µl) and avian tuberculin (12 µl). The tubes were centrifuged at the end of the incubation at 1500 rpm for 10 minutes. 200 µl were taken from the supernatant from each test tube and distributed in a 96-well plate. The reading was done with the Sumal PE2 spectrophotometer against saline, at a wavelength of 535 nm and the values were expressed in UDO.

**d. The in vitro blast transformation test for the evaluation of mononuclear cell activity**

The blood taken on heparin (50 IU/ml) was diluted with RPMI 1640 medium with the addition of 5% fetal calf serum (SFV) at a ratio of 1:5. The pH was adjusted to 7.2 – 7.4 with a 5% sodium bicarbonate solution and antibiotics (1000 I.U. penicillin and 1000 micrograms streptomycin/ml) were added to inhibit bacterial growth. The blood thus processed was distributed in sterile plates with 96 wells, each 200 µl/well. Several experimental variants were carried out: untreated control, treated with PHA (phytohemagglutinin, established mitogen), alcohol, alcoholic extracts of *Calendula officinalis, Arnica montana, Symphytum officinale, Echinacea angustifolia, Echinacea purpureea*, avian tuberculin and bovine tuberculin (each 1.5 µl), to compare the cellular response to different stimulating molecules. Each variant, including the untreated control, was tested in triplicate. The plates were incubated for 72 hours at 37°C in a $CO_2$ atmosphere. Initially, an average supernatant sample was obtained for each variant, homogenizing 12.5 µl from the three wells in a single tube. 12.5 µl of this mixture were passed on 0.5 ml ortho-toluidine reagent and after homogenization, the test tubes were kept in a water bath at 100°C for 8 minutes. A glucose standard (with a concentration of 100 mg%) and the initial medium were used

identically for each series of samples. Extinction was measured at a wavelength of 610 nm, in 96-well plates with the Sumal PE2 spectrophotometer.

The concentration of glucose in the samples to be tested is based on their extinction and the extinction of the glucose standard according to the formula:

$$C_P = \frac{E_P}{E_S} x100 \quad \text{where:}$$

$C_P$ = sample concentration in mg glucose %
$E_P$ = sample optical density
$E_S$ = standard optical density

Considering the residual concentrations of glucose after the cessation of cell growth and the initial concentration of the medium, blastogenic indices expressed as a percentage can be calculated, both for control and stimulated cultures. The reference value in the case of the control culture is the complete medium and for the stimulated culture it is the untreated control variant.

The following formulas are used:

$IS_M$ = (RPMI glucose – control glucose)/RPMI glucose x100,

$IS_{PP}$ = (control glucose – sample glucose)/control glucose x100

where
$IS_M$ is the spontaneous blastogenic index, and
$IS_P$ is the blastogenic index induced by phytohemagglutinin or other variants.

The blastogenic indices induced by PHA and the various extracts were calculated according to the formula.

**e. Statistical calculation**

All the results obtained through these tests were processed statistically to obtain the average values, standard deviations and variation using the usual formulas in the Microsoft Excel program. The significance of the differences was assessed using the t-Student test. For the positive and uncertain groups, the correlation coefficients between allergic skin reactivity and phagocytic activity were calculated at all three moments.

## RESULTS AND DISCUSSION

### a, b. Correlations between skin reactivity and humoral immune response in tuberculin-positive cattle

The results of the evaluation of total Ig and CIC levels were presented in table 1. It can be seen that in both sampling the CIC value is the highest in the positive group (0.235; 0.281 ± 0.017 UDO), it decreases in the uncertain group (0.232 ± 0.067 UDO; 0.250 ± 0.043 UDO) and is reduced in the negative group (0.215 ± 0.079 UDO; 0.213 ± 0.044 UDO). For the positive and uncertain batches at the second sampling (performed when reading the tuberculin test), the values were increased, the difference being obvious in the case of positive animals. On the other hand, in the negative animals, an insignificant decrease is found.

In several diseases, CIC levels provide a measure of the body's reactivity, on the one hand, and the severity of the disease on the other. In microbial diseases, the excess formation of CIC can lead to their deposition in various organs, with the activation of the membrane attack complex (complement) and the consequent destruction of the noble tissue, the release of new antigens and the formation of this time antibodies against the modified self .

