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CONTENT

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ACCESSORY CORPUS LUTEUM IN CATTLE ASSISTED REPRODUCTION

CORPUL LUTEAL ACCESORIU ÎN REPRODUCEREA ASISTATĂ A BOVINELOR

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Abstract

In dairy cows, current methods for managing reproduction still need improvement. Future advancements will require new strategies to minimize additional interventions and maintain acceptance among veterinarians. As a result, the development of new therapies in dairy cows' reproduction acts for a significant challenge for improving reproductive performances. In recent years, there has been an increasing interest in inducing accessory corpus luteum (aCL) formation in dairy cows, but the results have been inconsistent. It is still uncertain whether this strategy, of injecting gonadotropin-releasing hormone (GnRH) or human chorionic gonadotropin (hCG) early in the luteal phase following artificial insemination (AI), can be utilized as a herd management tool to enhance reproduction. Overall, our research supports the idea that giving GnRH agonists during the days 7–14 after AI to repeat breeder (RB) cows increases their fertility and farm profitability. The GnRH agonist treatment improved the odds that pregnant cows would develop an aCL, which could improve their fertility. The UW-DairyRepro$ decision support tool utilized in our study demonstrated that by implementing this approach, the net present value can be increased by US dollars (US$)30.2/RB cow/year. Therefore, our work suggests that implementing this strategy on the farm is feasible only for repeat-breeder dairy cows with low genetic merit for fertility. However, in the assisted reproductive technologies this strategy seems to improve reproduction in recipient heifers.

Keywords: accessory corpus luteum, dairy cows, embryos, reproductive biotechnologies.

Rezumat

La vacile de lapte, metodele actuale de gestionare a activității de reproducere necesită încă îmbunătățiri. Progresele viitoare presupun noi strategii pentru a minimiza intervențiile terapeutice și pentru a fi unanim acceptate de medicii veterinari. Ca urmare, dezvoltarea de noi strategii terapeutice în reproducerea vacilor de lapte reprezintă o provocare pentru îmbunătățirea performanțelor reproductive. În ultimii ani, a existat un interes din ce în ce mai mare pentru inducerea formării corpului galben accesoriu în reproducerea asistată a vacilor de lapte, dar rezultatele sunt controversate. Este încă incert dacă această strategie ce presupune injectarea de GnRH sau hCG la începutul fazei luteale, după inseminare artificială, poate fi utilizată ca instrument de management al efectivelor de taurine pentru a îmbunătăți reproducerea. În general, cercetarea noastră susține ideea că administrarea de agoniști GnRH în zilele 7-14 după inseminare artificială a vacilor infertile, crește fertilitatea și profitabilitatea fermei. Tratamentul cu agonist GnRH a îmbunătățit șansele ca vacile gestante să dezvolte un corp luteal accesoriu, care le-ar putea îmbunătăți fertilitatea. Programul de evaluare economică UW-DairyRepro$, utilizat în studiul nostru a demonstrat că prin implementarea acestei strategii, valoarea actualizată netă poate crește cu 30,2 USD/vacă infertilă/an. În concluzie, studiile noastre sugerează că implementarea acestei strategii în ferme este fezabilă numai pentru vacile de lapte infertile. În biotehnologiile de reproducere asistată, această strategie pare să îmbunătățească activitatea de reproducere doar a junicilor receptoare de embrioni.

Cuvinte cheie: biotehnologii, corpul luteal accesoriu, vacă de lapte, embrioni.

INTRODUCTION

In recent decades, new technologies and approaches have been developed to address reproductive performance issues in high-yielding dairy farms, stemming from a deeper understanding of contemporary reproductive physiology in these cows (Thatcher, 2017; Cardoso et al., 2021).

Progesterone deficiency is a significant factor contributing to embryo loss, which is a major cause of low reproductive performance in dairy cows. High-yielding cows experience increased metabolism, resulting in a higher rate of progesterone degradation and lower concentrations of progesterone in peripheral blood (Wiltbank et al., 2006; Robinson et al., 2008).

The increase in progesterone (P4) after ovulation is crucial for establishing and maintaining pregnancy (Forde et al., 2011; Lonergan and Sánchez, 2020), as it creates an optimal uterine environment for embryo elongation (Clemente et al., 2009).

Progesterone (P4) concentrations exceeding 1 ng/mL during the first five days following artificial insemination (AI) are significant indicators in cattle reproduction. Additionally, an increase in P4 levels after day 7 in recipient cows is strongly correlated with a higher likelihood of successful pregnancy. Monitoring these hormone levels is critical for predicting reproductive outcomes and can help optimize breeding practices in livestock management (Starbuck et al., 2001; Kenyon et al., 2013).

Low levels of circulating progesterone (P4) are strongly associated with diminished fertility in dairy cows, as noted by Lonergan (2011). To counteract the negative effects of low progesterone after AI, contemporary fertility programs often use a gonadotropin-releasing hormone (GnRH) injection to induce a surge of luteinizing hormone (LH). This surge is critical as it not only stimulates the initiation of a new follicular wave, which is essential for the development of new ovarian follicles, but it also triggers ovulation. Furthermore, this process promotes the formation of an accessory corpus luteum (aCL), which plays a vital role in increasing progesterone production. By enhancing progesterone concentrations, these fertility interventions aim to improve overall reproductive efficiency and increase the chances of successful conception and pregnancy.

This process significantly increases the circulating levels of progesterone (P4) during the critical growth phase of the pre-ovulatory follicle, which is essential for successful ovulation and subsequent embryo development (Pursley and Martins, 2011; Wiltbank and Pursley, 2014; Carvalho et al., 2018). Despite the biological reason for using P4 supplementation, various studies have shown that its administration during the embryo development stage does not consistently cause better fertility outcomes in dairy cows (Monteiro et al., 2015; Steichen and Larson, 2019; García-Guerra et al., 2020).

Furthermore, while some research has reported promising improvements in certain reproductive parameters, the variability in results suggests that a standardized protocol for enhancing the formation of large, functional aCL is still lacking. This gap in knowledge is particularly mandatory for high-yielding dairy cows, where optimizing fertility is vital for maximizing milk production and overall herd efficiency. Continued exploration of the methods for P4 supplementation and its role in reproductive physiology is necessary to establish best practices that can lead to improved fertility rates in this population of dairy cows.

RESULTS AND DISCUSSIONS

Induction of accessory corpus luteum formation using GnRH or hCG in high-yielding dairy cows

The study conducted by Schmitt et al. (1996) investigated the induction of an aCL in heifers and lactating cows through the administration of a GnRH agonist, specifically Buserelin at a dosage of 8 mg, or human chorionic gonadotropin (hCG) at a dosage of 3,000 IU. The primary aim was to boost plasma P4 levels, which are essential for establishing and maintaining pregnancy, and to enhance conception rates during the breeding season. The researchers found that both Buserelin and hCG had comparable effects on the formation of aCL, leading to significant increases in P4 concentrations in the blood. Despite these biochemical improvements, the study revealed that neither treatment led to increased pregnancy rates among heifers and dairy cows, particularly during the challenging summer season when heat stress can adversely affect reproductive performance. These findings suggest that while hormonal treatments can effectively stimulate progesterone production, external factors such as seasonal heat may still play a significant role in reproductive success.

Research shows that administering GnRH between days 5 and 15 following AI can significantly enhance conception rates, particularly during the warmer months. This intervention has been associated with an increase in conception rates by approximately 15%, as it was demonstrated in studies conducted by López-Gatius et al. (2006) and Willard et al. (2003). The timing of GnRH administration is crucial, as it aligns with the reproductive cycle of the animal, optimizing the hormonal environment for embryo implantation and overall fertility success during challenging seasonal conditions. Our previous study (Borș et al., 2023) demonstrated that administering GnRH 7 to 14 days after AI significantly enhanced reproductive activity in repeat breeder cows. This finding indicates that administering gonadorelin during the later stages of the luteal phase may improve embryo survival rates in the repeat breeder dairy cows, thereby potentially increasing overall reproductive efficiency (Borș et al., 2023).

In contrast, our follow-up research (Borș et al., 2025) revealed that administering GnRH on day 5 post-AI—whether it occurred naturally or as part of the OvSynch synchronization protocol—did not bring any significant improvements in reproductive outcomes for high-yielding dairy cows. This lack of effect suggests that early administration of GnRH may not be beneficial in enhancing fertility in this particular group, emphasizing the importance of precise timing and individual cow responses in reproductive management practices. Similar findings have been observed with exogenous progesterone administered via the CIDR intravaginal delivery device (Wolfenson et al., 1995).

According to the study by Doležel et al. (2017), administering gonadorelin to induce aCL formation is most effective in cows treated on day 5 after AI, compared to those treated on days 6 or 7. Yan et al. (2016) found that administering progesterone to dairy cows between 3 and 7 days after AI was beneficial, while treatments given earlier or later did not provide any advantages. Additionally, a study by Besbaci et al. (2020) indicated that administering GnRH 10 days after AI can also result in pregnancy.

A comprehensive study conducted across six farms investigated the effects of administering hCG on reproductive outcomes in cows. The findings indicated that administering hCG on day 5 after timed artificial insemination (TAI) significantly enhanced the pregnancy rate (P/AI) by 10% in primiparous cows compared to the untreated control group, and this increase suggests that hCG may play a crucial role in supporting reproductive success in this category of cattle. In contrast, the same treatment did not gire any beneficial effects on multiparous cows (Nascimento et al., 2013).

Furthermore, a more recent study explored the comparative efficacy of hCG and gonadorelin (GnRH) in enhancing ovulatory response. It found that administering at least 2,000 IU of hCG resulted in superior ovulatory efficiency, when compared to standard treatment with 86 μg of gonadorelin. This treatment was specifically applied on day 7 after AI in lactating dairy cows, highlighting the potential for hCG to optimize ovulation timing and improve reproductive outcomes in dairy farming practices (Cabrera et al., 2021). Additionally, Cabrera et al. (2021) found that cows treated with 2,500 IU of hCG exhibited a more significant increase in serum P4 concentrations from days 7 to 14 after AI, as well as a greater total luteal size on day 14 (Cabrera et al., 2021). These results may be attributed to the formation of a new aCL combined with the luteotropic effect of hCG on the original CL, since hCG binds to LH receptors in the CL and may increase the P4 secretion capacity of luteal cells (De Rensis et al., 2010).

Additionally, hCG decreased the number of multiparous cows returning to estrus (Cunha et al., 2021), indicating that the aCL induced by hCG may affect luteal and luteolytic dynamics.

Accessory corpus luteum regression

The treatment with GnRH or hCG administered between days 5 and 7 after AI led to the development of an aCL in 70 to 90% of the cows (Baez et al., 2017; Nascimento et al., 2013). This treatment also resulted in increased circulating progesterone (P4) levels (Nascimento et al., 2013). Several studies have demonstrated that the induction of aCL is associated with higher progesterone concentrations and improved conception rates in dairy cows (López-Gatius et al., 2004; Villarroel et al., 2004; Bech-Sàbat et al., 2009; Mehni et al., 2012; Pilz et al., 2012).

When aCL is induced via hCG or GnRH treatment early in the luteal phase, CL regression occurs in lactating dairy cows during pregnancy (Baez et al., 2017; Monteiro et al., 2021). Regression of the aCL primarily occurs in the aCL that is contralateral to the original CL. However, no clear explanation has been provided for this phenomenon (Baez et al., 2017; Monteiro et al., 2021; Hazano et al., 2021).

Accessory corpus luteum regression during either early (days 19–23) or late (>45 days) pregnancy resulted in a ~40% reduction in circulating P4 (Monteiro et al., 2021).

Consistent with the findings of Baez et al. (2017) and the study by Monteiro et al. (2021), there are two important periods during which the contralateral aCL underwent regression. The first period occurred during the first month of pregnancy, with 30.8% of cows experiencing earlier aCL regression. The second period was noted in the second month of pregnancy, where 69.2% of cows exhibited later aCL regression. The timing of aCL regression not only influenced circulating P4 concentrations, but also affected the incidence of pregnancy loss (Monteiro et al., 2021).

Some studies show that pregnancy loss is higher when aCL regression occurs early in pregnancy (Monteiro et al., 2021). This indicates that administering hCG to enhance conception rates may pose a risk of pregnancy loss. However, in the previous study, pregnancy diagnosis was conducted on Day 26 after AI, solely using the measurement of pregnancy specific protein B assay, without confirming the presence of a living embryo. It remains uncertain whether the regression of the aCL contributes to pregnancy loss. However, the study conducted by Bui et al. 2024, found that pregnancy loss might not occur even if the aCL regresses after fetal detection. When hCG administration is used to promote conception rates, it can be suggested that although regression of aCL occurs during pregnancy, it does not increase the risk of pregnancy loss (Bui et al., 2024).

During pregnancy, the ipsilateral aCL did not regress, while most of the contralateral CLs had regressed by 63 days. This provides evidence for local mechanisms that contribute to the regression of the aCL and the protection of the CL during pregnancy (Monteiro et al., 2021). Understanding the mechanism behind the regression of the aCL during pregnancy is important, as it may illuminate the processes that lead to luteal regression before pregnancy loss occurs. Gaining insight into how CL regression occurs during pregnancy could help reduce the risk of pregnancy loss.

Accessory corpus luteum in embryo transfer procedures

There have been few studies examining the effects of giving either GnRH or hCG on the day of embryo transfer (ET). Furthermore, most of these studies have small sample sizes and their results are inconsistent, with some revealing no change and others demonstrating beneficial impacts on pregnancy per embryo transfer (P/ET) (García-Guerra et al., 2020; Vasconcelos et al., 2011; Niles et al., 2019). A recent study by El Azzi et al. (2023), with a large sample size, investigates the impact of administering GnRH or hCG immediately before ET on pregnancy rates per embryo transfer (P/ET), pregnancy loss and calving outcomes in a significant number of recipient dairy heifers and cows receiving *in vitro* produced (IVP) embryos.

Vasconcelos et al. (2011) studied the effects of GnRH or hCG administered either 7 days after TAI or on the day of ET in lactating synchronized **Holstein** cows. In this study, the overall P/ET were

45.2% on day 28 and 37.4% on day 60. They found that administering GnRH or hCG 7 days post-ovulation improved conception rates in lactating dairy cows undergoing ET, but not in those subjected to TAI.

García-Guerra et al. (2020) found that administering GnRH on day 5 of the estrous cycle had no effect on pregnancy rates in heifers that received fresh IVP. However, they noted that GnRH treatment on day 5 reduced pregnancy losses between days 33 and 60 of gestation in heifers receiving expanded blastocysts, with loss rates of 15.2% in treated heifers, compared to 27.1% in untreated heifers. Similarly, Niles et al. (2019) reported that recipient heifers treated with hCG experienced lower pregnancy losses than controls, with rates of 10% and 22%, respectively.

In the study conducted by El Azzi et al. (2023), the overall pregnancy rate per embryo transfer at 37 ± 3 days of gestation was found to be 56.5%. The treatment for the formation of an aCL did not have an impact on the overall P/ET or calving rate per embryo transfer (El Azzi et al., 2023). This overall P/ET was considered high, compared to studies that induced aCLs in recipient dairy heifers or cows using GnRH or hCG (Vasconcelos et al., 2011; Niles et al., 2019).

Overall, the treatments for aCL formation did not significantly impact the P/ET or calving per embryo transfer, nor did they affect pregnancy loss. However, the high P/ET results observed in this study may have diminished any potential benefits of GnRH or hCG on the day of embryo transfer (El Azzi et al., 2023).

CONCLUSIONS

Administering GnRH or hCG early during the luteal phase has been shown to effectively induce the formation of aCL in both cows and heifers. Despite this efficacy, we do not recommend using this treatment as a standard practice for managing reproductive activities on dairy farms. For repeat breeder dairy cows, we recommend implementing this therapy 7 to 14 days after AI. This timing is important, as it can elevate these animals' overall reproductive success rate. The procedure is straightforward, can be performed weekly, and has a significant positive impact on the farm's economic performance due to improved fertility rates. However, it is important to note that the formation of aCL is not advisable during embryo-transfer procedures in assisted reproduction.

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RESEARCH ON THE RELATIONSHIP BETWEEN THE CALENDAR PERIOD OF CALVING AND THE AVERAGE DAILY GROWTH RECORDED OF ROMANIAN BUFFALO BREED

CERCETĂRI PRIVIND RELAȚIA DINTRE PERIOADA CALENDARISTICĂ A FĂTĂRII VIȚEILOR DIN RASA BIVOL ROMÂNESC ȘI SPORUL MEDIU ZILNIC ÎNREGISTRAT

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Abstract

Tracking and recording the average daily gain of ***Romanian buffalo*** *calves has a major impact on the profitability of Romanian buffalo farms, buffalo meat being successfully used in the preparation of sausages. The aim of this study was to investigate the average daily growth recorded in* ***Romanian buffalo*** *calves, calved in different calendar periods and the possible correlation between the recorded measurements and the calendar period of calving due to the different biochemical composition of the colostral phase and milk. The measurements were performed at birth, then monthly until the age of 18 months. They were accommodated in the same conditions and benefited from the same feeding conditions, thus they were given dry fiber, green meal, fodder corn, corn silage, water ad libitum. Average values of SMZ were recorded from the value of 742.6 g to 758.8 g.*

Key words: Romanian buffalo, calf, average daily gain.

Rezumat

Urmărirea și înregistrarea sporului mediu zilnic la viței de ***Bivol românesc*** *are un impact major asupra profitabilității fermelor de bubaline din România, carnea de bivol fiind folosită cu succes în prepararea mezelurilor. Scopul acestui studiu a fost investigarea sporului mediu zilnic înregistrat la viței de bivol românesc, fătați în perioade calendaristice diferite și eventuala corelare dintre măsurătorile înregistrate și perioada calendaristică a fătării datorită componenței biochimice diferite a fazei colostrale și a laptelui. Măsurătorile au fost efectuate la naștere, apoi lunar până la vârsta de 18 luni. Au fost cazați în aceleași condiții și au beneficiat de aceleși condiții de furajare, astfel le-au fost administrate fibroase uscate, masă verde, mălai furajer, porumb siloz, apă ad libitum. S-au înregistrat valori medii ale SMZ de la valorarea de 742,6 g până la 758,8 g.*

Cuvinte cheie: bivol românesc, vițel, spor mediu zilnic

INTRODUCTION

The breeding of buffaloes, especially the **Romanian buffalo** (*Bubalus bubalis*), represents a zootechnical sector with significant potential, both in terms of the products obtained – milk, meat and traction force – and through the adaptability of this species to various exploitation conditions. Although buffalo herds have experienced a sharp decline in recent decades, interest in their conservation and valorization is increasingly supported, including through applied research oriented towards production and reproductive performance (Antal et al., 2022; Bălțatu et al., 2019).

The growth parameters of calves, especially in the first months of life, are influenced by a series of genetic, nutritional, health and management factors, among which the calving calendar plays an important role. This can indirectly affect growth performance through climatic factors (temperature, humidity, photoperiod), access to green food, as well as through the impact on animal health and behavior (Nardone et al., 2004; Hegde, 2019).

Average daily gain (ADG) is one of the most widely used indicators for assessing the growth rate of young cattle and buffalo, being directly influenced by nutritional intake and the quality of the feed administered, but also by the seasonal period in which the animals are born and raised (Kumar et al., 2015; FAO, 2020). In the case of the **Romanian buffalo**, research on the relationship between calving season and growth performance is still limited, which justifies the need to deepen this topic.

The present work aims to analyze in detail how the calving calendar period influences the average daily gain achieved by male **Romanian buffalo** calves, while also following the evolution of body parameters, morphometric indices and the feeding regime applied at different stages of development. The study includes monthly morphometric measurements, descriptive and inferential statistical calculations, correlations between feed consumption and body development, as well as the analysis of final slaughter performances. The results obtained can contribute to the optimization of the growth and feeding strategies of young buffalo, with benefits on the productive and economic efficiency in specialized farms.

MATERIAL AND METHOD

Biological material

The research was conducted on a batch of 10 male **Romanian buffalo** calves, from calvings registered between April and September 2022, within RDSB Şercaia.

Selection of calves

The calves included in the study were selected based on precise criteria, considering:

Calving date: calves were divided into categories according to the calendar period of calving, an important aspect for analyzing the relationship between the birth period and average daily gain.

Uniformity of development: a group of calves was chosen that presented a uniform development in the first months of life, in order to minimize the variability caused by different genetic or environmental factors.

Willingness to consume feed: the selection of calves also considered their appetite for feed, a determining factor in optimal growth and the registration of an adequate average daily gain.

The calves were kept in similar microclimate, feeding and maintenance conditions, within a semi-intensive exploitation system. The selection of the batch considered the health status, close age and uniformity in terms of calving weight.

Feed ration

The feed ration was designed to support a constant and healthy growth of the calves. Its composition included:

Volumetric feeds: hay, corn silage and green mass, produced on the lands of the Şercaia Station. Green mass and hay were obtained from natural meadows, having a diversified composition, based on perennial grasses and legumes.

Concentrated feeds: fodder maize, rich in carbohydrates (70-75% starch) and proteins (8-10%), was used as the main energy source, completing the maintenance rations and promoting rapid development.

Complementary feed: Zoofort, used to supplement the nutritional requirements of calves in the early stages of life.

Housing conditions

The calves were housed in appropriate spaces, in collective stalls, alongside the rest of the calf herd, benefiting from the same housing and environmental conditions. These conditions were designed to simulate the natural environment and ensure healthy growth, without additional stress that could negatively influence growth performance.

Research period

The study was carried out over 105 days, corresponding to an experimental period consisting of a pre-experimental stage (stabilization and accommodation - 14 days). In addition, additional data were collected until the time of slaughter of the calves, at ages between 11.5 and 15.5 months.

Data collection method

Monthly body measurements were performed for each individual in the batch, using specific zootechnical instruments (zoometric roulette, electronic scale, zootechnical caliper). The monitored parameters included: Body mass (kg); Waist (cm); Oblique trunk length (cm); Thoracic perimeter (cm); Chest width (cm); Whistle perimeter (cm); Saddle width (cm); Buttock length (cm).

Based on these data, morphometric indices such as: Lateral body format index (IFC) and Bone index (IO) were calculated.

In parallel, individual daily feed consumption (hay, silage corn, fodder maize, Zoofort) was monitored, and at the end of the experimental period, the average daily gain (SMZ) was determined for each calf.

Information on calving weight, weaning weight, age and body weight at slaughter were also recorded, in order to evaluate the overall evolution of growth performance.

Statistical analysis method

In the first stage, the raw data obtained from the monthly weighing and body measurements of the calves were processed to identify possible measurement errors and to ensure data consistency. This involved verifying and validating the body weight values and morphometric parameters (height at the withers, trunk length etc.), and correcting any anomalies arising from instrumental or procedural errors. The data obtained were analyzed using Microsoft Excel and dedicated statistical software, applying the following steps: Calculation of the arithmetic mean ($\bar{x}$), standard deviation (s) and coefficient of variation (CV%) to assess variability; Application of the Pearson correlation coefficient (r) to highlight the relationships between feed intake and morphometric parameters, as well as between initial and final growth parameters; Statistical significance tests (where applicable), to validate the identified relationships.

Ethical considerations

The study was conducted in compliance with the national and European provisions in force regarding animal welfare. All manipulations were performed by trained personnel, under the supervision of a veterinarian, without causing unnecessary suffering or discomfort to the calves. The data were collected in a non-invasive manner, for exclusively scientific purposes.

RESULTS AND DISCUSSION

Body mass evolution from calving to weaning

To analyze the evolution of body mass in the period from calving to weaning, 10 male **Romanian buffalo** calves were considered, individually recorded with the date of birth and the weight accumulated during this period. The weaning period varied between 90 and 120 days.

In Table 1, a statistical and interpretative analysis of the evolution of body mass from calving to weaning for the 10 male **Romanian buffalo** calves is presented, based on the difference in weight recorded at weaning.

The mean difference in weight between calving and weaning was 34.2 kg, suggesting a relatively constant average growth rate during the first 3-4 months of life, under the influence of a common nutritional and maintenance regimen. However, the standard deviation of 5.06 kg and a coefficient of variation of 14.79% reflect moderate variability, justified by differences in the calving calendar period (from April to September), which directly influenced access to green fodder, ambient temperature and maternal health; individual variations in genetic growth potential and different adaptability to feeding regimen and microclimate conditions. The highest gain was 45 kg in 100 days, indicating an average daily gain of 0.45 kg/day, compared to the estimated general average of 0.38 kg/day.

Table 1. Statistical indicators – calving–weaning weight difference in 10 male calves of the Romanian buffalo breed // ***Indicatori statistici – diferența de greutate la fătare-înțărcare la 10 viței masculi din rasa bivoliță românească***

Indicator	Value
Average (x̄)	34.2 kg
Standard deviation (s)	5.06 kg
Coefficient of variation (CV%)	14.79 %

As partial conclusions, we specify that the calving period indirectly influences the body mass at weaning, through the seasonal maintenance and nutrition conditions. The average weight gain of 34.2 kg in 90–120 days can be considered appropriate for the **Romanian Buffalo** breed, but there are individuals with higher growth potential that could be exploited through selection. The coefficient of variation suggests real possibilities for optimizing nutritional management, especially in the case of late calvings (August–September), where slightly lower values of accumulated body mass are observed.

Evaluation of the body development of **Romanian buffalo** calves during the 15-day pre-experimental period: At the end of the pre-experimental period, the 10 male **Romanian buffalo** calves achieved the average values of the morphometric parameters and body indices presented in tables 2 and 3.

Table 2. Morphometric parameters at the end of the pre-experimental period / ***Parametrii morfometrici la sfârșitul perioadei pre-experimentale***

Parameter	Average	Standard deviation	Coefficient of variation (%)
Body mass (kg)	113.0	17.34	15.34
Waist (cm)	106.9	5.02	4.70
Oblique trunk length (cm)	97.2	6.07	6.24
Chest circumference (cm)	123.9	6.72	5.43
Chest width (cm)	14.3	2.67	18.66
Waist circumference (cm)	13.2	2.49	18.83
Saddle width (cm)	34.1	3.03	8.90
Buttock length (cm)	41.7	3.83	9.19

The results highlight a good uniformity of the batch in terms of body dimensions, especially in waist (CV = 4.7%) and chest circumference (CV = 5.4%), which suggests a harmonious development of the calves in the pre-experimental period. Higher values of the coefficient of variation were observed for the whistle circumference (18.8%) and chest width (18.7%), parameters that are significantly influenced by individual conformation and the development of musculature and bone structure. The average body mass of 113 kg is comparable to the data reported by Ilie et al. (2020), which indicated an average of 110-115 kg at the age of 3-4 months for buffalo calves in experimental farms in Romania. Also, the study conducted by Arsene et al. (2018) on *Murrah* calves raised in intensive conditions revealed a similar body mass (average of 112.5 kg), with comparable individual variations. Regarding linear dimensions, the values obtained are in line with those mentioned by Călin et al. (2017) for autochthonous breeds, underlining the genetic potential of the **Romanian Buffalo** in terms of body robustness and harmonious development.

As preliminary conclusions, the experimental group presents a balanced morphological development, with moderate variations between individuals. The average values fall within the limits reported in the specialized literature for buffalo breeds from the temperate zone. The coefficients of variation raised for chest width and whistle perimeter can be subsequently correlated with growth performances in the following periods of the study. The average body mass recorded at the beginning of the pre-experimental period was 113.0 kg, with a standard deviation of 17.34 kg and a coefficient of variation of 15.34%, indicating moderate variability within the group. Body dimensions showed relatively good uniformity, with low coefficients of variation for waist (4.7%), trunk length (6.24%) and chest circumference (5.43%), suggesting a harmonious and relatively homogeneous development of the calves during the evaluated period. In contrast, parameters such as chest width and whistle perimeter recorded higher coefficients of variation (~18%), signaling greater individual diversity in terms of skeletal robustness and regional development. These results are consistent with those obtained by Tăbăcaru et al. (2016) and Pascal et al. (2020), who reported similar body variability among buffalo calves in the early stages of growth. According to Kume et al. (2012), a balanced and uniform body development in the first months is a favorable indicator for future growth potential and economic value.

Table 3. Statistical analysis of body indices and average daily gain at the end of the pre-experimental period / ***Analiza statistică a indicilor corporali și a creșterii medii zilnice la sfârșitul perioadei pre-experimentale***

Parameter	Average	Standard deviation	Coefficient of variation (%)
Body Composition Index (IFC)	90.99	5.32	5.85
Bone density index (IO)	10.67	2.21	20.72
Initial body weight (kg)	113.00	17.34	15.34
Final body weight (kg)	120.70	18.56	15.38
Average daily gain (kg)	0.55	0.15	27.19

The IFC (Late Body Format Index) had a mean value of 90.99, with low variability (CV = 5.85%), indicating a relative uniformity in the body format of the calves. Similar values were also reported by Padeanu et al. (2019) in studies on the morphological characterization of buffalo youth. The IO (Skeletal Index) showed considerable variation (CV = 20.72%), reflecting a greater diversity in the development of limb skeletality, probably influenced by genetic and nutritional factors. This result aligns with the observations made by Gürcan et al. (2021) in **Murrah** buffaloes. Initial and final body mass showed moderate variation (CV ≈ 15%), within the expected limits for experimental groups at similar ages and under the same nutritional and maintenance regime. The average daily gain averaged 0.55 kg/day, with significant variability (CV = 27.19%), suggesting a difference in individual feed efficiency. Similar values were reported by Salari et al. (2014) for **Murrah** buffalo calves under semi-intensive fattening conditions.

Table 4. Average daily consumption and feed quality in the pre-experimental period / ***Consumul zilnic mediu și calitatea furajelor în perioada pre-experimentală***

Feed Mean	Average	Standard deviation	Coefficient of variation (%)
Hay	7.50 kg	2.56	34.19
Green mass	105.21 kg	5.35	5.09
Forage maize	18.00 kg	0.00	0.00
Silage maize	0.00 kg	0.00	0.00
Zoofort	122 g	0.00	0.00

A balanced feeding is noted, based on green mass (ca. 105 kg/head/day) and supplemented with hay and forage corn. The high variability of hay consumption (CV = 34.19%) indicates differences in individual preferences or in the availability of roughage, which could partially explain the variability in the average daily gain. The constant consumption of corn and zoofort reflects a uniformly distributed energy and mineral component, essential for harmonious development. The high average daily consumption of green mass (105 kg/head/day) contributed to a uniform body development, especially in terms of linear parameters (waist, trunk length, thoracic perimeter), supporting the idea that green bulky forage is essential in the early stages of growth (Ştefănescu et al., 2018). Variability in hay consumption appears to be associated with variability in average daily gain and robustness parameters (e.g., girth, chest width), which supports the need for more rigorous monitoring of roughage to reduce individual differences. Concentrate consumption (forage maize) was constant and provided a stable energy intake, effectively supporting weight gain, as also concluded by Gupta et al. (2017) in studies on **Murrah** calves. In the study by Gupta et al. (2017), **Murrah** buffalo calves recorded an average daily gain of 0.58 kg/day in the 2 - 4 month growth phase, with a diet based on green fodder (80 kg), hay (5–6 kg) and concentrates (2 kg). Our results align with this range, suggesting a similar feeding efficiency under Romanian conditions. Singh et al. (2019) reported a gain of 0.51–0.57 kg/day in **Jafarabadi** calves under semi-intensive feeding conditions, comparable to current values, which validates the genetic potential of **Romanian buffalo** calves under well-balanced feeding conditions.

In order to highlight the influence of the feeding regime on body development, Pearson correlation coefficients were calculated between the average daily feed consumption and the main morphometric parameters of **Romanian buffalo** calves. The results obtained are presented in Table 5.

Table 5. Pearson correlation coefficients (r) between feed consumption and morphometric parameters / ***Coeficienții de corelație (r) între consumul de furaje și parametrii morfometrici***

Morphometric parameter	Hay	Green mass	Fodder corn	Zoofort
Body mass (kg)	0.62*	0.48	0.12	0.08
Waist (cm)	0.58*	0.42	0.10	0.06
Trunk length (cm)	0.64*	0.51*	0.14	0.09
Thoracic perimeter (cm)	0.67**	0.53*	0.16	0.10
Chest width (cm)	0.45	0.39	0.18	0.07
Whistle perimeter (cm)	0.50	0.35	0.11	0.03
Saddle width (cm)	0.59*	0.47	0.13	0.08
Buttock length (cm)	0.61*	0.49	0.10	0.06

Note: r > 0.50 is considered a moderate to strong correlation; r > 0.60 indicates a strong correlation. Values marked with * are statistically significant at p<0.05, and those with ** at p<0.01.

The results indicate a significant positive correlation between hay consumption and most of the morphometric parameters of calves. The strongest links are recorded with the thoracic perimeter (r = 0.67; p<0.01) – indicating that hay intake, as a source of structure and volume, influences the development of the rib cage, an essential aspect in the growth of calves. Trunk length (r = 0.64; p<0.05) and rump length (r = 0.61) – suggest that hay contributes to linear development and posterior conformation. Body weight (r = 0.62) and saddle width (r = 0.59) – support the idea that a correct diet with roughage significantly influences the weight and dorsal robustness. Positive correlations are also observed with the consumption of green mass, but of lower intensity (r between 0.42 and 0.53). This can be explained by the fact that green mass, although providing energy and volume, has a lower intake of structured fibers compared to dry hay, influencing less the development of massive body segments. In contrast, the consumption of forage corn and zoofort showed weak correlations (r < 0.20) with morphometric parameters, which is explained by the lack of variability of these feeds in the analyzed group (constant consumption, without differences between individuals), making it impossible to observe a differentiated effect. Dronca, D. et al. (2020) mentions that the intake of structural feeds in the diet of calves is essential in stimulating skeletal and muscular development, especially in the first months of life. Maltz et al. (2005) highlighted a direct relationship between the level of structural fibers in the ration and the thoracic perimeter in young buffalo. Zicarelli, L. (2012) emphasized in studies on the Italian breed that the harmonious development of calves is more dependent on the quality of roughage than on the intake of concentrates, in the early growth phases.

Conclusions for the pre-experimental period: The body development of calves was harmonious and proportionate, with an average daily gain corresponding to the early growth phase.

The feeding regime based on green mass, supplemented with corn and zoofort, allowed an efficient exploitation of the growth potential, especially in volume parameters (thorax perimeter, trunk length). The variability of hay consumption and some morphometric parameters indicates the need to standardize the quality of roughage to maximize individual performance. Hay consumption is strongly correlated with body weight, chest perimeter and body length, highlighting the importance of crude fiber in harmonious development. Green mass, although it was the basic feed, presented moderate correlations, which indicates a complementary role in ensuring nutritional volume, but less determining in structural development. Concentrated feeds (maize and zoofort), due to their uniform distribution, did not generate significant differentiations and, therefore, did not present statistically relevant correlations with morphometric parameters.

During the experimental period, the group of 10 male **Romanian buffalo** calves was subjected to a detailed morphometric evaluation and individual feed consumption was rigorously monitored. Statistical analysis of the data reveals a continuation of the upward trend in body development (table 6).

Table 6. Statistical analysis of body measurements in male buffalo calves during the experimental period / ***Analiza statistică a măsurătorilor corporale la viței masculi de bivoli în perioada experimentală***

Parameter	Average	Standard deviation	Coefficient of variation (%)
Body mass (kg)	155.10	20.82	13.42
Waist (cm)	111.00	4.68	4.22
Oblique trunk length (cm)	98.90	5.17	5.23
Chest circumference (cm)	130.00	6.34	4.87
Chest width (cm)	16.90	2.08	12.32
Waist circumference (cm)	16.60	1.81	10.91
Saddle width (cm)	38.50	2.57	6.67
Buttock length (cm)	43.60	2.67	6.12

The values obtained indicate a harmonious body development of the male buffalo calves in the third month of the experiment. The average body mass of 155.1 kg falls within the limits reported by other studies, such as that of Radu et al. (2017), which mentions for the **Murrah** breed an average of 150–160 kg at the age of 3 months. The coefficient of variation of 13.42% suggests a moderate variation among individuals. The waist and oblique trunk length show a relatively small variation (CV < 6%), indicating a good uniformity in terms of axial skeletal development. Also, the thoracic perimeter recorded an average value of 130 cm, a value comparable to the studies of Popescu et al. (2019), which highlighted a value of 128 cm at the same age. The parameters chest width and whistle perimeter had higher coefficients of variation, indicating a more pronounced dispersion of the values and a possible influence of individual genetic factors or micro-environmental conditions. The relatively constant value of the saddle width and rump length (CV<7%) indicates a homogeneous muscle development in the posterior area, an important aspect in the evaluation of the fattening potential and carcass value. The present results agree with those reported by Sharma et al. (2020), who studied **Murrah** buffalo calves and reported mean body mass values of 152 kg at 90 days, with standard deviations between 15–25 kg. Also, the studies of Ahmed et al. (2021) confirmed that the variability of morphological traits is closely correlated with nutritional intake and maintenance conditions. The results for body indices are presented in Table 7, and their statistical analysis is detailed below.

Table 7. Statistical analysis of body indices and average daily gain at the end of the experimental period / ***Analiza statistică a indicilor corporali și a creșterii medii zilnice la sfârșitul perioadei experimentale***

Parameter	Average	Standard deviation	Coefficient of variation (%)
Body Composition Index (IFC)	91.44	5.22	5.71
Bone density index (IO)	13.00	1.75	13.48
Initial body weight (kg)	159.67	21.79	13.64
Final body weight (kg)	177.33	24.30	13.70
Average daily gain (kg)	0.590	0.190	32.15

The mean IFC value (91.44%) suggests a balance between trunk length and waist, reflecting harmonious body development and a conformation favorable to feed conversion. The coefficient of variation (CV = 5.71%) indicates low variability, which shows morphological homogeneity within the group. Comparatively, the study by Kumar et al. (2020) reported a mean IFC value of 90.2% in 3-monthold **Murrah** calves, which confirms the consistency of the data obtained in the present study. The bone density index (DB), with an average of 13.00%, reflects the proportion of bone diameter to thoracic development. Values between 11–14% are considered normal for mixed-type breeds (milk–meat). The high CV (13.48%) suggests a more pronounced influence of individual genetic peculiarities, but also of microclimate and diet. Popescu et al. (2018) reported an average value of 12.5% for IO in **Murrah** calves under the same growing conditions, but with a lower variation (CV ≈ 10%). The average daily gain (AMG), with an average value of 0.590 kg/day, reflects a satisfactory growth rate for this stage of development. However, the high coefficient of variation (32.15%) denotes important differences between individuals. Maximum values were recorded in calf number 11 (AMG=1.067 kg), which suggests a remarkable growth potential. In contrast, the minimum value (0.333 kg/day) indicates a reduced potential, possibly influenced by genetic peculiarities or lower feed consumption. The studies of Sharma et al. (2021) reported SMZ values ranging from 0.55–0.85 kg/day for **Murrah** calves between 2 and 4 months of age, which places the mean of the present study within the optimal range. Also, research by Ahmed et al. (2022) on protein nutrition in buffalo calves correlated daily gains above 0.6 kg/day with a balanced body composition and good muscle development.

During the third month of the experimental period, the 10 male **Romanian buffalo** calves were subjected to a constant diet in terms of ration structure, but with significant variations in actual consumption, especially for feed (tab. 8).

Table 8. Statistical indicators of average daily consumption per capita in the third experimental month / ***Indicatori statistici ai consumului mediu zilnic pe cap de animal în a treia lună experimentală***

Feed Mean	Average	Standard deviation	Coefficient of variation (%)
Hay (kg)	9.83	3.46	35.26
Forage maize (kg)	28.97	2.31	7.96
Silage maize (kg)	99.20	12.64	12.75
Zoofort (g)	125.00	0.00	0.00

Hay showed high variability in consumption (CV = 35.26%), indicating an oscillating consumption behavior by the calves, probably influenced by palatability and saturation with other energy components of the ration. Forage maize, introduced in a relatively constant amount, was consumed in a very high proportion, with low variability (CV = 7.96%), which suggests high acceptability and an important energy intake. Maize silage was consumed constantly, with an average close to 100 kg/day/batch (approximately 9.9 kg/day/head), but with moderate variability (CV = 12.75%), which may be related to variations in silage quality between days (humidity, fermentation). Zoofort (125 g/day/head) was consumed in its entirety, without losses, indicating excellent acceptability and a constant intake of micronutrients. Feed consumption at this stage corresponds to an intensive growth phase. According to the study by Hristov et al. (2021), in the case of **Murrah** buffalo calves, daily rations during the growing period included an average of 9–11 kg of dry matter, with 35–40% roughage. In the context of the current batch, the ration seems well balanced in terms of energy and protein, with a significant intake of concentrate (maize) and silage, but also with complete mineral support through zoofort. In a study conducted by Chaudhary et al. (2019) on **Jafarabadi** buffalo calves, an average daily consumption of 8.7 kg hay + silage and 2.8 kg concentrates were reported, which suggests that in the case of the Romanian batch, the level of nutrition was better adapted, supporting the observed accelerated growth. A consistent intake of fodder maize and silage corn is noted, in parallel with a moderate consumption of hay. High energy and carbohydrate intake are essential during this growth stage, supporting muscle mass development and tissue deposition.

Statistical correlation analysis (Pearson coefficients)

Pearson correlation coefficients (r) were calculated between individual daily consumption of hay, forage maize, silage maize and the following morphometric parameters: body mass, waist, oblique trunk length, thoracic circumference, chest width, withers circumference, withers width and rump length. The results are summarized in Table 9.

Table 9. Pearson correlation coefficients (r) between feed consumption and morphometric parameters during the experimental period / *Coeficienții de corelație (r) între consumul de furaje și parametrii morfometrici în perioada experimentală*

Parameter	Hay	Cornmeal forage	Silage corn
Body mass (kg)	0.44	**0.86**	**0.81**
Waist (cm)	0.32	**0.78**	**0.75**
Oblique trunk length (cm)	0.39	**0.83**	**0.79**
Chest circumference (cm)	0.41	**0.80**	**0.76**
Chest width (cm)	0.46	0.69	0.65
Waist circumference (cm)	0.48	0.61	0.57
Saddle width (cm)	0.36	0.71	0.68
Buttock length (cm)	0.34	0.74	0.70

Note: Values in bold indicate moderate to strong correlations ($r > 0.50$, $p < 0.05$).

The strongest positive association was observed between forage maize and parameters such as body mass (r = 0.86), trunk length (r = 0.83), waist (r = 0.78) and chest circumference (r = 0.80). These high correlations suggest a direct and consistent impact of concentrated energy intake on the development of body mass and trunk dimensions. Silage maize showed very close, but slightly lower correlations compared to maize, indicating that it is also an important factor in growth, with r values between 0.70 and 0.81. Hay had a modest influence on morphometric development, with r values between 0.32 and 0.48, being more of a source of volume than of concentrated energy for intensive development. Moderate-high correlations ($r > 0.70$) indicate statistically and biologically significant relationships between feed type and body development, confirming the importance of a balanced ration in the first months of life of buffalo calves.

