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Aims and Scope

An international scientific journal **"Veterinarija ir Zootechnika"** since 1924 publishes original research and review papers on all aspects of veterinary medicine, food safety and animal sciences. From 1952 to 1994 journal was published under the title: "Acta of Lithuanian Veterinary Academy". After decision of the Research Council of Lithuania under the auspices of the Government of Republic of Lithuania from year 1995 scientific journal „Veterinarija ir Zootechnika" (Vet Med Zoot) was re-established as the Official Organ of the Veterinary Academy (VA) in collaboration with Veterinary Academy, Veterinary Institute, LVA Animal Science Institute, Lithuanian University of Agriculture and Immunology Institute of Vilnius University.

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CONTENTS

The Influence of Ketosis as a Risk Factor on Mastitis Occurrence during Early Lactation Period in Dairy Cows

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Keywords: *bacteria, cows, ketosis, milk, Staphylococcus aureus.*

Abstract. *The study aimed to assess the impact of ketosis in cows during early lactation, immediately postpartum, on the development of mastitis as a secondary disease and its potential role as a risk factor for recurrent mastitis. This was achieved by monitoring affected udders throughout one lactation period. The research involved N = 156 Holstein Friesian and Simmental cows, divided into three groups of N = 52: the first group included cows with primary postpartum ketosis and secondary mastitis, the second group consisted of cows with mastitis but no ketosis, and the third served as a healthy control group. Ketosis was diagnosed through laboratory analysis of blood, milk, and urine samples for the presence of ketone bodies. Mastitis detection involved clinical evaluation of the udder and microbiological identification of causative pathogens from milk samples. Cows in the first group were monitored throughout lactation to determine the prevalence of recurrent mastitis and identify key risk factors contributing to its recurrence.*

The findings revealed that recurrent mastitis was diagnosed in 24 cows across both mastitis-affected groups, with Staphylococcus aureus identified as the primary pathogen responsible for recurrence in 87.5% of cases. Additionally, a statistically significant difference in milk yield was observed between the control group and the mastitis-affected groups (P < 0.05). These results suggest that metabolic disorders may contribute to the recurrence of mastitis caused by common pathogens and that mastitis has a significant impact on milk yield in dairy cows.

Introduction

The peripartum period and early lactation are marked by significant physiological adaptations that enable the body to adjust to its new state. These adaptations involve endocrine and metabolic changes, primarily driven by the increased energy demands in late pregnancy and the initial stages of lactation. A key metabolic alteration during this period is an energy deficit, resulting from heightened glucose utilization and decreased blood glucose levels. Concurrently, fat mobilization occurs through the release of unsaturated fatty acids to compensate for the increased energy requirement (Bernabucci et al., 2005; Sumathi et al., 2008). Excessive fat mobilization, coupled with the liver's limited capacity for catabolism, leads to an accumulation of acetyl coenzyme A (CoA) and ketone bodies. When these metabolic adjustments surpass the body's ability to utilize acetyl CoA and ketone bodies as energy sources, their concentration increases, resulting in ketosis. This condition is characterized by hyperketonemia, ketonuria, and the presence of ketone bodies in milk and exhaled air. Additionally, ketosis is associated with hypoglycemia and a reduction in hepatic glycogen stores. The interplay between negative energy balance, ketosis, and immunosuppression predisposes animals to secondary diseases linked to ketosis (Oetzel, 2007; Seifi et al., 2011; Ayano et al., 2013).

Mastitis is among the most prevalent conditions arising from metabolic disturbances during the peripartum period, particularly in early lactation. It is an inflammatory condition of the mammary gland, commonly caused by pathogenic microorganisms that enter through the teat canal and proliferate in the glandular tissue (Vikova et al., 2017). Recurrence of mastitis is particularly significant, as evidence suggests that negative energy balance and hypoglycemia during early lactation compromise both systemic and local immune defenses in the mammary gland. This impairment contributes to disrupted lactogenesis and a decline in milk production (Ahmadzadeh et al., 2009; Frey, 2013; Ruegg, 2017; Jamali et al., 2018).

The primary objective of this research is to identify the key risk factors associated with recurrent mastitis and to examine the impact of ketosis, as a metabolic disorder occurring post-parturition and during early lactation, on both the incidence of mastitis and overall milk yield in the studied cows.

Materials and methods

For the purpose of this scientific study, three groups of fifty-two cows (N = 52) were examined