**Table 1. Total Ig and CIC values in bovine based on their skin test results / Variația Ig totale și CIC la bovinele testate prin TCS, în fucție de categoria de reacție**

| Category | Sampling | CIC (UDO) | total Ig (UDO) |
|---|---|---|---|
| **positive** | I | 0,235 | 0,185 |
| | II | 0,281 ± 0,017 | 0,182 ± 0,004 |
| **uncertain** | I | 0,232 ± 0,067 | 0,145 ± 0,026 |
| | II | 0,250 ± 0,043 | 0,150 ± 0,047 |
| **negative** | I | 0,215 ± 0,079 | 0,143 ± 0,052 |
| | II | 0,213 ± 0,044 | 0,142 ± 0,041 |

Due to the chronic character of the disease and the presence of the antigen in the body over a long period of time, it was assumed that CIC can occur in high concentration. Their dosage, correlated with the hypersensitivity tests or supporting it, could serve as a diagnostic test. Comparing the two sampling, in the case of the positive batch, the CIC level emphasizes the presence of an infectious process, compared to the negative batch, in which case, the two sampling reveal similar results. Since the differences between the groups are quite small, it is difficult to conclude that tuberculosis infection significantly influences the formation of CIC, at the time of testing. However, the highest values, consistently obtained in the positive batch, suggests this possibility.

Microbial aggression induces in the body an immune response translated by the secretion of gamma globulins with antibody activity and the appearance of sensitized cells, mediators of cellular immunity. In tuberculosis, the diagnostic role of antibodies is less important, the type IV hypersensitivity reaction being cell mediated.

**Table 2. r values for the correlation between the skin test results and total Ig and CIC values / Coeficienţii de corelație între TCS și nivelele Ig totale și CIC**

| | Positive | Uncertain |
|---|---|---|
| $r_{\text{alergic test - CIC}}$ | **1, p<0.001** | **0,334 , NS** |
| $r_{\text{alergic test– total Ig}}$ | **1, p<0.001** | **0,133, NS** |

NS- non significant

Following the results obtained, in the case of the first sampling, it is observed that the positive animals present an increased concentration of immunoglobulins, compared to the animals in the R and N groups, which are at the same level. The concentration of gammaglobulins in the positive animals from the first sampling is almost identical to the average of the positive lot at the second sampling. Although the differences are not statistically significant, it can be observed that at both samplings, the positive animals showed increased serum immune globulin concentrations compared to the R and N groups, which suggests that the infection with *M.tuberculosis* induces an increase in the level of total gammaglobulins that can be detected by the zinc sulfate precipitation test. This is also confirmed by the obtained correlation coefficient, but due to the very small number of individuals, a firm conclusion in this direction cannot be issued. For the uncertain ( R ) group, the obtained results indicate a slight increase in gammaglobulins compared to the negative group, and the calculation of r indicates a positive correlation compared to allergic reactivity, without being statistically significant.

**c. Correlations between skin reactivity and phagocytic function in tuberculin-reactive cattle**

The average values and standard deviations of the optical densities, for all variants, groups and samplings performed are presented in tables 3 and 4.

**Table 3. Carbon particle inclusion in the presence ov bovine and avian PPD, 1st sampling / Activitatea de înglobare a particulelor de carbon in vitro în prezența tuberculinei bovine și aviare, recoltarea I**

| Variant | Time min | Positive | Uncertain | Negative |
|---|---|---|---|---|
| **Control** | **0** | 0,826 | 0,673±0,409 | 0,776±0,281 |
| | **30** | 0,894 | 0,740±0,376 | 0,811±0,233 |
| | **75** | 0,949 | 0,806±0,435 | 0,906±0,227 |
| **Avian T.** | **0** | 1,019 | 0,595±0,320 | 0,640±0,352 |
| | **30** | 1,164 | 1,050±0,290 | 0,943±0,264 |
| | **75** | 1,299 | 1,152±0,121 | 0,990±0,255 |
| **Bovine T.** | **0** | 1,271 | 0,529±0,358 | 0,783±0,252 |
| | **30** | 1,208 | 0.902±0,431 | 1,127±0,218 |
| | **75** | 1,133 | 0,875±0,394 | 1,170±0,229 |

Polymorphonuclear phagocytes (granulocytes) and mononuclear phagocytes (macrophages) are the key element of non-specific immune protection, which opposes microbial aggression from the entrance gate by embedding and destruction (microphages) or by embedding and processing with surface expression of specific antigens (Kumar et al., 2019). The overcoming of this barrier by the aggressors leads to the installation of the infection and subsequently to the appearance of the symptoms of the disease.