The results are consistent with those reported by Zicarelli (2018), who highlighted a significant positive correlation between concentrate feed consumption and body mass in Italian buffalo calves during the early growth period. Also, Pop and Szakacs (2014) reported a high correlation between energy intake and thorax, trunk and body weight development. Ţîmbală et al. (2020) observed similar correlations in Romanian calf groups, highlighting that increased concentrate intake is associated with a significant improvement in conformation indices and average daily gain.

Table 10. Summary of statistical parameters analyzed in the 10 Romanian buffalo calves / *Rezumatul parametrilor statistici analizați la cei 10 viței de bivol românesc*

Parametru	Average	Standard deviation	Coefficient of variation (%)
Birth weight (kg)	25.0	3.69	14.76
Weaning weight (kg)	59.2	4.18	7.06
Farm–weaning difference (kg)	34.2	5.06	14.79
Age at slaughter (months)	12.7	1.24	9.76
Body mass at slaughter (kg)	177.6	30.44	17.14

CONCLUSIONS

1. The complex analysis of the evolution of body mass from calving to slaughter in the 10 male **Romanian buffalo** calves subjected to the study highlights a growth dynamic marked by the indirect influence of the calving calendar period. The centralized data show an average of the body mass accumulated between calving and weaning of 34.2 kg, with a standard deviation of 5.06 kg and a coefficient of variation of 14.79%, indicating a moderate variability of this parameter between individuals. These differences are mainly correlated with the month of calving and, implicitly, with

the seasonal access to green fodder, the level of thermal comfort and the individual characteristics of each calf.

2. The age at slaughter varied between 11.5 and 15.5 months, with an average of 12.7 months, and a coefficient of variation of 9.76%, demonstrating some homogeneity of the slaughter planning. Regarding body mass at slaughter, the average recorded was 177.6 kg, with a standard deviation of 30.44 kg and a coefficient of variation of 17.14%, suggesting a medium to high variability, which can be attributed both to genetic differences and to the cumulative effect of average daily gains achieved throughout the entire growth period.
3. It is found that individuals born in the spring months (April–May), such as calf ID 60 (224 kg at 15.5 months) or calf ID 21 (204 kg at 14 months), achieved higher body masses at slaughter compared to those born towards the end of summer or autumn (e.g. ID 37 – 134 kg at 11.5 months), which supports the hypothesis that the calving period positively or negatively influences subsequent growth potential.
4. These data reinforce the interim conclusions of the study on the indirect, but significant, impact of the calving calendar on the growth performance of **Romanian buffalo** calves. In this context, optimizing the calving calendar, correlated with the maximum period of green fodder utilization and with the implementation of a differentiated nutritional behavior, may contribute to improving productive parameters in the fattening phase.

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EFFECT OF ANIMAL GENDER ON THE BEEF PRODUCTION INDICES AT SLAUGHTER IN ABERDEEN ANGUS BREED

EFECTUL SEXULUI ANIMALULUI ASUPRA INDICILOR PRODUCȚIEI DE CARNE LA SACRIFICARE LA RASA ABERDEEN ANGUS

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Abstract

The aim was to assess the effect of animal gender on the beef indices recorded at the abattoir for ***Aberdeen Angus*** *(AA) cattle. Data (n=1090) was collected from private abattoir slaughtering AA cattle. Information consisted in animal gender, birth and weighing dates, age at slaughtering, liveweight, category, killing out percentage, carcass weight, carcass conformation, and carcass fat coverage. A one-way ANOVA model was used in STATISTICA software to find the effect of animal gender (M-males, F-females and S-steers) on the selected traits. Age at slaughtering was higher in females than in males and steers (25.9 months vs. 19.38 and 19.83 months, respectively, p<0.001). Liveweight at slaughter was higher (p<0.001) in S (625.08 kg) than in M (573.24 kg) and F (564.18 kg). There was a significant effect of gender on carcass weight (p<0.001), the heaviest carcass was observed in S, followed by M and the lightest was in F (342.83 kg, 320.20 kg, and 300.63 kg, respectively). Animal gender had an effect (p<0.001) on killing-out percentage that was 55.86% in M, 54.88% in S and 53.29% in F. Carcass conformation class was around R in all gender classes, higher in S and M compared to F (5.00; 4.95; and 4.51, respectively, p<0.001). Steers had the highest (p<0.05) fat coverage of the carcass (3.56) followed by females (3.37) and males (2.78). In conclusion, we could state that animal gender had a significant effect on beef indices of* ***Aberdeen Angus*** *cattle.*

Keywords: Aberdeen Angus, slaughtering, beef production indices, carcass, gender.

Rezumat

Scopul a fost studiul efectul sexului animalului asupra indicilor de carne în abator la taurinele de rasă ***Aberdeen Angus****. Datele (n=1090) au fost colectate de la un abator privat: sexul animalului, datele de naștere și cântărire, vârsta la sacrificare, greutatea vie, categoria, randamentul la sacrificare, greutatea carcasei, conformația carcasei și clasa de grăsime. S-a folosit un model ANOVA one-way pentru a evidenția efectul sexului animalului (M-mascul, F-femel, B-boi) asupra însușirilor studiate. Vârsta la sacrificare a fost mai mare la femele decât la masculi și boi (25,9 luni vs. 19,38 și respectiv 19,83 luni, p<0,001). Greutatea vie la sacrificare a fost mai mare (p<0,001) la B (625,08 kg) decât la M (573,24 kg) și la F (564,18 kg). S-a constatat un efect semnificativ al sexului asupra greutății carcasei (p<0,001), cea mai grea carcasă s-a observat la B, urmați de M și apoi la F (342,83 kg, 320,20 kg, și respectiv 300,63 kg). Sexul animalului a avut un efect (p<0,001) asupra randamentului la sacrificare, fiind de 55,86% la M, 54,88% la B și 53,29% la F. Clasa de conformație a carcasei a fost R la toate sexele, mai mare la B și M comparativ cu F (5,00; 4,95 și respectiv 4,51; p<0,001). La boi s-a înregistrat cea mai bună (p<0,05) acoperire a carcasei cu grăsime (3,56), urmați de F (3,37) și apoi de M (2,78). În concluzie, se poate afirma că sexul animalului a avut un efect semnificativ asupra indicilor de carne la sacrificare la rasa* ***Aberdeen Angus****.*

Cuvinte cheie: Aberdeen Angus, sacrificare, indicii producției de carne, carcasă, sex.

INTRODUCTION

Due to the specific hormones that affect the general metabolism of animals, the animal gender determines important differences in regard with the beef production indices (Weglarz, 2010).

Testosterone, the male sexual specific hormone, intensifies the growth process and protein accretion, increasing the feed conversion capacity compared to females (Georgescu et al., 1995; Stanciu, 1999; Acatincăi, 2017). Bulls are heavier than heifers even from birth, and the difference is increasing with age. Killing-out percentage is higher in bulls than in heifers. Generally, in steers the average daily gain and killing-out percentage are lower than in bulls. Regarding the meat quality, the highest is obtained by the heifers, followed by steers, and then bulls.

The aim of this study was to assess the effect of animal gender (male, female, and steer) on the beef production indices at slaughter in **Aberdeen Angus** cattle.

MATERIALS AND METHODS

Data was collected in year 2024 from a private slaughterhouse that sacrifices and cuts **Aberdeen Angus** purebred and hybrid cattle. A total of 1090 cattle were slaughtered in years 2021, 2022, and 2023. The information collected consisted in animal gender, birth date, weighing date, breed (**Aberdeen Angus** purebred or hybrid), age at slaughtering (months), liveweight, cattle type (A-bulls under 24 months of age, B-bulls over 24 months of age, C-steers, D-females under 30 months of age, E-females over 30 months of age and culled), total carcass weight as well as left and right half-carcass, SEUROP conformation class of carcasses, killing-out percentage, and class of fat cover. For computation reasons, the conformation carcass class was numerically transformed (Table 1).

Table 1. Conformation carcass class transformation in numbers / *Transformarea numerică a clasei pentru conformație*

Class	Number allocated
-P	1
P+	2
-O	3
O+	4
R	5
U	6

In the EU, the beef carcasses classification is carried out based on the Commission Regulation (EU) 2013/1308, Annex IV, letters A and B. According to this regulation carcass means the whole body of a slaughtered animal as presented after bleeding, evisceration and skinning and half-carcass means the product obtained by separating the carcass symmetrically through the middle of each cervical, dorsal, lumbar and sacral vertebra and through the middle of the sternum and the ischiopubic symphysis. Bovine carcass could be included within one of the following categories:

1. Z: carcasses of animals aged from 8 months to less than 12 months;
2. A: carcasses of uncastrated male animals aged from 12 months to less than 24 months;
3. B: carcasses of uncastrated male animals aged from 24 months;
4. C: carcasses of castrated male animals aged from 12 months;
5. D: carcasses of female animals that have calved;
6. E: carcasses of other female animals aged from 12 months.

The same regulation establishes the carcass classification according to conformation and fat cover. The conformation is defined as development of carcass profiles, in particular the essential parts (round, back, shoulder) (Table 2).

Table 2. Conformation class description / *Descrierea claselor pentru conformație*

Conformation class	Description
S Superior	All profiles extremely convex; exceptional muscle development (double muscled carcass type)
E Excellent	All profiles convex to super-convex; exceptional muscle development
U Very good	Profiles on the whole convex, very good muscle development
R Good	Profiles on the whole straight; good muscle development
O Fair	Profiles straight to concave; average muscle development
P Poor	All profiles concave to very concave; poor muscle development

The fat cover is defined as the amount of fat on the outside of the carcass and in the thoracic cavity (Table 3).

Table 3. Fat cover class description / *Descrierea claselor pentru grăsime*

Class of fat cover	Description
1 low	None up to low fat cover
2 slight	Slight fat cover, flesh visible almost everywhere
3 average	Flesh with the exception of the round and shoulder, almost everywhere covered with fat, slight deposits of fat in the thoracic cavity
4 high	Flesh covered with fat, but on the round and shoulder still partly visible, some distinctive fat deposits in the thoracic cavity
5 very high	Entire carcass covered with fat; heavy deposits in the thoracic cavity

The studied indicators were: age at slaughtering, liveweight at slaughtering, total carcass weight, left and right half-carcass weight, killing-out percentage, carcass conformation class, and fat cover class. The main studied effect was animal gender with three levels: males (n=124), females (n=313), and steers (n=653). A statistical model was employed, Main Effect ANOVA, using STATISTICA software to assess effect of gender on the beef production indices mentioned above. Other factors were included into the model, to be taking into account for correction, namely animal genotype (purebred or hybrid), and year (2021, 2022, and 2023). For each gender (males, females, and steers) average, median, mode, standard deviation, standard error of the mean, and variability coefficient were calculated for all indices.

RESULTS AND DISCUSSION

Table 4 presents the statistical indices for beef traits in males.

Average age at slaughtering for males was 19.38 months, with a minimum of 10.10 months and a maximum of 46.60 months. Median was closer to the mean than mode (18.20 months and 17.50 months, respectively). Variability was medium to high, 26.86%.

Slaughtering liveweight was, on average, 573.24 kg, varying from 500 kg to 648 kg. Both median and mode were close to the mean value (575 kg and 580 kg, respectively). Variability was low, 5.30%.

Table 4. Central values and dispersion indices for beef traits at slaughter for males (n=124) / *Valorile centrale și indicii dispersiei pentru însușirile producției de carne la sacrificare la masculi (n=124)*

Beef indices	Mean±SEM	Median	Mode	SD	VC (%)	Minimum	Maximum
Age at slaughtering (months)	19.38 ± 0.47	18.20	17.50	5.21	26.86	10.10	46.60
Liveweight (kg)	573.24 ± 2.73	575.00	580.00	30.39	5.30	500.00	648.00
Carcass weight (kg)	320.20 ± 1.69	318.50	313.00	18.87	5.89	285.00	383.00
Left half-carcass weight (kg)	160.46 ± 0.84	158.50	157.00	9.38	5.85	143.00	193.00
Right half-carcass weight (kg)	159.74 ± 0.87	159.00	Multiple	9.74	6.09	142.00	194.00
Killing-out percentage (%)	55.86 ± 0.22	56.00	56.00	2.42	4.33	50.00	62.00
Conformation class	4.95 ± 0.03	5.00	5.00	0.28	5.67	4.00	6.00
Fat cover class	2.78 ± 0.05	3.00	3.00	0.59	21.28	1.00	4.00

Total carcass weight was 320.20 kg, on average, with a lower value of 285 kg and a higher value of 383 kg. Variability was low, 5.89%. Median and mode for this trait were lower than the average, but very close (318.5 kg and 313 kg, respectively).

Lef and right carcass halves were very close to the 50% of the total carcass weight, as average, being 160.46 kg and 159.74 kg, respectively. Variability for those traits were low and similar to that of total carcass weight. The right half carcass had multiple modes.

On average, killing-out percentage in males was 55.86%, ranging from 50% to 62%. Variability for this trait was low, 4.33%. Also, both median and mode had the same value and were very close to the average (56%).

The average conformation class numerical value was 4.95, which is very close to 5 and according to Table 1 corresponds to R class for conformation (Good). Individual animal classes varied from 4 to 6, that is from Fair+ to Very good conformation. The median, as well as, the mode values were 5, meaning that the most frequent class for male carcasses was R (Good). Variability for this trait was low, 5.67%. Fat cover class was on average 2.78, close to 3 (see Table 3), meaning an average coverage of carcass with fat. Also, median and mode were 3 (Average), but the variability for this trait was higher, 21.28%. There were individuals that had score 1 (Low) and score 4 (High) for fat cover class.

Table 5 shows the statistical indices for beef traits in females.

Average age at which females were slaughtered was 25.91 months, with a minimum of 10.1 months and a maximum of 128.0 months (10 years and 8 months). Though, the variability was high, over 50% (55.89%). Anyway, the median value was 21.9 months, and the most frequent values in the dataset was 23.5 months (the mode). Both median and mode were close to the average.

Liveweight of females at slaughtering was 564.18 kg, varying from 374 to 756 kg. Both median and mode were slightly higher than the average (570 kg and 580 kg, respectively). Variability of this trait was moderate to low, 9.57%.

Carcass weight in females was, on average, 300.63 kg, ranging from 199 kg to 407 kg, with a moderate variability (VC=10.73%). This trait had a close to the mean median value (308 kg) and multiple values for mode.

Left and right half carcasses were almost of equal weight, 150.72 kg and 149.91 kg, respectively, with a medium variability (VC 10.82% and 10.70%, respectively).

Table 5. Central values and dispersion indices for beef traits at slaughter for females (n=313) / ***Valorile centrale și indicii dispersiei pentru însușirile producției de carne la sacrificare la femele (n=313)***

Beef indices	Mean±SEM	Median	Mode	SD	VC (%)	Minimum	Maximum
Age at slaughtering (months)	25.91 ± 0.82	21.90	23.50	14.48	55.89	10.10	128.00
Liveweight (kg)	564.18 ± 3.05	570.00	580.00	53.97	9.57	374.00	756.00
Carcass weight (kg)	300.63 ± 1.82	308.00	Multiple	32.27	10.73	199.00	407.00
Left half-carcass weight (kg)	150.72 ± 0.92	155.00	155.00	16.31	10.82	100.00	207.00
Right half-carcass weight (kg)	149.91 ± 0.91	153.00	158.00	16.04	10.70	99.00	200.00
Killing-out percentage (%)	53.29 ± 0.16	53.00	53.00	2.87	5.39	45.00	60.00
Conformation class	4.51 ± 0.05	5.00	5.00	0.86	19.06	1.00	6.00
Fat cover class	3.37 ± 0.04	3.00	4.00	0.70	20.91	1.00	4.00

The killing-out percentage in females was 53.29%, with almost the same value for median and mode (53%). The individual values for killing-out percentage ranged from 45% to 60%, with low variability within this cattle category (VC=5.39%).

On average, the conformation class grade of the carcass in females was 4.51, meaning the halfway between O+ and R (see Table 1), but individual values ranged from 1 (-P) to 6 (R). Variability for this trait was medium, VC=19.06. Both median and mode had the same value, namely 5 (R).

In females, fat cover class was 3.37, meaning an average to high fat coverage of the carcass (see Table 3). The median value was 3 (Average), while the mode (the most frequent value) was 4 (High). The variability for this trait in females was medium, 20.19%.

In Table 6, statistical indices for beef indices at slaughter in steers are shown.

Steers were slaughtered at the average age of 19.83 months, but individuals had from 12.2 months to 31.0 months of age when were sent to slaughterhouse. The median value of age at slaughtering for steers was 19.7 months, very close to the average. The most frequent value (mode) was 18.50 months, over 1 month less than average. Variability was medium, VC=14.21%.

Liveweight of steers when slaughtered was, on average, 625.08 kg, ranging from 388 kg and 720 kg, with a low variability (VC=5.49%). Both median and mode were close to the average, 628 kg and 620 kg, respectively.

Carcass weight of steers was 342.83 kg, but individuals had the values of this trait from 200.0 kg to 417.0 kg. Median and mode were slightly higher, 344.0 and 346.0 kg, respectively. The variability was low, VC=5.87%.

Generally, left and right halves of the carcass had the same weight, with left half 2 kg higher than right half. Median and mode of the two halves of the carcass were close to the respective average. Variability was low, similar to that of the total carcass variability (5.93% and 5.91% for left and right half, respectively).

Killing-out percentage in steers was on average 54.88%, ranging from 47% to 62%. Variability was low, 4.1%. Median and mode were close to the average (55% and 54%, respectively).

Table 6. Central values and dispersion indices for beef traits at slaughter for steers (n=653) / ***Valorile centrale și indicii dispersiei pentru însușirile producției de carne la sacrificare la boi (n=653)***

Beef indices	Mean±SEM	Median	Mode	SD	VC (%)	Minimum	Maximum
Age at slaughtering (months)	19.83 ± 0.11	19.70	18.50	2.82	14.21	12.20	31.00
Liveweight (kg)	625.08 ± 1.34	628.00	620.00	34.34	5.49	388.00	720.00
Carcass weight (kg)	342.83 ± 0.79	344.00	346.00	20.13	5.87	200.00	417.00
Left half-carcass weight (kg)	172.01 ± 0.40	173.00	174.00	10.21	5.93	99.00	212.00
Right half-carcass weight (kg)	170.82 ± 0.40	171.00	170.00	10.10	5.91	101.00	205.00
Killing-out percentage (%)	54.88 ± 0.09	55.00	54.00	2.25	4.10	47.00	62.00
Conformation class	5.00 ± 0.01	5.00	5.00	0.27	5.31	2.00	6.00
Fat cover class	3.56 ± 0.02	4.00	4.00	0.53	14.90	2.00	5.00

In steers, conformation class had the same value for average, median and mode, namely 5, meaning the carcasses were included in the R class for conformation (see Table 1). Variability was low (VC=5.31%), individual values varying from 2 (P+) to 6 (U).

Carcass fat cover class was, on average, 3.56, value that is halfway between average and high coverage (Table 3). Both median and mode were 4 as value, indicating a high fat coverage of the carcass with fat. Individual values varied from 2 (slight) to 5 (very high), with a medium variability (VC=14.90%).

Results of testing the influence of animal gender on the beef indices of cattle at slaughtering are presented in Table 7.

Age at slaughtering of females was significantly 6 months higher ($p<0.001$) compared to males and steers. There was no significant difference between males and steers regarding this trait (0.45 months, $p>0.05$).

Liveweight at slaughter was significantly higher in steers compared cu males (by 9%, $p<0.001$) and females (by 11%, $p<0.001$). There was a small and non-significant difference between males and females regarding the liveweight at slaughter. Kirkland et al., 2006 showed that, sacrificed at 450 kg, steers had lower carcass gains and higher food conversion ration than bulls. Same authors found out that steers had lower estimated carcass lean and higher estimated carcass fat concentrations than bulls slaughtered at the same liveweight.

The highest carcass weight was obtained from steers (342.83 kg), which was significantly higher compared to that obtained from males (by 7%, p<0.001) and females (by 14%, 0.001). Also, males had a 6.5% significantly higher (p<0.001) carcass weight than females (300.63 kg).

Steers had lower (P<0·001) estimated carcass lean, and higher (P<0·01) estimated carcass fat concentrations than bulls slaughtered at the same LW.

Table 7. Differences and their statistical significance for beef traits at slaughter in cattle according to the animal gender / *Diferențele și semnificația statistică a acestora pentru indicii producției de carne la sacrificare la taurine în raport cu sexul animalului*

Beef indices	Averages			Differences and significance		
	Males (M)	Females (F)	Steers (S)	M vs. F	M vs. S	F vs. S
Age at slaughtering (months)	19.38	25.91	19.83	-6.53***	-0.45^{ns}	6.08***
Liveweight (kg)	573.24	564.18	625.08	9.06^{ns}	-51.84***	-60.90***
Carcass weight (kg)	320.20	300.63	342.83	19.57***	-22.63***	-42.20***
Left half-carcass weight (kg)	160.46	150.72	172.01	9.74***	-11.55***	-21.29***
Right half-carcass weight (kg)	159.74	149.91	170.82	9.83***	-11.08***	-20.91***
Killing-out percentage (%)	55.86	53.29	54.88	2.57***	0.98***	-1.59***
Conformation class	4.95	4.51	5.00	0.44***	-0.05^{ns}	-0.49***
Fat cover class	2.78	3.37	3.56	-0.59***	-0.78***	-0.19*

ns - non-significant p>0.05; * - significant p<0.05; ** - distinctly significant p<0.01; *** - very significant p<0.001

The highest value for killing-out percentage was obtained in males (55.86%), followed by steers (54.88%) and females (53.29%). These animal gender differences were statistically significant (p<0.001). Tagliapietra et al., 2018 showed that females calves obtained from different dams and Belgian Blue sires exhibited a very similar carcass conformation compared to males.

Males and steers had a significantly higher (p<0.001) conformation class for carcass than females (4.95 and 5 compared to 4.51, respectively). Thus, males and steers had carcasses classified as R while females were classified between R and O+ (Table 1).

The lowest fat cover of the carcass was observed in males (2.78), that was significantly lower (p<0.001) than in females and steers (3.37 and 3.56, respectively). Also, steers had a significantly higher (p<0.05) fat cover of the carcass than females. Tagliapietra et al., 2018 reported a higher fatness score in heifers than in males at slaughter.

Dimas et al., 2021 obtained higher liveweight at slaughter and carcass weight in steers compared to heifers, while killing-out percentage was the same irrespective of animal gender. Oliveira Cardoso et al., 2014 showed than when fed high grain diets, cattle males and females performed similarly, however females had a better fat coverage.

Mueller et al., 2019 found that heifers had increased fat thickness compared to males and steers, while steers and heifers had higher marbling scores than males.

Sadrettin et al., 2024 found that slaughter traits, carcass characteristics and carcass measurements were affected by sex when compared cattle of different genotypes.

CONCLUSIONS

1. Liveweight at slaughtering of females was similar to those of males, due to the fact that they were 6 months older.
2. Steers were slaughtered at similar age with males, but with higher liveweight. Also, steers had higher liveweight at slaughter than females.
3. Combination of age and liveweight at slaughtering resulted in significant differences between animal gender regarding carcass weight and killing-out percentage.

4. Males and steers produced carcasses classified in class R for conformation, while females' carcasses were classified lower, between R and O+.
5. Animal gender had an effect on fat cover of carcass. The highest fat cover of carcasses was obtained in steers, followed by females and the lowest was observed in males.
6. In conclusion all studied beef traits at slaughtering were significantly influenced by animal gender.

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REPRODUCTIVE INDICES OF COWS FROM INDIGENOUS BREEDS EXPLOITED AT RDSB TARGU MURES

INDICII DE REPRODUCȚIE A VACILOR DIN RASELE AUTOHTONE EXPLOATATE LA SCDCB TÂRGU MUREȘ

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Abstract

Milk and beef represent an important source of protein and essential nutrients worldwide, with a constant demand in both the food industry and international trade. Raising cattle for milk/meat plays an essential role in Romania's agricultural economy, and the efficient use of local resources is crucial for the sustainability of production. In farms for the exploitation of mixed-breed cattle, reproduction plays a special role in the efficiency of milk and meat production; this is achieved by obtaining female and male offspring that will ensure the replacement of reforms and the obtaining of new breeders; and the surplus provides products intended for fattening. The objective of this study was to present the reproduction indices, carried out on the population of cows from RDSB Targu Mures, from the ***Romanian Spotted****,* ***Brown of Maramures*** *and* ***Pinzgauer of Transylvania*** *breeds raised in purebred form.*

Keywords: cattle, indigenous breeds, reproduction

Rezumat

Laptele și carnea de vită reprezintă o sursă importantă de proteine și nutrienți esențiali la nivel mondial, având o cerere constantă atât în industria alimentară, cât și în comerțul internațional. Creșterea bovinelor pentru lapte/carne joacă un rol esențial în economia agricolă a României, iar utilizarea eficientă a resurselor locale este crucială pentru sustenabilitatea producției. În fermele pentru exploatarea taurinelor de rasă mixtă, reproducția are un rol deosebit în eficiența producției de lapte, carne; aceasta se realizează prin obținere de descendenți femeli și masculi care vor asigura înlocuirea reformelor și obținerea de noi reproducători; iar surplusul asigură produșii destinați îngrășării. Obiectivul acestui studiu a fost prezentarea indicilor de reproducție, efectuâdu-se pe populația de vaci de la SCDCB Târgu Mureș, din rasele ***Bălțată Românească****,* ***Brună de Maramureș*** *și* ***Pinzgauer de Transilvania*** *crescute în rasă curată.*

Cuvinte cheie: bovine, rase autohtone, reproducție

INTRODUCTION

Reproduction plays a special role in the perpetuation, multiplication of cattle and a special role in the production of meat and milk. The act of calving is the promoter of milk production but without calving we cannot talk about meat production either.

The potential reproductive capacity in taurine is the biological ability of cows to give birth to a product within a period of time "year and calf ".

Reproduction indicators express in absolute or relative values the level of reproductive activity and with the help of these indicators the reproductive activity is assessed, these values can be compared with established optimal values.

By estimating the value of reproduction indices, the reproduction process on a farm can be managed and monitored, and depending on their level, interventions can be made to eliminate technological deficiencies.

Reproduction in cattle differs greatly from the reproduction of other domestic species, sheep, goats, pigs, the multiplication and numerical growth of the cattle species being much slower than other species of zootechnical interest:

- They are monotocous animals – they give birth to a single product, and very rarely twins are born.
- Sexual maturity differs from a number of factors, but ranges between 12–20 months.
- The gestation period is 280-290 days.
- The generation gap is large.

Since milk production depends on a calving, but meat production is directly influenced by calving, the ideal situation at cattles is one calf a year, with an interval between two calvings of 365 days.

This paper aimed to analyze reproductive indices, providing a comprehensive perspective on the factors that influence fertility and reproductive performance at indigenous breeds.

MATERIALS AND METHODS

The study was conducted on the cow population at the Research and Development Station for Bovine in Târgu Mureș, and the reproductive indices were calculated for the **Romanian Spotted**, **Brown of Maramures** and **Pinzgauer of Transylvania** breeds.

Reproduction at the Research and Development Station for Bovine is ensured through artificial insemination using frozen semen. The improvement of the cow populations is carried out through purebred breeding.

The data processed in this activity were recorded for the years 2023–2024 and grouped by primiparous and multiparous cows for the three breeds.

Reproductive indices were calculated for the entire cow population and expressed as averages for the three indigenous breeds: **Romanian Spotted**, **Brown of Maramures** and **Pinzgauer of Transylvania**.

The data were collected from the farm's records, including: date of mating, date of calving, date of weaning, number of inseminations per gestation, and number of semen doses used per gestation.

By processing the aforementioned data, the calculated values were obtained – the reproductive indices, which are used to assess reproductive performance on the farm.
By comparing these indices with their respective optimal values, the performance level of the farm can be identified, and technological deficiencies can be addressed.

The calculated reproductive indices are:

- Insemination index
- Conception rate
- Service period
- Calving interval
- Gestation duration
- Dry period duration
- Age at first calving

Insemination index – represents the average number of inseminations performed for one gestation and is calculated by dividing the total number of inseminations by the number of pregnant cows. This value is considered very good when it is around 2.

Conception rate – represents the percentage of cows that became pregnant after the first insemination.

The normal value for this index is between 50–70%, and it is influenced by many technological and farm management factors such as: body condition, feeding level, milk production, microclimate

(temperature), reproduction management (monitoring calving, estrous cycle, optimal insemination timing), inseminator skill level, semen fertility, and the reproductive health of the cow.
If the conception rate as a reproductive index is within normal parameters, it positively influences other indices, as the aforementioned factors are correctly managed and controlled.

Service period – the period of uterine rest, expressed in days, between calving and the date of successful insemination (establishment of a new pregnancy).

Optimal values for this index range between 80–120 days. Any deviation above these values negatively impacts milk and meat production, requiring corrective measures to shorten the uterine rest period. Among the many influencing factors are: high milk production (which prolongs the time to conception), calf-at-foot management in beef production (delays estrus), and late return to estrus in lactating cows.

Calving interval – the interval between two successive calvings. It is calculated only for multiparous cows (those that have calved at least twice), either as the time in days between two calvings or by summing the gestation duration and the service period.
The optimal value for this index is under 400 days.

Gestation duration is considered the period from fertilization to parturition (calving). The average gestation length in cows is 285 days. However, many factors can influence this index in one direction or another. Calving is not considered pathological if it occurs between day 240 and day 311.

Weaning duration is a technological indicator and is set at 60 days before the estimated calving date. It can be shortened to 50 days with balanced feeding, or extended to 70–80 days in cases of poor nutrition or record-producing animals.

Age at first calving depends on the breed (precocity) and represents the period during which the animal does not produce. It is desirable to reduce this period without negatively affecting future production. It is calculated as the interval between the date of birth and the date of the first calving.

RESULTS AND OBSERVATIONS

The potential reproductive capacity in cattle is the biological ability of cows to give birth to a product within a period of time, the "year and calf".

Reproduction indices are expressed in absolute or relative values. With these indices we assess the reproductive activity of a population. The values obtained can be compared with the optimal values established as a result of zootechnical research activity.

The reproduction indices were studied and calculated across the entire cow population of the Research Station for the three native breeds: **Romanian Spotted** 200 heads of cattle, **Brown of Maramures** 80 heads of cattle and **Pinzgauer of Transylvania** 40 heads of cattle.

The study was conducted on groups of primiparous and multiparous cows during the period from October 1, 2023, to September 30, 2024, involving the three breeds raised at the Research Station.

The results obtained are presented in the table nr. 1, for each breed separately.

Table 1. Reproduction indices / *Indicii de reproducție*

Specification	Breed		
	Romanian Spotted 200 heads of cattle	Brown of Maramures 80 heads of cattle	Pinzgauer of Transylvania 40 heads of cattle
Age at first calving (months)	31.70	30.20	33.00
Gestation duration (days)	284.00	283.00	284.00
Duration of breast rest (days)	60.00	65.00	89.00
Service period (days)	124.00	143.00	131.00
Calving interval (days)	408.00	426.00	415.00
Seeding index (doses)	1.63	1.84	1.76
Conception rate (%)	63.0	65.0	60.0

The insemination index, namely the number of semen doses used per gestation, is very similar across the breeds: 1.63 for **Romanian Spotted** (B.R.), 1.84 for **Brown of Maramures** (B.M.), and 1.76 for **Pinzgauer of Transylvania** (P.T.).

The conception rate (natality) is 63% for B.R., 65% for B.M., and 60% for P.T.

The calving interval exceeds 400 days in all three breeds: 408 days for B.R., 415 days for B.M., and 426 days for P.T.; this index is correlated with the service period duration, which averages 132 days.

The age at first calving exceeds 30 months in all cases: 31.7 months for B.R., 30.2 months for B.M., and 33 months for P.T. Among the three breeds, **Brown of Maramures** is the most precocious.

According to the data presented in Table 1, the dry period for the indigenous breeds is 60 days for B.R., 65 days for B.M., and 89 days for P.T., due to the latter's shorter lactation period of 230 days compared to 305 days for the other two breeds.

Fig. 1 Romanian Spotted

Fig. 2 Brown of Maramures

Fig. 3 Pinzgauer of Transylvania

CONCLUSIONS

1. The conception rate for the three breeds is very similar, ranging between 60–65%, and the number of inseminations per gestation varies between 1.63 and 1.84.
2. The gestation duration is nearly identical for the three indigenous breeds: Romanian Spotted, Brown of Maramures, and Pinzgauer of Transylvania.
3. The calving interval varies proportionally with the length of the service period, which represents the reproductive inactivity phase.
4. The age at first calving is a reproductive index that falls within the range found in the literature regarding the precocity of mixed-purpose breeds.
5. The dry period duration is a technological indicator, influenced to a small extent by genetic factors.

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EFFECTS OF USING CANNABIS OIL (CBD) ON THE METABOLIC PROFILE OF CALVES DURING WEANNING

EFECTELE UTILIZĂRII ULEIULUI DE CANABIS (CBD) ASUPRA PROFILULUI METABOLIC LA VIȚEI ÎN PERIOADA ÎNȚĂRCĂRII

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Abstract

Cannabis oil is a product obtained from hemp plants and seeds (Cannabis sativa), with anti-inflammatory, analgesic, anxiolytic and anticonvulsant properties due to its high content of cannabidiol (CBD). The aim of this research was to evaluate the effects of CBD on haemato-biochemical profile in dairy calves during weaning. The study was conducted at the Experimental Farm of the Research and Development Institute for Bovine Balotesti, between March-June 2024, using 19 half-sibs ***Romanian Black and White*** *dairy calves, divided into two groups: the experimental group (E: n=10) and the control group (C: n=9). The experimental group received 5 ml of CBD oil/head/day in the first 2 days of experiment (pre-weaning) and 10 ml CBD oil/head/day for a period of 3 consecutive days post-weaning. Blood samples were collected during the weaning day (T_1), at 2 days (T_2) and 5 days (T_3) post-weaning. Mann-Whitney test was used to evaluate the influence of CBD on the blood parameters. The results showed significant differences for the mean corpuscular volume (MCV: p=0.0164) and mean corpuscular hemoglobin (MCH: p=0.0057) in the CBD group compared with the controls at T_1, and significant differences for platelets (PLT: p=0.0050) and alanine-aminotransferase (ALT: p=0.0328) in the CBD group compared to the control group at T_2. At 5 days post-weaning (T_3), the mean corpuscular hemoglobin (MCH: p=0.0018) differed significantly between calves' groups. The obtained results suggest that CBD addition in dairy calves' diets could lead to improvements in the metabolic profile, and as a result, to reducing the negative effects of weaning stress.*

Key word: weaning stress, dairy calves, cannabis oil, metabolic profile.

Rezumat

Uleiul de canabis este un produs obținut din plante și semințe de cânepă (Cannabis sativa), având proprietăți antiinflamatorii, analgezice, anxiolitice și anticonvulsive datorită conținutul ridicat de canabidiol (CBD). Studiul a fost realizat în Laboratorul Experimental Zootehnic al Institutului de Cercetare-Dezvoltare pentru Creșterea Bovinelor Balotești, în perioada martie-iunie 2024, pe un număr de 19 viței din rasa ***Bălțată cu Negru Românească*** *(loturi de semi-frați), grupați în două loturi: lotul experimental (E: n=10) și lotul de control (C: n=9). Lotul E a primit 5 ml ulei CBD/cap/zi în primele 2 zile ale experimentului (pre-înțărcare) și 10 ml ulei CBD/cap/zi timp de 3 zile post-înțărcare. Probele de sânge au fost recoltate în ziua înțărcării (T_1), la 2 zile (T_2) și la 5 zile (T_3) post-înțărcare. Pentru a evalua influența CBD-ului asupra parametrilor sanguini studiați, a fost utilizat testul Mann-Whitney. Rezultatele obținute au relevat diferențe semnificative pentru volumul eritrocitar mediu (VEM: p=0.0164) și hemoglobina eritrocitară medie (HEM: p=0.0057) între loturi, precum și diferențe semnificative pentru trombocite (PLT: p=0.0050) și alanin-aminotranzferaza (GPT: p=0.0328), la două zile post-înțărcare. La 5 zile după înțărcare, hemoglobina eritrocitară medie (HEM: p=0.0018) a înregistrat diferențe semnificative între cele două loturi. În concluzie, rezultatele obținute indică faptul că adăugarea uleiului de canabis în dieta vițeilor poate optimiza anumiți parametri sanguini, în vederea reducerii stresului de înțărcare.*

Cuvinte cheie: stres de înțărcare, viței, ulei de canabis, profil metabolic.

INTRODUCTION

The medical use of cannabis and cannabinoids in humans has increased interest in utilizing them as feed additives for livestock (Kleinhenz et al., 2020). Cannabidiol (CBD) oil, derived from hemp plants and seeds (*Cannabis sativa*), is recognized for its therapeutic properties, including anti-inflammatory, analgesic, anxiolytic, and anticonvulsant properties due to its high content of cannabidiol (Fallahi et al., 2022; Meyer et al., 2022). Authorized hemp varieties registered in the EU's Common Catalogue of Agricultural Plant Species must contain psychotropic delta 9-tetrahydrocannabinol (THC) levels below 0.2–0.3% (Bailoni et al., 2021), adhering to limits of <0.2% THC on a dry matter basis, ensuring safety and compliance (EPSA, 2011; EU Commission Regulation, 2017; EU Commission Regulation, 2023). Cannabinoids, including THC (tetrahydrocannabinol), CBD (canabidiol), cannabichromene (CBC), and cannabigerol (CBG), are plant-derived compounds (phytocannabinoids) synthesized within glandular trichomes of *Cannabis sativa* plants (Farinon et al., 2020; Kicman and Toczek, 2020). THC is the only psychoactive compound, whereas CBD exhibits non-psychoactive therapeutic effects (Atalay et al., 2020).

Hemp seeds and derivatives such as oil, cake and meal, are valuable supplements in ruminant diets, providing essential fatty acids and amino acids (Klir et al., 2019; Semwogerere et al., 2020). Including whole seeds and their co-products can transfer bioactive substances to human food. Incorporating these components into animal feed enhances the fatty acid profile of milk and cheese, increasing n-3 fatty acids and conjugated linoleic acid (Bailoni et al., 2021). Cannabidiol (CBD) oil shows therapeutic potential in reducing stress during weaning and transportation in calves (Austin, 2022), which are critical stress events affecting calves' immune function, growth, and overall performance (Lynch et al., 2019; Meyer, 2022).

This study aims to evaluate the impact of CBD oil supplementation on the haemato-biochemical profile of dairy calves during weaning, highlighting its potential to improve physiological responses to stress.

MATERIALS AND METHODS

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

The study was conducted at the Experimental Farm of the Research and Development Institute for Bovine Balotesti, between March-June 2024, using 19 half-sibs **Romanian Black and White** dairy calves, divided into two groups: the experimental group (E: n=10 head/group) and the control group (C: n=9 head/group). The experimental group received 5 ml of CBD oil/head/day in the first 2 days of the experiment (pre-weaning) and 10 ml CBD oil/head/day for 3 consecutive days post-weaning. The cannabis oil was purchased from certified suppliers, with a total cannabinoid content of 1350 mg/100ml.

The calves were weaned at an average age of 90±5 days. Prior to weaning, they were housed in individual hutches. Post-weaning, they were transitioned to group housing, accommodating 15-20 calves/group, which simulated typical social conditions. All calves were fed twice daily with a diet formulated to meet their nutritional requirements at this developmental stage. The diet/head/day consisted of 6 kg milk, quality alfalfa hay *ad libitum*, concentrates *ad libitum*, and water *ad libitum*.

Blood samples were collected from the jugular vein during the weaning day (T_1), at 2 days (T_2), and 5 days (T_3) post-weaning. The amount of blood was collected in vacutainer tubes with K_3EDTA for hemoleukogram (2 ml/tube, and chilled to +4 °C), and vacutainer tubes (9 ml/tube, centrifuged at 6000 rpm for 8 minutes for biochemical examination. Red blood cell (RBC), hemoglobin (HGB), hematocrit (HTC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT), total white blood cell (WBC), lymphocytes (LY), monocytes (MO), and neutrophils (NE) were performed using an automated hematology analyzer (Abacus Junior Vet 5). Total protein (TPro), blood urea nitrogen (BUN), creatinine (Cre), and gamma-glutamyltransferase (GGT) were determined using an automated biochemical analyser (Spotchem EZ SP-4430). Statistical analyses were carried out using Minitab Statistical Software (version 17).

The data were expressed as mean±standard error of the mean (SEM), standard deviation (SD), coefficient of variation3 (CV), minimum and maximum values. To test the influence of cannabis oil on the blood parameters studied, the Mann-Whitney test (one-tailed hypothesis) was used. Differences were considered statistically significant at $p \leq 0.05$, and trends $p \leq 0.10$. The correlations between blood parameters across different weaning periods (T_1, T_2, T_3) were estimated using Pearson's correlation coefficient with a statistical significance threshold of $p \leq 0.05$.

RESULTS AND DISCUSSIONS

The descriptive statistics of RBC, HGB, HTC, MCV, MCH, MCHC, and PLT are presented in Table 1. The obtained results showed a significant difference in mean corpuscular volume (MCV: p=0.0164) and mean corpuscular hemoglobin (MCH: p=0.0057) in the E group compared with the C group at T_1, and significant differences for mean corpuscular volume (MCV: p=0.0188), mean corpuscular hemoglobin (MCH: p=0.0232), and platelets (PLT: p=0.0050) in the E group compared to the C group at T_2.

These results suggest that CBD oil influences red blood cell size and haemoglobin content during weaning stress. Cannabinoids are known to modulate oxidative stress, which may impact erythrocyte morphology and oxygen transport efficiency (Radkowska and Herbut, 2014; Tathong et al., 2024). A tendency for platelet suggests that CBD oil may modulate coagulation and platelet activation during stress, as supported by studies on cannabinoid effects on thrombocyte function and reduce inflammatory responses (Atalay and Skrzydlewska, 2019; Kicman and Toczek, 2019).