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separately, all originating from the same farm (totaling 156 cows). These cows were raised under uniform hygienic conditions, maintained in a free-stall housing system, and fed according to standard nutritional guidelines for high-yield dairy cows in full lactation within an intensive milk production system. The study population consisted of high-producing Holstein Friesian (HF) and Simmental (S) breeds. The first group (N = 52; N = 31 HF, N = 21 S) comprised cows diagnosed with ketosis following parturition and during early lactation. Within this group, cases of both primary and recurrent mastitis were identified throughout the lactation period. Data on milk yield and its variations were recorded over the entire lactation cycle. The second group (N = 52; N = 31 HF, N = 21 S) consisted of cows that did not experience metabolic disorders post-calving or during early lactation but developed primary and recurrent mastitis at different stages of lactation. For this group, milk yield data and fluctuations during mastitis episodes were collected. The third group served as a control group and included healthy cows (N = 52; N = 31 HF, N = 21 S) that remained free from both metabolic disorders and mastitis throughout the lactation period. For these cows, daily milk yield data were recorded across the entire lactation. To diagnose ketosis, blood, milk, and urine samples were collected from all cows (N = 156) between the third and the twentieth day postpartum, with the sixth day post-calving identified as the most appropriate period for diagnosis due to the peak concentration of ketone bodies. Blood samples were analyzed for ketosis using an enzymatic catalysis method with a biochemical analyzer to measure beta-hydroxybutyric acid (BHB) concentration in serum. Blood was collected via puncture of the coccygeal vein (v. Coccygea), with subclinical ketosis defined as $\geqslant$ 1.2 mmol/L BHB and clinical ketosis as $\geqslant$ 2.8 mmol/L BHB (Seifi et al., 2011). Ketosis in milk was detected using the Rother-Ross test, which identifies the presence of acetone and acetoacetate by adding an alkaline sodium nitroprusside solution to milk. A positive reaction results in a color change to red or violet, allowing the detection of acetoacetic acid (1–5 mg/dL) and acetone (10–20 mg/dL) (Carrier et al., 2004; Seifi et al., 2011; Serenho et al., 2022). Ketosis in urine was confirmed using Lestradet's test, which detects ketone bodies (acetone and acetoacetate) through the addition of sodium nitroprusside, leading to a color change to red or purple in a positive reaction. This method can detect acetoacetic acid concentrations of 1–5 mg/dL and acetone concentrations of 10–20 mg/dL (Carrier et al., 2004; Serenho et al., 2022). Mastitis diagnosis and mammary gland health assessment in cows previously diagnosed with ketosis were conducted through standard clinical examination methods, including evaluation of visible changes in milk consistency. Cows displaying abnormal milk secretion without visible signs of mammary gland inflammation were further screened using the California Mastitis Test (CMT), which involved mixing 3 mL of milk with 3 mL of CMT reagent (containing alkyl sulfonate as the active component). The test results were determined based on gel viscosity and the color change of the indicator (Bartlett et al., 2001; Karimuribo et al., 2006; Heikkilä et al., 2012). For cows with a positive CMT reaction, sterile milk samples were collected for microbiological analysis. The laboratory procedure for isolating and identifying pathogenic microorganisms involved serial dilution in physiological saline (0.85% NaCl) followed by inoculation onto a solid nutrient medium. The samples were incubated at 37°C for 24 hours (Gezhagn et al., 2020; Kasravi et al., 2010; Bradley et al., 2005). Morphological characteristics of the resulting bacterial colonies (color, size, shape) were analyzed, alongside physiological properties such as enzymatic activity on proteins and carbohydrates and biochemical characteristics, including enzyme expression and pathogen susceptibility to specific substances. Cows diagnosed with additional health disorders during lactation, aside from ketosis as the primary condition and mastitis as the secondary condition (including mastitis recurrences), were excluded from further research. Ethical approval for this study was obtained from an ethics committee, which authorized sample collection and animal handling (ethics approval number: 001328298 2024 14841 002 001 323 022).

Statistical analysis of the collected data was performed using SPSS software version 19.0 for Microsoft Windows. Descriptive and inferential statistical methods were applied, including analysis of variance (ANOVA) with post-hoc Tukey HSD tests, as well as t-tests for comparing mean milk yields between breeds.

Results

Fig. 1 illustrates that 50% of the cows diagnosed with metabolic ketosis, equivalent to 26 of a total of 52 cows, also developed mastitis.

Based on the presentation of Fig. 2, it can be seen that of 26 cows with primary mastitis, in 50% or 13 cows, the pathogenic agent of mastitis was *Staphylococcus aureus*, in 15% or 4 cows, the pathogenic agent was *Streptococcus agalactiae*, in 11% or 3 cows, the pathogenic agent was *Streptococcus uberis*, while the pathogenic agents *Pseudomonas aeroginosa, Escherichia coli, Streptococcus dysgalactiae, Klebsiella* spp., *Streptococcus pyogenes* and *Enterobacter* spp. each separately caused mastitis in one cow and each represented 4% of the total number of primary mastitis.

From Fig. 3, it can be seen that of a total of 14 cows with recurrent mastitis, in 93% or 13 cows with recurrent mastitis, the pathological cause of mastitis was *Staphylococcus aureus*, while in 7% or one recurrent mastitis, the pathological cause was *Streptococcus agalactiae.*

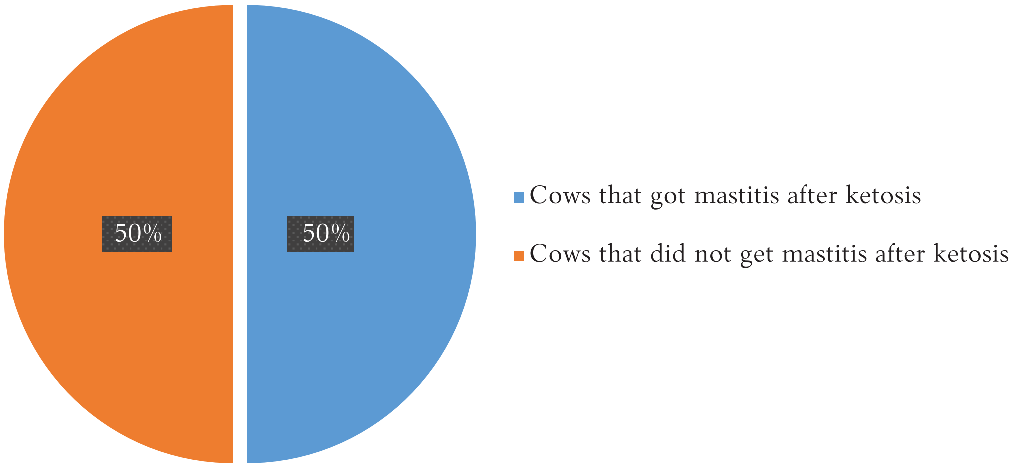

Fig. 1. Percentage of cows diagnosed with mastitis and cows with no mastitis after ketosis (%)

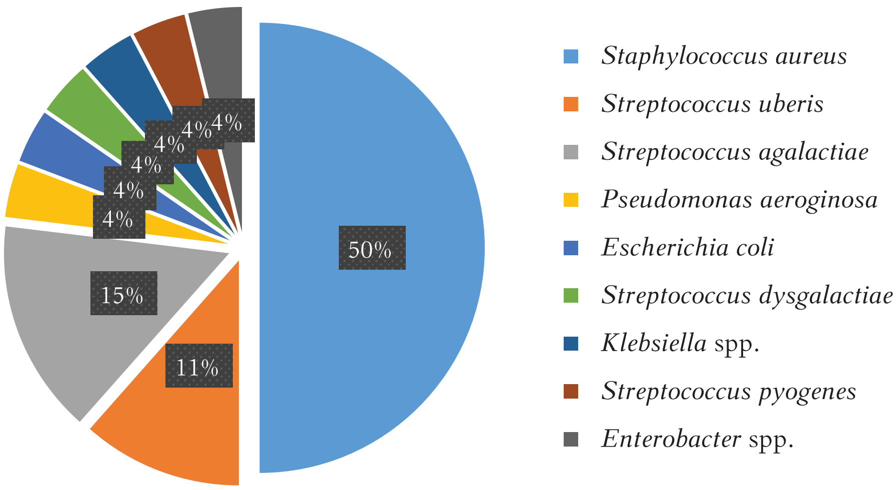

Fig. 2. Pathogenic agents of mastitis in cows that got mastitis after ketosis (%)