In the pathogenesis of tuberculosis, due to their particular structure, the bacteria remain confined to macrophages, thus favoring their spread in the body (Maphasa et al., 2021, Queval et al., 2017, Bîlbîie and Pozsgi , 1985).

Analyzing the data obtained at the first sampling for the positive batch, it is observed that the optical densities of the experimental variants increased towards the final reading (75 minutes) except for those treated with bovine tuberculin, but without the difference being statistically significant. It seems that the presence of tuberculin, regardless of its origin, inhibits phagocytic activity. In the case of the uncertain group, the dynamics are similar, with the mention that the values are somewhat lower compared to those of the positive animal.

In the negative batch, the values of the control variants and those treated with avian tuberculin are lower, so that those of the samples treated with bovine tuberculin are higher. Bovine tuberculin appears to have a weak phagocytosis-stimulating effect in this batch towards the end of the reading period.

In the case of the second sampling, in the untreated samples from the positive batch UDO are higher and in the uncertain batch even higher than in the previous one. This fact may also be due to the increase in cell friability towards the end of the reading with the release of embedded carbon. Treatment with tuberculin does not cause an increase in phagocytic activity in the positive group, while in the control group a slight improvement is observed in the case of treatment with bovine tuberculin.

**Table 4. Carbon particle inclusion in the presence ov bovine and avian PPD, 2nd sampling / Activitatea de înglobare a particulelor de carbon *in vitro* în prezența tuberculinei bovine și aviare, recoltarea II**

| Variant | Time min | Positiv | Uncertain | Negative |
|---|---|---|---|---|
| **Control** | **0** | 1,172±0,161 | 0,844±0,338 | 0,444±0,258 |
| | **30** | 1,393±0,174 | 1,141±0,360 | 0,763±0,395 |
| | **75** | 1,365±0,104 | 1,169±0,333 | 0,698±0,395 |
| **Avian PPD** | **0** | 0,794±0,030 | 0,511±0,246 | 0,360±0,138 |
| | **30** | 1,159±0,047 | 0,978±0,286 | 0,721±0,315 |
| | **75** | 1,160±0,001 | 1,132±0,293 | 0,580±0,233 |
| **Bovine PPD** | **0** | 1,192±0,068 | 0,695±0,349 | 0,311±0,161 |
| | **30** | 1,473±0,009 | 1,137±0,311 | 0,652±0,259 |
| | **75** | 1,481±0,080 | 1,061±0,367 | 0,451±0204 |

In the negative group, on the other hand, towards the end of the reading period for each experimental variant, a decrease in optical densities is observed, which indicates an improvement in

phagocytic activity. All the values recorded in this batch are lower, proving that the cellular fragility is not so pronounced and that the carbon incorporation activity takes place much faster.

Comparing the values obtained in the two samplings, in the batches and the corresponding variants, it can be mentioned that in the positive batch in the second phase the values are much higher than in the first, perhaps due to the small size of the batches. No significant differences were recorded in the case of the uncertain category, with the exception of the variant treated with bovine tuberculin, where at the second sampling the data obtained indicate a very weak phagocytic activity. This phenomenon can be caused by the different time of infection in the animals from the two batches and the period that has passed since samplinging. The differences recorded between the two samplings for the negative group were statistically significant in the case of treatment with bovine tuberculin.

Table 5 shows the correlation coefficients between allergic reactivity and phagocytic activity in all three variants for the uncertain group. The obtained values indicate a positive correlation for all the variables calculated, without this benefiting from statistical assurance. The exception is the variant with avian tuberculin where the values are very close to 0, the time 75 having a negative value (correlation), the results not being statistically covered.