Table 1. The effects of CBD oil diet addition on the haematological parameters in dairy calves during weaning / ***Efectele administrării uleiului de CBD asupra parametrilor hematologici în perioada înțărcării la viței***

Variable	Experimental group					Control group					p
	X±SEM	SD	CV	Min	Max	X±SEM	SD	CV	Min	Max	
RBC, T_1	9.60±0.45	1.43	14.90	6.65	12.32	9.72±0.36	1.08	11.13	7.66	11.25	0.3556
RBC, T_2	10.46±0.66	2.11	20.22	8.77	16.15	10.04± 0.20	0.61	6.08	8.84	10.79	0.4840
RBC, T_3	9.68±0.23	0.73	7.56	8.49	10.37	10.35± 0.21	0.63	6.12	9.56	11.47	**0.0951**
HGB, T_1	11.40±0.34	1.09	9.62	10.30	14.00	10.65±0.50	1.51	14.26	7.00	11.90	0.2451
HGB, T_2	11.17±0.22	0.69	6.22	10.10	12.40	10.80± 0.26	0.79	7.38	9.30	11.80	*0.1948*
HGB, T_3	11.00±0.15	0.48	4.41	10.20	11.70	11.01± 0.32	0.97	8.88	9.50	12.20	0.3556
HTC, T_1	30.82±1.42	4.49	14.57	20.38	37.65	30.37±1.11	3.32	10.93	23.10	32.99	0.3557
HTC, T_2	33.48±1.81	5.72	17.09	29.06	49.05	30.98± 0.78	2.34	7.55	26.76	33.71	*0.1949*
HTC, T_3	31.22±0.49	1.55	4.99	28.09	33.98	32.22± 0.51	1.53	4.76	29.51	34.43	0.7630
MCV, T_1	32.30±0.57	1.82	5.66	31.00	36.00	30.00± 0.33	1.00	3.33	29.00	32.00	**0.0016**
MCV, T_2	32.44±0.42	1.34	4.13	30.00	34.36	31.00± 0.44	1.32	4.27	30.00	33.00	**0.0188**
MCV, T_3	32.06±0.51	1.62	5.07	30.66	36.00	31.33± 0.28	0.86	2.76	30.00	33.00	0.2177
MCH, T_1	12.08±0.62	1.97	16.36	10.80	17.50	10.74± 0.21	0.63	5.91	9.20	11.40	**0.0057**
MCH, T_2	10.91±0.42	1.35	12.41	7.300	12.00	10.78±0.18	0.54	5.00	10.10	11.90	**0.0232**
MCH, T_3	11.57±0.18	0.57	4.97	10.90	12.40	10.72± 0.18	0.54	5.04	10.00	11.50	**0.0180**
MCHC, T_1	37.73±2.18	6.90	18.28	34.60	57.20	36.04±0.88	2.66	7.39	30.50	41.00	0.2709
MCHC, T_2	33.84±1.14	3.59	10.62	24.20	37.00	34.92±0.26	0.79	2.27	33.70	36.20	0.2327
MCHC, T_3	35.37±0.59	1.86	5.27	32.20	37.80	34.80±0.33	0.99	2.86	32.40	35.50	0.3557
PLT, T_1	383.3±36.2	114.6	29.89	191.0	575.0	450.9± 45.9	137.7	30.53	216.0	619.0	*0.1736*
3, T_2	374.0±52.6	166.3	44.47	139.0	697.0	526.4±21.9	65.6	12.45	409.0	607.0	**0.0050**
PLT, T_3	423.6±36.1	114.1	26.94	276.0	583.0	452.8±39.0	116.9	25.81	319.0	682.0	0.2980

RBC=red blood cells (10^3/µl), HGB=hemoglobin (g/dl), HTC=hematocrit (%), MCV=mean corpuscular volume (fl), MCH=mean corpuscular hemoglobin (pg), MCHC=mean corpuscular hemoglobin concentration (g/dl), PLT=platelet (10^3/µl); T_1=weaning day, T_2=2 days post-weaning, T_3=5 days post-weaning; Reference values – RBC: 5-8$10^3$/µl, HGB: 9-11 g/dl, HTC: 32-38 %, MCV: 40-60 fl, MCH: 11-17 pg, MCHC: 30-36 g/dl, PLT: 100-800 10^3/µl;
$p \leq 0.05$, trends $p \leq 0.10$.

At T_3 intervals, mean corpuscular hemoglobin (MCH: p=0.0018) differed significantly between groups. A tendency for red blood cell (RBC: p=0.0951) at T_3 between groups was also recorded. This indicates potential long-term effects of CBD oil on erythropoiesis, aligning with findings on cannabinoid effects on hematopoiesis (Neary et al., 2024).

For hemoglobin (HGB), hematocrit (HTC), and mean corpuscular hemoglobin concentration (MCHC), no significant differences were observed for these parameters across T_1, T_2, and T_3 (p>0.05), showing that CBD oil did not affect these haematological indicators between the studied groups. The obtained values have been found to be in contrast with values obtained by Austin (2022), who reported no statistically significant differences for these haematological parameters studied in 27 beef calves during weaning stress.

The descriptive statistics for white blood cells (WBC), lymphocytes (LY), monocytes (MO), and neutrophils (NE) are summarized in Table 2. At T_2 (2 days post-weaning), the E group showed significantly lower variability (CV of 15.66 %) and a lower mean WBC count compared to the control group (10.33±0.51 10^3/µl vs. 11.83±0.62 10^3/µl; p = 0.0050). This suggests that CBD may stabilize immune responses during stress periods, aligning with findings that cannabinoids modulate immune function (Tathong, 2024).

Across T_1, T_2, and T_3 intervals, no statistically significant differences between the experimental and control groups (p>0.05) were observed for LY, MO, and NE. However, trends in MO percentages suggest that cannabinoids may suppress monocyte activation during stress (Cabral et al., 2015; Maggirwar and Khalsa, 2021).

In contrast, Austin (2022) reported significant differences for monocyte count (p<0.0001) in a pilot study that investigated 27 beef calves during weaning stress.

Table 2. The effects of CBD oil diet addition on the withe blood cell and leucocytes formula in dairy calves during weaning / *Efectele administrării uleiului de CBD asupra leucogramei în perioada înțărcării la viței*

Variable	Experimental group					Control group					p
	X±SEM	SD	CV	Min	Max	X±SEM	SD	CV	Min	Max	
WBC, T_1	9.79±0.73	2.33	23.80	7.30	15.56	10.96± 1.39	4.17	38.06	2.96	16.78	*0.1736*
WBC, T_2	10.33±0.51	1.61	15.66	6.77	13.45	11.83±0.62	1.87	15.83	9.320	14.62	**0.0050**
WBC, T_3	10.38±0.38	1.22	11.76	8.68	12.53	11.10±0.69	2.07	18.66	8.650	14.45	0.2980
LY, T_1	70.42±1.99	6.31	8.96	58.70	77.70	68.80±3.88	11.65	16.94	53.50	83.80	0.4840
LY, T_2	67.73±2.28	7.22	10.66	55.90	81.00	64.00±3.04	9.13	14.26	46.20	76.80	0.2709
LY, T_3	63.62±1.77	5.60	8.81	52.60	72.10	63.94±3.49	10.48	16.38	44.20	78.90	0.4524
MO, T_1	2.31±0.88	2.80	121.37	0.60	9.90	1.48±0.48	1.45	97.93	0.50	5.20	0.2578
MO, T_2	1.92±0.440	1.39	72.54	0.80	5.10	1.61±0.36	1.08	67.17	0.60	3.50	0.2061
MO, T_3	1.86±0.40	1.27	68.30	0.50	3.80	2.65±0.93	2.81	105.99	0.50	9.70	0.3263
NE, T_1	27.30±2.34	7.41	27.16	16.10	40.30	29.87±4.00	12.01	40.22	11.00	44.40	0.3859
NE, T_2	30.36±2.17	6.86	22.60	17.80	43.10	34.39±3.05	9.15	26.61	21.30	52.90	*0.1736*
NE, T_3	34.51±1.96	6.21	18.00	25.30	46.70	33.43±2.78	8.35	24.96	20.60	46.10	0.4840

WBC=total white blood cells (10^3/µl), LY=lymphocytes (%), MO=monocytes (%), NE=neutrophils (%); T_1=weaning day, T_2=2 days post-weaning, T_3=5 days post-weaning; Reference values – WBC: 6.5-9.5 10^3/µl, LY: 45-61 %, MO: 0-4 %, NE: 15-41 %; p ≤ 0.05, trends p ≤ 0.10.

The descriptive statistics of serum biochemical parameters are presented in Table 3. Serum biochemical analysis found a tendency for gamma-glutamyltransferase (GGT) levels at T_2 and T_3 (p=0.0822 and p=0.0764), suggesting potential liver function modulation by CBD oil. Cannabinoids are known to influence hepatobiliary function and reduce oxidative stress in liver tissues (Mboumba Bouassa et al., 2021; Hassan et al., 2023; Huang et al., 2025). No significant differences were observed between experimental and control groups (p>0.05), however, trends in BUN levels suggest improved nitrogen metabolism in the experimental group.

Table 3. The effects of CBD oil diet addition on the biochemical parameters in dairy calves during weaning / *Efectele administrării uleiului de CBD asupra parametrilor biochimici în perioada înțărcării la viței*

Variable	Experimental group					Control group					p
	X±SEM	SD	CV	Min	Max	X±SEM	SD	CV	Min	Max	
TPro, T_1	6.16±0.11	0.35	5.69	5.70	6.90	6.26±0.14	0.42	6.72	5.70	6.90	0.2980
TPro, T_2	6.19±0.14	0.45	7.40	5.20	6.70	6.13±0.10	0.30	5.03	5.70	6.70	*0.1948*
TPro, T_3	6.08±0.10	0.31	5.25	5.60	6.60	6.211±0.05	0.16	2.72	6.00	6.50	*0.1356*
BUN, T_1	11.20±0.61	1.93	17.25	8.00	14.00	10.55±0.55	1.66	15.79	9.00	13.00	0.2177
BUN, T_2	10.40±0.37	1.17	11.29	9.00	13.00	10.33±0.62	1.87	18.10	7.00	13.00	0.4840
BUN, T_3	9.50±0.76	2.41	25.42	6.00	15.00	10.66±0.86	2.59	24.36	8.00	15.00	*0.1840*
Cre, T_1	3.28±1.63	5.15	156.87	0.70	14.00	0.82±0.03	0.09	11.82	0.60	0.90	*0.1538*
Cre, T_2	2.78±1.29	4.08	146.61	0.80	11.00	0.82±0.03	0.10	13.29	0.60	1.00	*0.1190*
Cre, T_3	2.26±0.95	3.02	133.87	0.80	8.00	0.87±0.03	0.09	11.07	0.80	1.10	0.4207
GGT, T_1	30.40±3.51	11.11	36.54	22.00	60.00	30.22±1.35	4.06	13.42	25.00	37.00	*0.1948*
GGT, T_2	30.40±3.94	12.45	40.94	20.00	62.00	30.22±1.15	3.46	11.44	26.00	36.00	**0.0822**
GGT, T_3	28.60±3.42	10.82	37.85	18.00	54.00	29.22±0.83	2.48	8.52	25.00	32.00	**0.0764**

TPro=total protein (g/dL), BUN=blood urea nitrogen (mg/dL), Cre=creatinine (mg/dL), GGT=gama-glutamyltransferase (IU/L); T_1=weaning day, T_2=2 days post-weaning, T_3=5 days post-weaning; Reference values –TPro: 5.8-8.5 g/dl, BUN: 10-25 mg/dl, Cre: 0.4-2.1 mg/dl, GGT: 10-39 IU/L; $p \leq 0.05$, trends $p \leq 0.10$.

Literature review indicates that cannabinoids can influence protein metabolism and nitrogen excretion (Landi et al., 2018; Saloner and Bernstein, 2022). Creatinine levels in the E group at T_1 and T_2 may reflect muscle metabolism adaptations during stress. Studies suggest that cannabinoids can modulate renal function and creatinine clearance (Di Salvo et al., 2024; Młynarska et al., 2024). In general, the experimental group showed more stability in serum biochemical indicators compared with the control group, suggesting better metabolic adaptation.

Table 4, presents Pearson correlation coefficients, showing the relationship between TPro levels and WBC count, LY, MO, and NE, across T_1, T_2, and T_3 intervals for the experimental group and the control group.

Total protein levels, which reflect nutritional and immune status, may correlate with WBC counts and leukocyte distribution. Higher total protein could indicate an immune response or inflammation, which is reflected in changes in leukocyte formula (neutrophils or lymphocytes).

For the E group, moderate positive correlations between TPro vs. MO at T_2 (r=0.558, p=0.094), as well as moderate negative correlations between TPro vs. WBC (r=-0.529, p=0.116), and TPro vs. NE (r=-0.532, p=0.113) at T_2 were found, suggesting potential relationships, even they are not statistically significant.

Table 4. Pearson correlation coefficient between WBC and leucocytes formula vs. TPro in dairy calves across T_1, T_2, and T_3 point time / *Coeficientul de corelație Pearson între WBC și formula leucocitară vs. TPro la viței*

Blood indicators	TPro		TPro		TPro	
	T_1		T_2		T_3	
	Experimental group					
	r	p	r	p	r	p
WBC	*-0.014*	0.970	-0.529	*0.116*	-0.345	0.329
LY	*0.049*	0.894	0.401	0.251	0.034	0.926
MO	0.273	0.445	0.558	0.094	-0.035	0.923
NE	-0.147	0.685	-0.532	*0.113*	-0.024	0.948
Control group						
WBC	-0.130	0.738	-0.006	0.987	-0.095	0.807
LY	-0.279	0.468	0.004	0.991	0.609	*0.082*
MO	0.083	0.832	0.542	*0.132*	-0.490	*0.181*
NE	0.154	0.693	-0.070	0.857	-0.598	*0.089*

WBC=total white blood cells, LY=lymphocytes, MO=monocytes, NE=neutrophils, TPro=total protein; T_1=weaning day, T_2=2 days post-weaning, T_3=5 days post-weaning; r=Pearson correlation coefficient; p-values.

For the C group, the correlation between TPro and LY (r=0.609, p=0.082) at T_3 indicated a potential link between total protein levels and lymphocyte activity under baseline conditions. These results highlight the interaction between total protein levels and immune indicators, particularly under stress or experimental conditions.

Blood urea nitrogen (BUN) is a critical marker of protein metabolism and renal function, and its correlation with hemoleucogram parameters like hemoglobin (HGB) levels and red blood cell (RBC) counts provides valuable information about metabolic efficiency and oxygen transport. Higher BUN levels may indicate excessive protein intake or inefficient utilization, which can impact these haematological parameters.

The Pearson correlation coefficients between BUN vs. RBC and HGB levels are summarised in Table 5.

For the E group, a positive moderate correlation between BUN and RBC at T_2 (r=0.601, p=0.066) suggests that BUN may influence RBC dynamics during this period, potentially reflecting adaptive metabolic responses. Additionally, the moderate positive correlation between BUN and HGB at T_3 (r=0.542, p=0.102) indicates some influence on hemoglobin levels, highlighting the connection between protein metabolism and oxygen transport.

For the C group, moderate negative correlations of BUN with RBC at T_2 (r=-0.509, p=0.162) and T_3 (r =-0.551, p=0.124) suggest a trend of inverse effects on red blood cell dynamics, possibly reflecting differences in protein utilization efficiency under baseline conditions.

Table 5. Pearson correlation coefficient between RBC and HGB vs. BUN in dairy calves across T_1, T_2, and T_3 point time / *Coeficientul de corelație Pearson între RBC și HGB vs. BUN la viței*

Blood indicators	BUN		BUN		BUN	
	T_1		T_2		T_3	
	Experimental group					
	r	p	r	p	r	p
RBC	- 0.218	0.545	0.601	*0.066*	- 0.041	0.911
HGB	0.073	0.840	0.044	0.905	0.542	*0.102*
Control group						
RBC	0.075	0.847	- 0.509	*0.162*	- 0.551	*0.124*
HGB	- 0.048	0.902	- 0.184	0.635	- 0.387	0.303

RBC=red blood cells, HGB=hemoglobin, BUN=blood urea nitrogen; T_1=weaning day, T_2=2 days post-weaning, T_3=5 days post-weaning; r=Pearson correlation coefficient; p-values.

Table 6 illustrates the relationship between Cre levels and WBC count, LY, MO, and NE. Creatinine may correlate with WBC counts and leukocyte formula, especially under stress or metabolic strain. Higher creatinine levels could indicate renal stress, which might also affect immune function.

Table 6. Pearson correlation coefficient between WBC and leucocytes formula vs. Cre in dairy calves across T_1, T_2, and T_3 point time / *Coeficientul de corelație Pearson între WBC și formula leucocitară vs. Cre la viței*

Blood indicators	Cre		Cre		Cre	
	T_1		T_2		T_3	
	Experimental group					
	r	p	r	p	r	p
WBC	0.550	*0.099*	0.518	*0.125*	0.324	0.361
LY	- 0.509	*0.133*	- 0.479	0.161	0.125	0.730
MO	- 0.279	0.435	- 0.380	0.279	- 0.481	0.159
NE	0.536	*0.110*	0.577	*0.081*	- 0.014	0.970
Control group						
WBC	0.566	*0.112*	- 0.233	0.547	-0.312	0.414
LY	- 0.248	0.519	0.127	0.746	0.180	0.642
MO	0.355	0.349	0.235	0.543	0.463	0.210
NE	0.211	0.585	**0.020**	0.959	0.643	*0.062*

WBC=total white blood cells, LY=lymphocytes, MO=monocytes, NE=neutrophils, Cre=creatinine; T_1=weaning day, T_2=2 days post-weaning, T_3=5 days post-weaning; r=Pearson correlation coefficient; p-values.

Creatinine levels show moderate positive correlations with WBC (r=0.550, p=0.099) and NE (r=0.536, p=0.110) at T_1 and T_2 (WBC: r=0.518, p=0.125; NE: r=0.577, p=0.081), suggesting potential links between renal function and immune activity during stress periods for the experimental group.

In contrast, the control group findings at T_3 highlight baseline immune activity, with neutrophils showing a moderate positive correlation with creatinine levels (r=0.643, p=0.062). These results underscore the complex relationship between renal function and immune activity, emphasizing the importance of monitoring creatinine levels in stress conditions.

Gamma-glutamyltransferase (GGT) often shows a correlation with red blood cell (RBC) and hemoglobin (HGB) levels (Table 7), as it reflects liver function and metabolic processes. GGT levels are an indicator of hepatic health. Changes in GGT levels may impact the synthesis and breakdown of hemoglobin in the liver, thereby influencing RBC and HGB levels. The liver plays a central role in iron storage and release, essential for RBC production and HGB synthesis. Elevated GGT levels can signal metabolic stress, which may directly or indirectly affect RBC and HGB values. These correlations suggest that GGT could serve as a marker for monitoring not just liver function but also the overall metabolic health, especially in contexts like disease, stress, or dietary interventions.

For the experimental group, RBC and GGT showed a strong negative correlation (r=-0.699) with statistical significance (p=0.025) at T_1, indicates a significant inverse relationship, suggesting metabolic stress affecting RBC dynamics. At T_2, a strong positive correlation (r=0.807) with high statistical significance (p=0.005) was found, highlights a significant direct relationship, reflecting adaptive changes in liver function and RBC levels under experimental conditions. HGB and GGT showed a moderate positive correlation (r=0.554), without statistically significant (p=0.096) at T_3, suggesting a potential relationship.

For the control group, RBC and GGT showed a moderate positive correlation (r=0.651), with statistical significance (p=0.058) and a moderate positive correlation (r=0.637) for HGB and GGT, without statistical significance (p=0.065) at T_1. Moderate positive correlations at T_1 for both RBC and HGB suggest potential baseline relationships between these parameters and liver function, even though statistical significance. These observations highlight how GGT might serve as a marker for liver function and its interplay with RBC and HGB levels under different conditions.

Table 7. Pearson correlation coefficient between RBC and HGB vs. GGT in dairy calves across T_1, T_2, and T_3 point time / *Coeficientul de corelație Pearson între RBC și HGB vs. GGT la viței*

Blood indicators	GGT		GGT		GGT	
	T_1		T_2		T_3	
	Experimental group					
	r	p	r	p	r	p
RBC	- 0.699	**0.025**	0.807	**0.005**	0.204	0.572
HGB	- 0.027	0.940	0.197	0.586	0.554	**0.096**
Control group						
RBC	0.651	**0.058**	- 0.187	0.630	- 0.149	0.701
HGB	0.637	**0.065**	- 0.132	0.736	- 0.171	0.661

RBC=red blood cells, HGB=hemoglobin, GGT= Gamma-glutamyltransferase; T_1=weaning day, T_2=2 days post-weaning, T_3=5 days post-weaning; r=Pearson correlation coefficient; p-values.

CONCLUSIONS

1. The obtained results suggest that CBD oil diet supplementation improves haematological and biochemical parameters stability in dairy calves during the weaning stress period, when both immune and metabolic markers are concerned.
2. Significant improvements in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), platelet (PLT), and total white blood cells (WBC) at specific time points (T_1, T_2, and T_3) highlight the

potential use of CBD oil as a natural and effective strategy to mitigate weaning stress and improve overall calf health during this critical transition phase.

3. Further research is necessary to explore the long-term effects of CBD oil supplementation, its impact on other physiological parameters, and its applicability across different dairy farming conditions. These future investigations could contribute to the understanding of CBD's effects and expand the benefits of CBD oil in livestock management for improving the overall animal health and welfare.

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MOLECULAR EPIDEMIOLOGICAL STUDY ON THE CIRCULATION OF PROTOZOAN *TOXOPLASMA GONDII* IN WILDLIFE FROM ROMANIA

STUDIU EPIDEMIOLOGIC MOLECULAR PRIVIND CIRCULAȚIA PROTOZOARULUI *TOXOPLASMA GONDII* ÎN FAUNA SĂLBATICĂ DIN ROMÂNIA

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Abstract

Toxoplasmosis is an important worldwide protozoan disease caused by coccidia belonging to the Toxoplasmidae family. The only species of the genus is Toxoplasma gondii*, a significant zoonotic parasite that can cause severe illness in immunocompromised humans. It parasitizes various organs and tissues in over 350 species of vertebrates, making it perhaps the most widespread protozoan in this regard. Wild animals play a significant role in the epidemiology of toxoplasmosis, particularly when contact with domestic animals is possible, through which humans can acquire infection with* T. gondii*. The aim of the study was to investigate the potential circulation of the protozoan* T. gondii *in wild hosts collected from various hunting grounds across eight counties in Romania. Tissue samples (heart, brain, liver, spleen, and muscle) from 96 animals, specifically 13 wild host species, were processed by artificial digestion with trypsin and analyzed by real-time PCR for parasite detection.* Toxoplasma gondii *was identified in 57.29% (55/96) of the examined wild animals. The present study provides important information on the circulation of* T. gondii *in Romania's wildlife and highlights the potential risk of human infection, especially in anthropized areas where domestic and wild animals may interact.*

Keywords: T. gondii, wildlife, Romania

Rezumat

Toxoplasmoza este o protozooză importantă la nivel mondial produsă de coccidii ce aparţin familiei Toxoplasmidae. Singura specie a genului este Toxoplasma gondii, un parazit zoonotic important care poate provoca boli severe la persoanele imunocompromise. Parazitează în diverse organe şi ţesuturi la peste 350 specii de vertebrate, fiind din acest punct de vedere, poate, cel mai răspândit protozoar. Animalele sălbatice joacă un rol important în epidemiologia toxoplasmozei, atunci când contactul cu animalele domestice este posibil, iar prin acestea, omul poate dobândi infecția cu T. gondii*. Studiul a avut ca scop investigarea circulației potențiale a protozoarului* T. gondii *la gazde sălbatice colectate din diverse fonduri de vânătoare, situate în opt județe din România. Probe de țesut (cord, tesut cerebral, ficat, splină și musculatură) rezoltate de la 96 de animale, mai exact 13 gazde silvatice au fost efectuate prin digestie artificială cu tripsină și analizate prin PCR în timp real pentru detectarea parazitului. Parazitul* T. gondii *a fost identificat la 57,29% (55/96) din animalele sălbatice examinate. Studiul prezent oferă informații importante despre circulația T. gondii în fauna sălbatică din România și subliniază riscul potențial de infecție umană, în special în zonele antropizate, unde animalele domestice și sălbatice pot interacționa.*

Cuvinte cheie: T. gondii, faună sălbatică, România

INTRODUCTION

The medical and veterinary significance of *T. gondii* has established this protozoan as one of the most extensively studied parasites. It is widely used as a model organism for studying apicomplexan cell biology due to several advantageous characteristics. *Toxoplasma gondii* is a protozoan of a size suitable for visualization under a light microscope, can be cultured in virtually any cell line, maintained indefinitely in mice and cell cultures, and consists of a single species capable of infecting all host types. Moreover, this parasite is highly amenable to genetic manipulation, with well-established protocols for classical genetics and high efficiency of experimental infections [11].

Toxoplasmosis is a systemic protozoan disease caused by coccidia belonging to the family Toxoplasmidae. The parasite infects various organs and tissues in more than 350 species of vertebrates, making it arguably the most widespread protozoan in this regard. The infection typically progresses asymptomatically in its definitive hosts - domestic and wild felids. However, in intermediate hosts, it can lead to abortions or even death. The resulting economic losses are significant, primarily due to reduced birth rates and abortions in economically important livestock species, particularly sheep and pigs. From a public health perspective, toxoplasmosis is of major importance, as it is one of the most critical zoonotic diseases, potentially causing congenital malformations or fetal loss [4, 12, 25].

Wild animals play an important role in the epidemiology of toxoplasmosis, particularly when contact with domestic animals is possible, thereby creating a potential route for human infection with *Toxoplasma gondii*.

Studies on the occurrence of toxoplasmosis in Romania indicate a seroprevalence (ELISA) ranging between 42.5% and 80.5% in cats [6, 14, 17], 6.5% in lambs slaughtered for Easter, and 65.7% in adult sheep. In pigs, infection rates range between 0.8% and 46.8% [28].

Research on human toxoplasmosis has been conducted by Coroiu Z. et al., 2009 [4]; Dubey J.P. et al., 2014 [10]; Mederle N. et al., 2008 [23]; Olariu T.R. et al., 2015 [25]; Radbea N. et al., 2006 [31], reporting a seroprevalence ranging from 10.3% to 59.5% in the human population.

Studies concerning the presence of *T. gondii* in wild animals in Romania remain scarce. Șuteu O. et al., 2014 [35], reported a seroprevalence of 6% in foxes, while in 2016, Morariu S. et al. [24] recorded a prevalence of 22.22% in European bison from the Armeniș-Plopu reserve.

Contact between necrophagous wild species (such as jackals, bears, and wild boars) and the carcasses of carnivores, including felids (e.g., the wildcat and lynx), occurs frequently and directly contributes to the non-trophic transmission of parasites in the wild. This aspect is particularly relevant in the study and analysis of diseases with zoonotic potential [13].

Considering the limited bibliographic data available in Romania and recognizing the role of wildlife in the life cycle of the protozoan, as ecological indicators in anthropogenic ecosystems and as sentinels for a broad range of zoonotic infections, the present study aimed to update current knowledge on the circulation of *T. gondii* in Romanian wildlife using real-time PCR (qPCR).

MATERIALS AND METHODS

The research was conducted over a two-year period on a total of 96 wild animals (jackal, fox, badger, wildcat, weasel, otter, pine marten, stone marten, European polecat, muskrat, wild rabbit, grey plover, and raccoon dog), originating from various hunting grounds across eight counties in Romania (Arad, Timiș, Bihor, Vâlcea, Olt, Ialomița, Iași, and Tulcea). The animals were either found dead (roadkill) or were legally hunted by the annual harvest quotas established by the Ministry of Environment, Waters and Forests. The hunting activities through which these wild animal specimens were collected were carried out in compliance with Law No. 407/2006 (regarding hunting and the protection of wildlife) [27] (Figure 1).

Figure 1. The map shows the counties where wild animals were collected / ***Harta arată județele de unde au fost recoltate animalele sălbatice***

The study was conducted in collaboration with the École nationale vétérinaire d'Alfort (ENVA), France, specifically with the reference laboratory UMR BIPAR ANSES "Foodborne Parasites", under the coordination of Professor Radu Blaga and Dr. Sandra Thoumire.

The first step of the research involved the collection of tissue samples of heart, brain tissue, liver, spleen, and muscle - carried out at the Parasitology and Parasitic Diseases Clinic of the Faculty of Veterinary Medicine in Timisoara/ University of Life Sciences ”King Mihai I” from Timisoara.

The sample collection process included the following procedures:

- necropsy examination performed using standard necropsy techniques;
- skinning of the carcass;
- opening of the carcass;
- sampling of each tissue.

The tissue samples used in the present study (heart, brain tissue, liver, spleen, and muscle) are by the recommendations of national and international authors. Specifically, brain and lung samples collected from European beavers and wildcats [15]; brain tissue from hedgehogs [16]; cardiac tissue from small rodents, foxes, jackals, and wolves [18, 38]; heart, brain, liver, spleen, and lung tissues collected from black-backed jackal, African wildcat, and rusty-spotted genet [29]; brain samples from foxes [35].

Following the collection of tissue samples, trypsin artificial digestion and molecular analyses for *T. gondii* DNA were performed at the UMR BIPAR ANSES “Foodborne Parasites” reference laboratory, within ENVA, France.

Trypsin artificial digestion

A total of 200 grams of tissue comprising heart, brain, liver, spleen, and muscle from each jackal, fox, badger, wildcat, weasel, otter, pine marten, stone marten, European polecat, muskrat, wild rabbit, grey plover, and raccoon dog were blended and incubated (90 minutes at 37°C, 200 RPM on a shaking plate) in trypsin solution (Trypsin (1:250), powder, Gibco™, Scotland, final concentration of 4 g/L). The mixture was filtered through a double layer of gauze, transferred to 50 mL sterile centrifuge tubes, and centrifuged at 1800 g for 10 minutes. The formed pellet was washed twice of leftover trypsin using a saline solution (0,9% NaCl, Sigma-Aldrich, US). The weight of the final pellet was recorded and samples were stored at - 20°C until DNA extraction and subsequent qPCR analysis (Figures 2, 3).

Figure 2. The mixture was filtered through a double layer of gauze into 50 ml tubes / ***Filtrarea amestecului printr-un strat dublu de tifon in tuburi de 50 ml***

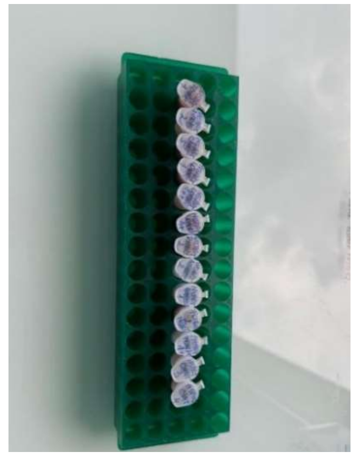

Figure 3. The final result of the trypsin digestion / ***Rezultatul final al digestiei cu tripsină***

Molecular analyses of T. gondii DNA

DNA was extracted from 80mg of digested pellet, using NucleoSpin DNA RapidLyse, Mini kit for rapid DNA purification (Macherey Nagel, France), according to the manufacturer's instructions. Extracted DNA was cleaned of PCR inhibitors, concentrated using ethanol precipitation protocol [41], and resuspended in 30µL of Milli-Q water. The detection and quantification of *T. gondii* DNA in each sample was performed in duplicate by amplification of a sequence within the 529 bp repetitive element, according to Opsteegh M. et al. (2010) [26] with minor modifications (25 µL total reaction mixture volume, 2X Premix Ex Taq™ (TakaraBio, Japan) and 5 µL of DNA as template) and competitive internal amplification control (CIAC) probe modification by Deng H. et al. (2021) [7], using LightCycler® 480 System 96-plate thermocycler (Roche, Germany) (Figure 4).

Genomic DNA from a suspension of 106 cultured RH-strain (Type I, ToxoDB Genotype #1) (ToxoDB, n.d.) was extracted using NucleoSpin DNA RapidLyse, Mini kit (Macherey-Nagel, France), according to the manufacturer's instructions. A standard curve was prepared with ten-fold serial dilutions of the genomic DNA, corresponding to 100 to 104 tachyzoites/µL [37]. Positive and negative controls, distilled H2O samples, and a standard curve were included in each qPCR run.

The qPCR results were analyzed using LightCycler® 480 System software, Version 1.2.9.11 (Roche, Switzerland) (Figure 5).

Figure 4. Molecular Biology Analyses / ***Analize de biologie moleculară***

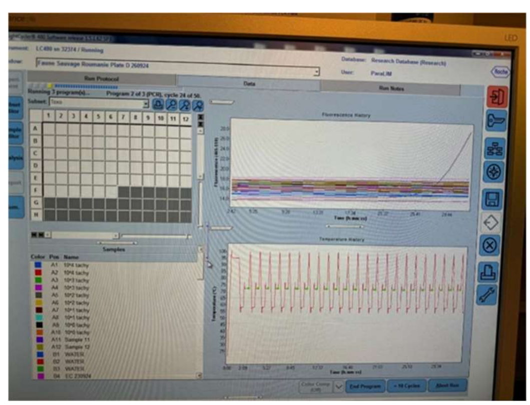

Figure 5. Software LightCycler® / ***Software LightCycler®***

RESULTS AND DISCUSSION

From the collected tissues (heart, brain, liver, spleen, and skeletal muscle) originating from 13 wild host species (jackal, fox, badger, wildcat, weasel, otter, pine marten, stone marten, European polecat, muskrat, wild rabbit, grey plover, and raccoon dog) and analyzed using the real-time PCR, 57.29% (55/96) were found to be infected with the protozoan *T. gondii* (Figure 6, Table 1).

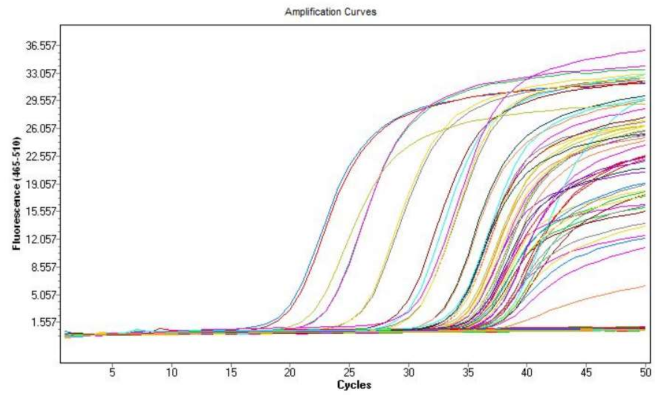

Figure 6. Amplification curves of positive samples derived from the examined wild animals / *Curbele de amplificare ale probelor pozitive provenite de la animalele sălbatice examinate*

Table 1. Prevalence of infection for *T. gondii* in wild animals examined / *Prevalența infecției cu T. gondii la animalele sălbatice examinate*

Host	Origin of positive samples [a]	Infected/examined (%)
Red fox (*Vulpes vulpes*)	AR, TM, IS, TL	31/49 (63.26)
Golden jackal (Canis aureus)	AR, TM	3/10 (30)
Badger (*Meles meles*)	AR, BH	3/8 (37.5)
European wild cat (*Felis silvestris*)	AR, TM	8/10 (80)
Weasels (*Mustela nivalis*)	AR, TM, BH	¾ (75)
Otters (*Lutra lutra*)	-	0/2
Pine marten (*Martes martes*)	AR, VL	2/2
Stone marten (*Martes foina*)	AR, TM	2/2
European polecat (*Mustela putorius*)	AR	2/2
Muskrat (*Ondatra zibethicus*)	AR	1/3 (33.33)
Wild rabbit (*Lepus europaeus*)	-	0/2
Grey Plover (*Pluvialis squatarola*)	-	0/1
Raccoon dog (*Nyctereutes procyonoides)*	-	0/1

[a] AR: Arad; TM: Timiș; IS: Iași: TL: Tulcea; BH: Bihor; VL: Vâlcea

Epidemiological research highlights that *T. gondii* is regarded as an unusual parasite due to its wide host range and the existence of a single species within the genus [11]. Toxoplasmosis is a globally significant zoonosis with potentially severe consequences for human health, as well as for certain domestic and wild animal species. The World Health Organization has recommended the collection of epidemiological data on *T. gondii*. However, despite the well-established links between infection in wildlife, domestic animals and humans, the prevalence of the parasite in wild animals is rarely monitored [3]. Consequently, studies concerning the presence of *T. gondii* in wildlife remain notably scarce.

There are reports of seroprevalence in foxes from Poland (85.3%) and Portugal (40%) [21, 39]. Additional studies have investigated seroprevalence in wildcats, with reported rates of 67% in France, 83.3% in Portugal, and 84.7% in Spain [1, 11, 39]. In the present study, by real-time PCR confirmed prevalence of *T. gondii* infection was 63.26% in foxes and 80% in wildcats.

PCR-RFLP analysis using nine genetic markers indicated that Type II *T. gondii* is the most common genotype infecting wildcats and beavers in Germany [15].

Similar results were reported by the authors of a study aimed at confirming the presence of the parasite in the wildlife of Serbia. Uzelac A. et al. (2019) [38] performed multiplex PCR-RFLP testing followed by genotyping on cardiac tissue from three species of wild canids: foxes, jackals, and wolves. The overall prevalence was 27.3%. The *T. gondii* population structure in wild canids from Serbia showed notable genetic diversity between foxes and jackals, with *T. gondii* genotype II being predominant.

Wild boars and wolves from the Apennine region of Italy can be considered important contributors to the transmission of toxoplasmosis in both peri-urban and wild areas, with a reported prevalence of 22.6% in wild boars and 26.6% in wolves. No statistically significant differences in seropositivity were observed based on age, sex, or geographic origin of the animals [9].

Similar findings were reported by researchers in southern Italy, who conducted a three-year molecular surveillance study and found a prevalence of 21.8% in examined wild mammals (including wolves, foxes, wild boars, badgers, and roe deer). The authors did not identify statistically significant differences in prevalence based on trophic level or host age, thereby rejecting the hypothesis that *T. gondii* infection is more prevalent in top predators and adult individuals [5].

In Slovakia, Kik M. et al. [20] reported a prevalence of 16.6% in red squirrels. Seltmann A. et al. (2020) [33] reported ELISA-based seroprevalence rates of 93.2% in African lions, 52.4% in cheetahs, and 10% in wildebeest from Namibia.

The Sabin-Feldman Dye Test, considered the gold standard in serological diagnostics, was used to monitor the spread of *T. gondii* in the United Kingdom, with the Eurasian otter - an important sentinel species for freshwater environments - serving as the intermediate host. This first spatial distribution study of *T. gondii* in UK wildlife reported a seroprevalence of 39.5%, with significantly higher infection rates in the eastern region and among adult animals. The findings suggest widespread fecal contamination of freshwater ecosystems with oocysts. Moreover, monitoring *T. gondii* infection in the Eurasian otter is of particular value for conservation research, given its status as a "near threatened" species [3].

In Romania, the present study reports the absence of the protozoan in tissue samples collected from otters and examined using real-time PCR.

Matas Méndez P. et al. (2023) [22] demonstrated that the wildcat and the Iberian lynx are among the most important definitive hosts susceptible to *T. gondii* infection in Spain. Indirect fluorescent antibody testing (IFAT) and molecular analysis revealed a prevalence of 85% in wildcats and 45% in lynxes.

Episodes of acute toxoplasmosis have been diagnosed in Slovak zoos, affecting wolves and zebras, with a prevalence reaching 43% [30].

A well-known mesocarnivore, the Eurasian badger, has been identified as a potential source of toxoplasmosis infection in the Izmir region of Turkey [19].

Similarly, in Romania, the present study indicates a molecular prevalence of 37.5% in badgers.

Hedgehogs, as indicator species in anthropogenic ecosystems and sentinels for a wide range of zoonotic infections, show susceptibility to *T. gondii* infection. PCR-confirmed prevalence of the protozoan ranged from 16.6% in the Northern white-breasted hedgehog (*Erinaceus roumanicus*) to 19.2% in the European hedgehog (*Erinaceus europaeus*) living in close proximity to urban areas in the Czech Republic [16].

Toxoplasma gondii is present in the tissues of wild animals, and the consumption of game meat may represent a potential source of infection for humans. During the 2009/2010 and 2010/2011 hunting seasons in Poland, Witkowski L. et al. (2015) [40] collected meat juice samples from hunted animals, including 552 red deer, 367 wild boars, and 92 roe deer. Using the ID Screen Toxoplasmosis Indirect kit (IDvet, Montpellier, France), the following prevalence rates of *T. gondii* infection were recorded: 24.1% of red deer tested positive, 37.6% of wild boars had *Toxoplasma*-specific antibodies, and 30.4% of roe deer were seropositive for *T. gondii* infection.

Black bears in the United States were tested serologically, with a reported seroprevalence rate of 76% [34].

Dini F.M. et al. (2024) [8] highlighted the crucial role of synanthropic rodents in the life cycle of *T. gondii* in anthropized regions, as well as their function as indicators of environmental contamination with oocysts.

In Romania, comprehensive studies addressing the identification of *T. gondii* in wildlife are lacking. Șuteu O. et al. (2014) [35] reported a seroprevalence of 6% in foxes, while in 2016, Morariu et al. indicated a prevalence of 22.22% in European bison from the Armeniș-Plopu reserve.

In wild animals (felines and ruminants) from a zoological garden in Romania, a high seroprevalence of anti-*Toxoplasma* antibodies was reported—73.1% [10].

Studies have been conducted in Romania to evaluate the influence of various factors—such as season, habitat, and origin (rural/urban areas) on *T. gondii* infection. Among 471 individuals belonging to 20 species of small wild mammals, cardiac tissue analysis by PCR revealed a prevalence of 7.3% positive for toxoplasmic infection. Neither season, habitat type, nor geographic origin had a significant influence on *T. gondii* infection rates, findings that are consistent with those reported by others researchers [18].

The present study, which aimed to identify the protozoan *T. gondii* by PCR in tissue samples collected from 13 host species (fox, jackal, badger, cat, weasel, otter, ferret, muskrat, hare, raccoon dog, pine marten, stone marten, bird of prey), revealed the presence of the parasite in 9 of them (fox, jackal, badger, cat, weasel, ferret, muskrat, pine marten, and stone marten), with prevalence rates ranging from 30% to 100%.

Surface water contaminated with oocysts and consumed by ungulate species, along with the accidental ingestion of contaminated soil during grazing, particularly in wild ruminants such as cervids represent key pathways for the transmission of various parasites to wild carnivores through the consumption of infected ruminant meat [2, 32].

Toxoplasmosis remains a zoonosis with a high risk of transmission to humans, with the golden jackal representing an important vector species for the spread of the parasite within Romania's wildlife. The results of the present study highlight a molecular prevalence of 30% in this key intermediate host involved in the *T. gondii* life cycle.

In comparison, research conducted in Hungary reported a *T. gondii* prevalence of 5% in jackals, based on a two-year study conducted between 2010 and 2012 [36].

The first study on *T. gondii* genotypes in golden jackals was conducted in Serbia in 2019. Given that anthropogenic food sources represent a major reservoir of archetypal strains from clonal lineages II and III of *T. gondii*, the authors suggest that the golden jackal may outcompete the red fox, forcing it to hunt wild mammals and birds for subsistence—thereby increasing its likelihood of acquiring variant *T. gondii* strains [38].

The protozoan has also been detected in tissue samples collected from jackals in Qatar and Senegal [11].

The first genetic characterization of *T. gondii* isolated from wild carnivores on the African continent, and the first report of the Africa 4 lineage (equivalent to RFLP genotype ToxoDB 20) was conducted in the black-backed jackal (*Canis mesomelas*). The study was based on a sample of 80 animals representing 20 species, with *T. gondii* DNA detected in 5% of the tested samples [29].