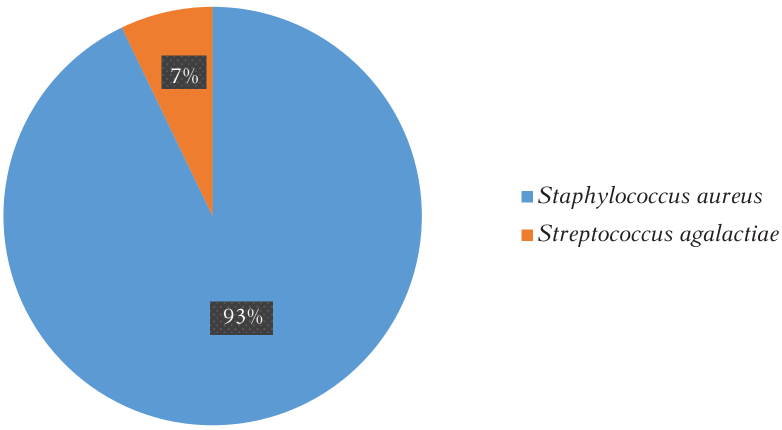

Fig. 3. Pathogenic agents of recurrent mastitis (%)

Fig. 4 indicates that among the 52 cows diagnosed with mastitis without a prior history of metabolic disorders, *Staphylococcus aureus* was identified as the causative agent in 56% of cases (29 cows). *Streptococcus uberis* was responsible for 13% of cases (7 cows), while *Streptococcus agalactiae* accounted for 11% (6 cows). Additionally, *Pseudomonas aeruginosa* was detected in 8% of cases (4 cows), and *Escherichia coli* was identified in 6% (3 cows). The remaining 2% of cases (one cow each) were attributed to *Streptococcus dysgalactiae*, *Klebsiella* spp.., and *Streptococcus pyogenes*.

In Fig. 5, it can be seen that of 10 cows with recurrent mastitis, in 80% or 8 cows, the main factor for mastitis was the bacterium *Staphylococcus aureus*, in 10% or in one cow, the bacterium isolated was *Streptococcus uberis*, and in 10% or in one cow, the

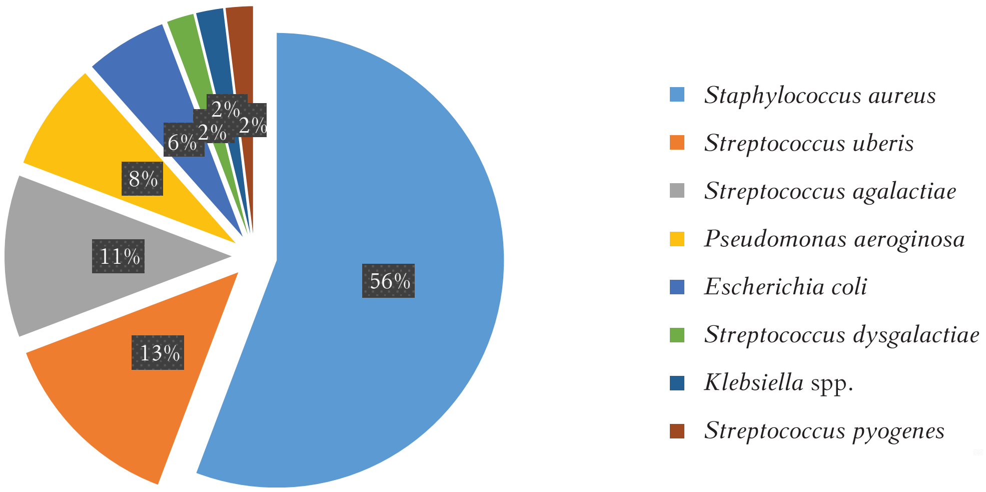

Fig. 4 Pathogenic agents of primary mastitis in cows without metabolic disease (%)

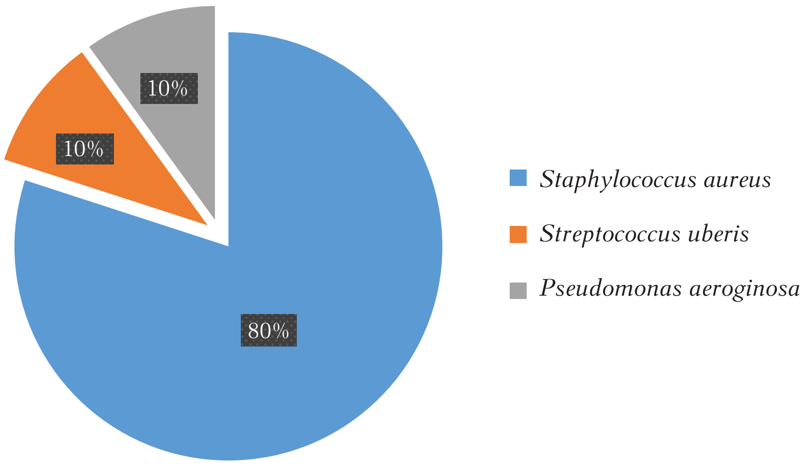

Fig. 5. Pathogenic agents of recurrent mastitis

cause was the bacterium *Pseudomonas aeruginosa*.

Table number 1 shows the results of the descriptive statistics for milk yield in the three groups of cows, where it is noted that the control group had the highest average milk yield (29.29 L/day), while the average milk yield in the group with mastitis and metabolic disease was 23.89 L/day and the average milk yield in the group with mastitis without metabolic diseases was 22.89 L/day.