**Table 5. r values for the correlation between the skin test results and phagocytosis in the uncertain group / Coeficienţii de corelaţie între TCS şi fogocitoză la grupul R**

| Variant | Time min | r |
|---|---|---|
| **Control** | **0** | 0,399 |
| | **30** | 0,124 |
| | **75** | 0,040 |
| **Avian T.** | **0** | 0,087 |
| | **30** | 0,074 |
| | **75** | -0,037 |
| **Bovine T.** | **0** | 0,367 |
| | **30** | 0,128 |
| | **75** | 0,207 |

Cellular reactivity in tuberculosis is one of the essential aspects of the immune response (Queval et al., 2017). The intervention of macrophages in the first stage of protection with sensitization and the subsequent intervention of lymphocytes, both antibody-secreting and those involved in delayed-type hypersensitivity, is known. These lymphocytes are the ones that determine the nodular or edematous reaction observable at the tuberculin inoculation site, when the allergen is inoculated into a sensitized animal (Kondratieva et al., 2022). The observation led to the idea of applying a test in which the presence of effectors can be monitored and which shows increased sensitivity. The *in vitro* lymphocyte blast transformation test is such a method.

Table 6 shows the average values and standard deviations of the stimulation indices, for each experimental variant, of the three lots in the two samplings. Following the results obtained during the first sampling, it is observed that the spontaneous cellular reactivity in the only positive animal is low compared to the average of the uncertain group (82.10 ± 9.84%) without the difference being statistically ensured. The obtained data correlate with those in the literature, which support the argumentation of the lymphocytic blast response in positive animals or uncertain for skin reactivity. It is also known that in positive animals there are fluctuations in immunity, so it is possible that the small difference between the positive batch and the uncertain, whose value exceeds the first, is given by such a situation.

The reactivity to PHA turns out to be the most intense in positive animals, following in order the average of the uncertain group (86.77 ± 16.80%) and negative (81.69 ± 15.78%). The observed response is the expected one.

The responses to the plant extracts and to avian and bovine tuberculin are variable. Thus, the presence of avian tuberculin stimulates the lymphocytes of the positive animal to the greatest extent, while bovine tuberculin acts with maximum intensity in the samples from the batch under uncertain. The

relatively weak reactivity to bovine tuberculin of the positive animals, less than that of the negative group, may be the indication of a habit in the animals in which the bacteria is a permanent "irritating thorn".

**Table 6. Blast transformation indices by experimental variant: a comparison of the two samplings / Indici de stimulare (valori medii şi deviaţii standard): evaluare comparativă la cele două recoltări**

| | Positive | | Uncertain | | Negative | |
|---|---|---|---|---|---|---|
| **Variant** | *Rec. I* | *Rec. II* | *Rec. I* | *Rec. II* | *Rec. I* | *Rec. II* |
| **M** | 76,63±9,31 | 81,17±10,35 | 82,10±9,84 | 84,02±13,39 | 74,9±9,53 | 81,04±18,08 |
| **PHA** | 98,45±10,22 | 88,28±10,94 | 86,77±16,8 | 88,30±15,12 | 81,69±15,78 | 87,01±14,48 |
| **Alcohol** | 61,76±8,35 | 88,49±12,42 | 85,89±15,35 | 88,30±14,35 | 81,0±10,86 | 85,12±12,88 |
| **Cal.** | 65,26±7,41 | 81,80±11,83 | 88,15±16,58 | 84,82±9,60 | 76,05±13,01 | 83,44±14,60 |
| **Ar.** | 56,51±8,99 | 93,51±14,79 | 69,85±8,64 | 77,13±14,08 | 77,70±13,23 | 68,13±9,97 |
| **Symph.** | 65,26±11,2 | 75,52±11,83 | 68,68±9,22 | 79,74±13,62 | 71,43±11,84 | 76,75±13,69 |
| **E. ang.** | 73,13±9,23 | 85,14±16,56 | 91,57±10,94 | 89,13±11,14 | 74,66±9,59 | 87,71±13,12 |
| **E. purp.** | 58,26±7,18 | 78,66±13,90 | 75,03±16,17 | 75,23±15,99 | 70,9±12,93 | 76,27±12,66 |
| **Avian PPD** | 92,96±10,11 | 93,51±24,26 | 85,59±10,8 | 89,65±17,12 | 77,99±10,85 | 84,64±14,22 |
| **Bovine PPD** | 70,80±8,67 | 91,21±18,04 | 92,37±7,45 | 93,16±11,61 | 78,38±8,14 | 85,92±11,51 |

Among the extracts, in the positive animal the maximum reactivity is induced by *E. purpurea*, known for its stimulating effects, while the rest of the extracts are weaker stimulants, but also strong enough (stimulation over 50%). The same situation is maintained for the group of animals under uncertain, with the mention that all the stimulation indices are increased. There is a difference of almost 20% between the biological effect of *E.angustifolia* (more intense) and *E.purpureea* (weaker). In the group of negative cattle, *Arnica montana* has the maximum effectiveness. In the case of this batch, the values are much more uniform than in the other batches.