In comparison, the present study, conducted on a sample of 96 wild animals from 13 species, reports the absence of the protozoan in otters, raccoon dogs, birds of prey, and hares, but confirms the circulation of *T. gondii* in foxes, jackals, badgers, weasels, wildcats, ferrets, muskrats, pine martens, and stone martens.

CONCLUSION

1. Systematic surveillance of wildlife is essential for preventing zoonotic infections that pose a threat to human health while also undermining biodiversity.
2. The real-time PCR (qPCR) was used to detect *Toxoplasma gondii* in tissue samples (heart, brain tissue, liver, spleen, and muscle) collected from nine wild animal species across six counties in Romania.
3. The high circulation rate of *T. gondii* (ranging from 30% to 100%) in jackals, foxes, badgers, wildcats, weasels, pine martens, stone martens, European polecats, and muskrats, underscores the potential risk of human infection, particularly in anthropized areas where domestic and wild animals may come into contact.

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RESEARCH ON THE INFLUENCE OF CALVING SEASON ON MILK YIELD, CHEMICAL COMPOSITION AND SOMATIC CELLS COUNT IN DAIRY COWS

CERCETĂRI PRIVIND INFLUENȚA SEZONULUI FĂTĂRII ASUPRA PRODUCȚIEI DE LAPTE, COMPOZIȚIEI CHIMICE ȘI A NUMĂRULUI DE CELULE SOMATICE LA VACILE DE LAPTE

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Abstract

This study aimed to evaluate the influence of the calving season on cow's milk yield during the first 100 days of lactation (100 DIM) and on milks chemical composition, including fat, protein, lactose, total solids, urea and casein. Additionally, the study assessed the dynamics of the somatic cells count (SCC) in relation to calving season (spring, summer, autumn and winter). The research was conducted at the Research and Development Institute for Bovine Balotești on 40 multiparous ***Romanian Black and White*** *cows (10 cows/calving season), kept under identical housing and feeding conditions. Statistical analysis included descriptive statistics and the Mann-Whitney U test to determine seasonal differences. Results indicated that cows calving in spring produced the highest 100DIM yields (2733.0 ± 144.0 kg), followed by summer (2599.2 ± 99.7 kg), whereas the lowest production was recorded in winter (2365.2 ± 82.7 kg) and autumn (2356.0 ± 230.0 kg) calving cows. Statistically significant differences ($p \leq 0.05$) were found between spring and autumn for parity corrected milk yield, as well as between spring and winter. The SCS was significantly higher in summer season ($p \leq 0.05$), while the lowest values were recorded in autumn, suggesting an increased risk of mastitis during periods of heat stress. The fat and protein percentages were not influenced ($p > 0.05$) by the calving season, likely due to the consistent feeding diet applied throughout the year. These findings highlight the effects of the calving season and it's potential to optimize production efficiency and udder health through reproductive planning and management.*

Keywords: calving season, milk yield, chemical composition, somatic cells count

Rezumat

Scopul prezentului studiu a constat în evaluarea influenței sezonului fătării asupra producției de lapte în primele 100 de zile de lactație, precum și asupra compoziției chimice a laptelui, urmărind indicatori precum procentul de grăsime, proteină, lactoză, substanță uscată totală, uree și cazeină. În plus, studiul a urmărit dinamica numărului de celule somatice (NCS) în funcție de sezonul fătării (primăvară, vară, toamnă și iarnă). Cercetările s-au efectuat în Laboratorul Experimental Zootehnic al I.C.D.C.B. Balotești, pe un număr de 40 vaci multipare din rasa ***Bălţată cu Negru Românească*** *(10 vaci/sezon), întreținute în condiții identice de furajare și management. Analiza statistică a inclus statistici descriptive și testul Mann-Whitney U. Rezultatele au arătat că vacile care au fătat în sezonul de primăvară au înregistrat cea mai mare producție de lapte (2733,0 ± 144,0 kg), urmate de cele care au fătat vara (2599,2 ± 99,7 kg), cele mai scăzute producții de lapte fiind înregistrate la vacile care au fătat iarna (2365,2 ± 82,7 kg) și toamna (2356,0 ± 230,0 kg). Diferențe semnificative ($p \leq 0,05$) au fost observate între sezonul de primăvară și toamnă pentru producția de lapte, precum și între sezoanele de primăvară și iarnă. Scorul celulelor somatice (SCS) a fost semnificativ mai ridicat în sezonul de vară ($p \leq 0.05$), în timp ce cele mai mici valori au fost înregistrate în sezonul de toamnă, sugerând un risc crescut de mastită în perioadele de stres termic. Conținutul de grăsime și proteină nu a înregistrat diferențe semnificative ($p > 0,05$) între sezoane, cel mai probabil datorită furajării din stoc (monodietă). Aceste rezultate subliniază efectele pe care sezonul fătării îl are asupra eficienței producției de lapte și sănătății ugerului la vacile de lapte.*

Cuvinte cheie: sezonul fătării, producția de lapte, compoziție chimică, număr de celule somatice

INTRODUCTION:

Milk production in dairy cows is influenced by multiple factors, including the individuality, lactation length, photoperiod, cold and heat stress, seasonal changes in forage availability and quality, lactation persistency and the cow's genetic background. It is well-established that approximately 75% of milk yield variation is attributed to environmental factors, while only 25% is due to genetics (Collier et al., 2017). Environmental factors include nutritional and herd management, housing technologies, disease occurrence, seasonal conditions and related climate-induced stressors. However, as milk yield continues to increase through genetic selection, improved nutrition and management, high-producing dairy cows become more vulnerable to thermal stress. Extreme temperatures can severely alter metabolic pathways in dairy cattle, as animals attempt to maintain homeostasis while adjusting their production (Baumgard & Rhoads, 2012).

The thermoneutral zone for dairy cattle ranges between 5 and 18°C, with heat stress signs appearing above 28°C. While adult cows are generally more tolerant of cold than heat, calves under 3 months of age are more sensitive to low temperatures. Furthermore, milk composition is also affected by various intrinsic and extrinsic factors, such as diet (Larsen et al., 2010), breed (Palladino et al., 2010), genetic variation (Soyeurt et al., 2008), lactation stage (Stoop et al., 2009), management (Coppa et al., 2013), calving season (Heck et al., 2009) and cow interactions (Stergiadis et al., 2013). Additionally, the seasonal transition from grazing to indoor housing alters both milk yield and composition. Milk fat, protein, lactose and solids content are all affected by changes in the feeding regime and environmental conditions. The somatic cell count (SCC), a key indicator of udder health, increases in response to intramammary infections and stress, with cows exceeding 200.000-400.000 cells/mL generally considered to have subclinical mastitis. Mineral content in milk (e.g., Ca, Mg, Zn, Cu) also varies with season, diet, housing system and region (Weiss et al., 2017; Orjales et al., 2018; Costa et al., 2021).

While molecular tools have been proposed to identify genes regulating macro- and micronutrient levels in milk, limited progress has been made so far (Bailey et al., 2015; Costa et al., 2021). In the context of climate change, dairy cows are increasingly exposed to heat stress, which negatively affects production, fertility and welfare. Moreover, heat tolerance has a negative genetic correlation with milk production traits (Aguilar et al., 2009; Pryce et al., 2022). A promising adaptation strategy is the SLICK1 mutation, which improves thermotolerance in cattle under heat stress without compromising milk yield (Dikmen et al., 2014). However, the physiological response of SLICK1 carriers to temperate conditions and cold stress, as found in Romania, remains largely undocumented.

The objective of this study was to evaluate the influence of calving season on milk yield and milk composition (fat, protein, lactose, total solids, urea and casein), as well as somatic cell score (SCS), during the first 100 days of lactation, in **Holstein** dairy cows managed under temperate continental conditions.

MATERIALS AND METHOD:

The present study was carried out at the Research and Development Institute for Bovine Balotesti (44°36′46″N 26°4′43″E) Romania, (altitude of site 92 m), where 40 purebred **Romanian Black and White** cows (Holstein-Friesian group, *Bălțată cu Negru Românească* national name) were managed under identical conditions. Cows taken into study were between 1st and 5th lactation, with age and parity balanced within the herd and representing a diverse sampling of genetic lines. Since 1987, the **Romanian Black and White** breed was selected for milk yield and fertility, with weights in the breeding scheme of 90% and 10%, respectively. All animals were included in the performance and recording scheme, being registered as a nucleus reference breeding herd. Cows were housed in a tie-stall barn (170/85 cm), using wheat straws as bedding, having *ad libitum* access to water, mineral blocks and during warm weather to outside paddocks (8 m^2/head, maximum 12 hours/day) and the feeding line outside ensuring minimum 0.75 cm/head. Cows were milked twice per day in the barn (starting at 5:00 and 17:00), using individual milking machines and received a daily feed ration of 30 kg corn silage, 6 kg of alfalfa hay and 6 kg of concentrates. The cow groups were established according to calving season (10 animals per season): spring (calvings in March–April), summer (calvings in June–July), autumn (calvings in September–October) and winter (calvings in December–January).

Milk yield (kg) during the first 100 days in milk (DIM) was taken from the results of the official performance recordings, according to the standardized International Committee for Animal Recording (ICAR) guidelines (2012). The alternative milking at 4 weeks (28AM/PM) recording intervals method was used for this purpose. The total milk production for the first 100 DIM was standardized for mature equivalent (cow's parity) using correction coefficients (Velea & Marginean, 2012). Milk fat content (% m/m), protein (% m/m), lactose (% m/m), total solids (% m/m), urea (mg/100g) and casein (g/L) were recorded based on the first three test-day records following calving. The somatic cell score (SCS) was calculated using the following formula: $SCS = \log_2 (SCC / 100.000) + 3$, where SCC = Somatic Cell Count (cells per millilitre of milk) to normalize its distribution and reduce skewness in statistical analyses. The transformation converts the typically skewed SCC distribution into an approximately normal distribution, facilitating parametric and non-parametric statistical analysis. An SCS of 4 corresponds to an SCC of approximately 200.000 cells/mL.

RESULTS AND DISCUSSIONS:

As shown in Table 1, calving season exerted a notable influence on milk yield during the first 100 days of lactation, when corrected for the mature equivalent coefficient. Cows that calved in spring recorded the highest average milk yield (2733.0 ± 144.0 kg), with values ranging from 1873.0 kg to 3346.0 kg. These were followed by cows calving in summer, with a mean production of 2599.2 ± 99.7 kg and a narrower range (2023.0–2983.1 kg). In contrast, cows that calved in autumn showed lower performance (2356.0 ± 230.0 kg), but with the widest variability, ranging between 1613.0 and 4081.0 kg. A similar average was observed in winter-calving cows (2365.2 ± 82.7 kg), although this group showed more consistent yields (CV = 11.05%). Statistical analysis using the Mann–Whitney U test revealed that cows calving in spring produced significantly more milk than those calving in autumn ($p \leq 0.05$) and winter ($p \leq 0.05$). Other pairwise comparisons were not statistically significant ($p > 0.05$), although numerical trends indicated a production advantage for cows calving in the warmer months.

Table 1. Calving season influence on milk yield in the first 100 DIM, corrected with mature equivalent coefficient (in kg) / *Influența sezonului fătării asupra producției de lapte în primele 100 de zile de la fătare, corectată cu coeficient echivalent maturitate (în kg)*

Season	X̄±SEM	SD	CV	MIN	Q1	Q3	MAX
Spring	2733.0±144.0	456.0	16.69	1873.0	2390.0	3154.0	3346.0
Summer	2599.2±99.7	315.2	12.13	2023.0	2325.7	2848.8	2983.1
Autumn	2356.0±230.0	729.0	30.94	1613.0	1813.0	2564.0	4081.0
Winter	2365.2±82.7	261.4	11.05	1902.4	2175.6	2600.6	2734.4
Differences with Mann-Whitney U Test, NS p>0.05; * p≤0.05; ** p≤0.01; *** p≤0.001							
Spring vs. Summer	NS (P= 0.4727)		***Summer vs. Autumn***			NS (P= 0.1041)	
Spring vs. Autumn	*** (P= 0.0452)**		***Summer vs. Winter***			NS (P= 0.1041)	
Spring vs Winter	*** (P= 0.0376)**		***Autumn vs. Winter***			NS (P= 0.3258)	

This seasonal pattern may be influenced by ambient temperature, as thermoneutral conditions for dairy cattle (5–18 °C) are generally present during spring, supporting optimal milk synthesis. Conversely, in colder seasons, a greater portion of dietary energy is diverted toward maintaining body temperature, reducing the energy available for lactation (Debnath et al., 2024). This physiological shift likely contributes to the lower milk yields observed in cows calving during cold seasons.

Table 2 presents the influence of calving season on somatic cell score (SCS) during the first 100 days in milk (DIM), along with descriptive statistics and results of pairwise comparisons using the Mann–Whitney U test. The results indicate that cows calving in summer exhibited the highest average SCS (5.1 ± 0.47), with values ranging from 2.8 to 8.1 and a moderate level of variation (CV = 29.00%). Cows that calved in spring and winter had identical mean SCS values of 4.8, but spring-calving cows showed a higher variability (SD = 1.5; CV = 32.01%) compared to those calving in winter (SD = 1.1; CV = 22.95%). The lowest somatic cell score was recorded in cows calving during autumn, with a mean of 3.5 ± 0.28, lower variability (CV = 25.44%) and a narrower range (2.3 to 5.5). The statistical analysis using the non-parametric Mann–Whitney U test revealed that the difference in SCS between summer and autumn was statistically significant (p = 0.0073), with lower values observed in autumn. A similar pattern was detected between autumn and winter season (p = 0.0091). In contrast, no significant differences were found between spring and any other season, nor between summer and winter, suggesting that autumn calving is more consistently associated with lower somatic cell counts and potentially better udder health during early lactation.

These findings support the hypothesis that seasonal environmental conditions, management practices and feed quality may influence udder health status. Autumn calving might be advantageous due to more favourable climate conditions and less heat stress at the beginning of the lactation, potentially reducing the risk of intramammary infections and inflammation, which are commonly reflected in elevated SCC. For instance, in a study conducted by Marinov et al. (2019) on **Holstein** cows, the authors reported the highest somatic cell count (SCC) during the summer season, while the lowest values were recorded in winter. This similar pattern with elevated SCS during summer could be attributed to heat stress and increased humidity, which compromised the cow's immune system and promoted the growth of mastitis-causing bacteria in the housing environment. These conditions increased the risk of intramammary infections and, consequently, elevate somatic cell counts in milk. These outcomes are most likely explained by the temperature and relative humidity conditions inside the barn, as during winter, cows were housed for extended periods on deep straw bedding in a high-humidity environment. Such conditions favoured the multiplication of environmental pathogens and prolonged contact between the mammary gland and the bedding, particularly immediately after milking, when the teat sphincter remained open and may had further increased the risk of intramammary infections.

Table 2. Calving season influence on somatic cells score in the first 100 DIM / ***Influența sezonului fătării asupra scorului de celule somatice din lapte în primele 100 de zile de lactație***

Season	X̄±SEM	SD	CV	MIN	Q1	Q3	MAX
Spring	4.8±0.49	1.5	32.01	2.4	3.7	6.1	6.7
Summer	5.1±0.47	1.4	29.00	2.8	4.4	5.8	8.1
Autumn	3.5±0.28	0.9	25.44	2.3	2.9	3.7	5.5
Winter	4.8±0.35	1.1	22.95	3.3	4.1	5.3	7.1
Differences with Mann-Whitney U Test, NS p>0.05; * p≤0.05; ** p≤0.01; *** p≤0.001							
Spring vs. Summer	NS (P= 0.9097)		***Summer vs. Autumn***			**** (P= 0.0073)**	
Spring vs. Autumn	NS (P= 0.0640)		***Summer vs. Winter***			NS (P= 0.7054)	
Spring vs Winter	NS (P= 0.9097)		***Autumn vs. Winter***			**** (P= 0.0091)**	

Table 3 shows the effect of calving season on milk fat percentage during the first 100 days of lactation. The highest average fat percentage was recorded in cows that calved in autumn, with a mean value of 4.280%, ranging from 3.557% to 4.783% and a relatively high variability (CV = 11.50%). Cows that calved during winter had an average fat percentage of 4.035% and values ranging between 3.55% and 4.973%. Cows that calved in the spring showed a moderate average fat percentage of 3.854%, with less variability (CV = 8.46%) and a range from 3.303% to 4.427%. The lowest average fat percentage was observed in cows calving during summer, at 3.718%, with values varying more widely between 2.683% and 5.067% (CV = 16.76%). The statistical analysis (Mann-Whitney U test) revealed no significant differences between any of the seasonal pairs ($p > 0.05$), although the comparison between summer and autumn ($P = 0.0757$) and summer and winter ($P = 0.0890$) approached the threshold of significance.

These results suggest a tendency for higher fat content in milk from cows calving in cooler seasons (autumn and winter), although this trend did not reach statistical significance in the current dataset. Previous research has shown that milk production in cattle tends to decrease with declining ambient temperatures and as milk volume decreases, fat percentage increases, due to a strong negative correlation between these two traits. This physiological relationship may partially explain the observed trend of higher fat percentages in cows calving during autumn and winter.

Table 3. Calving season influence on fat percentage in the first 100 DIM / ***Influența sezonului fătării asupra procentului de grăsime din lapte în primele 100 de zile de lactație***

Season	X̄±SEM	SD	CV	MIN	Q1	Q3	MAX
Spring	3.854±0.103	0.326	8.46	3.303	3.696	4.025	4.427
Summer	3.718±0.197	0.623	16.76	2.683	3.442	3.915	5.067
Autumn	4.280±0.156	0.492	11.50	3.557	3.806	4.731	4.783
Winter	4.035±0.126	0.399	9.90	3.55	3.808	4.237	4.973
Differences with Mann-Whitney U Test, NS p>0.05; * p≤0.05; ** p≤0.01; *** p≤0.001							
Spring vs. Summer	NS (P=0.2730)		***Summer vs. Autumn***			NS (P=0.0757)	
Spring vs. Autumn	NS (P=0.1212)		***Summer vs. Winter***			NS (P=0.0890)	
Spring vs Winter	NS (P=0.4727)		***Autumn vs. Winter***			NS (P=0.4727)	

Milk protein percentage during the first 100 days of lactation showed relatively minor fluctuations across calving seasons (Table 4). The highest average was recorded in cows that calved in autumn (3.324%), followed closely by those calving in winter (3.266%) and summer (3.252%), while the lowest average was observed in cows calving in spring (3.212%). All seasons showed a relatively low coefficient of variation (ranging from 5.55% to 10.59%), suggesting a relatively stable distribution of values within each group. Although slight numerical differences were present, no statistically significant variation in protein percentage was detected between seasons ($p > 0.05$ for all pairwise comparisons).

This relative consistency may reflect the greater physiological stability and stronger genetic regulation of milk protein content, as opposed to other milk components such as fat. While environmental factors like nutrition and metabolic stress can still influence protein synthesis, the data suggest that seasonal conditions exert a weaker short-term effect on this trait compared to fat percentage or overall milk yield.

Table 4. Calving season influence on protein percentage in the first 100 DIM / ***Influența sezonului fătării asupra procentului de proteină din lapte în primele 100 de zile de lactație***

Season	X̄±SEM	SD	CV	MIN	Q1	Q3	MAX
Spring	3.212±0.070	0.221	6.89	2.923	2.988	3.355	3.643
Summer	3.252±0.057	0.180	5.55	3.030	3.105	3.380	3.630
Autumn	3.324±0.111	0.352	10.59	2.680	3.070	3.612	3.803
Winter	3.266±0.099	0.313	9.59	2.896	2.997	3.495	3.820
Differences with Mann-Whitney U Test, NS p>0.05; * p≤0.05; ** p≤0.01; *** p≤0.001							
Spring vs. Summer	NS (P=0.5967)		***Summer vs. Autumn***			NS (P=0.3847)	
Spring vs. Autumn	NS (P=0.4057)		***Summer vs. Winter***			NS (P=0.9698)	
Spring vs Winter	NS (P=0.7913)		***Autumn vs. Winter***			NS (P=0.6232)	

The influence of calving season on milk lactose percentage during the first 100 days of lactation is summarized in Table 5. The highest average lactose content was observed in cows calving during the winter season, with a mean of 4.786% and values ranging from 4.483% to 4.916%. This was followed by cows calving in spring (4.722%; min–max: 4.436–4.946%), autumn (4.673%; min-max: 4.350–4.916%) and summer, which recorded the lowest average lactose content at 4.637%, with a narrower range of 4.540% to 4.826%. A statistically significant difference ($p \leq 0.01$) was detected between summer and winter, indicating a higher lactose percentage in milk from cows that calved in the colder season. No other seasonal comparisons reached the threshold of statistical significance ($p > 0.05$).

Previous studies investigating seasonal variation in milk lactose percentage reported similar patterns, with the highest values recorded during winter, followed by a gradual decrease throughout spring, autumn and summer (Bondan et al., 2018). One possible explanation is the increased inclusion of corn silage in winter diets, which contributes to a higher energy intake. Since milk lactose synthesis is tightly linked to energy availability, cows receiving more energy-dense rations during the cold season may exhibit increased lactose levels in early lactation.

Table 5. Calving season influence on lactose percentage in the first 100 DIM / ***Influența sezonului fătării asupra procentului de lactoză din lapte în primele 100 de zile de lactație***

Season	X̄±SEM	SD	CV	MIN	Q1	Q3	MAX
Spring	4.722±0.048	0.153	3.25	4.436	4.620	4.801	4.946
Summer	4.637±0.0241	0.076	1.64	4.540	4.598	4.650	4.826
Autumn	4.673±0.058	0.183	3.93	4.350	4.540	4.837	4.916
Winter	4.786±0.040	0.128	2.69	4.483	4.710	4.885	4.916

*Differences with Mann-Whitney U Test, NS p>0.05; * p≤0.05; ** p≤0.01; *** p≤0.001*			
Spring vs. Summer	NS (P=0.0890)	***Summer vs. Autumn***	NS (P=0.2899)
Spring vs. Autumn	NS (P=0.5453)	***Summer vs. Winter***	**** (P=0.0091)**
Spring vs Winter	NS (P=0.2265)	***Autumn vs. Winter***	NS (P=0.1509)

Milk total solids percentage during the first 100 days of lactation varied noticeably across calving seasons (Table 6). The highest concentration of milk solids was observed in cows that calved during the autumn, with a mean value of 14.234% and values ranging from 12.510% to 16.043%. This group also showed the widest variation (SD = 1.109, CV = 7.79%), suggesting a more heterogeneous metabolic response during this period. Cows calving in summer had the second-highest average (12.995%), followed closely by those calving in winter (12.847%) and spring (12.669%). Compared to autumn, the other seasons presented more homogeneous data distributions, with coefficients of variation between 3.28% and 4.58%. The Mann-Whitney U test revealed statistically significant differences between several seasonal pairs. Specifically, autumn-calving cows had significantly higher solids content than those calving in summer ($p = 0.0173$), spring ($p = 0.0036$) and winter ($p = 0.0082$). No significant differences were observed among the other comparisons ($p>0.05$).

These findings support the idea that autumn calving may enhance milk solids concentration, potentially due to a combination of post-summer nutritional recovery, favourable metabolic conditions, or stage of lactation aligning with cooler months.

Table 6. Calving season influence on solids percentage in the first 100 DIM / ***Influența sezonului fătării asupra procentului de solide din lapte în primele 100 de zile de lactație***

Season	X̄±SEM	SD	CV	MIN	Q1	Q3	MAX
Spring	12.669±0.131	0.415	3.28	11.773	12.447	13.040	13.213
Summer	12.995±0.176	0.556	4.28	11.883	12.753	13.251	14.053
Autumn	14.234±0.351	1.109	7.79	12.510	13.394	15.172	16.043
Winter	12.847±0.186	0.588	4.58	12.187	12.391	13.220	13.943

*Differences with Mann-Whitney U Test, NS p>0.05; * p≤0.05; ** p≤0.01; *** p≤0.001*			
Spring vs. Summer	NS (P=0.1405)	***Summer vs. Autumn***	*** (P=0.0173)**
Spring vs. Autumn	**** (P=0.0036)**	***Summer vs. Winter***	NS (P=0.4057)
Spring vs Winter	NS (P=0.9698)	***Autumn vs. Winter***	**** (P=0.0082)**

The influence of calving season on milk urea concentration (expressed in mg/100 g milk) during the first 100 days of lactation is shown in Table 7. The highest average levels were recorded in cows that calved in winter (28.90 mg/100 g) and spring (27.77 mg/100 g), with values ranging from 21.33 to 35.00 mg and 16.67 to 34.67 mg, respectively. These were followed by cows that calved in autumn, with a mean value of 23.83 mg/100 g (range: 18.00–29.33 mg) and summer-calving cows, which had the lowest average urea levels, at 18.97 mg/100 g, ranging between 14.00 and 26.00 mg. Statistical analysis revealed several significant differences between seasons. Summer-calving cows had significantly lower milk urea concentrations compared to spring (p = 0.0025), autumn (p = 0.0284) and winter (p = 0.0010). A significant difference was also observed between spring and autumn (p = 0.0494) and between autumn and winter (p = 0.0343), while no significant difference was found between spring and winter (p = 0.7337).

In our study, spring and winter calvings were associated with higher milk urea concentrations during early lactation. Similar findings were reported by Bendelja et al. (2011), who observed significantly higher urea levels in spring and summer compared to autumn and winter. Moreover, elevated milk urea concentrations have been reported in the period July–September, in connection with increased total and crude protein levels, particularly casein, which tend to rise in parallel with milk urea levels. For **Simmental** cattle, lower urea concentrations during summer and higher values in autumn were also observed by Marenjak and Poljicak-Milas (2007), potentially explained by the reduced dry matter intake due to heat stress, as well as greater access to fresh forage on pasture. These findings underline the complex interaction between seasonal feeding regimes, thermal comfort and protein metabolism, which together influence milk urea concentrations, a potential indicator of the protein-energy balance in the cow's diet.

Table 7. Calving season influence on milk urea in the first 100 DIM / ***Influența sezonului fătării asupra cantității de uree din lapte în primele 100 de zile de lactație (mg/100g)***

Season	X̄±SEM	SD	CV	MIN	Q1	Q3	MAX
Spring	27.77±1.61	5.09	18.34	16.67	25.00	30.83	34.67
Summer	18.97±1.36	4.32	22.76	14.00	15.58	24.17	26.00
Autumn	23.83±1.16	3.67	15.38	18.00	21.17	27.42	29.33
Winter	28.90±1.56	4.94	17.08	21.33	25.83	34.42	35.00

Differences with Mann-Whitney U Test, NS p>0.05; * p≤0.05; ** p≤0.01; *** p≤0.001			
Spring vs. Summer	**** (P=0.0025)**	***Summer vs. Autumn***	*** (P=0.0284)**
Spring vs. Autumn	*** (P=0.0494)**	***Summer vs. Winter***	***** (P=0.0010)**
Spring vs Winter	NS (P=0.7337)	***Autumn vs. Winter***	*** (P=0.0343)**

Milk casein content during the first 100 days of lactation was influenced by calving season, with clear variations in average concentrations and statistical significance between certain seasonal groups. The highest mean casein concentration was observed in cows calving during autumn (29.50 g/L), with values ranging from 22.83 to 36.30 g/L and the highest variability among all groups (CV = 13.51%). This was followed by cows calving in summer, which showed a more consistent distribution (mean = 26.52 g/L; CV = 4.50%) and cows calving in spring (25.45 g/L) and winter (25.37 g/L), both with moderate to low variation. Statistical comparisons using the Mann-Whitney U test revealed that autumn-calving cows had significantly higher casein concentrations than those calving in spring (p = 0.0173), summer (p = 0.0452) and winter (p = 0.0233). No significant differences were found between spring and summer, spring and winter, or summer and winter (Table 8).

Table 8. Calving season influence on milk casein in the first 100 DIM / *Influența sezonului fătării asupra cantității de cazeină din lapte în primele 100 de zile de lactație (g/L)*

Season	X̄±SEM	SD	CV	MIN	Q1	Q3	MAX
Spring	25.45±0.54	1.711	6.72	23.46	23.55	26.85	28.43
Summer	26.52±0.37	1.194	4.50	24.70	25.55	27.72	28.30
Autumn	29.50±1.26	3.990	13.51	22.83	26.95	32.26	36.30
Winter	25.37±0.80	2.550	10.05	22.36	23.08	27.45	29.60
Differences with Mann-Whitney U Test, NS p>0.05; * p≤0.05; ** p≤0.01; *** p≤0.001							
Spring vs. Summer	NS (P=0.1736)			***Summer vs. Autumn***		*** (P=0.0452)**	
Spring vs. Autumn	*** (P=0.0173)**			***Summer vs. Winter***		NS (P=0.2413)	
Spring vs Winter	NS (P=0.9097)			***Autumn vs. Winter***		*** (P=0.0233)**	

These results align with the previously observed trends in milk urea levels, as elevated urea concentrations are often associated with an increase in total milk protein, particularly casein. The autumn peak in casein could be linked to improved post-summer feeding strategies, enhanced dry matter intake, or altered nitrogen metabolism.

CONCLUSIONS

1. Calving season had a clear impact on early lactation performance. Spring calvings were associated with higher milk yields, while winter and autumn calvings resulted in the lowest production.
2. Autumn-calving cows showed the best udder health, with the lowest somatic cell scores, significantly better than those calving in summer and winter.
3. Milk fat and solids contents were highest in cows calving during autumn and winter, while lactose was significantly higher in winter compared to summer.
4. Overall, autumn calving emerged as optimal for both milk quality and udder health, while spring calving was most favourable for milk yield.

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DYNAMICS OF THE CHEMICAL COMPOSITION OF MILK FROM ROMANIAN BUFFALOES COWS DEPENDING ON THE LACTATION CURVE

DINAMICA COMPOZIȚIEI CHIMICE A LAPTELUI PROVENIT DE LA BIVOLIȚELE DIN RASA ROMÂNEASCĂ ÎN FUNCȚIE DE CURBA DE LACTAȚIE

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Abstract

The aim of this study was to analyze the dynamics of fat, protein and lactose content in fresh milk from ***Romanian Buffaloes*** *cows according to the different stages of lactation. Samples were collected from 20 buffaloes in four phases of lactation: early lactation (1-30 days), plateau phase (30-50 days), end of plateau (180-200 days) and end of lactation period (200-270 days). The variation of the parameters monitored in this study was also highlighted by following their changes throughout the lactation. The results indicated a decreasing trend of all three parameters as lactation progressed. The fat content decreased slightly but steadily, from 7.87% in early lactation to 7.46% in the last phase. Protein levels followed a similar pattern, decreasing from 4.51% to 4.22%, while lactose content decreased from 4.56% to 4.19%. These results highlight the natural variations in the composition of fresh buffalo raw milk during lactation, with implications for optimizing management, increasing efficiency in buffalo milk production, both for milk processing and for its nutritional value.*

Key words: buffaloes, milk, lactation, chemical composition, lactation curve.

Rezumat

Scopul acestui studiu a fost de a analiza dinamica conținutului de grăsimi, proteine și lactoză din laptele proaspăt de bivoliță românească în funcție de diferitele stadii de lactație. S-au recoltat probe de la 20 de bivolițe în patru faze de lactație: lactație timpurie (1-30 de zile), faza de platou (30-50 de zile), sfârșitul de platou (180-200 de zile) și sfârșitul perioadei de lactație (200-270 de zile). Variația parametrilor monitorizați în acest studiu a fost evidențiată și prin urmărirea modificărilor acestora pe parcursul lactației. Rezultatele au indicat o tendință de scădere a tuturor celor trei parametri pe măsură ce lactația a progresat. Conținutul de grăsime a scăzut ușor, dar constant, de la 7,87% la începutul lactației la 7,46% în ultima fază. Nivelurile de proteine au urmat un model similar, scăzând de la 4,51% la 4,22%, în timp ce conținutul de lactoză a scăzut de la 4,56% la 4,19%. Aceste rezultate evidențiază variațiile naturale ale compoziției laptelui crud proaspăt de bivoliță în timpul lactației, cu implicații pentru optimizarea managementului, creșterea eficienței în producția de lapte de bivoliță, atât pentru prelucrarea laptelui, cât și pentru valoarea sa nutritivă.

Cuvinte cheie: bivolițe, lapte, compoziție chimică, curba lactației.

INTRODUCTION

The domestic buffalo (*Bubalus bubalis*) is a valuable species in animal husbandry, appreciated for its adaptability and for its ability to produce milk with a superior chemical composition, rich in fat, protein and minerals (Borghese, 2005). Buffalo milk is preferred in the dairy industry for obtaining refined and high-quality products, such as mozzarella and matured cheeses.

The chemical composition of milk varies throughout the lactation period. Studies show that in the first weeks after calving, fat and protein levels are higher, gradually decreasing as lactation progresses

(Kashwa, 2016). These variations reflect the physiological changes that occur in the mammary gland and influence both the quality of milk and its economic value.

The lactation curve is essential in production studies, providing a clear picture of how milk production varies during lactation. This includes an initial phase of growth, followed by a plateau and then a final phase of decline. The shape of the curve is mainly determined by physiological factors, such as the activity and number of secretory epithelial cells in the mammary gland (Penchev et al., 2011).

In Romania, studies on the variation of milk composition according to the lactation curve are still limited, especially in the case of the local population. This work aims to analyze the variations of fat, protein and lactose content in **Romanian buffalo** milk, according to the different stages of the lactation curve, thus contributing to a better valorization of this indigenous genetic resource.

MATERIAL AND METHOD

The study was conducted on a batch of 20 buffaloes of the **Romanian Buffalo breed**, in different stages of lactation. All milk samples were taken from buffaloes in lactations between the first and fourth, to allow a balanced assessment of the variation in composition depending on the stage of lactation. The animals were maintained under similar feeding and housing conditions, within the Research and Development Station Buffaloes in Şercaia.

The breeding system applied was semi-intensive. During the summer, the buffaloes were taken out to pasture, and in the cold season they were fed with pickled and coarse fodder.

Milk was collected twice a week, at both milkings (morning and evening), and the samples were analyzed immediately after collection. The determinations were made for the following parameters: fat, protein and lactose.

Physico-chemical analyses were performed using an EKOMILK-Ultra device, and the data obtained were centralized for each lactation phase. The stages analyzed were:

- phase 1: beginning of lactation (1–30 days),
- phase 2: plateau phase (30–50 days),
- phase 3: end of plateau (180–200 days),
- phase 4: end of lactation (200–270 days).

The values obtained were expressed in percentages (%) and statistically processed by calculating means and standard deviations.

RESULTS AND DISCUSSION

FAT

The fat content values in buffalo milk varied according to the stage of lactation, following the profile of the lactation curve. A higher concentration at the beginning of lactation, a period of relative stability and a decrease towards the end of lactation. These changes reflect the physiological changes that occur in the mammary gland during lactation and are influenced by the volume of milk produced, the metabolic state of the animal and the activity of the secretory cells. These are represented by the alveolar epithelial cells, located in the alveoli of the mammary gland, which have the role of synthesizing and secreting the main components of milk.

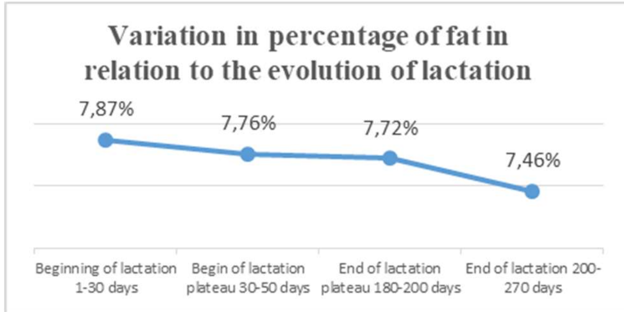

Figure 1. Evolution of fat content (%) according to lactation stage / ***Evoluția conținutului de grăsime (%) în funcție de stadiul lactației***

At the beginning of lactation (1–30 days), the highest fat content was recorded with an average of 7.87 ± 0.05%. This increased content is specific to milk from the postpartum period, when the amount of milked milk is reduced, but its composition is rich in concentrated nutritional components, intended to support the development of newborn calves. The activity of alveolar epithelial cells is intense, which are responsible for the synthesis and secretion of the main components of milk, especially fat, proteins and lactose. This stage reflects the ascending phase of the lactation curve, in which the mammary gland activates its physiological mechanisms for optimal milk production. During the plateau period of lactation (30–50 days), the fat content recorded a slight decrease compared to the first phase, with an average of 7.76 ± 0.33%. This decrease is mainly influenced by the increase in the volume of milk produced, a phenomenon that determines the physiological dilution of solid components. However, the fat content remains high, reflecting an efficient secretory activity of the mammary gland during a period of maximum milk production. Studies by Yadav et al. (2013) and Gunathilake et al. (2024) confirm that variations in fat content can be influenced by both the stage of lactation and the breed, but in general, slightly lower values are observed in the phases of intensive production, followed by progressive increases towards the end of lactation. At the end of the lactate curve (180–200 days), fat content continued to decrease slightly, with a mean value of 7.72 ± 0.37%. This decrease can be attributed to the progressive decrease in mammary gland activity, influenced by hormonal changes and physiological adaptations that occur as lactation progresses. As milk production decreases, the activity of alveolar epithelial cells slows down, leading to changes in milk composition, including a slight decrease in fat content. Similar results were reported by Nayak et al. (2017) in **Murrah** buffaloes, where a decreasing trend in fat was observed towards the end of lactation.

In the last phase of lactation (200–270 days), the lowest fat content was recorded, with an average of 7.46 ± 0.07%. This decrease is associated with the end of the lactation curve, a period in which milk production is minimal, and the body redirects metabolic energy towards body maintenance, to the detriment of milk production. The activity of alveolar epithelial cells is significantly reduced, which directly affects fat synthesis. In addition, the decrease in prolactin levels and the increase in progesterone levels towards the end of lactation influence the secretory capacity of the mammary gland. Similar results were reported by El-Tarabany et al. (2016), who observed a decrease in fat content in the last months of lactation in Egyptian buffaloes, under conditions of decreased production and metabolic adaptation. These results highlight the dynamics of fat content depending on the stage of lactation, an aspect analyzed in parallel for the other parameters of milk composition. The decrease in fat content in the advanced stages of lactation is also associated with changes in the fatty acid composition of milk.

Table 1. Evolution of fat content (%) depending on lactation stage / *Evoluția procentului de grăsime în funcție de stadiul lactației*

FAT %			
Beginning of lactation 1-30 days	**Begin of plateau 30-50 days**	**End of plateau 180-200 days**	**End of lactation 200-270 days**
X±Sx	**X±Sx**	**X±Sx**	**X±Sx**
7.87±0.05	7.76±0.33	7.72±0.37	7.46±0.07

During periods of low milk production, studies have shown a tendency to decrease short-chain fatty acids, such as butyric acid, but also essential polyunsaturated acids, such as linoleic acid (C18:2) and alpha-linolenic acid (C18:3). These variations affect not only the nutritional value of milk, but also its behavior in industrial processing, influencing the texture, taste and final quality of dairy products. In addition, the reduction in the total amount of lipids entails a lower intake of unsaturated fatty acids, which may indirectly modify the characteristics of buffalo milk fat in the final stage of the lactation curve.

PROTEINS

The proteins in buffalo milk represent an essential parameter of its nutritional value and its ability to be used in the dairy industry. It is mainly composed of casein and serum proteins, which have a fundamental role in the development of calves in the first days of life, but also in the coagulation of milk for the production of dairy products. The protein content varies during lactation, being influenced by the physiological stage of the animal, nutrition and endocrine activity. As a rule, the values are higher in the early phases of lactation and tend to decrease towards the end of it.

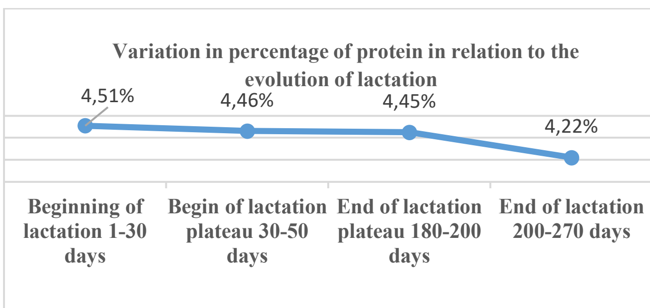

Figure 2. Evolution of protein content (%) depending on the stage of lactation / *Evoluția procentului de proteină în funcție de stadiul lactației*

In the first 30 days of lactation, the protein content recorded the highest value, with an average of 4.51 ± 0.07%. This increased level is characteristic of the beginning of lactation, when the milk has a high nutritional density, with a significant protein intake. These play an essential role in supporting the vital functions of calves, being involved in both nutrition and immunity. The high amount of casein facilitates the absorption of calcium, while serum proteins contribute to protection against infections. These particularities reflect the physiological adaptation of the body to the postpartum period and support the superior quality of milk at this stage. At the beginning of the plateau phase of lactation (30–50 days), the protein content presented an average value of 4.46 ± 0.09%, marking a slight decrease compared to the previous stage. This variation is associated with the metabolic changes that occur with reaching a constant level of milk production. Unlike the onset of lactation, when protein secretion is influenced by intensely stimulating hormonal factors (e.g. prolactin), in this phase milk protein synthesis stabilizes, being regulated more by physiological needs and the efficiency of nutrient use. At the end of the lactation curve (180–200 days), protein content remained at a relatively constant level, with an average value of 4.45 ± 0.36%. Although close to the previous phase, this stage is marked by a slight reduction in secretory activity, along with a gradual decrease in milk production. The greater variation in protein value in this interval may reflect the influence of individual factors such as the number of calvings, the physiological state of the animal or differences in nutritional intake. Milk protein synthesis remains active, but registers a slightly downward trend, characteristic of the middle to late phases of lactation. At the end of lactation in the decline phase (200–270 days), the protein content recorded the lowest value, with an average of 4.22 ± 0.18%. This reduction is associated with the end of the lactation curve, a period in which milk production decreases significantly. The decrease in protein content can be explained by the reduction in the activity of alveolar epithelial cells in the mammary gland, responsible for the synthesis of milk proteins. In parallel, the body redirects metabolic resources to the maintenance of vital functions, and the lower frequency of milking causes a hormonal decline that affects secretion. All these changes contribute to the progressive decrease in protein concentration, characteristic of the end of lactation.

Table 2. Evolution of protein content (%) depending on the stage of lactation / *Evoluția procentului de proteină în funcție de stadiul lactației*

PROTEIN %			
Beginning of lactation 1-30 days	**Begin of plateau 30-50 days**	**End of plateau 180-200 days**	**End of lactation 200-270 days**
X±Sx	**X±Sx**	**X±Sx**	**X±Sx**
4.51±0.07	4.46±0.09	4.45±0.36	4.22±0,18

Beyond quantitative variations, the quality of the protein in buffalo milk remains high throughout lactation, due to its complete profile of essential amino acids, such as leucine, valine or methionine (Coroian, 2009). These amino acids play an essential role in protein synthesis, supporting the immune system and the growth and regeneration of the body. Even in the final stages of lactation, milk continues to provide important nutritional benefits, which supports its value both from a nutritional and qualitative point of view.