From the values of standard deviations and coefficients of variation, it can be observed that the highest variability of milk yield was in the group that had mastitis and metabolic diseases (27.75%), and the lowest (17.92%) was in the control group of cows.

The results of the one-way analysis of variance revealed statistically significant differences ($P \leqslant 0.05$) in milk yield among the three observed groups of cows. To identify specific groups with significant differences in milk yield, a post hoc Tukey HSD test was conducted (Table 3).

The findings indicate a statistically significant difference in average milk yield between the control group and the group affected by both mastitis and metabolic disorders ($P \leqslant 0.05$), as well as between the control group and the mastitis-only group ($P \leqslant 0.05$). However, no statistically significant difference was observed in the average daily milk yield between the mastitis-only group and the group with both mastitis and metabolic disorders ($P > 0.05$).

According to the results presented in Table 4, the control group, which remained free of disease, exhibited the highest average milk yield on the final day of measurement, reaching 29.21 liters per day. In comparison, the group with both mastitis and metabolic disorders demonstrated a slightly lower average yield of 25.15 liters per day, while the mastitis-only group had the lowest average yield at 19.38 liters per day.

The lowest recorded milk yield on the final day was 6 liters, observed in a cow from the mastitis and metabolic disorder group, whereas the highest yield within the same group reached 41 liters. When examining standard deviations and coefficients of variation, the greatest variability in milk yield on the last measurement day was observed in the group affected by both mastitis and metabolic disorders (20.61%), while the lowest variability (14.31%) was recorded in the mastitis-only group.

Table 1. Descriptive statistics of the average milk yield of the studied cows

Group of cows	%	Min	Max	Standard deviation	Coefficient of variation (%)
Control group	29.29	18.00	47.00	5.25	17.92
Cows with mastitis and metabolic	23.89	1.00	45.00	6.63	27.75
Cows with mastitis	22.89	6.00	43.00	5.88	25.68

Table 2. ANOVA test for the average daily milk yield in the studied groups of cows

Effect	SS	df	MS	F	*P* value
Intercept	100 297.3	1	100 297.3	5952.691	0.000
Tretman	1231.0	1	615.5	36.532	0.000

Table 3. Results of the post-hoc Tukey HSD test for the average milk yield of the studied groups of cows

Treatment	Control group of cows	Cows with mastitis and metabolic disturbances (ketosis)	Cows with mastitis
Control group of cows		0.000	0.000
Cows with mastitis and metabolic disturbances (ketosis)	0.000		0.424
Cows with mastitis	0.000	0.424	

Table 4. Descriptive statistics of daily milk yield in the studied cows on the last day of measurement

Group of cows	Average	Min	Max	Standard deviation	Coefficient of variation (%)
Control group of cows	29.21	21.00	40.00	4.47	15.31
Cows with mastitis and metabolic disturbances (ketosis)	25.15	6.00	41.00	5.18	20.61
Cows with mastitis	19.38	11.00	24.00	2.77	14.31

Table 5. ANOVA test for average daily milk yield in the studied groups of cows

Effect	SS	df	MS	F	*P* value
Intercept	94 277.08	1	94 277.08	5181.641	0.000
Treatment	2536.17	1	1268.08	693 696	0.000

Table 6. Results of the post-hoc Tukey HSD test for the average milk yield of the studied groups of cows

Treatment	Control group of cows	Cows with mastitis and metabolic disturbances (ketosis)	Cows with mastitis
Control group of cows		0.000	0.000
Cows with mastitis and metabolic disturbances (ketosis)	0.000		0.000
Cows with mastitis	0.000	0.000	

The results of the one-factor analysis of variance showed the existence of statistically significant differences ($P \leqslant 0.05$) in milk yield on the last day of measurement in the observed three groups of cows. To determine exactly which cows had a statistically significant difference in milk yield on the last day, the post-hoc Tukey HSD test was applied, as in the previous case (Table 6).

Based on the results of the Tukey HSD test, it can be noted that there is a statistically significant difference in milk yield on the last day of measurement between all three groups of cows ($P < 0.05$).

Discussion

The statistical analysis of the research findings confirmed the thesis proposed by Fleischer et al.

(2001), which suggests an intrinsic correlation between ketosis and mastitis. This relationship was also validated in the present study, as 50% (26 of 52) of the cows diagnosed with ketosis subsequently developed mastitis at a certain stage of lactation. Mastitis was attributed to various bacterial strains, with *Staphylococcus aureus* being the predominant causative agent in both mastitis-affected groups. Specifically, *Staphylococcus aureus* accounted for 50% of cases in the first group and 56% in the second group, aligning with the findings of Saidi (2013), where *Staphylococcus aureus* comprised 40% of the total isolated mastitis pathogens. Similarly, Abrahamsen (2013) reported that *staphylococci* were responsible for 54.7% of mastitis cases.

Regarding recurrent mastitis (N = 24), *Staphylococcus aureus* was identified as the causative agent in 87.5% (N = 21) of cases, leading to the conclusion that this pathogen represents a primary risk factor for recurrent mastitis. These findings are consistent with studies by Bradley et al. (2007), Roesch et al. (2007), and Kalmus et al. (2011), which identified *staphylococci* and *streptococci* as the most frequently isolated pathogens in subclinical mastitis. Additionally, research by Dingwell et al. (2003) highlighted *Staphylococcus aureus*, *Streptococcus* spp., and *Escherichia coli* as the predominant pathogens during early lactation.

The impact of ketosis and mastitis on milk yield, as demonstrated through statistical analysis, closely aligns with the research of Dohoo and Martin (1983), who documented a reduction in milk yield ranging from 1 to 1.4 liters per day. In the present study, similar findings were observed concerning standard deviation values and coefficients of variation. The highest variability in milk yield (27.75%) was observed in the group affected by both mastitis and metabolic disorders, while the lowest variability (17.92%) was recorded in the control group. The group with mastitis but no metabolic disease exhibited a milk yield variability of 25.68%. These findings further support the mild influence of ketosis on milk production, as indicated by Oetzel (2015) and Dohoo and Martin (1983), while also confirming the significant impact of mastitis on milk yield variability.