In the case of the second sampling, the index of spontaneous blastogenic is somewhat higher than at the first sampling, without the difference being statistically significant. Both in the uncertain group and in the negative one, the spontaneous blastogenic indices were very close to those of the positive group. Even in the case of stimulated cultures, no differences were observed and comparing these values with those of the first sampling, a slight increase is observed. In the positive batch, in the case of the second sampling, the *Arnica* extract was the most effective (93.51 ± 14.79 %). Even alcohol in the dose used proves to be a stimulant. Both tuberculins acted stimulating, with a similar intensity to that of *Arnic*a. The blastogenic index at the second sampling in the presence of bovine tuberculin is much higher compared to the first sampling.

In the uncertain category, the bovine tuberculin had a more pronounced effect than the avian one, even more pronounced than in the positive group. This fact could be attributed to the small number of cases included in the positive group. Negative animals respond less well to in vitro tuberculin stimulation, regardless of type, than those in the positive or uncertain category. *E.angustifolia* was the extract with the maximum efficiency in the negative group, most of the extracts having less pronounced effects than in the positive or uncertain group, with lower stimulation indices.

## CONCLUZII

1. Positive reactivity to the skin test was correlated with an increase in the level of circulating gammaglobulins, although they do not represent the essential mediator of the hypersensitivity reaction.

2. The precipitation test with PEG 4.2% allows detection of level differences in CIC concentration and although these are not statistically significant, the maximum values recorded in positive animals suggest an increase in immune complexes following infection, with diagnostic potential.

3. The carbon particle inclusion test proved to be useful in tracking the phagocytic activity in tuberculin-reactive cattle, with the detection of differences between categories. Although compared to the control, the phagocytic activity was less intense, the positive influence of bovine tuberculin was observed, especially in the case of the second sampling.

4. The blast transformation test allowd the investigation of sensitivity to tuberculin *in vitro*, blastogenic indices in its presence, regardless of type, being higher in the positive group, of a slightly reduced amplitude in the uncertain group and close to the value of the spontaneous index in the group negative.

5. The plant extracts used to verify their immunomodulatory effect prove to be effective stimulants with few exceptions (*Arnica* at the first sampling in the positive lot 69.85 ± 8.64% or *Symphytum* 68.68 ± 9.22%, the value below that of the spontaneous index).

6. *Echinacea purpureea* has an inhibitory effect on all samplings and batches, the induced blastogenic indices being lower than those from spontaneous transformation.

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# CREATION OF A NEW BREED OF HIGH PERFORMANCE MEAT SHEEP WITH HIGH GROWTH RATE AT FATTENING OF MALE LAMBS, HIGH SLAUGHTER YIELD AND HIGH QUALITY CARCASSES

# CREAREA UNEI NOI RASE DE OVINE DE CARNE PERFORMANTĂ CU VITEZĂ MARE DE CREȘTERE LA ÎNGRĂȘARE A MIEILOR MASCULI, RANDAMENT RIDICAT LA SACRIFICARE ȘI CARCASE DE CALITATE SUPERIOARĂ

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***Abstract***

*The research was carried out on fattened young male sheep of the **Palas Meat Breed**, the **Palas Prolific Breed** as well as on 2 hybrids F1 **Rouge de L'Ouest** x **Palas Meat Breed** and F1**Texel** x **Palas Meat Breed**. Weight gain to live weight of 40.0 kg/head, slaughter yield (R1 and R2), feed consumption efficiency (Ef), carcass tissue structure and carcass quality grading according to the EUROP grid were monitored. All data were statistically processed and interpreted.*