LACTOSE

Lactose is the main carbohydrate present in buffalo milk, being composed of two monosaccharides glucose and galactose. It is responsible for the slightly sweet taste of fresh milk and represents an essential source of energy for calves in the early stages of life. At the same time, lactose contributes to the development of beneficial intestinal flora, having an important prebiotic role. From a technological point of view, lactose is indispensable in fermentation processes, being a substrate for lactic acid bacteria used in obtaining acidic products. Although fluctuations in lactose content during lactation are lower compared to other components, they may indicate metabolic changes in the mammary gland, the health status of the animal or the occurrence of functional imbalances, such as subclinical inflammations (e.g. mastitis) or the redirection of metabolic resources in contexts of stress or inadequate nutrition.

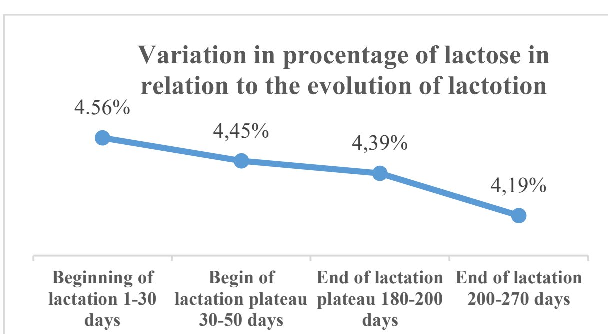

Figure 3. Evolution of lactose content (%) depending on the stage of lactation / *Evoluția procentului de lactoză în funcție de stadiul lactației*

At the beginning of lactation (1–30 days), lactose content reached the maximum value recorded during the study, with an average of 4.56 ± 0.16%. This high concentration can be associated with the physiological specificity of the onset of lactation, when the mammary gland has an intense secretory activity, and the composition of the milk reflects a formula adapted to the initial needs of the maternal organism and the newborn calf. The increased lactose level contributes to maintaining a balanced milk secretion, both in terms of quantity and quality. Higher values were reported by Kashwa (2016) in the case of Egyptian buffaloes, with an average of 5.08% in the first 30 days of lactation. The differences between the two studies can be attributed to the genetic influence (breed), Mediterranean climatic conditions and the intensive feeding system used in that area, compared to traditional conditions in Romania.

In the early stage of the lactation plateau (30–50 days), the lactose content remained at a relatively constant level, with an average of 4.45 ± 0.17%, registering a slight decrease compared to the first stage of lactation. This stabilization reflects the achievement of a functional and constant milk production, characterized by a balance between milk production and its chemical composition..

In the late stage of the lactation plateau (180–200 days), the lactose content continued to decrease slightly, reaching an average value of 4.39 ± 0.07%. This reduction can be explained by the physiological changes characteristic of advanced lactation, which occurs concurrently with gestation. Part of the body's metabolic resources are directed towards supporting fetal development, which causes a gradual decrease in mammary gland activity. At the same time, hormonal changes associated with this period, such as a decrease in prolactin levels and an increase in progesterone, can negatively influence lactose synthesis, by reducing the production of α-lactalbumin, an enzyme directly involved in its formation.

In the last stage of lactation (200–270 days), lactose content recorded the lowest value in the entire analyzed interval, with an average of 4.19 ± 0.03%. This decrease reflects the physiological decline in milk production, at a time when the buffalo's body prioritizes maintaining gestation and conserving metabolic resources. The reduction in the activity of secretory cells in the mammary gland, especially the alveolar epithelial ones, leads to a general decrease in milk synthesis, including lactose. Also, towards the end of lactation, its level can be influenced by structural changes in the mammary tissue, associated with the gradual involution of the gland. Similar results were reported by Kashwa (2016) in the case of Egyptian buffaloes, where lactose content decreased from 5.08% to 4.64% in the seventh month of lactation, confirming the downward trend observed in the present study.

Table 3. Evolution of lactose content (%) depending on the stage of lactation / ***Evoluția procentului de lactoză în funcție de stadiul lactației***

LACTOSE%			
Beginning of lactation 1-30 days	**Begin of plateau 30-50 days**	**End of plateau 180-200 days**	**End of lactation 200-270 days**
X±Sx	**X±Sx**	**X±Sx**	**X±Sx**
4.56±0.16	4.45±0.17	4.39±0.07	4.19±0.03

CONCLUSIONS

The lactation curve expresses how milk production and composition change during a full lactation, reflecting the physiological, hormonal and metabolic influences on the mammary gland. In this study, a gradual decrease in fat, protein and lactose content was observed, with maximum values in the first 30 days after calving and minimum values in the late lactation phase (200–270 days). Specifically, fat content ranged from 7.87 ± 0.05% to 7.46 ± 0.07%, protein from 4.51 ± 0.07% to 4.22 ± 0.18%, and lactose from 4.56 ± 0.16% to 4.19 ± 0.03%. These variations are determined by functional changes in the mammary gland, by the redistribution of metabolic resources in the body, but also by the gradual decrease of hormonal stimuli specific to lactation. Fat content is the first to be affected in the advanced stages of lactation, while protein and carbohydrate content follow a slower but constant decline. The results obtained emphasize the importance of careful monitoring of the stage of lactation in order to maintain milk quality. Adapting feeding and selection strategies according to these variations can significantly contribute to increasing efficiency and to the superior valorization of production in buffalo farms in Romania. In this context, an in-depth knowledge of the lactation curve becomes an essential tool for optimizing productivity and supporting a sustainable breeding system, with high technological and economic results. Deepening these aspects allows the development of modern management strategies, the adaptation of genetic selection programs, the adjustment of feed rations depending on the lactation phase and the careful monitoring of biochemical parameters of milk, all of which contribute to improving the performance of buffalo milk production in Romania.

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PRELIMINARY STUDY OF THE QUALITATIVE PARAMETERS OF RAM SEMINAL MATERIAL PRESERVED BY FREEZING

STUDIUL PRELIMINAR AL PARAMETRILOR CALITATIVI AI MATERIALULUI SEMINAL DE BERBEC PREZERVAT PRIN CONGELARE

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Abstract

*The interest of sheep and goat breeders in increasing productivity and implicitly profitability has materialized through the modernization of the traditional exploitation system. To increase productive performance, breeders resort to natural mating with breeding males. The epizootic situation of recent years, respectively the sanitary-veterinary restrictions as a result of the evolution of infectious diseases (Bluetongue, PMM) have limited the circulation of males with implications for reproduction management. An alternative to solving this impediment is to resort to artificial insemination with semen from breeding males. In this sense, Palas Sheep and Goat Breeding Research and Development Institute, in partnership with National Association of Goat Breeders in Romania "CAPRIROM", carries out research activities in order to establish methodologies for preserving ram and goat seminal material in frozen form that would allow the creation of a sperm cryobank from valuable males. When examining frozen-thawed semen from **Palas Merino** rams, processed during the breeding season, a total motility ranging from 43.2 to 64.1% and a progressive motility ranging from 31.8 to 47.7% were recorded, while in the off-breeding season, values of 39.4-44.5% for total motility and 27.1-32.5% for progressive motility were obtained. The total and progressive motility values from **Palas Milk** rams had a very high variability.*

Keywords: ram semen, total/progressive motility

Rezumat

*Interesul crescătorilor de oi și capre pentru creșterea productivității și implicit a profitabilității s-a materializat prin modernizarea sistemului de exploatare tradițional, iar pentru creșterea performanțelor productive crescătorii apelează la monta naturală cu masculi amelioratori. Situația epizootologică a ultimilor ani, respectiv restricțiile sanitar-veterinare ca urmare a evoluției unor boli infecțioase (Bluetongue, PMM) au limitat circulația masculilor cu implicații în managementul reproducției. O alternativă de rezolvare a acestui impediment este apelarea la inseminarea artificială cu material seminal provenit de la masculi amelioratori. În acest sens, Institutul de Cercetare – Dezvoltare pentru Creșterea Ovinelor și Caprinelor Palas, în parteneriat cu Asociația Națională a Crescătorilor de Capre din România "CAPRIROM" desfășoară activități de cercetare în sensul stabilirii unor metodologii de prezervare a materialului seminal de berbec și țap sub formă congelată care să permită crearea unei criobănci de spermă de la masculi valoroși. La examinarea materialului seminal congelat-decongelat de la berbeci rasa **Merinos de Palas**, procesat în sezon de reproducție, s-a înregistrat o motilitatea totală care a variat între 43,2 și 64,1 % și o motilitate progresivă cuprinsă între 31,8 și 47,7, iar în contrasezon de reproducție s-au obținut valori de 39,4-44,5% pentru motilitatea totală și 27,1-32,5% pentru motilitatea progresivă. Valorile motilităților totală și progresivă de la berbecii din **Rasa de Lapte Palas** au avut o variabilitate foarte mare.*

Cuvinte cheie: material seminal berbec, motilitate totală / progresivă

INTRODUCTION

Sheep reproduction in temperate zones is seasonal, occurring in the autumn period, respectively with the decrease in daylight hours, for which reason they are also called "short-day" animals (Chemineau, P. et al., 2017, 1988). During the period with a daylight duration of over 10-12 hours, seasonal anestrus (lack of sexual activity, respectively of estrus and ovulation) is observed in ewes and a reduction in sexual activity is observed in rams. Photoperiod is a decisive factor in triggering sexual activity in sheep, which influences the general management of farms, by placing the calving campaign in the period December-March. Thus, the production of lamb meat and sheep milk is seasonal, not meeting the demands of the human population and bringing insufficient benefits to sheep farmers.

Off-season sheep breeding is increasingly practiced as producers adopt accelerated lamb rearing programs to better supply lamb markets and ensure a consistent supply of sheep milk throughout the year. Coupling this method with other biotechnical methods of sheep reproduction (induction and synchronization of estrus, early diagnosis of pregnancy, embryo transfer) amplifies the usefulness of artificial insemination. The intensive use of tested rams of high zootechnical value or the use of the gene pool offered by imported rams of various specialized breeds, leads to obtaining a superior quality offspring and intensifies the pace of genetic improvement of sheep flocks. By artificial insemination of a large number of sheep, approximately 600-1000 sheep / ram, the pace and degree of improvement is accelerated by using breeding rams of superior genetic value, tested according to their own performance or that of their offspring (Zamfirescu S., Sonea A., 2004).

Ways to intensify the reproductive activity of males in order to be able to mount during the breeding season include the application of non-hormonal treatments, namely maintaining them in photoperiod conditions (Nadolu D., Anghel A., 2007, José Abecia et al., 2020) or the application of melatonin implants (Șogorescu E et al., 2012).

By using artificial insemination, the main advantages for the breeder are the increase in the rate of genetic progress in terms of milk quantity, milk protein quantity and quality, obtaining a large number of daughters from a breeding ram from one's own farm or from another farm. By de seasonalizing reproduction and concentrating calving periods, the products demanded by the market (milk, meat) obtained with a reduced manpower requirement are ensured throughout the year. In addition, the danger of transmitting diseases through reproduction is reduced and the transport of animals is avoided, which is important, especially during the period of sanitary and veterinary restrictions, a situation of utmost topicality. By imposing quarantine measures and prohibiting the movement of animals, rational matings are prevented, and in situations when restrictions provide for the elimination of sick animals, the risk of losing valuable breeders is very high. By freezing the semen from valuable males in a cryobank, the genetic fund of each breed is protected.

MATERIALS AND METHODS

The present study was conducted at the Palas Sheep and Goat Breeding Research and Development Institute, Constanta, Reproductive Biotechnologies Laboratory, on semen collected from 2 **Palas Merino** rams and 4 **Palas Milk** rams, monitoring the variation of sperm parameters after freezing-thawing.

The results presented are part of a complex study currently underway, regarding the establishment of a freezing protocol and assessment of the suitability for freezing of ram semen under the conditions of using the C.A.S.A. (Computer - Assisted Sperm Analysis) system. The research is carried out through the collaboration of the institute with the National Association of Goat Breeders of Romania CAPRIROM, which purchased the complete semen freezing line, namely an automatic straw stamping system, an automatic straw loading system, an automatic station with a freezing program and connection to a liquid nitrogen tank and the C.A.S.A. (Computer - Assisted Sperm Analysis) computerized sperm analysis system.

To determine sperm indices, 48 ejaculates were analysed, 8 ejaculates from each ram, 4 ejaculates collected during the off-season (last week of March 2024) and 4 ejaculates collected during the normal breeding season (last week of September 2024) (table 1).

Table 1. Semen collection scheme and frozen-thawed semen analysis / ***Schema de recoltarea materialului seminal și analiza materialului seminal congelat-decongelat***

Specification	**Merino Palas** breed 1	**Merino Palas** breed 2	**Milk Palas** breed 1	**Milk Palas** breed 2	**Milk Palas** breed 3	**Milk Palas** breed 4
September 2024	4	4	4	4	4	4
March 2024	4	4	4	4	4	4
Total	8	8	8	8	8	8

The six rams, 2 of the **Palas Merino** breed (MP1 and MP2) and 4 of the **Palas Milk** breed, from which semen was collected benefited from the same maintenance and exploitation conditions. 2 ejaculates/day were collected from each male, at intervals of 2 days, and the sperm was processed in parallel, benefiting from the same environmental conditions. The only difference is 10 minutes between the semen collections from the two males, a difference that does not significantly influence the results of the research. It should be noted that from all males, collected during the off-season, semen was collected one week before the start of the study, in order to eliminate the sperm reserve with a high content of denatured spermatozoa due to non-use in mating and to stimulate spermatogenesis in the presence of females in hormonally induced estrus.

In the activity of the Reproduction Laboratory, the procedures provide for the cryobank of frozen semen which, when checking viability by the vital staining method, has values over 35%. Frozen-thawed semen with viability between 30 and 35% is kept as a buffer stock, as a reserve. It should be noted that the method of establishing viability has a degree of subjectivity depending on the experience, skill and conscientiousness of the operator performing the control. By using the CASA automatic system, these disadvantages are eliminated, the results being much more complex and precise. Thus, total motility indicates the percentage of live spermatozoa, regardless of the type of movement they perform (circular movements, backwards, zig-zag etc.), a parameter similar to the viability determined by vital staining. Therefore, following the verification of sperm parameters, the semen retained in the cryobank must register a total motility of over 35%.

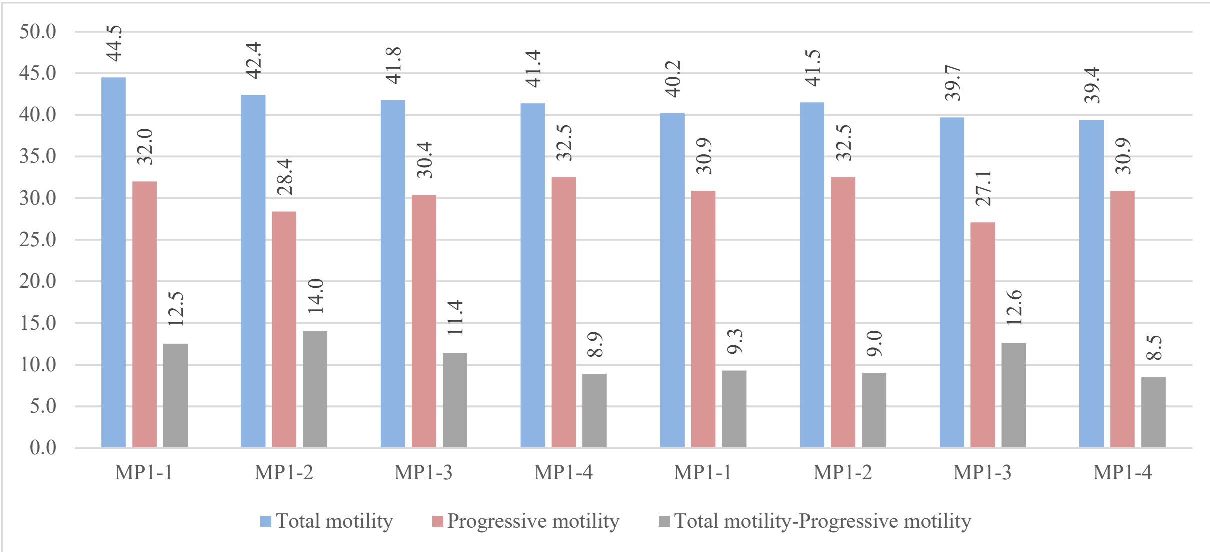
The research of the Laboratory of Reproductive Biotechnologies is aimed at finding a correlation between total motility and progressive motility and the factors that may influence this correlation. In establishing the minimum level of progressive motility of frozen semen, the verification of the fertilizing capacity of the semen following artificial insemination of sheep must also be taken into account.

Frozen preservation of semen was verified by analysing total motility and progressive motility within 24 hours of freezing for the same male in normal and off-season breeding.

RESULTS AND DISCUSSION

The results of the qualitative assessment of semen from **Palas Merino** rams harvested in the off-season and preserved in frozen form are presented in Graph 1.

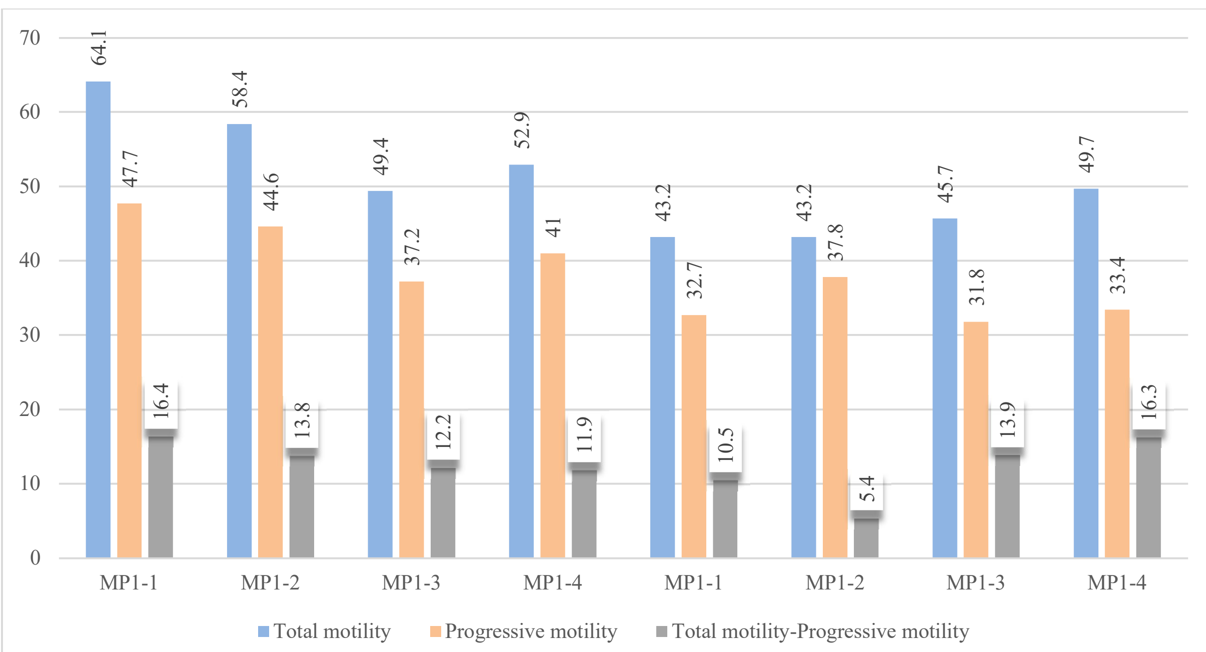
Total motility varies between 39.4 and 44.5%, with higher values recorded in the first ram (41.4 – 44.5%) and progressive motility is between 27.1 and 32.5%, with the differences between the two motilities being between 8.3 and 14%. What is notable about the semen from the first ram is the greater difference between the two motilities, between 8.9 and 14% compared to the ejaculates of the second ram, where the difference between total and progressive motility is between 8.5 and 12.6%.



MP1–first Merino de Palas ram, MP2- second Merino de Palas ram

Graph 1. Variation of total motility and progressive motility of frozen-thawed semen from Palas Merino rams, collected in the off-season / ***Variația motilității totale și a motilității progresive a materialului seminal congelat-decongelat de la berbeci Merinos de Palas, recoltat în contrasezon de reproducție***

The results of the assessment of motility after freezing-thawing of semen from **Palas Merino** rams, harvested during the breeding season, are presented in graph 2.



MP1–first Merino de Palas ram, MP2- second Merino de Palas ram

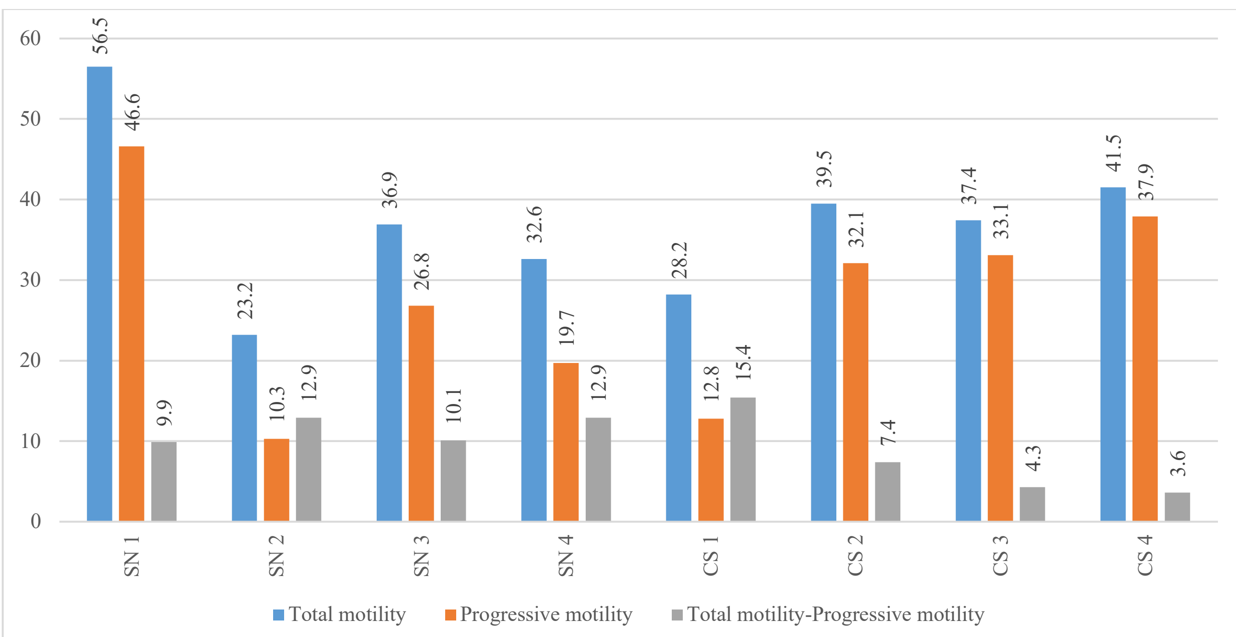
Graph 2. Variation of total motility and progressive motility of frozen-thawed semen from Merinos de Palas rams, harvested during the breeding season / ***Variația motilității totale și a motilității progresive a materialului seminal congelat-decongelat de la berbeci Merinos de Palas, recoltat în sezon de reproducție***

The situation observed in the evaluation of semen collected in the off-breeding season and processed by freezing is also found in those collected in the breeding season. Thus, the ejaculates from the first **Palas Merino** ram, after freezing-thawing, recorded total motility values ranging between 49.4 and 64.1%, while in the second ram these were between 43.2 and 49.7%. The second ram stands out by obtaining close values of total (43.2-49.7%) and progressive (between 31.8 and 37.8%) motility, while the semen of the first ram has a greater variability between the sperm parameters determined in successive collections and with a greater difference between the motility values.

Ejaculates collected during the breeding season and cryopreserved recorded total values of over 35% and were stored in the semen cryobank. And progressive motility has values of over 30% in all 8 ejaculates collected, following that by insemination of the sheep the fertility rate will be increased. Therefore, the two **Palas Merino** males are suitable for semen collection for cryopreservation.

The other ejaculates analysed in this study came from 4 males of the **Milk Palas** breed. Graph 3 contains the total and progressive motility values of the ejaculates collected from the first male of the Milk Palas breed. It is observed that, in the normal breeding season harvests, only the first ejaculate collected in September 2024 and frozen had, after thawing, a high total and progressive motility of 56.5% and 46.6%, respectively.

The other ejaculates collected had, after freezing-thawing, total motilities ranging between 32.6% (harvest 4 of September) and 36.9% (harvest 3) and only in harvest 2 was a total motility below 30% (23.2%) obtained, which would lead to the removal of the frozen straws from the cryobank. Regarding progressive motility, except for the first collection with very good values and the 2nd collection in the case of removed ejaculate, it has values below 30%, respectively 26.8% (3rd collection) and 19.7% (4th collection).



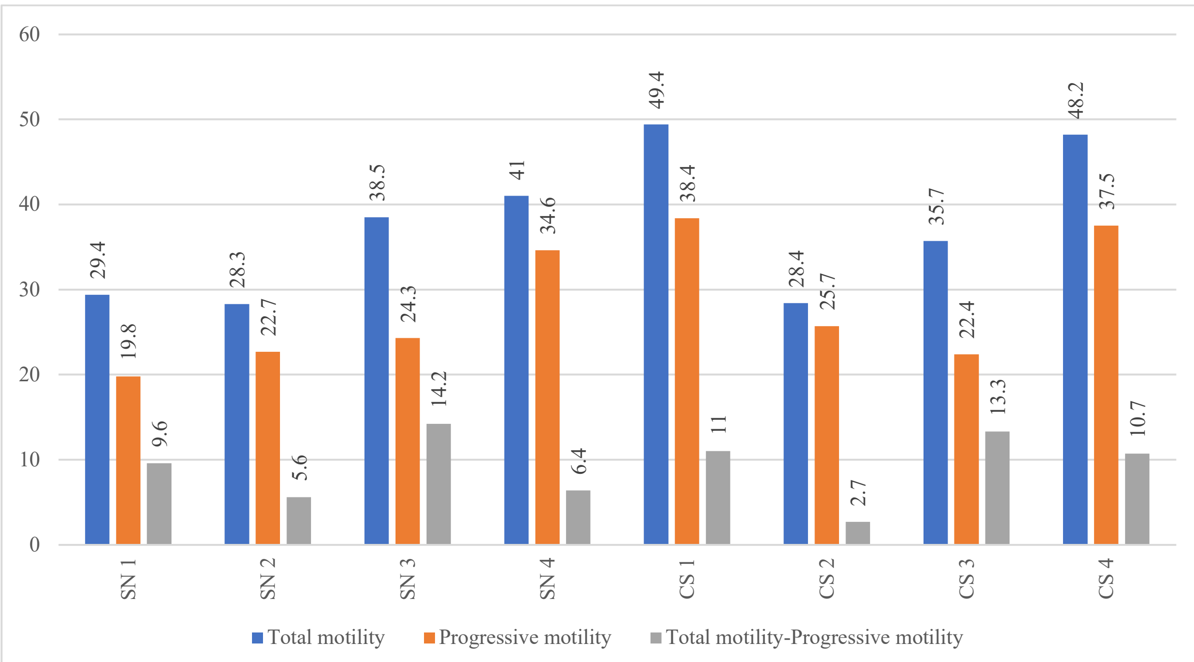
SN – normal season, CS – off-season

Graph 3. Variation of total motility and progressive motility of frozen-thawed semen collected from the Milk Palas 1 ram in the breeding season and off-season / ***Variația motilității totale și a motilității progresive a materialului seminal congelat-decongelat recoltat de la berbecul de rasa Lapte Palas 1 în sezon și contrasezon de reproducție***

During the off-season breeding harvests (in March 2024), only the first ejaculate collected was removed from the cryobank, with a total motility of 28.2%. The other ejaculates with total motilities ranged between 39.5% (harvest 2 in off-season breeding) and 41.5% (harvest 4 in March 2024). In the case of these 3 ejaculates retained in the cryobank, a progressive motility lower by 3.6% to 7.4% compared to the total motility is observed. These small differences in progressive motility compared to the total motility indicate a good suitability for freezing of the semen from the first analysed **Milk Palas** male, which will also be correlated with the fertility rate following artificial inseminations of sheep.

Motility after freezing-thawing of straws with ejaculate from the 2nd **Milk Palas** ram is presented in graph 4.

During the normal breeding season harvests, from September 2024, it is observed that the first 2 ejaculates have total motility below 30% and were removed from the cryobank. The other 2 ejaculates have total motility of 38.5% and 41%, values of over 35%, which leads to their preservation in the semen cryobank stock. As can be seen, the progressive motility of these ejaculates is good, with the largest decrease recorded by the ejaculate from the 3rd harvest, by 14.2%, the last harvest recording progressive sperm motility of over 30%.



SN – normal season, CS – off-season

Graph 4. Variation of total motility and progressive motility of frozen-thawed semen harvested from the Milk Palas 2 ram in the breeding season and off-season / ***Variația motilității totale și a motilității progresive a materialului seminal congelat-decongelat recoltat de la berbecul de rasa Lapte Palas 2 în sezon și contrasezon de reproducție***

In the off-season collections, a large variation in total sperm motility is observed when checking after freezing-thawing, between 28.4% and 49.4%. The same variation is also recorded in progressive motility, which varies from 22.4% to 38.4%.

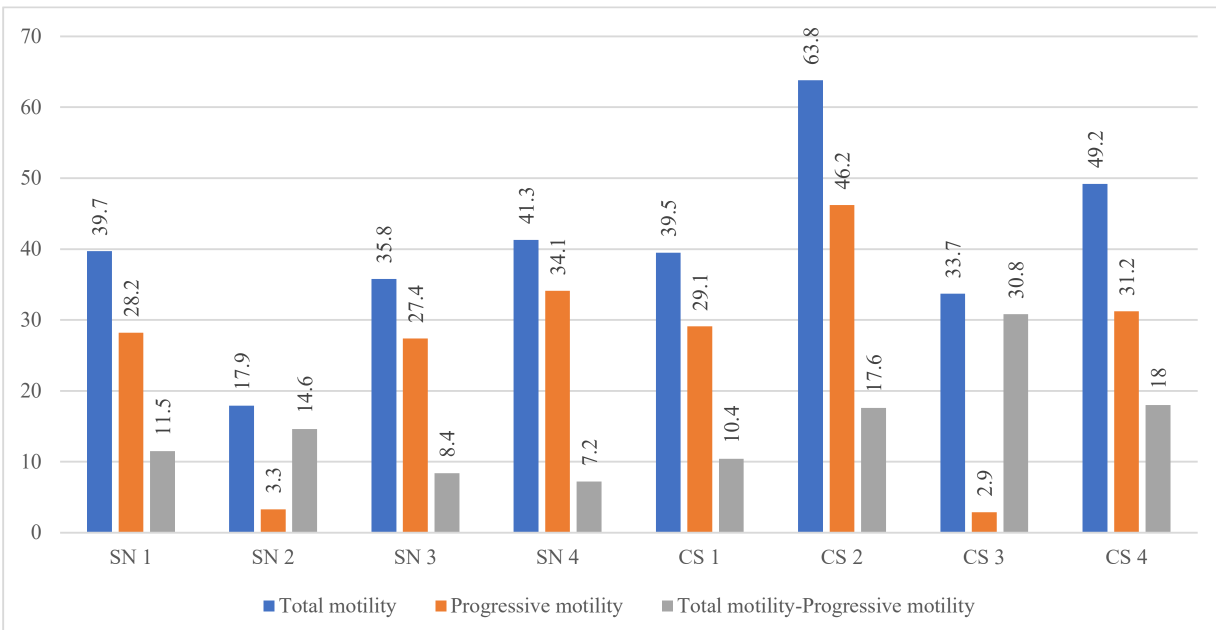
From the analysis of the evolution of motilities in the 8 ejaculates, we can conclude that the semen of the second male is suitable for freezing and storage in the sperm cryobank, specifying that in the off-season, the greater variation in results may be due to a lower rate of sperm production and therefore it must be monitored whether a lower frequency of collections is necessary with the interspersion of rest days in the collection to restore the sperm reserve.

Graph 5 shows the variation in total motility and progressive motility of frozen-thawed semen collected from the 3rd ram of the **Milk Palas** breed.

During the normal breeding season, a very high variability is observed in both total and progressive motility, and the differences between the 2 motilities are between 7.2% and 14.6%.

Of the 4 ejaculates collected in September, one ejaculate was removed and 3 were kept in the cryobank stock, and the fertility rate after artificial insemination was to be correlated with progressive motility to evaluate the lowest value of progressive motility at which acceptable fecundity indices are obtained.

In the breeding season (March 2024), 3 ejaculates had total motility of over 35% (39.5%- first harvest, 63.8%- 2nd harvest and 49.2%- 4th harvest). Of these, in one ejaculate, respectively in the 3rd harvest, a progressive motility of only 2.9% is observed. The decrease by 30.8% is probably due to a processing error, since on the monitor image it is observed that the spermatozoa have fast but static movements or with a reduced forward speed, which was not observed in the other ejaculates. This ejaculate was kept in the cryobank reserve, to be used for artificial insemination of goats to check the gestation rate and confirm whether it was just a processing error.



SN – normal season, CS – off-season

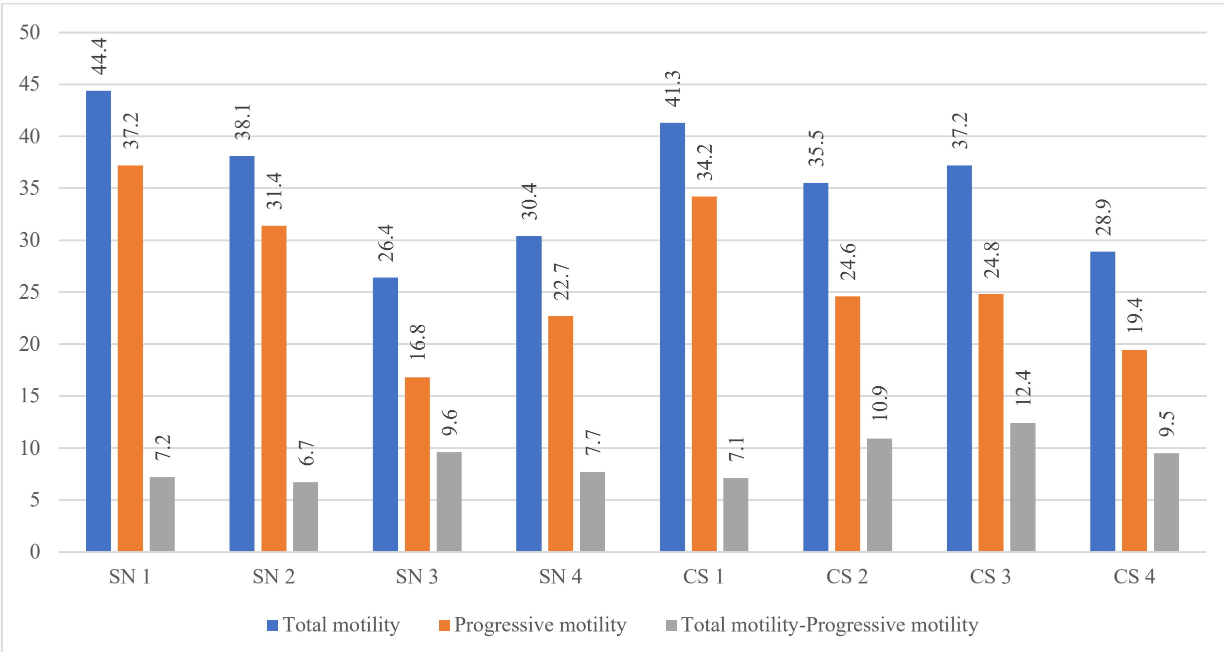
Graph 5. Variation of total motility and progressive motility of frozen-thawed semen collected from the Milk Palas 3 ram in the breeding season and off-season / ***Variația motilității totale și a motilității progresive a materialului seminal congelat-decongelat recoltat de la berbecul de rasa Lapte Palas 3 în sezon și contrasezon de reproducție***

From the analysis of the quality of the semen collected in the two seasons, it can be concluded that the 3rd ram of the **Milk Palas** breed is suitable for freezing ejaculates, with better results being obtained in the off-season when the rams are not used for mating. It is recommended to check the ejaculates of this ram in the breeding season but when he does not also mate the ewes, thus having a control regime of ejaculations.

Graph 6 presents the evolution of the total motility and progressive motility of the frozen-thawed semen collected from the 4th ram of the **Milk Palas** breed.

In the ejaculates collected during the breeding season, the one corresponding to the 3rd collection had a motility after freezing-thawing of 26.4% and was removed from the semen cryobank and the 4th ejaculate, with a total motility of 30.4% was kept in the cryobank reserve stock. The other 2 ejaculates had a total motility of 44.4% (first collection during the breeding season) and 38.1% (2nd collection). In these 2 ejaculates with total motility of over 35%, a progressive decrease in motility is observed with small percentages of 7.2% and 6.7%.

In the ejaculates collected in the off-season of reproduction, it is observed that only the 4th collection has a total motility of 28.9% and is removed from the sperm cryobank. The other 3 ejaculates have a total motility of over 35% and the progressive motility is between 24.6% and 34.2%.



SN – normal season, CS – off-season

Graph 6. Variation of the total motility and progressive motility of the frozen-thawed semen, collected from the Milk Palas 4 ram in the breeding season and off-season / ***Variația motilității totale și a motilității progresive a materialului seminal congelat-decongelat, recoltat de la berbecul de rasa Lapte Palas 4 în sezon și contrasezon de reproducție***

The analysis of the motility values obtained in the two reproduction seasons, with values of over 35% in total motility and values of 25% in progressive motility, recommends keeping this ram for collecting semen for freezing and is also a candidate for research on establishing the minimum level of progressive motility depending on the fertility rate of inseminated females.

Based on the analysis of the evolution of total and progressive motility of semen preserved in frozen and refrigerated form and by analysing the fertility rate obtained following artificial insemination, the minimum threshold for progressive motility will be established so that the sperm thus processed can be used or stored in the cryobank.

CONCLUSIONS

1. The quality and suitability for freezing of semen is influenced by the individual and the breeding season.
2. Semen collected from **Palas Merino** rams recorded higher values of total and progressive motility in the breeding season (total motility: 64.1%-43.2%, progressive motility: 47.7%-31.8%) compared to the non-breeding season (total motility: 44.5%-39.4%, progressive motility: 32.5%- 27.1%).

3. Semen from **Milk Palas** rams showed large differences between total and progressive motility and a high total motility does not guarantee a proportional progressive motility (example: total motility: 63.8% and progressive motility: 46.2%, total motility: 38.5% and progressive motility: 24.3%).
4. In the semen of **Milk Palas** rams, large differences in total motility are observed between successive collections (56.5% - 23.2%) from the breeding season compared to collections from the counter-breeding season (41.5% - 28.2%).
5. No direct correlation was established between total motility and progressive motility of frozen-thawed semen regardless of the breeding season in which the sperm was collected and processed. Research activity to establish the minimum value of progressive motility for the two forms of ram semen preservation is in progress.

ACKNOWLEDGEMENTS

The results presented in this paper are part of the research on establishing a technology for freezing ram and goat semen using state-of-the-art equipment. The purpose of the project, which was started with funding from the state budget - ASAS contract no. 7674 of 06.12.2023, is to establish a sperm cryobank from males of local or imported breeds raised in our country.

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COMPARATIVE RESEARCH ON MEAT PRODUCTION PERFORMANCE IN INTENSIVELY FATTENED LAMBS FROM NATIVE BREEDS

CERCETĂRI COMPARATIVE PRIVIND PERFORMANȚELE PENTRU PRODUCȚIA DE CARNE LA TINERETUL OVIN DIN RASELE AUTOHTONE ÎNGRĂȘAT INTENSIV

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Abstract

*In recent years, there have been significant changes in the breeding direction of native sheep breeds, with increasing concern for enhancing meat production. Thus, sheep breeders in our country have turned to crossbreeding with specialized meat breeds without thoroughly evaluating the current productive potential of the native sheep breeds.In this context, the present study aims to assess the main morpho-productive indices, as well as the performance of intensively fattened male lambs from native breeds (**Tsurcana, Tsigai, Palas Merino, Palas Prolific breed**, and **Palas Meat breed**) and crossbreeds (**Rouge de L'Ouest** × **Palas Prolific breed**, **Rouge de L'Ouest** × **Palas Meat breed**). The average birth weight of male lambs ranged between 3.82 kg/head and 5.29 kg/head, regardless of breed. At the end of the growth and fattening period, the lowest body weights were recorded in the **Tsigai** and **Tsurcana** breeds, at 30.55 kg/head and 31.67 kg/head, respectively. The highest body weights at the end of the growth and fattening period were observed in the crossbreeds **Rouge de L'Ouest** × **Palas Prolific breed** (45.16 kg/head) and **Rouge de L'Ouest** × **Palas Meat breed** (48.16 kg/head). The daily average gain of lambs during the fattening period was 320 g/day in the **Palas Meat breed**, while the crossbreeds achieved a daily average gain of 253.0 g/day (**Rouge de L'Ouest** × **Palas Meat breed**) and 245.71 g/day (**Rouge de L'Ouest** × **Prolific Palas breed**).*
*Among the experimental groups, the **Palas Merino** and **Palas Meat breed** lambs exhibited the lowest feed conversion ratio, both in terms of energy intake (4.37 – 4.78 N.U./kg gain) and crude protein intake (745.01 – 789.06 g D.C.P./kg gain). The obtained results highlight the good current potential of native breeds for meat production and shows the beneficial effect of crossbreeding with specialized meat breeds to improve the economic efficiency of sheep farming operations.*

Key words: breeds, carcass, crossbreeds, fattening.

Rezumat

*În ultimii ani au existat schimbări privind direcția de creștere a raselor autohtone de ovine, crescând semnificativ preocuparea pentru sporirea producției de carne. Astfel, crescătorii de ovine din țara noastră au apelat la aplicarea de încrucișări cu rase specializate pentru producția de carne, fără a testa însă potențialul real actual al raselor autohtone de ovine. În acest sens, lucrarea de față are drept scop studierea principalilor indici morfo-productivi, precum și performanțele obținute la îngrășarea intensivă a tineretului ovin mascul din rasele autohtone (**Țurcană, Țigaie, Merinos de Palas, rasa Prolifică Palas, rasa de Carne Palas**) și a unor metiși (**Rouge de L'Ouest** x **rasa Prolifică Palas**, **Rouge de L'Ouest** x **rasa de Carne Palas**). Greutatea corporală medie a mieilor masculi la fătare a fost cuprinsă între 3,82 kg/cap și 5,29 kg/cap., indiferent de rasă. La finalul procesului de creștere și îngrășare, valoarea cea mai redusă a greutăților corporale a fost întâlnită la rasele **Țigaie** și **Țurcană**, acestea fiind de 30,55 kg/cap și respectiv 31,67 kg/cap. Cele mai mari greutăți corporale înregistrate la sfârșitul perioadei de creștere și îngrășare s-au întâlnit la metișii **Rouge de L'Ouest** x **rasa Prolifică Palas** și **Rouge de L'Ouest** x **rasa de Carne Palas**, acestea fiind de 45,16 kg/cap respectiv 48,16 kg/cap. Sporul mediu zilnic realizat de miei în perioada de îngrășare a fost de 320 g/cap la **rasa de Carne Palas**, în timp ce metișii au realizat un spor de 253,0 g/zi (**Rouge de L'Ouest** x **rasa de Carne Palas**) și respectiv 245,71 g/zi (**Rouge de L'Ouest** x **rasa Prolifică Palas**). În cadrul loturilor luate în studiu, lotul de miei din rasa **Merinos de Palas** și din **rasa de Carne Palas** au realizat cel mai redus consum specific atât la energie (4,37 – 4,78 UN/kg spor), cât și la proteină (745,01 – 789,06 g PBD/kg spor).*

Rezultatele obținute evidențiază potențialul actual bun pentru producția de carne al raselor autohtone și ilustrează efectul benefic al încrucișării cu rase specializate pentru producția de carne, în vederea rentabilizării exploatațiilor de ovine.