The one-way analysis of variance revealed statistically significant differences ($P \leqslant 0.05$) in milk yield among the three observed groups. The application of the Tukey HSD test confirmed a statistically significant difference in average milk yield between the control group and the group with both mastitis and metabolic disorders ($P \leqslant 0.05$), as well as between the control group and the mastitis-only group ($P \leqslant 0.05$). However, no statistically significant difference was detected in average daily milk yield between the mastitis-only group and the group with both mastitis and metabolic disorders ($P > 0.05$), partially aligning with the findings of Oetzel (2015) and Dohoo and Martin (1983), who reported a minor impact of ketosis and a significant influence of mastitis on milk yield.

Based on the final measurement of average milk yield, the lowest production was recorded in the mastitis-only group, with an average of 19.38 liters per day. This suggests that mastitis, when not preceded by metabolic disorders, has the most pronounced effect on the physiological condition of the mammary gland and milk yield, consistent with the findings of Sanford et al. (2006). Conversely, the group affected by both ketosis and mastitis had a higher average milk yield of 25.15 liters per day. This result indicates that milk production in this group may be attributed to the correlation between mastitis and ketosis-induced immunosuppression, leading to a higher incidence of mastitis in the postpartum period, as described by Spears and Weiss (2008) and Sordillo and Aitken (2009).

Lastly, the one-way analysis of variance and Tukey HSD test confirmed statistically significant differences ($P \leqslant 0.05$) in milk yield among all three groups of cows on the final day of measurement.

Conclusion

The findings of this study confirm a significant correlation between ketosis and mastitis, supporting the hypothesis proposed by Fleischer et al. (2001). The results indicate that ketosis is a predisposing factor for the onset of mastitis, with *Staphylococcus aureus* emerging as the predominant pathogenic agent in both mastitis-affected groups. This aligns with previous research, reinforcing the role of *Staphylococcus aureus* as a major contributor to both primary and recurrent mastitis.

Furthermore, the study highlights the impact of ketosis and mastitis on milk yield, demonstrating that mastitis exerts a stronger influence on milk production variability compared with ketosis. The statistical analysis revealed significant differences in milk yield among the three groups, with the lowest production recorded in cows with mastitis alone. These findings suggest that mastitis, particularly in the absence of prior metabolic disorders, has severe consequences for the physiological condition of the mammary gland. The observed lowering of milk yield in cows affected by both ketosis and mastitis compared with the control group suggests that immunosuppressive effects associated with ketosis may contribute to a higher incidence of mastitis, particularly in the postpartum period. This aligns with previous studies on the relationship between metabolic disorders and immune function. Overall, this study provides valuable insights into the interplay between ketosis, mastitis, and milk yield, emphasizing the need for effective management strategies to mitigate the economic and physiological impacts of these conditions. Future research should further investigate the underlying mechanisms linking metabolic diseases with infectious conditions to develop targeted prevention and treatment strategies.

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First Molecular Identification of *Ehrlichia chaffeensis* in Dogs

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Abstract.*The species Ehrlichia chaffeensis is important for public health as it is the etiological agent of human monocytic ehrlichiosis (HME). The objective was to detect Ehrlichia chaffeensis in dogs from León, Nicaragua. Thirty-nine dogs with symptoms consistent with hemoparasitosis and tick infestation were evaluated and underwent a blood smear, SNAP® 4Dx® Plus immunochromatography test, and nested PCR. The presence of Ehrlichia spp. morulae in blood smears was detected in 87.2% (95% CI, 72.6–95.7) of the samples, while 53.9% (95% CI, 36.9–70.8) of E. canis / E. ewingii seropositive canines were detected with SNAP. Ehrlichia spp. was detected by PCR in 87.2% (95% CI, 72.6–95.7) of the samples. In the nested analysis, 24.6% (95% CI, 10.7–40.6) of canines were positive for Ehrlichia chaffeensis. This is the first report of molecular detection of this important zoonotic agent causing canine ehrlichiosis in Nicaragua.*

Introduction

Most neglected zoonotic and vector-borne diseases go undiagnosed in humans and animals, often because they are subclinical. In some cases, however, they are associated with serious pathological manifestations. Canine vector-borne diseases are often widespread in tropical and subtropical regions due to climatic conditions favorable for the survivability of arthropod vectors and the development of vector-borne canine pathogens (Kilpatrick and Randolph, 2012; Selim et al., 2021)and many endemic diseases have increased in incidence. Although introductions and local emergence are frequently considered distinct processes, many emerging endemic pathogens are in fact invading at a local scale coincident with habitat change. We highlight key differences in the dynamics and disease burden that result from increased pathogen transmission following habitat change compared with the introduction of pathogens to new regions. Truly in situ emergence is commonly driven by changes in human factors as much as by enhanced enzootic cycles whereas pathogen invasion results from anthropogenic trade and travel and suitable conditions for a pathogen, including hosts, vectors, and climate. Once established, ecological factors related to vector characteristics shape the evolutionary selective pressure on pathogens that may result in increased use of humans as transmission hosts. We describe challenges inherent in the control of vector-borne zoonotic diseases and some emerging non-traditional strategies that may be more effective in the long term.","container-title":"Lancet","DOI":"10.1016/S0140-6736(12.

An increase in *Ehrlichia* infections has been observed due to factors such as the presence and number of animal reservoirs and vector ticks in the endemic area *(Mogg et al.*, 2020 ; Forero-Becerra et al., 2021)506 healthy residents and 114 dogs from four municipalities (Cauca, Colombia. *Ehrlichia chaffeensis*, *E. ewingii*, and *E. canis* species have been identified as causative agents of emerging zoonotic infections in humans (Thomas et al., 2009; Bouza-Mora et al., 2017)including divergent tandem repeat sequences. Nucleotide sequences of dsb and trp36 amplicons revealed a novel genotype of E. canis in blood bank donors from Costa Rica. Indirect immunofluorescence assay (IFA. *E. chaffeensis* is the etiological agent of human monocytic ehrlichiosis (HME) (Guillemi et al., 2019), a disease described in 1987 in the United States. Although *E. canis* was initially implicated as the responsible bacterium (serological cross-reaction) in HME conditions, it is now known that the causal agent is *E. chaffeensis*, due to its isolation in Fort Chaffee, which birthed the name *E. chaffeensis* (Dolz et al., 2013). The diagnosis of ehrlichiosis by PCR has shown greater effectiveness in blood samples, presenting more specific results, since there are no cross-reactions, and it also detects *Ehrlichia* spp. in any of its phases and offers a definitive diagnosis (Franco-Zetina et al., 2019).