***Key words: sheep, meat, fattening, slaughter, carcass***

***Rezumat***

*Cercetările s-au efectuat pe tineret ovin mascul îngrășat din **Rasa de Carne Palas, Rasa Prolifică Palas** precum și pe 2 hibrizi $F_1$ **Rouge de L'Ouest** x **Rasa de Carne Palas** și $F_1$ **Texel** x **Rasa de Carne Palas**. S-au urmărit sporul de creștere în greutate până la greutatea vie de 40,0 kg/cap, randamentul la sacrificare ($R_1$ și $R_2$), eficiența consumului de furaje ($E_f$), structura tisulară a carcaselor și încadrarea în clase de calitate a acestora după grila EUROP. Toate datele au fost prelucrate și interpretate statistic.*

***Cuvinte cheie: oi, carne, îngrășare, sacrificare, carcase***

## INTRODUCTION

The transformations that have taken place over the last decade in the field of sheep breeding and the alignment with existing practices at European level have led to a change of vision regarding local breeds and the application of crossbreeding with specialised breeds, and Romania can make an important contribution in this field due to the large number of sheep it has.

In the European Union there is a multitude of high performance meat sheep breeds with high growth rate at fattening, high yield at slaughter and high quality carcasses. Almost every country pays attention to the production of lambs that are marketed for meat on the domestic or foreign market, using the system of crossbreeding between two or more specialised breeds (Dickerson G.E). Slaughter weight performance varies with genotype, sex, age and fattening conditions (Martynuk E s.a). By combining and crossbreeding two breeds, higher slaughter lambs can be obtained due to the manifestation of the phenomenon of heterozys (Zupp W ).

In Romania there is only one high performance meat breed, created at RDISGB Palas Constanta, comparable to the best meat breeds in the world. Research on the use of the **Rouge de L'Ouest** and **Texel**

breeds in crosses with the **Prolific Palas breed** has been reported by Vartic A. and collaborators, who reported average daily fattening gains of 302.47 g for F1 **Rouge de L'Ouest** x **Prolific Palas** hybrids and 304.78 g for F1 **Texel** x **Prolific Palas** hybrids compared to 235.47 g for **Prolific Palas** young. The two hybrid variants were also superior to the maternal breed in terms of thigh muscle index (TMI) which was 33.92% higher in the **Rouge de L'Ouest** hybrids and 28.43% higher in the **Texel** hybrids, the differences being highly significant compared to the maternal breed.

## MATERIAL AND METHODS

The research was carried out on batches of fattened male lambs of the **Palas Meat Breed**, **Palas Prolific Breed**, $F_1$ **Rouge de L'Ouest** x **Palas Meat Breed** hybrids and $F_1$ **Texel** x **Palas Meat Breed** hybrids.

They were fattened to an average weight of 40 kg/head, followed by weighing, growth rate, specific feed consumption and feed efficiency expressed in grams of growth gain for 1 kg dry matter consumption.

Three animals were slaughtered from each variant and the slaughter yield, carcass quality indices and carcass quality classes were calculated according to the EUROP grid.

All data were processed and statistically interpreted (Snedecor G.W).

## RESULTS AND DISCUSSION

Table no. 1 shows the values of daily weight gain

**Table 1. The values of daily weight gain (g ) depending of genotype / Valoarea sporului mediu zilnic (g) în funcție de genotip**

| Genotype | Daily weight gain (g) | ± Differences between genotypes | |
|---|---|---|---|
| | | g | % |
| **Palas Prolific Breed** | 299.13 ± 15.6616 | - 38.54 | - 11.41 |
| **Palas Meat Breed** | 337.67 ± 4.4700 | $p < 0.001$ | |
| $F_1$ **Rouge de L'Ouest** x **Palas Meat Breed** | 331.13 ± 12.7185 | Between **Palas Meat Breed** and $F_1$ **Rouge de L'Ouest** x **Palas Meat Breed** | |
| | | + 6.54 | + 1.98 |
| | | $p > 0.05$ | |
| $F_1$ **Texel** x **Palas Meat Breed** | 335.20 ± 12.900 | Between **Palas Meat Breed** and $F_1$ **Texel** x **Palas Meat Breed** | |
| | | + 2.47 | + 0.074 |
| | | $p > 0.05$ | |

It results that the highest yield was obtained by **Palas Meat Breed** lambs followed by $F_1$ **Texel** x **Palas Meat Breed** (fig.1) , $F_1$ **Rouge de L'Ouest** x **Palas Meat Breed** (fig. 2) and **Palas Prolific Breed**.