Cuvinte cheie: carne, îngrășare, metiși, rase.

INTRODUCTION

Native sheep breeds are generally breeds with high production mixed, presenting a high degree of variability of individuals, characterized through the existence of certain specimens with visible traits for meat or milk production.

The continuous scientific research in the field, relating to the improvement of native sheep breeds in the direction of meat production, seeking methods to increase the degree of capitalization of the potential productive by selection or by creating lines with consolidated genetics for meat production.

Many studies made in Romania (Ciolcă N. et al. 1972, Mireșan E. et al. 1989, Pascal C. 2007) have proven the fact that biological potential for meat production in sheep differs significantly, the differences existing between breeds due both specific precocity as well as fattening technology used.

The local breeds traits, **Tsurcana** and **Tsigai**, for meat production was studied by Pascal C. et al. (2012) for the semi-intensive fattening. In this way, by fattening for 175 days of lambs, the indigenous sheep breeds have achieved a daily average gain of 140.0 g for lamb's males of the **Tsigai breed**, 125.0 g in females of the **Tsurcana** breed, the variety black and 116.0 g in lambs males of the **Tsurcana breed**, the variety white.

After slaughter, the carcasses were evaluated, and the comparison of the results obtained with other scientific references led to the conclusion that both breeds have limited possibilities to produce high-quality carcasses, because the proportion of carcasses in the U and R categories of the EUROP grid was below 10%, those classified in the P category were over 20%, and the classification in the higher categories was null.

Another study conducted within Palas - Constanța Institute followed the evolution of body weight, feed conversion and slaughter results on batches of lambs from local breeds subjected to intensive fattening. In this way, the research conducted by Călătoiu A. et al. (1975, 1977, 1980) on fattening lambs from the **Merino, Spancă, Tsigai** and **Tsurcana breeds**, tested 3 energy levels (2500, 2750 and 3000 Kcal digestible energy/kg combined feed) and within each energy level three protein levels were ensured. The results obtained revealed that local breeds best utilized feed recipes that provided 2750 kcal E.D./combined feed, the differences between breeds being due to the protein level. In this way, Merino and Spancă lambs better utilized feed recipes with 15.95% protein, while **Tsigai** and **Tsurcana** lambs better utilized recipes with protein levels of 12.65% and 9.35%, respectively.

In recent years, concerns have grown in Romania for increasing the meat production of indigenous sheep breeds, frequently resorting to their crossbreeding with other specialized meat breeds. In this context, it appears appropriate to establish the current potential of indigenous breeds as well as of some crossbreeds obtained for meat production that can be capitalized on various markets.

MATERIAL AND METHOD

The research was carried out within the Palas Constanța Institute on lambs from the current year belonging to the following breeds and genotypes:

- **Palas Merino Breed**
- **Palas Meat Breed**
- **Palas Prolific Breed**
- **Tsurcana Breed**
- **Tsigai Breed** (rusty variety)

- **Rouge de L'Ouest x Palas Prolific Breed**
- **Rouge de L'Ouest x Palas Meat Breed**

The present work aims to study the main morphoproductive indices (body weight at calving, weaning), as well as the performances achieved during intensive fattening of young male sheep from the mentioned breeds and crossbreeds.

After weaning the lambs, the experimental groups were formed of 20 male lambs from each breed, after which they were introduced for fattening, the groups being homogeneous in terms of age and body weight.

The feeding of the batches was done with the same recipe, namely combined feed with an energy value of 2751 Kcal and a protein value of 16%, as well as with alfalfa hay granules, which had 2740 Kcal and 18.5% protein.

The administered feeds were weighed daily, and the unconsumed residues were harvested after a period of 3-5 days for each batch, thus determining the consumability of the administered rations.

Based on the average daily consumption of nutrients within each batch, the energy consumption (UN) and protein (PBD)/kg live weight gain was calculated.

The growth rate of lambs was thus monitored by individually weighing each batch at the beginning of the experimental period, and then monthly until the end of fattening and the achievement of body weights of 30-40 kg.

RESULTS AND DISCUSSIONS

Table 1 presents the evolution of body weight and the average daily gain achieved by **Palas Merino** lambs subjected to intensive fattening.

Table 1. Morphoproductive indices in Palas Merino lambs subjected to intensive fattening / *Indicii morfoproductivi la mieii din rasa Merinos de Palas supuși îngrășării intensive*

Breed	Body weight (kg)			Daily average gain (grams)
	Birth	Weaning	End of fattening period	
	$X \pm S_x$	$X \pm S_x$	$X \pm S_x$	$X \pm S_x$
Palas Merino	3.96 ± 0.13	22.88 ± 0.55	40.63 ± 0.91	290.33 ± 0.64

The data presented show that **Palas Merino** lambs had an average body weight of 3.96 kg at calving and 22.88 kg at weaning. During the intensive fattening period, male lambs achieved an average daily gain of 290.33 g, with a live weight at the end of fattening of 40.63 kg/head. Table 2 presents the morphoproductive indices of male lambs of the **Palas Meat Breed**, subjected to intensive fattening.

Table 2 presents the morphoproductive indices of male lambs of the **Palas Meat Breed**, subjected to intensive fattening.

Table 2. Indices morphoproductive characteristics in lambs of the Palas Meat breed stall intensive / *Indicii morfoproductivi la mieii din rasa de Carne Palas îngrășați intensiv*

Breed	Body weight (kg)			Daily average gain (grams)
	Birth	Weaning	End of fattening period	
	$X \pm S_x$	$X \pm S_x$	$X \pm S_x$	$X \pm S_x$
Palas Meat Breed	3.82 ± 0.13	22.75 ± 0.81	40.35 ± 0.82	320.0 ± 0.64

The lambs of the **Palas Meat Breed** weighed 3.28 kg and 22.75 kg at birth and weaning, respectively. During the fattening period, the young sheep achieved an average daily gain of 320.0 g/head, at the end of the period having a final body weight of 40.35 kg/head.

The morpho-productive indices achieved by male offspring of the **Palas Prolific Breed** subjected to intensive fattening are presented in Table 3.

Table 3. Morpho-productive indices in intensively fattened lambs of the Prolific Palas breed / *Indicii morfoproductivi la mieii masculi din rasa Prolifică Palas îngrășați intensiv*

Breed	Body weight (kg)			Daily average gain (grams)
	Birth	Weaning	End of fattening	
	$X \pm S_x$	$X \pm S_x$	$X \pm S_x$	$X \pm S_x$
Prolific Palas Breed	3.83 ± 0.27	22.38 ± 0.91	38, 46 ± 1.74	191.56 ± 0.81

At birth and weaning, the lambs of the **Palas Prolific Breed** had a body weight of 3.83 kg and 22.38 kg, respectively. The body weight at the end of the fattening period was 38.46 kg, achieving a daily gain during the aforementioned period of 191.56 g/day/head.

In the crossbreeds **Rouge de L'Ouest** x **Prolific Palas Breed**, the morphoproductive indices presented in table 4 were obtained.

Table 4. The main morpho-productive indices in crossbred Rouge de L'Ouest x Prolific Palas breed subjected to intensive fattening / *Principalii indici morfoproductivi la metișii Rouge de L'Ouest x Rasa Prolifică Palas supuși îngrășării intensive*

Breed	Body weight (kg)			Daily average gain (grams)
	Birth	Weaning	End of fattening	
	$X \pm S_x$	$X \pm S_x$	$X \pm S_x$	$X \pm S_x$
Rouge de L'Ouest crossbreed x Palas Prolific Breed	4.17 ± 0.10	22.52 ± 1.16	45.16 ± 0.83	245.71 ± 0.61

The table shows that the crossbreeds **Rouge de L'Ouest** x **Palas Prolific Breed** achieved an average daily gain of 245.71 g/head, with a final body weight of 45.16 kg. Also, at calving and weaning, the crossbred lambs had average body weights of 4.17 kg/head and 22.52 kg/head, respectively.

In the **Rouge de L'Ouest** x **Palas Meat Breed** crossbreds, the body weight of lambs at calving is 3.87 kg, as shown in the data presented in Table 5.

Table 5. Morpho-productive indices in intensively fattened Rouge de L'Ouest x Palas Meat Breed crossbreds / *Indicii morfoproductivi la metișii Rouge de L'Ouest x Rasa de Carne Palas îngrășați intensiv*

Breed/Genotipe	Body weight (kg)			Daily average gain (grams)
	Birth	Weaning	End of fattening	
	$X \pm S_x$	$X \pm S_x$	$X \pm S_x$	$X \pm S_x$
Rouge de L'Ouest x Palas Meat Breed	3.87 ± 0.22	22.97 ± 1.14	48.16 ± 1.17	253.0 ± 0.54

Average body weight at weaning of **Rouge de L'Ouest** x **Palas Meat Breed** crossbreds was 22.97 kg, and after the fattening period. The final weight was 48.16 kg, the daily average gain by lambs' crossbreds being 253.0 g/cap.

In the **Tsurcana breed**, the average body weight of lambs at birth and at weaning was 5.29 kg and 24.98 kg, respectively, as shown in Table 6.

Table 6. Morphoproductive indices in Tsurcana lambs subjected to intensive fattening / ***Indicii morfoproductivi la mieii din rasa Țurcană supuși îngrășării intensive***

Breed	**Body weight (kg)**			**Daily average gain (grams)**
	Birth	**Weaning**	**End of fattening**	
	$X \pm S_x$	$X \pm S_x$	$X \pm S_x$	$X \pm S_x$
Tsurcana	5.29 ± 0.30	24.98 ± 1.02	31.67 ± 0.94	228.16 ± 0.01

During the growing and fattening period, the lambs achieved a daily average weight gain of 228.16 g/head, having at the end fattening an average body weight of 31.67 kg/head.

In the **Tsigai breed** (rusty variety), the average daily gain achieved by male lambs is presented in Table 7.

Table 7. Morphoproductive indices in Tsigai breed (rusty variety) lambs subjected to intensive fattening / ***Indicii morfoproductivi la mieii din rasa Țigaie supuși îngrășării intensive***

Breed	**Body weight (kg)**			**Daily average gain (grams)**
	Birth	Weaning	End of fattening	
	$X \pm S_x$	$X \pm S_x$	$X \pm S_x$	$X \pm S_x$
Tsigai (Rusty variety)	4.28 ± 0.14	16.12 ± 0.30	30.55 ± 0.63	206.45 ± 0.51

At birth and at weaning **Tsigai breed** lambs' rusty variety had an average body weight of 4.28 kg and 16.12 kg/head, respectively. The daily average gain achieved during the growth and fattening period was 206.45 g/head, the final body weight being 30.55 kg/cap.

Energy (N.U) and protein (D.P) consumption per kilogram of live body weight of the batches taken into consideration is presented in table 8.

Table 8. Specific energy and protein intake obtained in lambs undergoing the fattening process / ***Consumul specific de energie și proteină obținut la mieii supuși procesului de îngrășare***

Breed/ Genotype	**Specific energy consumption (N.U)**	**Specific protein intake (D.P) (g)**
Palas Merino breed	4.37	745.01
Palas Meat breed	4.78	789.06
Prolific Palas breed	6.72	915.12
Crossbreds Rouge de L'Ouest x Prolific Palas Breed	7.79	1032.58
Crossbreds Rouge de L'Ouest x Palas Meat breed	7.72	990.05
Tsurcana breed	8.39	1059.34
Tsigai breed	5.27	899.98

Among the breeds taken into studies, the lowest specific consumption was found in the **Palas Merino** and **Palas Meat breeds**. Thus, the specific energy consumption in the above-mentioned breeds was between 4.37 N.U and 4.78 N.U, while the specific protein consumption had values of 745.01 – 789.06 g D.P, being lower in the **Palas Merino**. In the other groups, the specific nutrient consumption had values between 5.27 N.U and 899.98 g D.P/kg live weight gain and 8.39 UN and 1059.34 g PBD/kg gain.

CONCLUSIONS

Through comparative research on the performance of meat production in young sheep from native breeds (**Tsurcana, Tsigai, Palas Merino**) and some crossbreeds (**Rouge de L'Ouest** x **Palas Prolific Breed, Rouge de L'Ouest** x **Palas Merino Breed**) subjected to intensive fattening, the following conclusions can be drawn:

1. The body weight of lambs at calving was between 3.82 kg and 5.29 kg, regardless of breed. The late breeds (**Tsurcana** and **Tsigai**) had the highest weight of male lambs at calving, 4.28 kg for the **Tsigai** breed and 5.29 kg for the **Tsurcana** breed. In the other breeds and crossbreeds, the weight of male lambs at calving was between 3.82 kg/head and 4.17 kg/head.
2. At the end of the growing and fattening process, body weight was variable, depending on the breed or genotype. Thus, within the **Tsigai** and **Tsurcana** breeds, the average body weights were 30.55 kg and 31.67 kg/head, respectively.
3. The highest body weights recorded at the end of the growing and fattening period were found in the **Rouge de L'Ouest** x **Palas Prolific Breed** and the **Rouge de L'Ouest** x **Palas Meat Breed**, which were 45.16 kg/head and 48.16 kg/head, respectively. The other breeds achieved final body weights ranging between 38.46 kg/head (**Prolific Palas Breed**) and 40.63 kg/head (**Palas Merino**). The average daily gain achieved by lambs during the fattening period was 320 g in the **Palas Meat Breed**, while the crossbreeds achieved an increase of 253.0 g/day (**Rouge de L'Ouest** x **Palas Meat Breed**) and 245.71 g/day (**Rouge de L'Ouest** x **Palas Prolific Breed**), respectively.
4. The specific nutrient consumption was the lowest in the **Palas Meat Breed** and the **Palas Merino** breed, in which between 4.37-4.78 UN and 745.01-789.06 g PBD were consumed to achieve one kilogram of weight gain.
5. The results obtained highlight the current good potential for meat production of local breeds and illustrate the beneficial effect of crossing with specialized meat breeds in order to make sheep farms more profitable.

ACKNOWLEDGEMENTS

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RESEARCH ON THE PRODUCTIVE PERFORMANCE RECORDED IN WHITE AND PINK LINE LAMBS OF THE KARAKUL OF BOTOSANI BREED

CERCETĂRI PRIVIND PERFORMANȚA PRODUCTIVĂ ÎNREGISTRATĂ LA MIEII DIN LINIILE ALB ȘI ROZ ÎN CADRUL RASEI KARAKUL DE BOTOȘANI

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Abstract

*During the period 2022-2024, on a representative flock of lambs belonging to the **Karakul of Botosani** breed, data on lambing weight and the score obtained in the breeding sheet were processed, representative data within the framework of the control of productive performance in the case of skin production. The biological material studied belongs to RDSSGB Popauti and is registered in the Genealogical Register of the **Karakul of Botosani** breed, being the subject of the multiplication and consolidation of individuals from the two lines in order to homologate them. Currently, the degree of homogeneity of individuals from the White and Pink lines is quite high in terms of curl quality and birth weight, with an average score of 490.8 points in White lambs for breeding 490.3 points for Pink lambs for breeding in 2024, aspects favorable to their homologation.*

Key words: pelts, sheep, Karakul of Botosani, breeding sheet

Rezumat

*În perioada 2022-2024, pe un efectiv reprezentativ de miei aparţinând rasei **Karakul de Botoșani**, au fost prelucrate datele referitoare la greutatea la fătare și punctajul obținut în fișa de bonitare, date reprezentative în cadrul controlului privind performanța productivă în cazul producției de pielicele. Materialul biologic studiat aparține SCDCOC Popăuți și este înscris în Registrul Genealogic al rasei **Karakul de Botoșani**, făcând obiectul înmulțirii și consolidării indivizilor din cele două linii în vederea omologării acestora. În momentul actual, gradul de omogenitate al indivizilor din liniile Alb și Roz este destul de ridicat în ceea ce privește calitatea buclajului și greutatea la naștere, cu un punctaj mediu de 490,8 puncte la mieii de prăsilă din linia Alb și 490,3 puncte la mieii de prăsilă din linia Roz în anul 2024, aspecte favorabile omologării acestora.*

Cuvinte cheie: pielicele, ovine, Karakul de Botoșani, fișă de bonitare

INTRODUCTION

The activity of forming the **Karakul of Botosani** breed began a long time ago, namely in 1948, when the first specimens of **Karakul** males from the USSR, Austria and Germany were brought to RDSSGB Popăuți to be crossed with **Tsurcana** sheep. After an extraordinary work, in 1989 the Certificate of Approval was issued for the recognition of the **Karakul of Botosani** breed with the two varieties (black and greyish). Later, in 2010 the approval of the Brown Line within the breed was achieved and in 2018 the approval of the Gray Line within the **Karakul de Botosani** breed.

The **Karakul of Botosani** breed is a Romanian breed specialized in production of pelts, formed by combining the **Karakul** breed (males from the Bukhara basin – Turkmenistan, Kazakhstan, Germany, Austria, Bessarabia) with females from the black and grayish **Tsurcana** breed, an indigenous breed with coarse wool well adapted to the specific conditions of northeastern Moldova. Of the 7 color lines within the breed, the first four are homologated till now, as follows: black, greyish, brown and grey (Alexandru M.F. et al., 2020, Nechifor I. 2019, Pascal C. et all 1994-2001, Albertyn, L.R. et all 1993, Alemneh T., et. al., 2019).

This breed was selected and subjected to the improvement process almost exclusively for the characters that influence the quality of the pelts. Regarding body maturity, expressed by weight at mating, many publications specify that in Merino breeds, reproduction can proceed without subsequent negative effects if the weight, at the time of mating, represents at least 60–70% of that of adults (Moise et al 2012, Sandu 1993, Stăncescu 2009, Pascal et al. 2005)

Individuals from the White and Pink lines are in the breeding and consolidation phase in view of their homologation, and production performances and compliance with the breed standard contribute significantly to achieving the objective, with homologation expected in 2026.

MATERIALS AND METHODS

The research was conducted on 382 individuals from the active population of the White and Pink lines of the **Karakul of Botosani** breed, representing lambs obtained in 2022, 2023 and 2024. The individuals are registered in the Official Control of production performances of the **Karakul of Botosani** breed and using the official control data, supplemented with direct personal observations, the following were analyzed: total score and birth weight.

Figure 1. Individuals from White and Pink lines of the Karakul of Botosani breed / ***Miei din liniile Alb și Roz, Rasa Karakul de Botoșani***

Once systematized, the data were processed and interpreted using methods specific to such research - arithmetic mean, error of the mean. standard deviation, coefficient of variability, significance tests, using the GraphPad Prism and SPSS Amos statistical programs. Statistics were noted with Latin letters: arithmetic mean (± s), standard deviation (σ).

The complexity of the aspects pursued required the use of a diversified work methodology depending on the aspects pursued, using and respecting the investigation methodology recommended by the specialized literature (Drucker, A.G., et al, 2001).

RESULTS AND DISCUSSIONS

The **Karakul of Botosani** lamb's performance is given by the breeding sheet score, which for lambs from the White and Pink lines was centralized in tables no. 1 and no. 2. The study on the total score highlighted an average score of 480.4 points for those from the Alb line and 486.5 points for those from the Roz line in 2022, with no statistical differences with the scores recorded in 2022 and 2023. This fact shows us that the populations of the two lines are close in terms of productive performance and the individuals from the two lines tend towards uniformity of the quality characteristics of the curl, thus creating opportune premises for their homologation as distinct lines within the breed. Of course, the quality of the curl can always be improved, taking into account the fact that the score recorded in the **Karakul of Botosani** breed can have a maximum of 725 points, but which is currently only reached in some individuals from the Brack and Greyish lines (lines approved precisely in 1989), which subsequently, through selection and improvement work, have currently reached this performance, but which is still manifested in a limited number of individuals within the two lines.

Table 1. Statistics for the characters studied in White and Pink lambs of the Karakul of Botosani breed in 2022-2024 / *Statisticii pentru caracterele studiate la mieii Alb și Roz din cadrul rasei Karakul de Botoșani în anii 2022-2024*

Scores (points)	White line			Pink line		
	2022	2023	2024	2022	2023	2024
N	36	72	55	73	73	73
Minimum	450	410	390	415	385	400
Maximum	545	575	555	600	550	555
± s	**480.4 ± 3.69 ns**	**489.2 ± 4.35 ns**	**481.7 ± 4.66 ns**	**486.5 ± 4.37 ns**	**482.5 ± 4.17 ns**	**485 ± 4.58 ns**
σ	22.14	28.22	34.58	37.33	35.59	39.17

ns - insignificant statistically differences

Table no. 2 summarizes the data on the average performance of curls quality of lambs destined for breeding, belonging to the White and Pink lines of the **Karakul of Botosani** breed. From the data summarized in table no. 2, we can see that the average score of the lambs retained for breeding was generally higher than the total average score, with the exception of 2023 for the Pink line, where an average score of 473.7 points was recorded for breeding lambs, due to our desire to multiply the breeding flock with a larger number of lambs than we initially planned. The performance of future generations of products depends on lamb's quality retained for breeding, and therefore lambs with superior curl quality are predominantly retained for breeding.

Table 2. Statistics for the characters studied in White and Pink lambs retained for breeding within the Karakul of Botosani breed in 2022-2024 / *Statisticii pentru caracterele studiate la mieii Alb și Roz reținuți pentru prăsilă din cadrul rasei Karakul de Botoșani în anii 2022-2024*

Lambs for breeding socres (points)	White line			Pink line		
	2022	2023	2024	2022	2023	2024
N	27	26	36	58	29	55
Minimum	450	460	420	420	425	415
Maximum	545	540	575	600	545	555
± s	**481.8 ± 4.52 ns**	**490.8 ± 4.38 ns**	**490.8 ± 5.17 ns**	**493.5 ± 4.73 ns**	**473.7 ± 6.2 ns**	**490.3 ± 5.02 ns**
σ	23.49	22.35	31	35.99	33.4	37.2

ns - insignificant statistically differences

The selection of lambs for breeding based on birth weight, in the **Karakul of Botosani** breed, will influence, in addition to the size of the skin surface, their subsequent development. Table no. 3 summarizes the data regarding the average birth weight of lambs retained for breeding from the two-color lines.

The retention of lambs for breeding is not only conditioned by the quality of the curl but also by the birth weight, as a higher lamb weight will generate a skin with a larger surface area.

For the years 2022 and 2023, according to the data summarized in table no. 3, there are no significant differences in the average birth weight of both the White and Pink line lambs, with a minimum of 4.037 kg in 2022 for the White line lambs and 4.44 kg in 2023 for the Pink line lambs. In 2024, due to the selection and improvement practiced throughout the period, an average weight of 5.1 kg was obtained for the White line lambs and 5.29 kg for the Pink line lambs, with a maximum of 6.4 kg for the White line and 8.1 kg for the Pink line. By retaining for breeding products with a higher average birth weight, we ensure the improvement of future generations of lambs.

Table 3. Evaluation of the average weight of lambs retained for breeding from the White and Pink lines in 2022-2024 / *Evaluarea greutății medii a mieilor reținuți pentru prăsilă din linii Alb și Roz în anii 2022-2024*

Lambs for breeding birth weight (points)	White line			Pink line		
	2022	2023	2024	2022	2023	2024
N	27	26	36	58	29	55
Minimum	2.3	2.8	3.7	3.2	2.5	2.9
Maximum	5.6	5.4	6.4	6.5	7.2	8.1
± s	4.037 ± 0.17 ns	4.1 ± 0.17 ns	**5.1 ± 0.12** ****	4.641 ± 0.10 ns	4.44 ± 0.17 ns	**5.29 ± 0.14** ****
σ	0.897	0.92	0.71	0.78	0.92	1.02

ns - insignificant statistically differences
**** - very significant statistically differences (*P<0,0001)*

In order to highlight the degree of improvement of the two lines, regarding birth weight, information on the average weight of all products obtained in the years 2022-2024 within the White and Pink lines was centralized in table no. 4 and figure no. 2 shows the evolution of the average birth weight of lambs from the two lines during the studied period.

Table 4. Evaluation of the average weight of lambs from the White and Pink lines in 2022-2024 / *Evaluarea greutății medii a mieilor din linii Alb și Roz în anii 2022-2024*

Birth weight (kg)	White line			Pink line		
	2022	2023	2024	2022	2023	2024
N	36	72	55	73	73	73
Minimum	2.6	2.7	3	2	2	2.9
Maximum	5.6	6	6.4	6.5	7.2	8.1
± s	3.76 ± 0.16 ns	3.962 ± 0.12 *	**4.782 ± 0.11** ****	4.3 ± 0.12 ns	4.364 ± 0.11 ns	**5.085 ± 0.12** ****
σ	0.96	0.7997	0.8336	1.03	0.9334	1.015

ns - insignificant statistically differences
** - statistically significant differences (P <0.05);*
***** - very significant statistically differences (P<0,0001)*

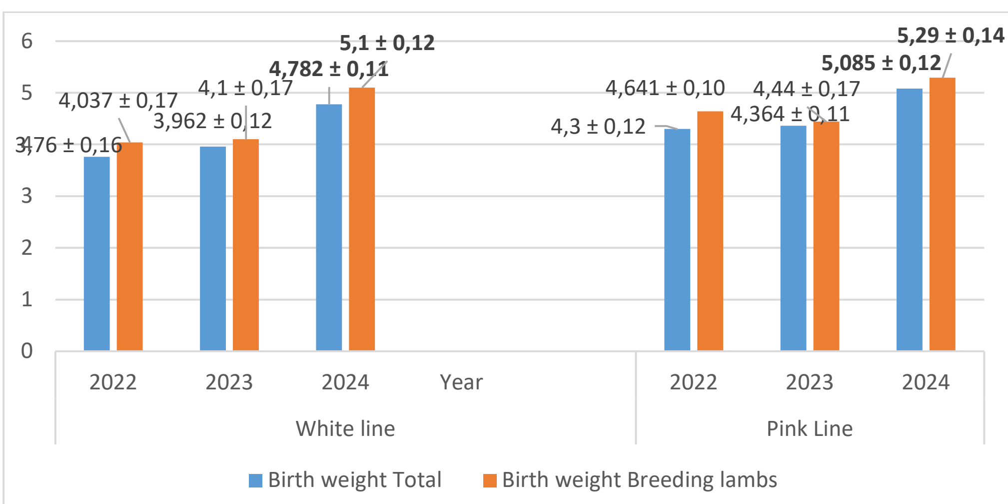

Figure 2. Average birth weight evolution of White and Pink lambs' lines during the analyzed period / *Evoluția greutății medii la naștere a mieilor din liniile Alb și Roz în perioada analizată*

According to the graph in fig. no. 2 and comparing the data centralized in tables no. 3 and 4, we can state that the individuals from the White and Pink lines obtained in 2024 had a higher average birth weight compared to those obtained in previous years, obtaining an average of 5.085 kg for the lambs retained for breeding from the Pink line and 5.1 kg for the lambs retained for breeding from the White line. The selection of breeding individuals based on body weight ensures the massiveness of the two lines and the increase in the development capacity of the next generations.

The individuals from the White and Pink lines are part of a research project aimed at their homologation, and based on the results obtained regarding birth weight and skin quality, we can state that we are on the right track regarding the opportunity of their homologation as soon as possible, respectively in 2026.

CONCLUSIONS

The following conclusions can be drawn from the study:

1. Individuals from 2024, both those from the White and Pink lines, had improved results in terms of birth weight, with an average weight of 4.78 kg for white lambs and 5.09 kg for pink lambs.
2. In each analyzed season, the average weight of individuals retained for breeding was higher than the average weight of the total lambs obtained in the season, and this aspect confirms the fact that a very important criterion in individuals selection for breeding is birth weight, the higher it is, the larger skin surface area the lambs will have.
3. The quality of the pelts is given by the score obtained in the breeding sheet and during the analyzed period, although there were no significant differences between seasons, the individuals retained for breeding had a higher score than the group average.
4. The fact that the lambs from the White and Pink lines do not record significant differences in the quality of the curl during the analyzed period confirms that the individuals from the two lines are homogeneous in terms of the quality of the curl, being a genetically consolidated population with the possibility of homologation.
5. Considering that the populations of the two lines are consolidated, we can focus our attention in the future on improving the production traits and improving the aspects related to the quality of the curl, because the maximum score limit is 725 points for the **Karakul of Botosani** breed.

ACKNOWLEDGEMENTS

White and Pink lines are an important category of **Karakul of Botosani** sheep, and this study helps us to complete the documentation for their homologation, documentation developed within the ADER 7.3.1 project.

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PRECISION LIVESTOCK FARMING AND REPRODUCTIVE BIOTECHNOLOGIES – MODERN SOLUTIONS FOR OPTIMIZING REPRODUCTIVE MANAGEMENT AND ENSURING NATIONAL FOOD SECURITY

ZOOTEHNIA DE PRECIZIE ȘI BIOTEHNOLOGIILE DE REPRODUCERE – SOLUȚII MODERNE PENTRU OPTIMIZAREA MANAGEMENTULUI REPRODUCTIV ȘI ASIGURAREA SECURITĂȚII ALIMENTARE NAȚIONALE

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Abstract

Precision livestock farming and reproductive biotechnologies, such as the use of sexed semen in dairy cows, play a crucial role in improving reproductive performance and increasing the economic efficiency of farms. A preliminary study was conducted in two dairy farms in northeastern Romania, which implemented the SenseHub® Monitoring system, based on sensors, to optimize reproductive management. The study included 40 nulliparous heifers undergoing their first artificial insemination, with procedures carried out by trained personnel based on data generated by the monitoring system. The results showed a statistically significant increase in conception rates in heifers inseminated using this system compared to conventional estrus detection methods. The implementation of such solutions—based on continuous monitoring, artificial intelligence, and real-time data analysis—allows for the precise identification of the optimal insemination time, reducing economic losses associated with inefficient reproductive cycles. Furthermore, optimizing herd genetic structure by increasing the proportion of female calves contributes to enhancing the productivity of future dairy cow generations and ensuring farm sustainability. Thus, applying modern monitoring and reproductive strategies in farms represents a viable solution for increasing the competitiveness of the livestock sector and ensuring national food security.

Keywords: **precision livestock farming, reproductive management, biotechnologies, sexed semen.**

Rezumat

Zootehnia de precizie și biotehnologiile de reproducere, precum utilizarea materialului seminal sexat la vacile de lapte, joacă un rol esențial în îmbunătățirea performanțelor de reproducție și creșterea eficienței economice a fermelor. Studiu preliminar a fost efectuat în două ferme de vaci de lapte din nord-estul României, care au implementat sistemul SenseHub® Monitoring, bazat pe senzori, pentru optimizarea managementului reproductiv. În studiu au fost incluse un număr de 40 de femele nulipare aflate la prima însămânțare artificială. Procedurile au fost realizate de personal specializat, pe baza informațiilor generate de sistemul de monitorizare. Rezultatele au indicat o creștere semnificativă statistic a ratei de concepție la femelele însămânțate, comparativ cu metodele convenționale de detecție a estrului. Implementarea unor astfel de soluții – bazate pe monitorizare continuă, inteligență artificială și analiza datelor în timp real – permite identificarea precisă a momentului optim pentru inseminare, reducând pierderile economice asociate cu ciclurile de reproducție ineficiente. În plus, optimizarea structurii genetice a turmelor prin creșterea procentului de vițele contribuie la îmbunătățirea productivității viitoarelor generații de vaci de lapte și la sustenabilitatea fermelor. Astfel, aplicarea *strategiilor moderne de monitorizare și reproducere în ferme reprezintă o soluție viabilă pentru creșterea competitivității sectorului zootehnic și asigurarea securității alimentare naționale.*

Cuvinte cheie: zootehnie de precizie, management reproductive, biotehnologii, material seminal sexat.

INTRODUCTION

Beef and dairy play a key role in food security and agricultural development globally and nationally. Increased competition for land and environmental water brings long-term structural sustainability issues for the global livestock sector (Bewley et al., 2017). In this context, enhancing efficiency within livestock production systems is paramount. Precision livestock farming (PLF) has emerged as a transformative approach, defined as the use of integrated technology to obtain relevant information from animals, thereby assisting in their management; it may also be referred to as precision farming, precision agriculture, or precision animal agriculture (Menendez et al., 2023; Monteiro et al., 2021; Tadele et al., 2025). This definition encompasses the broad applications of sensing technology in diverse production settings. Although pig and poultry production are commonly regarded as intensive systems, the dairy sector has been a particularly active adopter of PLF technologies, with a significant portion of PLF research originating from the bovine industry (Tzanidakis et al., 2023).

The core objective of PLF systems is the continuous, automated monitoring of animals or their environment, focusing on data collection, subsequent analysis, and the development of diagnostics pertinent to the overall production system, such as fertility status, lameness detection, or health alerts, ultimately providing actionable feedback to farm management (Morrone et al., 2022; Tzanidakis et al., 2023). The ultimate goal of PLF is to enhance the productivity and well-being of individual animals and the herd by continuously monitoring key parameters related to the producer, the production site, and the animals themselves (Simitzis et al., 2021). This involves tracking housing conditions, pen or herd parameters, and individual animal productivity metrics, often integrating data from existing farm management software (Bramley et al., 2022). While not always utilized for real-time on-farm decisions, individual performance data can be aggregated voluntarily or through paid services, allowing the industry to better understand production dynamics and respond to regulatory and consumer pressures. Continuous monitoring and subsequent data analysis enable the quantification of how various factors translate into bulk tank parameters and milk quality traits, facilitating targeted feedback to producers for enhanced performance while upholding animal well-being (Bramley et al., 2022).

The dairy industry is one of the most dynamic and innovative agribusiness sectors. Dairying significantly contributes to food security, economic development, livelihoods, and the alleviation of rural poverty. Modern dairy herds have been intensely selected and managed for high milk yield and production efficiency. High-yielding breeds, sophisticated management practices, advanced reproductive, nutritional, and health strategies, alongside stringent biosecurity measures, are increasingly common. However, these advancements often come with a trade-off: compromised fertility and reproductive efficiency, which represent major challenges in many herds worldwide (Bragança & Zangirolamo, 2018; Tolosa et al., 2021). Reproductive efficiency is a fundamental cornerstone for the economic viability and sustainability of dairy production systems, yet it is often suboptimal in high-producing herds. Improving fertility has been an ongoing challenge, with many herds experiencing declines in reproductive performance over time, often linked to environmental, management, nutritional factors, or complex interactions thereof.

Accurate and timely estrus detection is critical for successful artificial insemination (AI) and overall reproductive success (Rial et al., 2022; Sharifuzzaman et al., 2024). Traditional methods, primarily based on visual observation of estrus behaviors, are labor-intensive, time-consuming, and prone to error, especially in large herds or with animals exhibiting subtle or short estrus periods. Missed heats lead to extended calving intervals, reduced milk production per lifetime, and increased culling rates, resulting in significant economic losses. PLF systems, equipped with sensors monitoring activity levels, rumination patterns, body temperature, or other physiological indicators, offer a promising solution for automated, continuous, and accurate estrus detection (Neculai-Valeanu et al., 2025).

Furthermore, reproductive biotechnologies, such as the use of sex-sorted semen (sexed semen), provide tools to accelerate genetic progress and optimize herd structure. Sexed semen allows producers to preferentially generate female offspring from genetically superior dams for replacements, while potentially using beef semen on lower-merit cows to produce more valuable crossbred calves. However, conception rates with sexed semen can sometimes be lower than with conventional semen, making precise insemination timing even more critical for success (Neculai-Valeanu AS & Ariton AM, 2021).

This study aims to evaluate the effectiveness of a commercially available PLF system (SenseHub®) for optimizing reproductive management, specifically focusing on improving conception rates following AI in nulliparous dairy heifers under typical farm conditions in northeastern Romania. We hypothesize that utilizing sensor-based estrus detection leads to more accurate identification of the optimal insemination window, resulting in higher conception rates compared to conventional detection methods. The findings are discussed in the context of enhancing farm efficiency, leveraging reproductive biotechnologies, and contributing to national food security objectives.

MATERIAL AND METHODS

Study Location and Animals This preliminary study was conducted on two dairy farms located in the northeastern region of Romania. Both farms utilized modern dairy management practices. A total of 40 nulliparous dairy heifers (predominantly **Holstein-Friesian** or local breeds crossed with **Holstein-Friesian**) approaching their first breeding cycle were enrolled in the study. The animals were housed and fed according to the standard operating procedures of the participating farms. The study period spanned approximately 6 months during 2024.

Precision Livestock Farming System Both participating farms had implemented the SenseHub® Monitoring system (Allflex Livestock Intelligence/MSD Animal Health). This system utilizes neck-worn sensors that continuously monitor animal activity (e.g., steps, head movements, rest) and rumination patterns. Data collected by the sensors are transmitted wirelessly to a central base station and processed by proprietary algorithms using artificial intelligence to detect deviations from individual baseline behavior. The system generates alerts indicating suspected estrus events, providing an estimated optimal insemination window for each alerted animal via farm management software and mobile applications (Figure 1).

Reproductive Management and AI Procedure. Estrus detection for the 40 study heifers was based primarely on the alerts generated by the SenseHub® system. Farm personnel responsible for reproduction were trained to interpret system alerts and inseminate heifers within the recommended optimal time window provided by the system. Artificial insemination was performed by certified AI technicians following standard protocols established on the farms, using conventional semen from proven bulls. All AI events, including date, time, and heifer ID, were recorded.

Conception Rate Determination. Pregnancy diagnosis was performed on all inseminated heifers approximately 45 days post-AI using transrectal ultrasonography, conducted by experienced veterinarians. The first-service conception rate (FSCR) was calculated as the number of heifers confirmed pregnant after the first AI divided by the total number of heifers inseminated for the first time (n=40), expressed as a percentage.

Comparison Data. The FSCR achieved using the SenseHub® system in the study group (n=40) was compared to the historical first-service conception rate data obtained from the records of the same two farms for nulliparous heifers of similar breed types inseminated during the preceding two years

(2022-2023). During that period, estrus detection relied primarily on conventional visual observation methods by farm staff (e.g., observing mounting behavior, restlessness, mucus discharge).

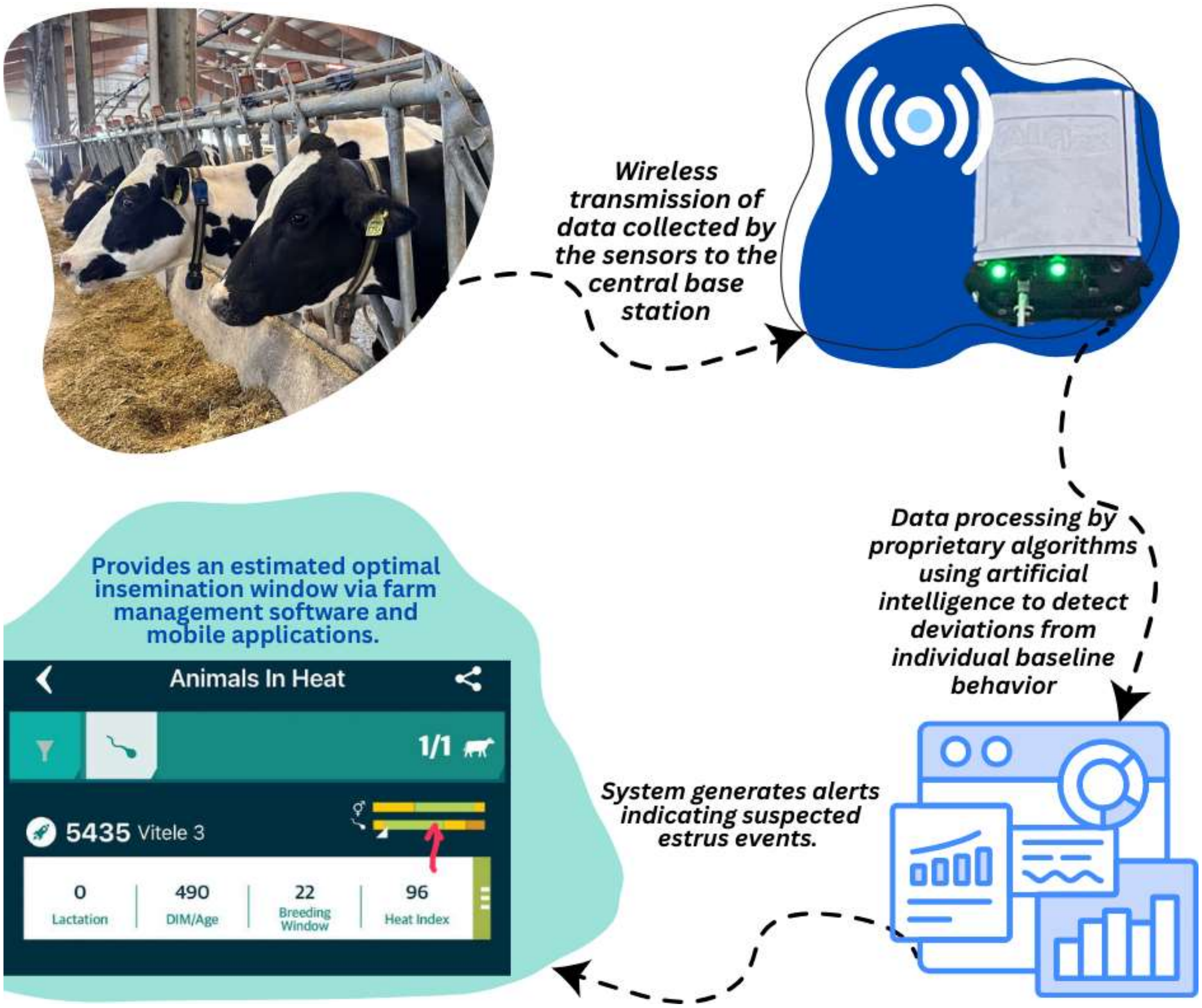

Figure 1. Schematic representation of the Allflex ecosystem / ***Prezentarea schematică a ecosistemului Allflex***

Statistical Analysis. The conception rate observed in the SenseHub® group was statistically compared to the expected conception rate based on the historical farm data using a Chi-square goodness-of-fit test. A p-value less than 0.05 was considered statistically significant. Statistical analyses were performed using GraphPad software.