E. chaffeensis has been extensively studied as a cause of acute febrile illness and an emerging tick-

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borne zoonosis present throughout the Americas (Chikeka et al., 2016)the etiologic agent of human monocytic ehrlichiosis (HME. In the United States, the main vector of *E. chaffeensis* is the tick of the *Amblyomma americanum* species; however, other species that can transmit this bacteria have also been described, such as *Amblyomma parvum*, which has already been found in domestic animals in Nicaragua (Düttmann et al., 2016)3299 were parasitized, which represent 68 % of the bovines and 67 % of the equines in study: 59 cows and 25 horses were parasitized by more than one species. In addition, 280 specimens of the entomological museum in León were examined. The ticks found on cattle were Rhipicephalus microplus (75.2 % of the ticks collected. In addition, serological evidence of *E. chaffeensis* infection in humans has been reported (Chikeka et al., 2016) the etiologic agent of human monocytic ehrlichiosis (HME, that could be associated with the role of dogs as a zoonotic reservoir for human infection. Despite these various reports, there are no studies that report the presence of anti-bodies against *E. chaffeensis* or its DNA in canines, thus suggesting the need for the study. The objective was to detect *Ehrlichia chaffeensis* in dogs from León, Nicaragua.

Materials and methods

Blood sampling and *Ehrlichia* spp. screening

An observational study was carried out on 39 tick-infested dogs that attended the UNAN-León veterinary clinic, with a mean age of 48 months (Range 24–84), 49% of Creoles and 51% of other breeds. Following the previously described protocol (Overall, 2013), blood sampling was performed by experienced veterinarians according to established routine practice for laboratory diagnosis. Venous blood (3 mL) was collected aseptically from the cephalic vein and mixed with ethylenediaminetetraacetic acid (EDTA). A blood smear was performed for an examination of intracytoplasmic inclusion bodies compatible with *Ehrlichia*. The SNAP® 4Dx® Plus immunochromatographic assay (IDEXX Laboratories, Inc., Westbrook, Maine) was also applied for antibody detection.

Molecular diagnosis

For molecular detection, DNA extraction was performed using the QIAamp DNA Mini Kit QIAGEN (Hilden, Germany), according to the manufacturer's instructions. For the first PCR reaction, the primers described in Table 1 were used in a reaction volume of 20 µL, containing 8.5 µL of nuclease-free water, 10 µL of Master Mix 2X (Promega, EE. UU), 0.5 µL (5000 nmol) of Forward (F) primer for *Ehrlichia* spp., 0.5 µL (5000 nmol) of Reverse (R) primer for *Ehrlichia* spp. and 0.5 µL of extracted DNA.

Positive samples of the first reaction underwent the nested analysis. A volume of 25 µL was prepared, containing 10.5 µL of nuclease free water, 12.5 µL of Master Mix 2X (Promega, USA), 0.5 µL (5000 nmol) of primer Forward (F) for *E chaffeensis*, 0.5 µL (5000 nmol) of Reverse (R) primer for *E. chaffeensis*, and 1 µL of DNA from the primary PCR product was used. Amplification was performed at 94°C for 5 minutes, followed by 40 cycles of 94°C for 30 seconds, 60°C for 30 seconds, 55°C for 30 seconds, a final extension at 72°C for 10 minutes and 4°C until the moment of disassembling the reactions. The amplified products were separated using electrophoresis on a 1.2% agarose gel that was stained with ethidium bromide. Negative and positive controls were included in each DNA extraction and PCR reaction.

Ethical consideration

Dogs included in this study were managed according to the *Law for the protection and welfare of domestic animals and domesticated wild animals (747)* (Normas Jurídicas de Nicaragua, 2011), and this study was previously approved by the Research Commission of the School of Agricultural and Veterinary Sciences (ECAV), National Autonomous University of Nicaragua, Leon (UNAN-Leon). Informed consent was obtained from each dog owner.

Data analysis

In statistical analysis, descriptive statistics are reported first. Laboratory results were treated as categorical variables and reported as frequency and 95% confidence intervals (95% CI). Continuous variables (e.g., hematological laboratory values) were reported as mean. To determine hematological parameters associated with *Ehrlichia* positive test results, the Student *t* test for independent samples was employed, and statistical significance was determined if $P < 0.05$. To determine concordance between the

Table 1. Primers used in the nested PCR for the detection of *Ehrlichia* spp. and *Ehrlichia chaffeensis*

Pathogen	Expected product	Primers	Reference
Ehrlichia spp.	490 pb	Primary: ECC (5′AGAACGAACGCTGGCGGCAAGCC) Primary: ECB (5′-CGTATTACCGCGGCTGCTGGCA)	(Gleim et al., 2016) (Dawson et al., 1994) (Anderson et al., 1992)
Ehrlichia chaffeensis	380 pb	Secondary: HE1 (5′CAATTGCTTATAACCTTTTGGTTATAAAT) Secondary: HE3 (5′TATAGGTACCGTCATTATCTTCCCTAT) (51)	(Gleim et al., 2016) (Dawson et al., 1994) (Anderson et al., 1992)

blood smear, SNAP and PCR tests, the Cohen Kappa concordance test was used.

Results

Of the 39 canines in this study, intracytoplasmic morulae were observed in the blood smears of 87.2% (95% CI, 72.6–95.7) (Fig. 1). These were observed in neutrophils (43.6%, 17/34), platelets (35.9%, 14/34), and monocytes (7.7%, 3/34).

SNAP 4Dx plus technique. Additionally, 6 dogs co-infected with *Ehrlichia* spp. / *Anaplasma* spp., 2 dogs co-infected with *Ehrlichia* spp., *Anaplasma* spp. and *Diroflaria immitis* were also observed.