There were very significant differences ($p < 0.001$) between the **Palas Meat Breed** and the **Palas Prolific Breed**. Between the **Palas Meat Breed** and the 2 hybrids the differences were statistically insignificant ($p > 0.05$).

**Figure 1. $F_1$ Texel x Palas Meat Breed / $F_1$ Texel x Rasa de carne Palas**

**Figure 2. $F_1$ Rouge de L'Ouest x Palas Meat Breed / $F_1$ Rouge de L'Ouest x Rasa de carne Palas**

Feed conversion efficiency ($E_f$ - g/kg S.U.) is shown in Table no. 2.

**Table 2. Feed conversion efficiency / Eficiența de conversie a nutrețurilor**

| Genotype | Feed conversion efficiency ( g/kg S.U.) | ± Differences between genotypes | |
|---|---|---|---|
| | | g | % |
| Palas Prolific Breed | 260 | + 39 | + 13 |
| Palas Meat Breed | 299 | p < 0.05 | |
| $F_1$ Rouge de L'Ouest x Palas Meat Breed | 287 | Between Palas Meat Breed and $F_1$ Rouge de L'Ouest x Palas Meat Breed | |
| | | + 12 | + 4 |
| | | p > 0.05 | |
| $F_1$ Texel x Palas Meat Breed | 291 | Between Palas Meat Breed and $F_1$ Texel x Rasa de Carne Palas | |
| | | + 8 | + 2.7 |
| | | p > 0.05 | |

There were significant differences ( $p < 0.05$) only between the **Meat Breed** and the **Prolific Palas Breed**.

Table no.3 shows the slaughter yield.

**Table 3. Slaughter yield depending on genotype / Randamentul la sacrificare în funcție de genotipul ovinelor**

| Genotype | $R_1$ (%) | $R_2$ (%) | ± Differences between genotypes | | |
|---|---|---|---|---|---|
| | | | $R_1$ | $R_2$ | Significance |
| Palas Prolific Breed | 44.84±0.0084 | 54.36±0.0068 | Palas Meat Breed and Palas Prolific Breed | | |
| Palas Meat Breed | 50.97±0.0115 | 58.46±0.0070 | + 6.33 | + 4.1 | $p < 0.05$ |
| $F_1$ Rouge de L'Ouest x Palas Meat Breed | 49.46±0.0085 | 57.54±0.0022 | Palas Meat Breed and $F_1$ Rouge | | |
| | | | + 1.51 | + 0.92 | $p > 0.05$ |
| $F_1$ Texel x Palas Meat Breed | 46.13±0.0090 | 56.03±0.0121 | Palas Meat Breed and $F_1$ Texel | | |
| | | | + 4.84 | + 2.43 | $p > 0.05$ |

$$R_1 = \frac{\text{Weight of cooled carcass}}{\text{Live weight}} \text{ x } 100$$

$$R_2 = \frac{\text{Weight of cooled carcass}}{\text{Live empty weight}^{*}} \text{ x } 100$$

* Live weight from which the digestive tract content has been deducted

It can be seen that the **Palas meat breed** lambs had the highest yield, followed by the 2 hybrids. There were significant differences only between the **Palas Meat Breed** and the **Palas Prolific Breed**.

Table no. 4 shows the tissue structure of the carcasses.

**Table 4. Tissue structure of the carcasses / Structura tisulară a carcaselor**

| Genotype | Muscles | | Bones | | Fat | | Meat | | ± Differences between genotypes | |
|---|---|---|---|---|---|---|---|---|---|---|
| | g | % | g | % | g | % | g | % | | |
| 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | |
| **Palas Prolific Breed** | 4663.33 | 55.2 | 2208 | 26.1 | 1586.47 | 18.8 | 6280 | 73.9 | **Palas Meat Breed** and **Palas Prolific Breed** | |
| | | | | | | | | | g | % |
| **Palas Meat Breed** | 5998.33 | 60.73 | 2075 | 21.00 | 1952.50 | 19.77 | 7950.83 | 80.50 | + 1701 | + 27.22 p<0.001 |
| $F_1$ **Rouge de L'Ouest** x **Palas Meat Breed** | 5835.00 | 62.19 | 2283.33 | 24.33 | 1265.00 | 13.48 | 7100 | 75.67 | **Palas Meat Breed** and $F_1$ **Rouge** | |
| | | | | | | | | | + 850.83 | + 11.98 p<0.01 |
| $F_1$ **Texel** x **Palas Meat Breed** | 5821.67 | 61.70 | 2203.33 | 23.35 | 1410.00 | 14.94 | 7231.67 | 76.65 | **Palas Meat Breed** and $F_1$ **Texel** | |
| | | | | | | | | | + 719.16 | + 11.98 p<0.01 |