RESULTS AND DISCUSSIONS

Of the 40 nulliparous heifers monitored and inseminated based on SenseHub® system alerts, 26 were confirmed pregnant at the first service diagnosis. This resulted in a first-service conception rate (FSCR) of 65.0% for the SenseHub® group. Analysis of historical farm records from the preceding two years (2022-2023) for similar nulliparous heifers managed with conventional visual estrus detection indicated an average FSCR of 52.5% on these farms. The Chi-square goodness-of-fit test comparing the observed conceptions (26 out of 40) in the SenseHub® group against the expected conceptions based on the historical rate (expected 21 out of 40). This indicates that the conception rate achieved using the SenseHub® system was significantly higher ($p < 0.05$) than the historical baseline performance achieved with conventional visual estrus detection methods on these farms. The SenseHub® system successfully identified estrus events and provided timely alerts, enabling farm personnel to perform AI procedures within the suggested optimal insemination window for the study animals. No major technical issues hindering the system's performance were reported during the study period.

The results of this preliminary study strongly support the hypothesis that utilizing PLF technology, specifically the SenseHub® monitoring system, significantly improves first-service conception rates in nulliparous dairy heifers compared to the historical performance achieved with conventional estrus detection methods on the same farms. The observed percentage point increase in FSCR (from 52.5% to 65.0%) demonstrates the system's practical efficacy in accurately detecting estrus and, crucially, identifying the optimal time for insemination. This enhanced precision likely overcomes the inherent limitations of visual observation, which can miss estrus events (especially silent or nocturnal heats) or misinterpret behavioral signs, leading to inseminations performed outside the optimal fertility window (Valenza et al., 2012; Stevenson, 2014; Endo, 2022). The significant improvement provides strong evidence for the system's positive impact.

The economic implications of improved conception rates are substantial. A higher FSCR translates directly into reduced days open, fewer inseminations required per pregnancy (lowering semen and labor costs), and ultimately, a shorter calving interval, moving heifers into production more quickly. These factors collectively enhance the overall economic efficiency and profitability of the dairy operation (Burgers et al., 2022, 2023; Krpálková et al., 2020) By minimizing reproductive inefficiency, PLF systems contribute to the farm's sustainability. The successful application of the SenseHub® system highlights the practical benefits of integrating continuous monitoring, data analysis, and artificial intelligence into routine farm management. Such systems provide objective, data-driven insights into individual animal status, allowing for more timely and precise interventions. This aligns with the broader goals of PLF, which aims to optimize production while potentially improving animal welfare through early detection of health or reproductive issues (Bianchi et al., 2022; Das et al., 2023; Trapanese et al., 2025).

Furthermore, the ability of PLF systems to pinpoint the optimal insemination time is particularly relevant when considering the use of reproductive biotechnologies like sexed semen. While sexed semen offers the advantage of controlling offspring sex for targeted herd development, it often exhibits slightly reduced fertility compared to conventional semen (Neculai-Valeanu & Ariton, 2021). Although conventional semen was used in this specific trial, the demonstrated improvement in timing accuracy suggests that PLF systems could be crucial for achieving acceptable conception rates when using sexed semen, thereby fully leveraging its potential for accelerating genetic gain and optimizing herd demographics. This contributes to enhancing the productivity of future generations and supports long-term farm planning.

In the context of Romania and national food security, improving the efficiency of the domestic dairy sector remains vital in 2025. Technologies that boost reproductive performance, like the PLF system evaluated here, can increase milk production efficiency, reduce reliance on imports, and enhance the competitiveness of local farms (Bianchi et al., 2022; Trapanese et al., 2025). By enabling more efficient reproduction and potentially facilitating better use of advanced genetics (including sexed semen in the future), these modern solutions contribute to a more resilient and secure national food supply chain.

This study, being preliminary, has certain limitations. The sample size (40 heifers) is relatively small, and the study was confined to two farms in northeastern Romania. The comparison relied on historical data rather than a concurrent, randomized control group; while common in farm studies, this approach cannot entirely rule out confounding factors related to time (e.g., subtle changes in nutrition or management between the historical period and the study period). Future research should ideally involve larger, multi-farm studies encompassing a randomized controlled trial design if feasible. Further investigation into the specific algorithms and sensor parameters most critical for estrus detection accuracy would also be beneficial. Additionally, a detailed economic analysis quantifying the return on investment for implementing such PLF systems under Romanian conditions, specifically assessing the cost-benefit when used in conjunction with sexed semen strategies, is warranted.

CONCLUSIONS

This study demonstrated that the implementation of the SenseHub® PLF system significantly improved first-service conception rates (65.0% vs. 52.5%) in nulliparous dairy heifers on two Romanian farms compared to reliance on conventional visual estrus detection methods. The findings highlight the substantial potential of modern technological solutions, based on continuous monitoring and real-time data analysis, to optimize reproductive management in dairy cattle by precisely identifying the optimal time for artificial insemination. Improving reproductive efficiency directly impacts farm profitability and sustainability. When potentially combined with reproductive biotechnologies like sexed semen, PLF systems can further enhance genetic progress and herd management strategies. The adoption of such modern monitoring and reproductive approaches represents a viable pathway for increasing the competitiveness of the Romanian livestock sector and contributing positively to national food security.

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THE INFLUENCE OF DIFFERENTIATED FERTILIZATION OF NATURAL GRASSLANDS ON GREEN MASS PRODUCTION AND QUANTITATIVE AND QUALITATIVE MILK PRODUCTION IN SHEEP OF THE TSIGAI BREED

INFLUENȚA FERTILIZĂRII DIFERENȚIATE A PAJIȘTILOR NATURALE ASUPRA PRODUCȚIEI DE MASĂ VERDE ȘI A PRODUCȚIEI CANTITATIVE ȘI CALITATIVE DE LAPTE LA OILE DIN RASA ȚIGAIE

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Abstract

*According to the data provided by the last Agricultural Census carried out in our country, permanent pastures and hayfields lost approx. 21% of their existing area in 1990 as a result of their abandonment and improper use. Efficient management of natural grasslands is essential for optimal animal nutrition, mitigation and adaptation to climate change, biodiversity conservation, landscape management and soil fertility. This study aimed to investigate the effect of differentiated fertilization of permanent pastures in the Moldavian Plateau area on green mass production and quantitative and qualitative milk production in sheep of the **Tsigai** breed, the rust variety. The experiments were carried out within the RDSSGB Secuieni Bacău during 2023-2024. The best results in terms of pasture productivity and quality were obtained when using manure fertilization (15 tons/hectare) associated with over-seeding with valuable fodder plants (perennial grasses and legumes). In terms of quantitative and qualitative milk production, the best results were achieved by sheep fed with green mass from pasture fertilized with chemical and organic fertilizers ($N_{80}P_{50}K_{50}$/hectare + 7.5 tons of manure/hectare).*

Key words: *fertilization, green mass, natural meadows, milk production, sheep*

Rezumat

*Conform datelor furnizate de ultimul Recensământ Agricol efectuat în țara noastră pășunile și fânețele permanente au pierdut cca. 21% din suprafața existentă în anul 1990, ca urmare a abandonării și a utilizării lor defectuoase. Gestionarea eficientă a pajiștilor naturale este esențială pentru hrănirea optimă a animalelor, atenuarea și adaptarea la schimbările climatice, conservarea biodiversității, gestionarea peisajului și fertilitatea solului. Acest studiu și-a propus să investigheze efectul fertilizării diferențiate a pășunilor permanente din zona Podișului Moldovei asupra producției de masă verde și a producției cantitative și calitative de lapte la oile din rasa **Țigaie**, varietatea ruginie. Experiențele au fost efectuate în cadrul SDCOC Secuieni Bacău în perioada 2023-2024. Cele mai bune rezultate din punct de vedere al productivității și calității pășunilor s-au obținut în cazul utilizării fertilizării cu gunoi de grajd (15 tone/hectar), asociată cu supraînsămânțarea cu plante furajere valoroase (graminee și leguminoase perene). Sub raportul producției cantitative și calitative de lapte rezultatele cele mai bune le-au realizat oile hrănite cu masă verde provenită de pe pășunea fertilizată cu îngrășăminte chimice și organice ($N_{80}P_{50}K_{50}$/hectar + 7,5 tone de gunoi de grajd/hectar).*

Cuvinte cheie: *fertilizare, masă verde**,** pajiști naturale, producție de lapte, ovine*

INTRODUCTION

Permanent grasslands represent 34% of the agricultural area of the European Union, playing a key role in providing a wide range of ecosystem services vital to society. In recent decades, the area of these grasslands has decreased significantly (between 30 and 50%) in many Member States, and is still threatened by land-use change (Schils et al., 2022; Peeters, 2009).

In Eastern Europe, including Romania, political transformations since the late 1980s led to the massive abandonment of permanent grasslands, many of which were left unused and subsequently degraded (Torok et al., 2020). Thus, in the period 2010–2020, the area of pastures and hayfields in Romania decreased by 17.4% (INS, 2024).

The grassland is a multifunctional system with different utilities: fodder production, plant and animal biodiversity, stopping soil erosion, water storage, maintaining groundwater quality, ensuring landscape quality, carbon storage, supplying the soil with biologically fixed nitrogen etc. Permanent grasslands – natural or/and semi-natural – are an important part of the natural heritage, and their use through grazing represents the cheapest source of fodder.

Current research in the field of permanent grasslands focuses on sustainable management and conservation methods, focused on soil health, carbon sequestration and production efficiency. For the sustainable use of these ecosystems, an interdisciplinary approach that integrates climatic, edaphic and vegetation and animal management factors is essential.

The experiences within this work were carried out at RDSSGB Secuieni Bacău during 2023-2024. The aim of this study was to investigate the effect of differential fertilization of permanent pastures in the Moldavian Plateau area on green mass production and quantitative and qualitative milk production in **Tsigai** sheep, the rusty variety.

MATERIAL AND METHOD

The research began in 2023, with the completion of a study on the natural conditions in the Moldavian Plateau area, the identification of permanent grasslands suitable for conducting experiments and the organization of the experimental device (demarcation, fencing and establishment of fields). To achieve the proposed objectives, four experimental plots were set up, each with an area of 1.4 ha, with the following treatments applied: Plot 1 – control, without fertilization; Plot 2 – combined fertilization: chemical fertilizers ($N_{80}P_{50}K_{50}$/ha) + organic fertilizers (7.5 t manure/ha); Plot 3 – organic fertilization: 25 t manure/ha; Plot 4 – organic fertilization (15 t manure/ha) + overseeding.

Plots 3 and 4 were additionally fertilized with simple phosphate (P_2O_5 – 30 kg/ha), given the low phosphorus content of the manure. In experimental plots 2, 3 and 4, agro-ecological maintenance works were also carried out – stone removal, clearing of woody vegetation, weed control – to improve the quality of the available green mass.

To achieve the main purpose of the research, the following steps were taken: analysis of the quantitative and qualitative milk production performances of the **Tsigai** breed (rusty variety) sheep breed; evaluation of the productivity of the meadows and the quality of the forages in the experimental variants. The working methods adopted were those specific to research of this type.

Organization of animal lots. Four batches of sheep were organized, one control batch and three experimental batches. When establishing the batch size, the estimated green mass production to be obtained from the four permanent grassland areas was taken into account, as well as the following factors: breed, age, lactation stage, growing environment etc. Consequently, four batches of animals were established, in the amount of 10 sheep for the control batch and 15 sheep for each experimental batch, eliminating all variation factors.

The 2024 animal experiments were initially planned to be carried out over a period equivalent to the grazing period specific to the area (approx. 6 months). For objective reasons (delay in the acquisition of chemical fertilizers and implicitly the application of fertilization with this type of fertilizer, the difficult acquisition of the plant mixture used for over-seeding and the delay in carrying out this work), the experiments could only be carried out over a period of 4 months, respectively in the months of June-September. The experiments started with monitoring the quantitative and qualitative milk production performances of the sheep in the 4 batches, as well as evaluating the productivity of the meadows and the quality of the fodder in the experimental variants.

Monitoring the quantitative and qualitative milk production performances. The working methods used in the research were appropriate for the objectives of this kind. Thus, the evaluation of milk production performances was based on the application of successive productive controls, at 30-day intervals. At each control interval, the standard method was used, namely AT4, respecting the technical specifications recommended by the International Committee for Animal Recording (ICAR). At each control applied, the amount of milk milked from each female was determined by weighing using an approved instrument with an accuracy of ±5 g, and the estimation of the average total milk production obtained per lactation considered was carried out using the method described by Fleischmann (ICAR, 2018).

Research on milk quality was carried out only during the experiments (4 months), which coincided with the milking period of the sheep. For the analysis of milk quality, determinations were made at each control (5 milk samples/control/control batch and 7 milk samples/control/experimental batches) regarding the chemical composition of the milk in the main constituents, namely dry matter, and within the dry matter the content of fat, protein, lactose and mineral salts. The determination of the chemical composition of the milk samples was carried out in the own laboratory of RDSSGB Secuieni Bacău using the Funke Gerber milk analyzer type LactoStar.

Evaluation of green mass production. The evaluation of the green mass production obtained for each of the 4 permanent grassland plots was done according to the classical methodology (Bărbulescu et al., 1983), by harvesting at each mowing or grazing cycle (once a month), areas of 10 m^2 from 5 points (depending on the degree of uniformity of the vegetation and the area considered) located on the diagonal of the meadow. The production resulting from all the control points is weighed, then divided by the total harvested area and the green mass production per square meter (m^2) is obtained, denoted by G. By multiplying the green mass production per square meter in kilograms from each mowing (G1,2,3....) by the area of one hectare (10,000 m^2), the production per hectare per cycles (C1,2,3....) or mowing is obtained and is expressed in kg/ha. Subsequently, by adding all cycles (C1 + C2 + C3 + ...) or scythes, the total annual green mass production per hectare (C) is obtained, which can be expressed in kg/ha or tons/ha.

Calculation formulas:

a. $C_{1,2,3\,.....} = G_{1,2,3......} \times 10000$
b. $C = C_1 + C_2 + C_3 + ...$

Statistical analysis. The data obtained from the determinations performed were processed according to the classical methodology of each type of determination, and subsequently they were interpreted by comparing them with the data from the specialized literature. The results are presented as mean values ± standard errors of the mean. Microsoft Office Excel 2016 was used to calculate all statistical parameters (mean, standard deviation, coefficient of variability and standard error of the mean values) and the Tukey test to determine the significance of the difference between the mean values. Differences were considered statistically significant at $P<0.05$ and indicated by specific superscripts.

RESULTS AND DISCUSSION

Quantitative and qualitative analysis of milk production

Daily, monthly and total milk yield. The **Tsigai** breed sheep, which constituted the experimental groups, achieved during the 4 months of control an average daily milk production relatively similar to the data available in the literature (Taftă et al., 1997). The results of the average daily milk production by groups and lactation months, recorded following the official control, are presented in Table 1.

The highest average daily production was recorded by the sheep in group 2 (388±17.18 ml), closely followed by the production achieved by the sheep in group 4, then by those in the control group, and in last place by the production achieved by the sheep in group 3. It should be noted that the differences between the groups are insignificant (P>0.05).

Table 1. Evolution of the average daily milk quantity during the period June-September (ml/day) / *Evoluţia cantităţii medii zilnice de lapte pe perioada iunie-septembrie (ml/zi)*

Specification	Control Lot	Lot 2	Lot 3	Lot 4
June	590±19.76	609±30.55	504±12.46	617±14.99
July	498±29.87	505±30.50	574±23.84	508±20.59
August	264±24.11	272±26.53	249±12.96	268±12.23
September	131±8.39	165±10.98	150±8.79	152±7.58
Mean	**371±16.49**	**388±17.18**	**369±9.10**	**386±8.16**
S	**52.11**	**66.50**	**45.48**	**40.80**
CV%	**14.05**	**17.15**	**12.31**	**10.56**

NS – non-significant differences (P>0.05); *significant differences (P<0.05); **distinctly significant differences (P<0.01); ***highly significant differences (P<0.001).

Following the ranking based on the average daily production achieved by the ewes in the experimental batches, the total milk production during the 4 months of the experiment followed the same path in terms of batch ranking (Table 2). The differences between batches in terms of total milk production are also insignificant (P>0.05).

Table 2. Evolution of monthly and total milk quantity during June-September (liters) / *Evoluţia cantităţii lunare și totală de lapte pe perioada iunie-septembrie (litri)*

Specification	Control Lot	Lot 2	Lot 3	Lot 4
June	17.70±0.59	18.28±0.92	17.24±0.72	18.52±0.45
July	15.44±0.93	15.66±0.65	15.62±0.39	15.74±0.61
August	8.20±0.75	8.43±0.82	7.72±0.40	8.31±0.38
September	3.94±0.25	4.94±0.33	4.51±0.26	4.55±0.23
Mean	**45.27±2.03**	**47.30±2.10**	**45.16±1.12**	**47.13±1.00**
S	**6.41**	**8.13**	**5.59**	**4.99**
CV%	**14.15**	**17.18**	**12.38**	**10.59**

NS – non-significant differences (P>0.05); *significant differences (P<0.05); **distinctly significant differences (P<0.01); ***highly significan differences (P<0.001).

The highest total milk production was recorded by the sheep in batch 2 (47.30±2.10 liters), which benefited from green mass from the mixed fertilized pasture (pasture fertilized with chemical fertilizers $N_{80}P_{50}K_{50}$/hectare + organic fertilizers, 7.5 tons of manure/hectare), and the lowest level by the sheep in batch 3 (45.16±1.12 liters), which had as a source of volume feed the green mass obtained from the pasture fertilized only with organic fertilizers (25 tons of manure/hectare).

The results regarding the total milk production obtained during the period analyzed in our experiment are similar to those obtained by Tsiplakou et al. (2010), who found that sheep from the group fed with conventionally obtained fodder recorded an average daily milk production 37.5% higher than that achieved by sheep from the group fed with organic manure-based fodder.

Contrary to the results obtained in the present experience, the results communicated within the ADER 7.1.1/2015-2018 project, carried out within RDSSGB Caransebeș, show that sheep that were fed green mass from the pasture fertilized with manure recorded a milk production with only 2.8% higher (78.95 liters versus 76.81 liters) compared to sheep that were fed green mass obtained from the pasture fertilized with chemical fertilizers. The data on the specific consumption for obtaining one liter of milk are presented in Table 3.

Table 3. Specific consumption per liter of milk / *Consumul specific pe litru de lapte*

Specification	Control Lot	Lot 2	Lot 3	Lot 4
Specific energy consumption (UNL/liter of milk)	4.031	4.029	4.222	4.168
Differences (%) compared to the control group	-	-0.05	4.74	3.40
Specific protein consumption (g PDIN/liter milk)	410.606	427.619	448.173	429.264
Differences (%) compared to the control group	-	4.14	9.15	4.54
Specific concentrate consumption (kg/liter milk)	0.567	0.685	0.718	0.688
Differences (%) compared to the control group	-	20.81	26.63	21.34

From the data presented in table 3, it is found that the best energy consumption was recorded in group 2, which is lower by 0.05% compared to the control group, while the consumption in groups 3 and 4 is higher by 4.74% and 3.4% respectively. From the analysis of protein consumption, it is found that it is higher by 4.14-9.15% in experimental groups 2, 3 and 4 compared to the control group. Regarding the consumption of concentrates, it is found that in experimental groups 2, 3 and 4 the consumption is also higher by 20.81-26.83% compared to the control group.

In conclusion, it appears that the most efficient batch in terms of specific energy consumption is batch 2, which received green mass from the mixed fertilized pasture as feed (pasture fertilized with chemical fertilizers $N_{80}P_{50}K_{50}$/hectare + organic fertilizers, 7.5 tons of manure/hectare), and in terms of protein and concentrate consumption it is the control batch that received green mass from the unfertilized pasture as feed.

Qualitative analysis of milk production. Milk quality is understood as its ability to satisfy consumer requirements. This is expressed through 6 basic and 3 additional components. The basic components of quality are: sensory, nutritional, hygienic-sanitary, psycho-social, technological and commercial. The additional components are: quality constancy, quality perception and quality-price ratio. Data on milk quality in experimental sheep flocks are presented in Table 4.

Table 4. Milk quality results (%) (n=5 in the control group and n=7 in groups 2, 3 and 4) / *Rezultate privind calitatea laptelui (%) (n=5 la lot martor și n=7 la loturile 2, 3 și 4)*

Specification	Control Lot		Lot 2		Lot 3		Lot 4	
	$\overline{X} \pm s_{\overline{X}}$	CV%	$\overline{X} \pm s_{\overline{X}}$	CV%	$\overline{X} \pm s_{\overline{X}}$	CV%	$\overline{X} \pm s_{\overline{X}}$	CV%
Fat	7.95±0.11	2.98	8.17±0.11	3.44	8.08±0.06	2.06	8.15±0.03	2.31
Protein	5.90±0.04	1.35	5.81±0.05	2.34	5.49±0.04	2.01	5.82±0.02	2.10
Lactose	4.48±0.04	1.89	4.61±0.05	3.14	4.58±0.06	3.28	4.56±0.02	3.73
Minerals	0.90±0.02	4.57	0.95±0.02	4.57	0.94±0.02	5.03	0.93±0.01	5.99
Dry Mattter	19.24±0.08	0.90	20.15±0.13	1.71	19.39±0.11	1.53	19.45±0.04	1.57

The analysis of the data in Table 4 shows the following:

- the highest average fat percentage is recorded in group 2 (8.17±0.11%), and the lowest percentage in the control group (7.95±0.11%);
- the highest average protein percentage is recorded in the control group (5.90±0.04%), and the lowest percentage in group 3 (5.49±0.04%);
- the highest average dry matter percentage is recorded in group 2 (19.24±0.08%), and the lowest one in the control group (19.24±0.08%);

These values are within the normal limits of raw whole sheep milk, specific for the **Ţigaie** breed (Taftă, 2008). Following the analysis of the significance of the differences in dry matter content, it is found that it is very significantly higher (P<0.001) in group 2 compared to the control group, very significantly higher (P<0.001) in group 2 compared to group 3 and distinctly significantly higher (P<0.01) in group 2 compared to group 4 (Table 5).

Table 5. Difference in dry matter content (%) and statistical significance between batches / *Diferența privind conținutul în substanță uscată (%) și semnificația statistică între loturi*

Tukey Test	Control Lot	Lot 2	Lot 3	Lot 4
Control Lot	0	0.91***	0.15^{NS}	0.21^{NS}
Lot 2	-	0	0.76***	0.70**
Lot 3	-	-	0	0.06^{NS}
Lot 4	-	-	-	0

NS – non-significant differences (P>0.05); *significant differences (P<0.05); **distinctly significant differences (P<0.01); ***highly significant differences (P<0.001).

The results regarding milk quality obtained during the period analyzed in our experience are similar to those obtained by Tsiplakou et al. (2010), who found that the sheep in the group fed with conventionally obtained feed recorded a dry matter content 8.1% higher than that achieved by the sheep in the group fed with organically obtained feed based on manure, the difference being significant (P<0.05). Similar results to those obtained in the present experience are also those communicated in the ADER 7.1.1/2015-2018 project, carried out within RDSSGB Caransebeș, where it is found that the sheep that were fed with green mass from the chemically fertilized pasture had a dry matter content higher by approx. 2% compared to the sheep that were fed with green mass from the pasture fertilized with manure.

Analysis of green mass production and consumption

The green mass requirement for animals was ensured by administering daily feed rations to the manger. The daily feed ration was weighed in the morning before administration, being administered in two doses/day, respectively in the morning and in the evening. At the same time, uneaten leftovers were weighed every morning in order to calculate feed consumption. Data on the amount of feed administered and the amount of uneaten leftovers were entered daily in the experiment record book, separately for each type of feed and for each batch of animals.

The rations administered to the animals during the experiment are presented synthetically in Tables 6 and 7. During the August-September period, due to the drought, green mass production was considerably reduced and we had to reduce the amount of green mass in the animals' rations by almost half, and the need for nutrients was covered by administering alfalfa hay.

From the analysis of the two tables it can be seen that the ration used for the control group is different from the rations of the experimental groups in terms of the amount of green mass administered, while the amount of concentrates and alfalfa hay is identical for all four groups, the rations differing only in the amount of green mass and the origin of the green fodder (each group of animals received mowed green mass and administered to the manger from the plot specific to the group).

The height of the plants at harvest was approx. 15-20 cm. The indicative daily delimitation of the pasture area to be mowed was made as follows: 75-100 m^2 for 1 UVM (1 large cattle unit = 6.6 sheep) for the first two grazing cycles (mowing); 150-300 m^2 for 1 UVM for the following grazing cycles (mowing).

Table 6. Feed rations used in animal experiments (sheep) in 2024 (kg/head/day) during June-July / ***Rații furajere utilizate în experiențele cu animale (oi) din anul 2024 (kg/cap/zi) în perioada iunie-iulie***

Crt. No.	Specification	Meadow (green mowed grass)	Corn grain	Wheat grain
1.	Control lot (green mass source: unfertilized pasture)	7.0	0.15	0.15
2.	Lot 2 (green mass source: chemically fertilized pasture + manure)	6.7	0.15	0.15
3.	Lot 3 (green mass source: pasture fertilized with manure)	6.7	0.15	0.15
4.	Lot 4 (green mass source: pasture fertilized with manure + overseeding)	6.7	0.15	0.15

Table 7. Feed rations used in animal experiments (sheep) in 2024 (kg/head/day) during August-September / ***Rații furajere utilizate în experiențele cu animale (oi) din anul 2024 (kg/cap/zi) în perioada august-septembrie***

Crt. No.	Specification	Meadow (green mowed grass)	Alfalfa hay	Corn grain	Wheat grain
1.	Control lot (green mass source: unfertilized pasture)	3.3	1.0	0.15	0.10
2.	Lot 2 (green mass source: chemically fertilized pasture + manure)	3.0	1.0	0.15	0.10
3.	Lot 3 (green mass source: pasture fertilized with manure)	3.0	1.0	0.15	0.10
4.	Lot 4 (green mass source: pasture fertilized with manure + overseeding)	3.0	1.0	0.15	0.10

The biomass quantity was calculated according to the classical methodology (repeated mowing method) (Bărbulescu et al., 1984). The biomass obtained per hectare after monitoring the 4 mowing cycles, carried out during the months of June, July, August and September, is presented in Table 8.

From the analysis of the data recorded in Table 8, it is found that the green mass production was different depending on the type of plot. Thus, during the analyzed period (June-September), the highest green mass production was recorded in lot 4 (8840 kg/ha), which is approx. 65.85% higher than the production of the control lot, followed by lot 2 (8050 kg/ha), then by lot 3 (6560 kg/ha) and by the control lot in 4th place (5330 kg/ha).

The results regarding green mass production expressed in dry matter are presented in Table 9, taking into account the 5:1 ratio between green mass and dry matter (Marușca et al. 2014). Regarding the monthly amount of green mass consumed, the results obtained are presented in Table 10.

Table 8. Green mass production during June-September (kg/ha) / ***Producția de masă verde pe perioada iunie-septembrie (kg/ha)***

Specification	Cycle 1 (June)	Cycle 2 (July)	Cycle 3 (August)	Cycle 4 (September)	Production (June-September)	Comparative production (%)
Control meadow	1900	1510	1110	810	5330	100
Meadow Lot 2	2720	2500	1610	1220	8050	151.03
Meadow Lot 3	2310	1950	1300	1000	6560	123.07
Meadow Lot 4	3010	2710	1820	1300	8840	165.85

Table 9. Influence of fertilization type on dry matter (DM) production of pasture plots within the experiment / ***Influența tipului de fertilizare asupra producției de substanță uscată (SU) a loturilor de pășune din cadrul experienței***

Specification	Dry matter (t/ha)	Absolute differences (t)	Percentage differences (%)
Control lot (green mass source: unfertilized pasture)	1.066	-	100
Lot 2 (green mass source: chemically fertilized pasture + manure)	1.610	0.544	151.03
Lot 3 (green mass source: pasture fertilized with manure)	1.312	0.246	123.07
Lot 4 (green mass source: pasture fertilized with manure + overseeding)	1.768	0.702	165.85

Table 10. Monthly quantity of green mass consumed during the analyzed period /
Cantitatea consumată lunar de masă verde pe perioada analizată

Specification	Lots	Amount of green mass consumed (kg/head/day)
June	Control Lot	6.76
	Lot 2	6.57
	Lot 3	6.57
	Lot 4	6.57
July	Control Lot	6.77
	Lot 2	6.58
	Lot 3	6.58
	Lot 4	6.58
August	Control Lot	3.09
	Lot 2	2.80
	Lot 3	2.80
	Lot 4	2.80
September	Control Lot	3.19
	Lot 2	2.90
	Lot 3	2.90
	Lot 4	2.90

The monthly amount of green mass consumed was relatively close in all 4 groups, namely in June it was 6.76 kg/head/day in the animals in the control group and 6.57 kg/head/day in the animals in the experimental groups, in July it was 6.77 kg/head/day in the animals in the control group and 6.58 kg/head/day in the animals in the experimental groups, in August it was 3.09 kg/head/day in the animals in the control group and 2.80 kg/head/day in the animals in the experimental groups, and in September it was 3.19 kg/head/day in the animals in the control group and 2.90 kg/head/day in the animals in the experimental groups.

In conclusion, the best influence on green mass production per hectare had a fertilization variant applied to lot 4 – pasture fertilized with organic fertilizers (15 tons of manure/hectare) + overseeding. The results obtained in our experience are similar to those obtained in the ADER 7.1.1/2015-2018 project carried out within RDSSGB Caransebeș, as well as with other research in the specialized literature (Marușca, 2012).

CONCLUSIONS

Following the activities carried out to achieve the objective of this stage, the following conclusions can be drawn:

1. The highest average daily milk production was recorded by the sheep in batch 2 (388±17.18 ml), closely followed by the production achieved by the sheep in batch 4, then by those in the control batch, and in last place by the production achieved by the sheep in batch 3, the differences between batches being insignificant ($P>0.05$).
2. The highest total milk production was recorded by the sheep in batch 2 (47.30±2.10 liters), which benefited from green mass from the mixed fertilized pasture (pasture fertilized with chemical fertilizers $N_{80}P_{50}K_{50}$/hectare + organic fertilizers, 7.5 tons of manure/hectare), and the lowest by the sheep in batch 3 (45.16±1.12 liters), which had as a source of bulk feed the green mass obtained from the pasture fertilized only with organic fertilizers (25 tons of manure/hectare).
3. The most efficient batch in terms of specific energy consumption is batch 2, which received green mass from the mixed fertilized pasture as volume feed (pasture fertilized with chemical fertilizers $N_{80}P_{50}K_{50}$/hectare + organic fertilizers, 7.5 tons of manure/hectare), and in terms of protein and concentrate consumption it is the control batch that received green mass from the unfertilized pasture as volume feed.

4. Following the analysis of the significance of the differences in milk quality, it was found that the dry matter content is very significantly higher (P<0.001) in batch 2 compared to the control batch, very significantly higher (P<0.001) in batch 2 compared to batch 3 and distinctly significantly higher (P<0.01) in batch 2 compared to batch 4.
5. The highest green mass production over the 4 mowing cycles (June-September) in the pasture plots within the experiment was recorded in batch 4 (8840 kg/ha), which is approx. 65.85% higher than the production of the control batch, followed by batch 2 (8050 kg/ha), then by batch 3 (6560 kg/ha) and by the control batch in last place (5330 kg/ha).
6. The best influence on green mass production per hectare was that by a fertilization variant applied to lot 4 – pasture fertilized with organic fertilizers (15 tons of manure/hectare) + overseeding with a mixture of perennial grasses and legumes.
7. The monthly amount of green mass consumed was relatively close in all 4 groups, respectively in June being 6.76 kg/head/day in the animals in the control group and 6.57 kg/head/day in the animals in the experimental groups, in July being 6.77 kg/head/day in the animals in the control group and 6.58 kg/head/day in the animals in the experimental groups, in August being 3.09 kg/head/day in the animals in the control group and 2.80 kg/head/day in the animals in the experimental groups, and in September being 3.19 kg/head/day in the animals in the control group and 2.90 kg/head/day in the animals in the experimental groups.
8. The research carried out highlighted the fact that the technology of free-range animal maintenance in collective stalls with an outdoor paddock, used in the experiment, led to the good performance of all feeding, watering, milking operations, as well as the possibility of making detailed observations on the behavior of the sheep, the way of utilizing the feed, the assessment of their health status etc.

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MODERN MANAGEMENT STRATEGIES TO REDUCE LABOR DEPENDENCY ON DAIRY FARMS

STRATEGII MODERNE DE MANAGEMENT PENTRU REDUCEREA DEPENDENȚEI DE FORȚA DE MUNCĂ ÎN FERMELE DE VACI DE LAPTE

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Abstract

Labor shortage is one of the biggest challenges for dairy farms in Romania, having a direct impact on their productivity and sustainability. In this context, digitalization and automation become essential solutions to optimize processes and reduce dependence on manual labor. This paper analyzes the organizational and human resources management in six dairy cow farms with different farming systems (intensive, semi-intensive and extensive) in Northeast Romania. The study examines the organization of the daily work schedule on the farm, the efficiency of specialized teams compared to generalized service, and the impact of management strategies on productivity. It also discusses digitization and automation solutions applied in managerial decision-making, highlighting the advantages of using modern technologies to increase operational efficiency. The results of the study demonstrate that the implementation of these technologies contributes to optimizing production processes, reducing costs and improving the safety and quality of dairy products, as well as food security.

Keywords: dairy farms, food safety, automation, precision livestock farming, artificial intelligence.

Rezumat

Lipsa forței de muncă reprezintă una dintre cele mai mari provocări pentru fermele de lapte din România, având un impact direct asupra productivității și sustenabilității acestora. În acest context, digitalizarea și automatizarea devin soluții esențiale pentru optimizarea proceselor și reducerea dependenței de muncă manuală. Această lucrare analizează managementul organizațional și al resurselor umane în șase ferme de vaci de lapte, cu diferite sisteme de exploatare (intensiv, semi-intensiv și extensiv) din nord-estul României. Studiul examinează organizarea programului zilnic de lucru în fermă, eficiența echipelor specializate comparativ cu deservirea generalizată, precum și impactul strategiilor de management asupra productivității. De asemenea, sunt discutate soluțiile de digitalizare și automatizare aplicate în luarea deciziilor manageriale, evidențiindu-se avantajele utilizării tehnologiilor moderne pentru creșterea eficienței operaționale. Rezultatele studiului demonstrează că implementarea acestor tehnologii contribuie la optimizarea proceselor de producție, reducerea costurilor și îmbunătățirea siguranței și calității produselor lactate, precum și a securității alimentare.

Cuvinte-cheie: ferme de vaci de lapte, securitate alimentară, automatizare, zootehnie de precizie, inteligență artificială.

INTRODUCTION

In recent years, the livestock sector in Romania, especially dairy farms, has been facing a major structural challenge: the acute shortage of specialized labor. This problem affects both large farms with intensive production systems and small and medium-sized farms, particularly in rural areas where the migration of the active population has led to a significant decline in available human resources. The

Romanian dairy sector stands at a critical juncture, facing profound challenges that threaten its productivity, competitiveness, and long-term sustainability. Foremost among these is a deepening dependency on and shortage of agricultural labor.

This issue is significantly amplified by the sector's structural characteristics, notably its high degree of fragmentation and the overwhelming prevalence of small, often subsistence-level farms. These farms typically operate with limited resources and face difficulties in achieving economies of scale, making them particularly susceptible to labor market pressures. Farms grapple not only with diminishing labor availability, driven by demographic shifts such as an aging rural population and significant outward migration (especially of younger, potentially more skilled individuals), but also with a qualitative gap in the skills required for modern agricultural practices.

In this context, the economic efficiency, food security, and sustainability of farms are increasingly influenced by their capacity to adapt to new socio-economic and technological conditions. This complex labor situation necessitates an urgent exploration of contemporary solutions capable of mitigating dependency on manual work while enhancing operational efficiency and overall farm performance.

Against this scenario, modern management strategies that include digitalization, process automation, and the use of artificial intelligence are becoming not just innovative options, but imperative necessities for maintaining competitiveness. Technologies ranging from robotic milking systems (AMS) to sophisticated Precision Livestock Farming (PLF) tools offer the potential to automate tasks, optimize resource use, and provide valuable data for improved decision-making.

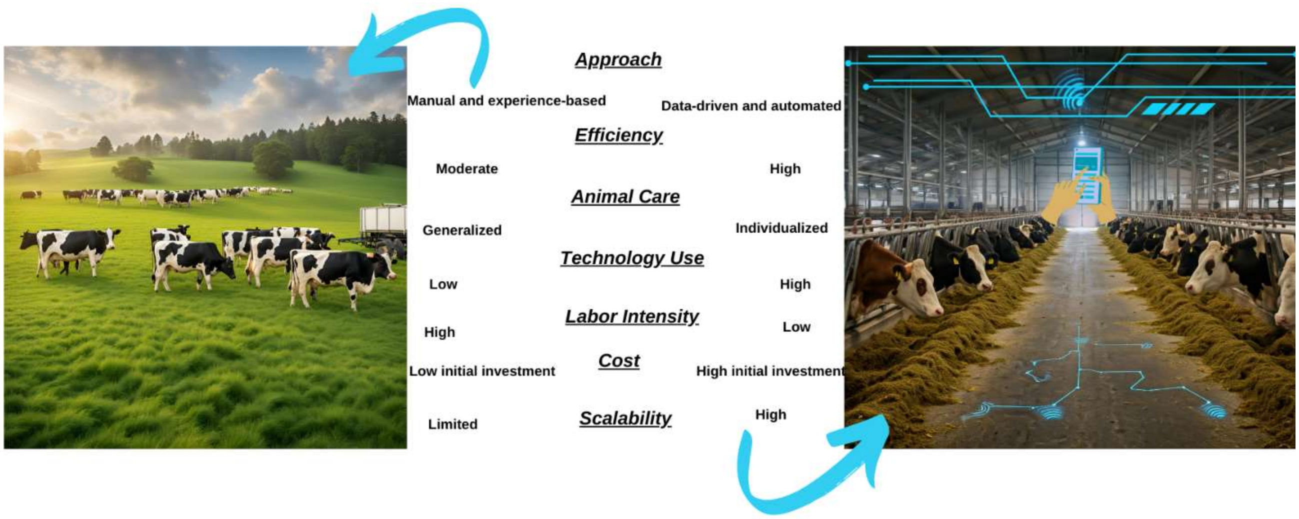

Figure 1. Livestock Management / *Managementul efectivelor de animale*

Precision livestock farming technologies offer effective solutions for optimizing daily activities, reducing operational costs, and increasing product quality, while at the same time reducing dependence on constant human intervention (Giordano, 2024).

The present paper aims to analyze the impact of modern technologies on the organization of work on dairy cow farms, with a focus on organizational and human resource management strategies in the context of reducing the need for physical labor and to assess how digitalization and automation can contribute to reducing labour dependency on dairy farms, to identify the most effective management practices adapted to the Romanian context. The case study is carried out on a selected set of six farms in north-eastern Romania, representing different farming models (intensive, semi-intensive, and extensive).

The research questions clarify how digital and automated technologies affect dairy farm organization and performance. The first question identifies key differences in work organization between automated and traditional farms, addressing task structure, standardization, labor requirements, and responsibility delegation. The second explores digitalization's role in managerial decisions, emphasizing the benefits of real-time data, automated monitoring, and computerized management systems for faster, informed choices. Finally, the third question examines how modern tech impacts productivity and product quality, focusing on digitalization's economic and qualitative effects and potential competitive advantages from these innovations.

Through a comparative analysis of these farms, the paper provides concrete insights into the potential for transforming Romanian farms into a more sustainable, efficient, and resilient agricultural system.

MATERIAL AND METHOD

This paper was based on an applied study conducted on six dairy farms in the north-eastern region of Romania, strategically selected to cover a variety of farming systems - intensive, semi-intensive and extensive. The main objective of this research was to assess the impact of modern management strategies and digital technologies on the organization of daily farm activities, human resource efficiency and productivity in the context of reduced labour dependency.

Table 1. General characterization of the analyzed farms / *Caracterizarea generală a fermelor supuse analizei*

Farm	General location	Size (heads)	Farming system	Technologies used
F1	Iasi county (plain area)	>2.000	Intensive	Multiple milking parlours, mechanized feeding, automated manure management, specialized teams
F2	Neamt county (hilly area)	~1.000	Intensive	DeLaval SenseHub™, DeLaval feeding robot, automatic sprinklers, audio system, Afimilk, microclimate sensing
F3	Iasi county (peri-urban area)	~890	Semiintensive	Mechanized feeding, standard milking parlours, mechanized manure treatment, team organization, strict schedule
F4	Iasi county (Siret river valley)	~1.000	Semiintensive	Mechanized feeding and cleaning systems, natural and forced ventilation, own feed base
F5	Rural region - Iasi (low hills area)	<90	Extensive	Traditional forage, minimal technological equipment, predominantly manual labor
F6	Sirețel commune, Iasi county	<30	Extensive	Basic equipment, lack of automation, mainly manual work

The six farms included in the study were grouped as follows: two intensive farms (F1 and F2), two semi-intensive farms (F3 and F4) and two extensive farms (F5 and F6). They were selected taking into account several factors: location, size, diversity of technologies used, openness to innovation and accessibility for research.

Data collection was mainly through field visits and direct observation, supplemented by semi-structured interviews with farm managers and staff involved in day-to-day activities. Technological sheets and internal farm documents were also used to validate data on work organization, livestock structure and investments in modern equipment.

The aspects analysed included: the structure of human resources (number of employees, qualifications, team organization), the daily work schedule, the technologies used in milking, feeding, animal health and welfare monitoring, and the degree of automation of activities. The level of digitalization of each farm, the presence of real-time monitoring systems (e.g. SenseHub, Afimilk, mobile environmental monitoring applications), as well as management strategies for future investments in technologies were also taken into account.

Comparisons between farms were made according to the type of farming system, linking data on technological inputs with operational outcomes such as labor productivity, resource consumption, dairy product quality, and the ability of the farm to operate efficiently in the absence of an abundant labor force. The analysis aimed to highlight good practices, but also barriers encountered in the transition to digitized and automated farms.

RESULTS AND DISCUSSIONS

Agriculture continues to be a cornerstone of Romanian employment, accounting for a substantial 20.9% of the total workforce in 2020, a figure dramatically higher than the EU average of 4.2%. This highlights the sector's socio-economic importance but also underscores its potential vulnerability to labor market fluctuations and suggests potentially lower labor productivity compared to EU counterparts. Despite this high employment share, the agricultural labor force in Romania is experiencing a rapid decline, potentially shrinking 50% faster than in other sectors like tourism. This contraction points to significant challenges in retaining workers within the agricultural domain.