Ehrlichia spp. were detected by PCR in 87.2% (34/39) of dogs, of which 10 (29.4%) were positive for *E. chaffeensis* DNA. Of the 10 dogs positive for *E. chaffeensis*, 9 presented monocytosis as a common hematological alteration.

No hematological parameters were associated within *Ehrlichia* positive and negative animals by blood smears, PCR tests for *Ehrlichia* spp., or PCR tests for *E chaffensis*, (Table 2).

The test concordance between blood smear and PCR for *Ehrlichia* spp. demonstrated that 29 samples were positive in both tests, 5 dogs were negative by smear but positive by PCR, and 5 dogs were positive in smear but negative in PCR. No sample was negative in both tests (Kappa = –0.147, *P* = 0.358).

From the concordance of SNAP serology vs PCR for *Ehrlichia* spp. diagnosis, 18 samples were positive in both tests, 17 canines were negative by SNAP but positive in PCR, 3 dogs were positive in SNAP but negative in PCR, and 2 samples were negative in both tests (Kappa = –0.033, *P* = 0.768) (Table 3).

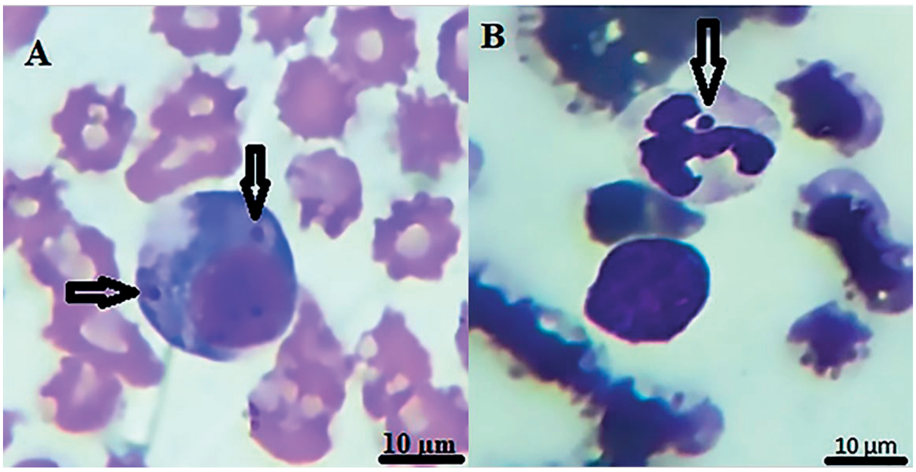

Fig. 1. Morulae of Ehrlichia in blood smear of canine (100 X)
Arrows indicate morulae of monocytic *Ehrlichia* (A) and granulocytic *Ehrlichia* (B)

Table 2. Comparison of blood parameters with respect to the detection of hemoparasites in the blood smear, *Ehrlichia* spp. and *Ehrlichia chaffeensis*

Blood parameter	Hemo-parasites	Blood smear		PCR-*Ehrlichia* spp.		PCR-*E. chaffeensis*	
		Means	P^*	Means	P^*	Means	P^*
Hematocrit (%)	Negative	39.30	0.427	39.20	0.440	36.26	0.483
	Positive	34.94		34.96		33.31	
White blood cells count (10^3 cells/mL)	Negative	9.44	0.272	8.15	0.148	12.93	0,417
	Positive	14.24		14.43		15.66	
Red blood cell count (10^6 cells/mL)	Negative	3.65	0.184	4.70	0.537	5.17	0.374
	Positive	5.92		5.77		6.98	
Lymphocytes (%)	Negative	7.14	0.729	7.20	0.747	7.17	0.327
	Positive	8.17		8.16		10.56	
Neutrophils (%)	Negative	58.46	0.749	65.86	0.324	62.20	0.077
	Positive	60.55		59.46		54.70	
Eosinophils (%)	Negative	5.18	0.917	6.30	0.659	5.28	0.796
	Positive	5.43		5.26		5.74	
Monocytes (%)	Negative	21.20	0.798	18.66	0.479	20.60	0.126
	Positive	22.86		23.23		28.59	

Table 3. Concordance analysis between the smear/SNAP/PCR techniques for the diagnosis of Erhlichia spp. in dogs

<table>
<tr><th rowspan="2"></th><th rowspan="2"></th><th colspan="2">PCR-Erhlichia spp.</th><th rowspan="2">Total</th><th rowspan="2">Kappa</th></tr>
<tr><th>Negative</th><th>Positive</th></tr>
<tr><td rowspan="3">Smear</td><td>Negative</td><td>0</td><td>5</td><td>5</td><td rowspan="2">–0.147</td></tr>
<tr><td>Positive</td><td>5</td><td>29</td><td>34</td></tr>
<tr><td>Total</td><td>5</td><td>34</td><td>39</td><td></td></tr>
<tr><td rowspan="3">SNAP</td><td>Negative</td><td>2</td><td>16</td><td>18</td><td rowspan="2">–0.033</td></tr>
<tr><td>Positive</td><td>3</td><td>18</td><td>21</td></tr>
<tr><td>Total</td><td>5</td><td>34</td><td>39</td><td></td></tr>
</table>

Discussion

In this observational study of canines in Nicaragua, a high prevalence (87.2%) of ehrlichiosis was documented. Furthermore, *E. chaffeensis* was specifically identified by PCR in a high percentage of dogs, the species with the greatest zoonotic involvement. Prior to this study, there had been no reports of molecular detection of *E. chaffeensis* in ticks or domestic animals from Central America. Despite this lack of evidence in vectors and animal hosts, *E. chaffeensis* was previously highlighted as an unrecognized cause of acute febrile illness in humans in Nicaragua (Chikeka et al., 2016)the etiologic agent of human monocytic ehrlichiosis (HME. Additionally, 5 humans with a history of tick bites and general arthralgia were diagnosed with *E. chaffeensis* infection by PCR in neighboring Costa Rica (Rojas et al., 2015). This new evidence confirms *E. chaffeensis* in canine populations in Central America and highlights the need for heightened awareness of ehrlichiosis epidemiology in the veterinary and human health domains.