It results that the breed with the highest amount of meat in the carcass was the **Palas Meat Breed** followed by the 2 hybrids.

There were very significant differences ($p<0.001$) between the **Palas Meat Breed** and the **Palas Prolific Breed**, respective distinct significant differences ($p<0.01$) between the **Palas Meat Breed** and the 2 hybrids.

Table 5 shows the quality grading of the carcasses.

**Table 5. Carcass quality grading according to the EUROP grid / Calitatea carcaselor conform grilei EUROP**

| Genotype | Quality grid (%) | | | | |
|---|---|---|---|---|---|
| | $E_{2-3}$ | $U_{2-3}$ | $R_{2-3}$ | $O_{2-3}$ | $P_{2-3}$ |
| **Palas Meat Breed** | 33.3 | 66.7 | - | - | - |
| **Palas Prolific Breed** | - | - | 100.00 | - | - |
| **$F_1$ Rouge de L'Ouest** x **Palas Meat Breed** | - | 100.0 | - | - | - |
| **$F_1$ Texel** x **Palas Meat Breed** | - | 100.00 | - | - | - |

It results that in the **Palas Meat Breed** one third of the carcasses were classified in class $E_{2-3}$ (excellent, lean and medium-fatty carcasses, figure 3 ) and two thirds in class $U_{2-3}$ (very good, lean and medium-fatty carcasses. The carcasses of the two hybrids fell entirely into class $U_{2-3}$ (very good, poor and medium fat carcasses figure 5 – 6) and carcasses of the **Palas Prolific Breed** are entirely classified into class $R_{2-3}$ (figure 4).

**Figure 3. Palas Meat Breed Carcass E $_{2-3}$ class / Carcasă de Rasa de carne Palas clasa E 2-3**

**Figure 4. Palas Prolific Breed Carcass R $_{2-3}$ class / Carcasă de Rasa prolifică de Palas clasa R 2-3**


**Figure 5. $F_1$ Rouge de l Quest x Palas Meat Breed Carcass U $_{2-3}$ class / Carcasă de $F_1$ Rouge de l Quest x Rasa de carne Palas Clasa U 2-3**


**Figure 6. $F_1$ Texel x Palas Meat Breed Carcass U $_{2-3}$ class / Carcasă de $F_1$ Texel x Rasa de carne Palas Clasa U 2-3**

Table no. 6 shows the thigh muscularity index (TMI) according to Purchas formulae .

$$TMI = \frac{\text{Weight of thigh muscles}}{\text{Length of femur}} \text{ x } 100$$

**Table 6. Thigh Muscularity Index (TMI ) / Indicele de muscularitate a coapsei**

| **Genotype** | **T.M.I** | **± Differences between genotypes** | |
|---|---|---|---|
| | | **Value** | **%** |
| $F_1$ **Rouge de L'Ouest** x **Palas Meat Breed** | 0.581 ± 0.0177 | + 0.005 | + 0. 9 |
| $F_1$ **Texel** x **Palas Meat Breed** | 0.567 ± 0.0084 | | |
| | | p > 0.05 | |
| **Palas Meat Breed** | 0.592 ± 0.0078 | **Palas Meat Breed** and $F_1$ **Rouge** | |
| | | + 0.011 | + 1.9 |
| | | p > 0.05 | |
| | | **Palas Meat Breed** and $F_1$ **Texel** | |
| | | + 0.016 | + 2.7 |
| | | p > 0.05 | |

From the table it can be seen that there were no significant differences (p > 0.05) between the values of the T.M.I index between the 3 genotypes.

## CONCLUSION

The results obtained are encouraging in terms of creating the precursors of a new breed of high performance meat sheep comparable to the best breeds in the world.

It is estimated that the new meat breed could be homologated around 2036.

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