A critical dimension of this challenge is the demographic profile of the agricultural workforce. It is predominantly elderly, with approximately 59.2% of workers aged between 40 and 64, and a further 9% aged 65 or over. Young people constitute only a small fraction of the workforce, and the proportion of farm managers under 40 is notably low at 10.4%. While aging is an EU-wide agricultural concern, with only 11.9% of EU farm managers under 40, the figures for Romania suggest a particularly acute demographic imbalance. This aging workforce not only poses a critical succession problem for family farms, which dominate the Romanian landscape, but also potentially limits the sector's adaptability to new technologies and practices. The difficulty in performing labor-intensive tasks, such as conserving forages for winter milk production, has been directly linked to the advanced age of many traditional dairy farmers.

In a global context where agriculture is facing multiple challenges, from climate change and diminishing natural resources to labor shortages and economic volatility, the livestock sector is under increasing pressure to adopt advanced technological solutions. In the literature (Yılmaz, 2023), several modern directions are outlined that can help reduce the dependence on manual labor on dairy farms, among which the following stand out: digitalization of processes, automation of repetitive tasks, and the use of artificial intelligence in decision-making.

According to recent studies (Sangode, 2024; Espinoza-Sandoval et al., 2024), labor shortages are one of the most important barriers to the sustainable development of livestock farms. The phenomenon is aggravated by the migration of young people from rural areas and the unwillingness of the working population to work in physically labor-intensive and irregular working hours environments.

In Romania, research shows that this deficit is more acute in rural northeastern regions, where farms are often family-run and have limited access to training and capital for technologization (Popescu et al., 2022). The structure of the Romanian dairy sector, characterized by extreme fragmentation, makes it acutely vulnerable. Over 90% of farms are essentially households with one or two cows, primarily for self-consumption, and family holdings constitute around 87-92% of all farms. These small units rely heavily on family labor, which, as established, is aging and diminishing. This structure inherently limits efficiency, prevents effective farm management, and weakens farmers' bargaining power in the market, often forcing them to accept low prices from processors. Many small dairy farms struggle with low profitability, outdated facilities, lack of investment, and difficulties accessing markets and finance, contributing to a decline in cow numbers and overall milk production.

The implementation of digital technologies - such as sensors for animal health monitoring, milking robots, automatic feeding systems - has become a practical solution to reduce the need for human labor in many European countries. Studies such as the one by Eastwood et al., (2015) showed that farms equipped with milking robots reduced the time needed for daily animal care by up to 30-40%, while increasing milk productivity and quality.

In Romania, the adoption of these solutions is still timid but growing. According to a report by Ministry of Agriculture and Rural Development - Romania (2023), more than 120 farms have integrated "smart farm" technologies, most of them in the central and north-eastern areas.

Classical models of work organization, based on a generalist workforce and manual routines, are gradually being replaced by specialized teams, in which each employee has precise responsibilities and is assisted by technological equipment. The literature (Kimmerer et al., 2024) emphasizes the importance of continuous training of staff and managers who understand the digital agricultural ecosystem.

A key point highlighted by the researchers is that technology does not completely replace human work, but transforms it, requiring new skills (digital, data interpretation, technical maintenance etc.).

The use of artificial intelligence (AI) in agriculture is on the rise, especially for monitoring animal behavior, predicting productivity and optimizing feed. Algorithms can analyze real-time data from a variety of sources (sensors, video cameras, production records) to support quick and accurate decisions by farmers (Tangorra et al., 2024). A relevant example is the CowManager system, which provides alerts on cow health and fertility. Case studies (Doidge et al., 2024, Priyan, 2024) show a reduction in veterinary treatment costs and an improvement in economic returns through early interventions.

The primary goals driving the development and adoption of these technologies include:

✓ Reducing Labor Dependency: Automating repetitive and time-consuming tasks like milking and feeding directly addresses labor shortages and reduces labor costs.

✓ Increasing Efficiency and Productivity: Optimizing processes like milking frequency, feed conversion, and health management can lead to significant gains in milk yield and overall operational efficiency.

✓ Improving Animal Health and Welfare: Continuous monitoring allows for early detection of health issues (e.g., mastitis, lameness, metabolic disorders), enabling prompt intervention and potentially reducing morbidity and mortality. Automation can also offer animals greater freedom and reduced stress.

✓ Optimizing Resource Use: Precision feeding minimizes feed wastage, and optimized health management can reduce veterinary expenses and medication use. Efficient operations may also lower energy and water consumption.

✓ Enhancing Decision-Making: Data gathered by sensors and automated systems, when analyzed effectively, provides farmers with actionable insights for improving breeding strategies, nutritional plans, health protocols, and overall farm management.

✓ Improving Sustainability: By enhancing efficiency, optimizing resource use, and potentially reducing the environmental footprint per unit of milk produced, these technologies can contribute to the economic and environmental sustainability of dairy farming.

The main categories of technologies comprising the PLF/PDF toolkit are Automated Milking Systems (AMS), Automated Feeding Systems, various Herd Monitoring Sensors (wearable and non-wearable), and integrated Farm Management Software (FMS) powered by data analytics, often incorporating Artificial Intelligence (AI) and Machine Learning (ML) (Oliveira et al., 2024).

Organization of work

The differences between the six farms analysed (F1-F6) clearly highlight the direct relationship between the level of technologization and the degree of specialization of the staff.

In intensive farms (F1 and F2), the work is mainly carried out by specialized teams (e.g. milking, feeding, calf care, cleaning, technical maintenance), with a high number of employees with higher education and regularly trained staff. For example, in F1, more than 15% of the employees have university education (veterinarians, engineers), while in F2 rigorous sanitation, milking and management procedures are implemented.

In contrast, the semi-intensive farms (F3 and F4) show a mixed organization: there is a tendency towards specialization, but in practice many employees cover multiple tasks, depending on needs. The lack of specialized staff is compensated by the use of basic technologies (partial mechanization, strict working hours).

On extensive farms (F5 and F6), the work is carried out almost exclusively by non-specialized staff, with an informal organization of tasks. Milking, feeding and sanitation are carried out manually and tasks are not clearly divided into functional teams.

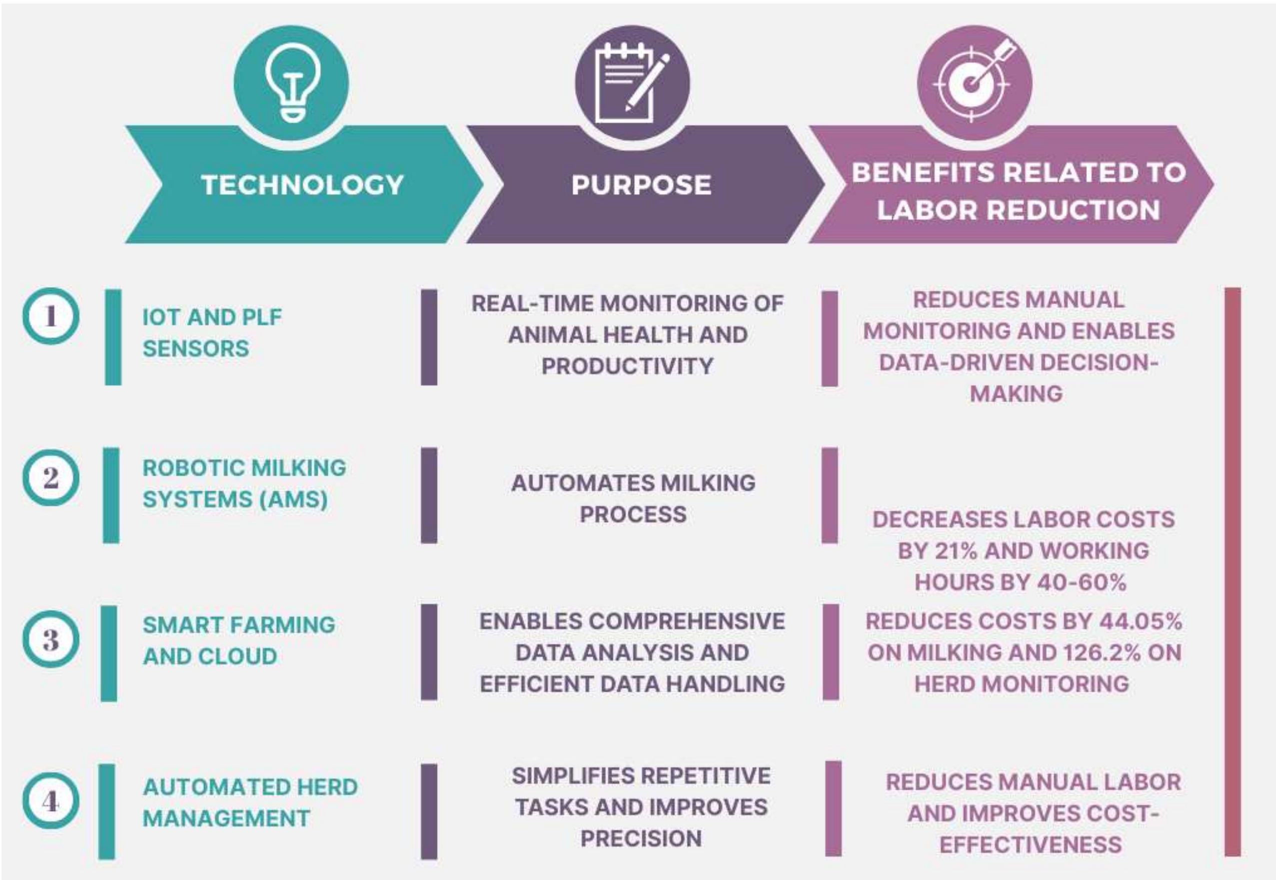

Figure 2. Comparative Analysis of Key Technologies / ***Analiza comprataivă a tehnologiilor cheie***

Addressing labor dependency involves not only exploring technological solutions but also optimizing the management of the existing human capital. Effective labor management practices are crucial for enhancing productivity, ensuring consistency, improving employee retention, and maximizing the return on labor investments, which constitute a significant portion of farm expenses. As farms grow and increasingly rely on non-family employees, the need for structured and professional human resource management becomes paramount. Poor management practices can undermine the potential benefits of even advanced technologies.

Labor efficiency

Labor efficiency varies directly proportional to the level of technologization. On F1 and F2 farms, average labor time/man/day for key activities is significantly lower due to automation. Mechanized feeding and push robots (F2) reduce the time required by 30-40% compared to traditional methods.

Automation demonstrably reduces labor input, especially for milking. Surveys and studies report significant reductions in daily milking time, such as a 62% decrease (from 5.2 to 2.0 hours/day) or savings of 2.5-3.4 hours/day. Overall labor savings associated with automated milking systems are often cited in the range of 20% to 50%, with some estimates suggesting potential savings up to 70%. Automation can also lead to a reduction in the number of employees required; one Canadian survey found a decrease from 2.5 to 2.0 employees (a 20% reduction) after automated milking system adoption. In a striking example, one US farm added 1,200 cows after installing 30 robots without increasing its labor force. However, the nature of labor changes; time is shifted from manual milking to tasks like fetching reluctant cows (a median of 3-4% of the herd per day needed fetching in one survey) and, more significantly, to managing the technology, interpreting data, and troubleshooting. Some studies found that while labor hours decreased, the reduction was not always statistically significant, particularly on larger or non-family farms, suggesting that labor might be reallocated rather than purely eliminated.

Reducing labor dependency and promoting a sustainable future for Romanian dairy farming requires a dual strategy. Smaller farms should focus on improving management skills, access to finance, and infrastructure (like connectivity and irrigation) while adopting low-cost digital tools through enhanced advisory services and cooperatives. For larger, commercial farms, targeted support in advanced automation and PLF technologies, along with data management skills, can yield significant efficiency gains. A uniform, high-tech approach across the diverse sector is unlikely to succeed and may worsen existing inequalities.

The impact of modern technologies

The implementation of digital and automated technologies has brought measurable benefits on both intensive and, in part, semi-intensive farms. The use of monitoring sensors, such as SenseHub™ on the F2 farm, has enabled early detection of health problems and the optimal time for insemination, helping to reduce productivity losses by up to 25% and increase gestation ratesIn parallel, management software, such as Afimilk used in F2 and F1, has supported fast and accurate decision making in terms of treatment application, animal selection and herd reform. The introduction of automatic feeding systems and push robots in F2 also led to an increase of more than 15% in feed consumption per head, which was directly reflected in increased daily milk production.

Farm Management Software serves as the central hub, integrating data streams from milking systems, sensors, automated feeders, and potentially manual inputs. Advanced software utilizes algorithms, often incorporating AI and machine learning, to analyze this vast amount of data, identify patterns, generate alerts for cows needing attention (e.g., for heat, health issues, overdue for milking), and provide comprehensive reports. Examples include Lely Horizon, DeLaval DelPro™, AfiMilk, GEA's platform, and DTM feed management software. These systems empower farmers with data-driven insights for making more informed decisions regarding breeding, feeding strategies, health treatments, culling, and overall herd management. Cloud-based platforms offer the additional benefit of remote access and monitoring via smartphones or computers.

Farmers have seen a number of clear benefits from the adoption of digital and automated technologies, including a reduction in labor requirements by up to 30-40%, a decrease in disease incidence due to better hygiene and constant monitoring, increased predictability in production, and stabilization of work teams by reducing physical effort. However, implementing these solutions is not without its limitations (Kaur et al., 2023). Among the main challenges are the high upfront costs associated with the purchase of equipment and software, the need for continuous training of staff to effectively use the new technologies, the increased dependence on specialized technical support and IT maintenance, and the reluctance to change, especially on traditional farms, where adoption of new technological solutions is often slower.

Pletsch et al. (2024) highlights that technologically advanced farms tend to adopt simple but clear hierarchical structures, in which tasks are delegated to specialized teams, which increases efficiency and allows scaling of the activity without proportionally increasing the number of employees.

The automated farms (F1, F2 - intensive system) are characterized by a clear organization, structured in specialized teams with well defined tasks: milking team, feeding team, maintenance team, veterinary team, etc. The schedule is standardized and correlated with the biological needs of the animals (e.g. compliance with milking, feeding, resting times).

In contrast, in traditional farms (F5, F6 – extensive system), work is organized informally, generalist, with most activities being carried out by a small number of people, often without specialization. Tasks overlap, are distributed based on availability, and activity planning is flexible but less efficient.

The key differences between modern and traditional farms are evident both in terms of organization and daily operations. In large farms, the activity is characterized by increased efficiency, continuity in processes, standardization of operations and a clear delegation of tasks, aspects that contribute to predictable and controlled production management. In contrast, traditional farms often rely on improvisation and multitasking, and the success of activities depends largely on the physical effort and individual experience of staff, which makes processes less homogeneous and more vulnerable to fluctuations in human resources and working conditions.

According to Burda et al. (2021), dairy farms implementing modern technologies (e.g. milking robots, automated management systems) require a profound reorganization of work: new roles emerge (AI operators, maintenance technicians), and farmers become “data managers” rather than physical executors.

Digitalization plays a key role in managerial decision-making, especially in high-tech farms, completely transforming the way data and interventions are managed. By using integrated monitoring systems, such as SenseHub™ or Afimilk, alongside herd management applications and software dedicated to sanitary and veterinary records, managers can access detailed information on production, animal health, reproduction and performance in real time. Thus, digitalization directly contributes to the substantiation of rapid and well-argued decisions, optimizing interventions and reducing the risks associated with uncertainty or delayed reactions. In this context, the research question "What role does digitalization play in the decision-making process at the managerial level?" becomes essential to understand the impact of new technologies on modern livestock management.

This rapid data flow allows for:

• prompt and data-driven decisions, not intuition
• preventive (not reactive) interventions in case of illness or production decline
• optimization of resource planning (feeding, employee rotation, weaning, reform)

Steeneveld et al. (2012) show that farms equipped with sensors and management software make faster decisions based on real data (data-driven decisions), rather than subjective observations. Thus, decisions regarding treatments, reproduction or selection are more accurate, with reduced risk and better economic results.

In traditional farms, where digitalization is lacking, decisions are often made empirically, based on the farmer's direct experience and visual observations. This can lead to delayed interventions and loss of efficiency. Gargiulo et al. (2018) mention that digitalization transforms the role of the agricultural manager into a data analyst, emphasizing the importance of continuous training in related fields: agricultural IT, biological data interpretation, economic analysis.

The use of modern technologies significantly influences the productivity, costs and quality of dairy products, having a direct impact on the overall performance of farms. In terms of productivity, automated farms manage to achieve 30–40% higher average yields per cow, due to the optimization of feeding, milking processes and ensuring increased comfort for animals, which reduces stress and stimulates lactation (Liu et al., 2023). Implementing modern technologies in the dairy sector requires a high initial investment, but it significantly reduces long-term operational costs, particularly labor, by up to 25–35%. Automation minimizes downtime and technological losses, enhancing economic efficiency. The quality of dairy products benefits from better hygiene in milking, early mastitis detection, and consistent maintenance of quality parameters like fat content and protein levels in milk. This raises the question of how technological innovation impacts productivity, costs, and product quality, emphasizing the importance of understanding its role in sustainable performance in livestock.

Dayoub et al. (2024) emphasize that the implementation of technologies such as milking robots, automated feeding systems and AI significantly contributes to increasing productivity per animal, reducing waste and increasing the quality of dairy products, through better control over hygiene and nutrition.

SWOT ANALYSIS

Farm type	STRENGTHS S	WEAKNESS W	OPPORTUNITIES O	THREATS T
F1 – F2 (Intensive)	- High level of technologization - Automation of key processes - High labor efficiency - Specialized teams - Consistent, high-quality production	-High investment and maintenance costs - Need for qualified staff - Dependency on IT infrastructure	- Full robotization extension - Access to European funds - AI integration for decision optimization	- Vulnerability to technological disruptions (IT, energy) - High costs in times of crisis - Unwillingness of older workforce to change
F3 – F4 (Semiintensive)	- Own fodder base - Flexible organization - Moderate costs - Potential for gradual modernization	- Average level of technologization - Sometimes undersized teams - Insufficiently qualified staff	- Efficiency gains through targeted investments - Adoption of core digital solutions - Partnerships with research centers	- Lack of young workforce - Limited access to advice - Competition with fully technologized farms
F5 – F6 (Extensive)	- Low operating costs - Adaptability to natural conditions - Direct links with the rural community	- Lack of modern technologies - High dependence on manual labor - Informal organization - Variable and low output	- Possible conversion to eco or traditional products - Support through agro-rural measures - Re-profiling on local niches	- Depopulation of rural areas - Lack of seasonal labor - Difficulties adapting to EU standards

Figure 3. Comparative SWOT analysis of the three types of farms / ***Analiza comparativă SWOT a celor trei tipuri de ferme***

Concrete examples from practice support the impact of modern technologies on farm performance. In the F2 farm, the introduction of the feeding robot led to an increase of over 15% in voluntary feed consumption per animal, which was directly reflected in a higher milk production per cow. On the other hand, in the F1 farm, the automation of the milking process contributed to maintaining a constant level of milk quality, through a more rigorous control of hygiene and process parameters, which led to a reduction in the incidence of mastitis and, implicitly, to obtaining milk with higher quality standards. These examples highlight how modern technologies support both economic efficiency and improved dairy product quality.

Barkema et al. (2015) show that farms with advanced digitalization have been able to reduce total production costs by up to 20%, even though they initially involved large investments. These savings come from optimizing resource consumption (water, energy, feed) and reducing disease incidence through continuous monitoring.

CONCLUSIONS

The Romanian dairy sector faces a profound labor crisis characterized by an aging workforce, significant emigration, critical skills gaps, and exacerbated by extreme farm fragmentation. This situation poses a direct threat to the sector's productivity, viability, and long-term sustainability. Modernization, through the adoption of improved management strategies and advanced technologies like automation and Precision Livestock Farming (PLF), offers a potential pathway to mitigate labor dependency, enhance efficiency, and improve animal welfare.

Our study demonstrates that technologies such as automated milking systems, automated feeding, and sensor-based monitoring can deliver quantifiable benefits, including significant reductions in labor requirements, potential increases in milk yield, and opportunities for improved animal health management. Modern human resourses management practices, including structured training and standard operating procedures, are equally important for optimizing workforce performance and ensuring operational consistency.

However, the impact and suitability of these solutions are highly context-dependent. High-cost automation is often only economically viable for larger, typically more intensive operations, presenting a significant challenge for the predominantly small-scale Romanian dairy farms. Furthermore, the successful implementation of technology is heavily reliant on the quality of farm management and the ability to effectively utilize the data generated. Profitability is not guaranteed and requires careful planning and adaptation.

In Romania, the adoption of these modern solutions remains very low. Progress is severely hampered by a complex web of interconnected barriers, including prohibitive investment costs, limited access to finance, critical infrastructure deficits (especially rural connectivity and irrigation), pervasive skills gaps and low digital literacy, structural impediments related to small farm size and weak farmer organization, and potentially inadequate or poorly implemented policy support. This systemic lock-in makes modernization exceptionally difficult for the majority of the sector.

To forge a sustainable path forward, a dual strategy is essential. Improving basic management practices, skills, access to finance, and infrastructure is crucial for Romanian dairy farms. Targeted support should also aid smaller farms in adopting advanced technologies. Strengthening the Agricultural Knowledge and Innovation System (AKIS) is vital for training and support, while farmer cooperation can enhance scale and bargaining power.

Ultimately, addressing labor dependency and ensuring the future resilience of Romanian dairy farming demands more than just technological fixes. It requires strategic investment in human capital, infrastructure, and institutional capacity, alongside policies that recognize the diversity of the sector and support a transition towards greater efficiency and sustainability for all types of farms. Failure to address these systemic challenges risks further decline and consolidation, potentially undermining rural economies and food security objectives. The successful integration of management and technology, tailored to context and supported by a robust ecosystem, holds the key to navigating the challenges and unlocking the potential of the Romanian dairy sector.

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RESEARCH ON THE EVOLUTION OF THE MAIN GENETIC FACTORS IN THE NEW BIOLOGICAL CREATIONS FROM R.D.I.S.G.B. PALAS CONSTANTA

CERCETĂRI PRIVIND EVOLUȚIA PRINCIPALILOR FACTORI GENETICI LA NOILE CREAȚII BIOLOGICE DE LA I.C.D.C.O.C. PALAS CONSTANȚA

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Abstract

This paper presents the genetic history of the three new breeds created by the scientific research at the Research and Development Institute for Sheep and Goat Breeding Palas Constanta, namely: ***Palas Milk Breed, Palas Meat Breed*** *and* ***Palas Prolific Breed****. For each breed are presented: index of reproductive isolation, genetic similarity with the founding breeds, the evolution of the interval between generations, inbreeding and subdivision of the breed and the genetic size of each breed (N_e). The study showed that the sheep breeds created at R.D.I.S.G.B. Palas are genetically stable and do not present any danger of degeneration.*

Keywords: reproductive isolation, genetic similarity, generation interval, inbreeding, genetic size.

Rezumat

Lucrarea prezintă istoria genetică la cele trei rase nou create de cercetarea științifică de la Institutul de Cercetare – Dezvoltare pentru Creșterea Ovinelor și Caprinelor Palas Constanța și anume: ***Rasa de Lapte Palas, Rasa de Carne Palas*** *și* ***Rasa Prolifică Palas****. Pentru fiecare rasă sunt prezentate: indicele de izolare reproductivă, asemănarea genetică cu rasele fondatoare, evoluția intervalului între generații, a consangvinizării și subdivizării rasei și mărimea genetică a fiecărei rase (N_e). Urmare a studiului efectuat a rezultat faptul că rasele de ovine create la ICDCOC Palas sunt stabile genetic și nu prezintă pericol de degenerare.*

Cuvinte cheie: izolare reproductivă, asemănare genetică, consangvinizare, mărimea genetică.

INTRODUCTION

The integration of the sheep farming sector in the EU has involved the rapid completion of mandatory steps regarding the improvement of meat and milk production and the quality of the products obtained, a context in which the problem of the exploitation of specialized breeds has become particularly relevant.

Using the local genetic pool and the genetic pool of some imported breeds, research has been carried out at the Palas Research and Development Institute for Sheep and Goat Breeding in Constanta (R.D.I.S.G.B. Palas), in order to create new populations of sheep specialized for meat, milk and high prolificacy.

The concept of breed development based on cross-breeding, which was practiced many decades ago, has today become very successful in the field of improvement of desirable traits of sheep breeds.

After selection, cross-breeding is the second factor that exploits the genetic variation that exists both between and within populations.

The main breeds used for crossbreeding to create the three new breeds are the following: **Friesian**, **Awassi** (for milk), **Ile de France** (for meat) and **Romanov**, **Border Leicester** and **Finish Landrace** (for prolificacy).

Imported specialized sheep breeds have been used in cross-breeding programs in many countries in Europe, resulting in hybrid populations with traits close to the intended objective.

The data on the performance of the breeds used for the creation of prolific populations by crossing and their morphoproductive parameters were communicated by Maijala K., et al. (1977), Nitter (1977), Ricordeau G. (1978). Radu R. et al. (2011) presents data on the improvement of milk production in Romanian sheep breeds following crossbreeding with specialized breeds. Vicovan G. et al. (2009) presents in a paper, aspects regarding the improvement of meat production in Romanian sheep breeds by crossing with specialized breeds. The **Palas Merino** breed, a mixed wool-meat breed, considered the most valuable autochthonous sheep breed created by scientific research in the field, was the basis for the creation of three specialized sheep breeds by crossing with imported specialized breeds, namely: the **Palas Milk Breed**, the **Palas Meat Breed** and the **Palas High Prolificity Breed**, breeds that have been homologated in the last 15 years in Romania.

These breeds have become part of the national genetic heritage, they are bred at R.D.I.S.G.B. Palas Constanta and from them breeding animals with high breeding value are delivered annually to improve meat and milk production in sheep breeding units in the country.

In terms of flocks, they are relatively few in number, and it is necessary to find a breeding approach that leads to the preservation of their genetic structure under the conditions of maintaining inbreeding below 1% per generation.

This paper presents important aspects of the creation of the three breeds and their genetic history.

MATERIAL AND METHOD

The research was carried out on three breeds of sheep, specialised for milk, meat and high fertility, certified as new breeds and bred at the Institute. Each of the three breeds is studied in terms of morpho-productive and reproductive indices, and each year they offer breeding animals of high breeding value to breeders interested in improving their flocks.

The work carried out presents, for each breed, the mode of formation and the results obtained after lambing. The reproduction of the flocks of the three breeds is organised in such a way that inbreeding does not increase by more than 1% per generation, so the flocks of each breed are divided into ram families (10-12), with rotational mating between unrelated or slightly related ram families.

After weaning, a number of lambs are intensively fattened each year until they reach a body weight of 40 kg, and the males with the highest weight gains, over 320 g/day in the **Palas Meat Breed** and over 230 g in the **Palas Prolific Breed**, provide data for testing the progeny of the sires and are used to decide whether to keep the sires for breeding.

The **Palas Milk Breed** is used to breed rams whose mothers, grandmothers by dam and grandmothers by sire, have produced large quantities of milk, 20-30% more than the average of the population, and have had long lactations, more than 270 days.

Palas Milk Breed

The main economic characteristic of the **Palas Merino** breed is its milk production, which is higher than that of local breeds.

The breed was created in 1975 by crossing the **Palas Merino** with the **East Frisian** breed, then with the **Awassi** (from Israel), reproductive isolation for 5 generations and genetic consolidation.

The *coefficient of reproductive isolation (R.I.C.)* is the basic criterion for considering a population of sheep as a breed and is determined according to the formula:

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

where: AA - the number of individuals admitted for reproduction with both parents from their own flock;

AI - the number of individuals with one immigrant parent acquired through purchase or acquisition outside their own flock;

II - the number of individuals with both parent's immigrant.

The *breeding isolation coefficient* of the **Palas Milk Breed** is shown in Table 1.

Table 1. The reproductive isolation coefficient in the Palas Milk Breed / ***Coeficientul de izolare reproductivă la Rasa de Lapte Palas***

Specification	Years				
Reproductive Isolation Coefficient	1988	1993	1998	2003	2022
	+0.137	+0.52	+1	+1	+1

Until 1993, the population had a lower degree of reproductive isolation (1988) and a relative degree of reproductive isolation (1993), and since 1998 a total reproductive isolation was reached, the RIC being +1. Under these conditions, according to Drăgănescu C, 1970, the analysed population with CIR values close to +1 can be considered a breed.

It was homologated as a new indigenous sheep breed in 2010.

At present, the **Palas Milk Breed** is genetically 55% similar to the Friesian, 35% to the Palas Merino and 10% to the **Awassi**, as shown in Table 2, which shows the *degree of similarity* to the founding breeds in the period 1988-2022.

Table 2. The genetic similarity of the Palas Milk Breed with the founder breeds / ***Asemănarea genetică a Rasei de Lapte Palas cu rasele fondatoare***

Breed	R_{xy} (%) of sheep born between 1988-2022 R_{xy} % al oilor născute în intervalul anilor 1988-2022				
	1988	**1993**	**1998**	**2003**	**2022**
Frisian	56	59	57	55	55
Palas Merino	34	30	34	35	35
Awassi	10	11	9	10	10

The genetic similarity of the **Palas Milk Breed** to the founding breeds has remained relatively constant since 1988 and throughout the evolution of the breed. The data on the evolution of the *interval between generations* in this breed (Table 3) show that in the period 1988-2003 the interval on father-son and father-daughter lines was 3.16 and 3.32 years, respectively, lower values compared to the interval on mother-son and mother-daughter lines, which had values of 4.03 and 4.43 years, respectively. For the entire period analysed and for all filiations, the average intergenerational interval was 3.84 years.

Table 3. Evolution of the interval between generations of Palas Milk Breed (1988-2003) / ***Evoluția intervalului între generații la Rasa de Lapte Palas (1988-2003)***

Year	Yearly generation interval				
	Father-son	Father-daughter	Mother-son	Mother- daughter	Average
1988	4.38	4.04	3.40	4.67	4.04
1993	3.05	3.10	4.12	4.05	3.32
1998	2.50	2.32	4.0	3.88	3.13
2003	2.90	2.25	4.58	4.82	3.57
Average 1988-2003	3.16	3.32	4.03	4.43	3.84

The inbreeding and subdivision of the **Palas Milk Breed** (Table 4) shows that in 2003 the breed had low *inbreeding* -3.52%, similar to the **Palas Merino Breed** from which it derives.

Table 4. Evolution of inbreeding of the Palas Milk Breed in the period 1988-2003 / ***Evoluția consangvinizării Rasei de Lapte Palas în perioada anilor 1988-2003***

Specification	Year			
	1988	1993	1998	2003
Total inbreeding – F (%)	0,51	3,69	8,50	3,52
Average inbreeding per generation - ΔF (%)	0,17	0,97	1,75	0,73

The average inbreeding per generation increased from 0.17% in 1988 to 1.75% in 1998 and then decreased to 0.73% in 2003.

The total milk production of the **Palas Milk Breed** is shown in Table 5.

From the table it can be seen that the total milk yield of the **Palas Milk Breed** was 214.57 litres, which was significantly ($p<0.001$) higher ($p<0.001$) than the maternal **Palas Merino** breed by 95 litres and 179.5%, respectively, and the lactation duration of the dairy breed was 69 days longer than that of the **Palas Merino** breed.

Table 5. Total milk production in Palas Milk Breed ewes compared Palas Merino ewes / ***Producția totală de lapte la oile din Rasa de Lapte Palas comparativ oile din rasa Merinos de Palas***

Breed	Total milk yield (litres/head)			Total milk yield litres/head)		Lactation duration (days)			Lactation duration (days)	
	n	X±sx	V(%)	Minim	Maxim	n	X±sx	V(%)	Minim	Maxim
Palas Milk Breed	25	214,57± 8,7813	20,46	155,8	325,7	25	264±2,0704	3,91	248	299
Palas Merino	25	119,53± 5,5340	23,23	83,5	181,1	25	195±2,3452	6,01	155	2

The *genetic size (Ne)* of the breed is given by the formula:

$$Ne=\frac{4Nm \text{ x } Nf}{Nm+Nf}$$
,

where:

Ne – effective (genetic) size;

Nm – the number of reproductively active males;

Nf – the number of females.

The breed has a genetic size of Ne = 60.3 individuals, which gives it a high level of security in the evolution of future generations.

The flock of this breed bred at the Research and Development Institute for Sheep and Goat Breeding Palas Constanta is currently 700 ewes, of which 350 are ewes, 48 are rams and 302 are young males and females.

Palas Meat Breed

A cross between the **Palas Merino** and **Ile de France** breeds, created through rigorous selection.

It was recognized as a native breed in 2012.

After 1989, the population was reproductively isolated, crossbreeding was stopped and the population itself was bred and selected for increased meat production.

Reproductive isolation of the **Palas Meat Breed** was determined using the same formula as presented for the **Palas Milk Breed** (Table 6).

Table 6. The reproductive isolation coefficient in the Palas Meat Breed / ***Izolarea reproductivă la Rasa de Carne-Palas***

Specification	Year								
	1973	1976	1981	1989	1994	1999	2003	2009	2022
Reproductive Isolation Coefficient	-1	+ 0.54	+ 0.81	+1	+ 0.94	+ 0.98	+ 1	+ 1	+ 1

During the period 1973-1981 the breed had a relative degree of reproductive isolation.

Since 1989 and until now reproductive isolation is total.

The *genetic similarity* of the **Palas Meat Breed** to the founding breeds is shown in Table 7.

Table 7. The genetic similarity of the Palas Meat Breed with the founder breeds / ***Asemănarea genetică a Rasei de Carne-Palas cu rasele fondatoare***

Breed	R_{xy} (%) of sheep born between 1989-2009 R_{xy} (%) al oilor născute în intervalul anilor 1989-2009				
	1989	1994	1999	2003	2009
Ile de France	55.67	58.45	53.29	56.67	56.78
Palas Merino	41.25	40.15	39.71	34.10	36.28
Other breeds*	3.03	1.39	6.98	9.19	6.90

* Other breeds (**Romanov, East Friesian, Suffolk**)

The similarity of the **Palas Meat Breed** with the **Palas Merino** was 41.25% in 1989 and decreased progressively in all the analysed periods, reaching about 36% (36.28%) in 2009.

After 2009, the **Palas Meat Breed** did not change its genetic structure, remaining reproductively isolated and genetically stable.

The values of the *interval between the generations* in the period from 1989 to 2009 in the **Palas Meat Breed** are shown in Table 8.

Analysing the evolution of the interval between generations in the period 1989- 2009, it was observed that the average interval for father-son and father-daughter filiations was 3.24 years, respectively 3.12 years, lower than for mother-son and mother-daughter filiations, where it resulted 4.15 years and 4.38 years. For the total period analysed and for the total progeny, the average intergenerational interval for the **Palas Meat Breed** was 3.65 years.

Table 8. The interval between generations of the Palas Meat Breed in the period 1989-2009 / *Intervalul între generații la Rasa de Carne Palas în perioada anilor 1989-2009*

Year	The range in years				
	Father-son	Father-daughter	Mother-son	Mother- daughter	Average
1989	3.31	3.19	4.51	4.30	3.77
1994	4.10	4.01	3.75	3.46	3.83
1999	2.60	2.49	4.65	4.83	3.33
2003	2.85	2.81	4.40	4.70	3.53
2009	3.32	3.12	3.44	4.63	3.78
Average 1989-2009	3.24	3.12	4.15	4.38	3.65

Inbreeding of the ***Palas Meat Breed***

The relatedness of a population (breed) itself means the average relatedness of all its individuals to each other and is due to reproductive isolation, limited size and selection. The individuals of a population (breed) are related to each other at all times, meaning that they are descended from a series of common ancestors that existed previously (in ancestry).

The evolution of total inbreeding and average inbreeding per generation of the **Palas Meat Breed** is presented in table 9.

Table 9. The evolution of inbreeding of the Palas Meat Breed in the period 1989-2009 / *Evoluția consangvinizării Rasei de Carne-Palas în perioada anilor 1989-2009*

Specification	The years analysed				
	1989	1994	1999	2003	2009
Pedigree number	163	33	41	37	62
Total inbreeding – F (%)	5.06	17.42	9.15	14.19	10.00
Average inbreeding per generation - ΔF (%)	1.06	3.30	1.48	2.38	1.04

Total inbreeding increased from 5.06% in 1989 to 17.42% in 1994 and then decreased to 14.19% in 2003 and 10% in 2009.

The average inbreeding per generation was 1.06% in 1989 and after increasing to 3.30% in 1994, it decreased to 1.04% in 2009.

The body weight of ewes and rams of the **Palas Meat Breed** is shown in Table 10.

The table shows that the difference in weight in favor of the **Palas Meat Breed** was 4.26 kg for ewes (7.54%) and 5.63 kg for rams (6.40%). The differences were statistically very significant for ewes ($p<0.001$) and significant for rams ($p<0.05$).

Table 10. The body weight of ewes and rams of the Palas Meat Breed / *Greutatea corporală a oilor și berbecilor din Rasa de Carne Palas*

Breed	Category	Body weight (kg) X±sx	V(%)
Palas Meat Breed	rams	95,23±1,1732	6,28
	ewes	60,75±0,3232	7,41
Palas Merino	rams	89,50±2,8893	13,70
	ewes	56,49±0,5849	13,46

The *genetic size* of the **Palas Beef Breed** *(Ne)* was calculated using the formula presented above for the **Palas Milk Breed**.

Calculation of the genetic size of the breed with 338 females gives a value of 93.11 individuals.

The **Palas Meat Breed** has a herd of 300 ewes, 20 rams, 20 young male and 44 young female.

Palas Prolific Breed

The **Palas Prolific Breed** was created in the period 1973-1988 through a complex program of crossbreeding between the **Palas Merino** breed and major prolific breeds. Many combinations of **Palas Merino** with **Romanov, Friesian, Border Leicester** and **Finnish Landrace** breeds were tested and in 1989 the desired type was obtained.

It was homologated in 2020.

The population has been reproductively isolated since 1990 and has been self-breeding for 7 generations. During this time, selection has been aimed at maintaining a fertility of over 150%, a high lactation capacity and an increase in inbreeding of less than 1%.

The value of the *Reproductive Isolation Coefficient* (RIC) is presented in Table 11.

Table 11. Reproductive isolation in the Prolific Palas Breed / *Izolarea reproductivă la Linia cu Prolificitate Ridicată Palas*

Specification	Year 1973	Year 1978	Year 1988	1989 up to the present
Reproductive Isolation Coefficient (RIC)	- 1.0	- 1.0	- 1.0	+ 1.0

In the period 1973-1988, the value of the Reproductive Isolation Coefficient (RIC) was -1, which led to the fact that the population was opened and used for the reproduction of rams of different breeds until the "desired type" was obtained.

Since 1989 and until now, for more than 7 generations, the breed's (line's) reproductive isolation is total, with a RIC value of +1.

The *genetic similarity* with the founding breeds is presented in Table 12.

To establish the genetic similarity with each of the founding breeds, the following formula was used:

$$\text{Similarity of breed X} = \frac{\text{Number of occurences in the pedigree of X breed}}{\text{Number of pedigree analyzed x 4}}$$

Table 12. Genetic similarity of the Prolific Palas Breed to the parent breeds / *Asemănarea genetică a Rasei Prolifice Palas cu rasele parentale*

Breed	Breed genetic similarity of individuals born in 2015-2017 to the founder breeds (%)		
	Rams	Ewes	Total population
Romanov	38.46	39.22	39.07
Palas Merino	27.08	28.44	28.13
East Frisian	18.69	14.73	15.63
Border Leicester	12.31	8.51	9.36
Ile de France	2.92	7.23	6.25
Finnish Landrace	0.85	1.77	1.56

It can be noted that **Palas Prolific Breed** breed is most genetically similar to the **Romanov** breed (39.07%), then to the **Palas Merino Breed** (28.13%), to the **Friesian** breed (15.63%), to the Border **Leicester** breed (9.36%), to the **Ile de France** breed (6.25%) and to the **Finnish Landrace** (1.56%).

Evolution of the *interval between generations* in the **Prolific Palas Breed** is presented in Table 13.

Table 13. Evolution of the interval between generations / ***Evoluția intervalului între generații***

Specification	Father - son	Father - daughter	Mother - son	Mother - daughter	Average
Intergenerational interval (in years)	3.49	3.94	5.18	5.12	4.49

The intergenerational intervals for father-son and father-daughter consanguinity were 3.49 and 3.94 years, respectively, lower than those for mother-son and mother-daughter lineages, which were 5.18 and 5.12 years, respectively. The average intergenerational interval for total lineages was 4.49 years. The size of this interval indicates that in this breed, as generations evolved, young sheep were retained for breeding from older ewes that had to have at least 5 progenies at first lambing.

The *inbreeding* of the **Palas Prolific Breed** is presented in Table 14.

Table 14. Inbreeding of the Palas Prolific Breed / ***Consangvinizarea Rasei Prolifice Palas***

Specification	Mean values
Total inbreeding (%)- F	4.39
Inbreeding per generation (%)	0.57

Total inbreeding was 4.39% and inbreeding over generations has the value of 0.57%.

The prolificacy value for the **Palas Prolific Breed** is shown in Table 15.

The data in the table show that the prolificacy of primiparous ewes of the **Palas Prolific Breed** was 146.43% and that of multiparous ewes was 153.80%, with ewes weaning 1.40 lambs per ewe lambed.

Table 15. Prolificacy in the Palas Prolific Breed compared with the Palas Merino / ***Valoarea prolificității la Rasa Prolifică Palas comparativ cu rasa Merinos de Palas***

Breed / Category		Prolificacy (%)	Weaned lambs %	Weaned lambs On ewe (head)
Prolific Palas Breed	Primiparous ewes	146.43	91.46	1.34
	Multiparous ewes	153.80	92.81	1.43
	Total ewes	151.13	92.34	1.40
Palas Merino	Primiparous ewes	100.0	95.16	0.95
	Multiparous ewes	115.0	96.0	1.10
	Total ewes	111.0	95.83	1.00

Palas Merino ewes from a breeding flock of the Association for Sheep Breeding in Dobrogea achieved a lower fertility of 46.43 percentage points in primiparous ewes, 36.13 percentage points in multiparous ewes and 49. 13 points on the total population of Merino sheep.

The *genetic size* of the **Palas Prolific Breed** (Ne) determined was 87,7192 individuals.

Currently the total flock of sheep existing at R.D.I.S.G.B. Palas- Constanța is 700 heads, of which 350 mother ewes, 40 rams and 310 young sheep.

CONCLUSIONS

1. As a result of the study it was found that the sheep breeds created at R.D.I.S.G.B. Palas - Constanta are genetically stable and can be further used for the production of rams for milk, meat and lambs per 100 ewes lambed.
2. These breeds produce 100-120 rams per year.
3. The use of **Palas Milk Breed** rams is estimated to increase the milk production of their daughters by 50-53%.
4. The use of **Palas Meat Breed** rams for breeding purposes increases the body weight of the progeny by 17.54% in females and 6.4% in males.
5. The use of **Palas Prolific Breed** rams increases the prolificacy of daughters by 69%.

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