This study also indicates a high seroprevalence of *E. canis / E. ewingii* according to the SNAP® 4Dx® Plus. This is higher than the 38.20% found in Costa Rica (95% CI: 32.8–43.4%), which also reported the highest prevalence, reaching up to 62%, was in the province of Guanacaste, the area closest to Nicaragua (Montenegro et al., 2017). This finding is similar to a prior report of 62.90% canine seroprevalence in Nicaragua, with authors attributing the high frequency to the fact that the dogs were sampled in the western parts of Nicaragua, the same origin of the canines analyzed in this study. This area is characterized by less rainfall and higher human population density than elsewhere in the country (Springer et al., 2018).

The application of SNAP® 4Dx® Plus identified canines with serological evidence of multiple concomitant exposures (6/39 (15%) for *Ehrlichia/Anaplasma* and (2/39 (5.12%) for *Ehrlichia/Anaplasma/Dirofilaria*), unlike in the study of Montenegro et al. (2018) where double exposure to pathogens was only 8.9%. These results support the high seroprevalence of hemoparasites in dogs from Western Nicaragua, as well as the reproducibility when SNAP® 4Dx® Plus is applied.

A high percentage of dogs had morulae compatible with *Ehrlichia* spp., compared with what has been reported by others in Nigeria (1.5%) (Daramola et al., 2022)Nigeria by microscopy and nested PCR. Blood samples were collected from 205 dogs, thin smears were made, field-stained, and DNA was extracted from the blood samples. A partial region of the 16S rRNA gene was amplified by polymerase chain reaction (PCR and Thailand (36.7%) (Rucksaken et al., 2019). This could be attributed to the fact that we sampled dogs with symptoms compatible with ehrlichiosis and tick infestations, biasing our selection toward clinically ill canines. No significant differences were observed in hematological parameters between positive and negative dogs for *Ehrlichia* spp. by PCR. This lack of association could be due to the small number of the analyzed samples, while other studies demonstrated a higher percentage of monocytes in canines positive for *Ehrlichia* spp. and *E. chaffeensis* (Lara et al., 2020; Thongsahuan et al., 2020)tick-borne rickettsial pathogens of dogs that may cause life-threatening diseases. In this study, we assessed the usefulness of PCR and a widely used commercial antibody-based point-of-care (POC.

PCR is one of the most sensitive and specific techniques for the diagnosis of *Ehrlichia* spp., and has even been considered the gold standard (Franco-Zetina et al., 2019). By comparison to PCR, the blood smear technique had a low concordance, reflecting a low accuracy for the detection of *Ehrlichia*. Due to its low cost, microscopy in Nicaragua and most countries is the only method available for routine use for diagnosing hemoparasites in dogs (Harrus & Waner, 2011)caused by the rickettsia Ehrlichia canis, an important canine disease with a worldwide distribution. Diagnosis of the disease can be challenging due to its different phases and multiple clinical manifestations. CME should be suspected when a compatible history (living in or traveling to an endemic region, previous tick exposure; nevertheless, this technique lacks specificity due to the need for experienced examiners to distinguish between *Ehrlichia* spp. infections and other cytoplasmic inclusions (Kaur et al., 2020). Although the blood smear is a simple, rapid, and inexpensive technique to routinely detect the bacteria, visualization of morulae in peripheral blood cells is also the least sensitive and nonspecific technique, because of the low circulating amounts of bacteria, morulae are not detected in the blood smear, and sometimes it is possible to find inclusions not related to *Ehrlichia* spp. That can cause diagnostic confusion such as the identification of false positives (Dolz et al., 2013). Its low sensitivity could be a factor why some studies have reported low prevalence of canine *Ehrlichia* (Happi et al., 2018; Daramola et al., 2022) Nigeria by microscopy and nested PCR. Blood samples were collected from 205 dogs, thin smears were made, field-stained, and DNA was extracted from the blood samples. A partial region of the 16S rRNA gene was amplified by polymerase chain reaction (PCR. No concordance was observed between PCR *vs* SNAP, a rapid test that has become essential to improve diagnosis. However, similar to the natural history of infection with many pathogens, antibodies are generally absent during the first two weeks and may persist after removal of the agent from the body (Wong et al., 2011)there was 85.2% concordance. The 24 discordant results between serology and PCR occurred in tests involving Ehrlichia canis (14. Cross-reactions between members of the Anaplasmataceae family that result in false positive results are also possible (Dolz et al., 2013).

This study documents an important veterinary and zoonotic pathogen in dogs of Central America and provides new evidence that dogs may be a source of *E. caffessis* infection in Nicaragua. Veterinary and human public health professionals should pay special attention to its zoonotic potential, and clinicians should consider ehrlichiosis in acute febrile conditions in humans if other causes have been ruled out.

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Comparative Effects of Herbal Essential Oils, Organic Acids, and Medium-Chain Fatty Acids on Laying Hens: Performance, Egg Quality, and Microbial Activity

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Abstract. *This study compared the effects of herbal essential oils, organic acids, and medium-chain fatty acids, and their sole and decreased mixture on performance, egg quality, blood constituents, and fecal microbial activity in laying hens. A total of 162 Hy-line W80 38-week-old laying hens were allocated into 6 treatment groups with 9 replicates. The treatment groups were as follows: 1) control (C, basal diet, no supplement); 2) supplemented with a herbal essential oil mixture (EOs); 3) 400 mg/kg humic acid (HA); 4) 15 mg/kg medium-chain fatty acids mixture (MCT); 5) EOs + HA + MCT mixture (EHAM); 6) A decreased mixture of EOs + HA + MCT (Mix 1/2).*

The results showed that diet additives did not have a significant effect on the final body weight (BW) of hens ($P < 0.05$). The feed conversion ratio (FCR) was significantly higher in the control group compared with the groups with supplemented diets ($P < 0.01$). The egg yield (number and percentage) and the egg mass in supplemented diets were greater than those of the control group ($P < 0.01$). The Haugh unit, Roche color scale value, eggshell ratio, eggshell surface area, and eggshell unit weight did not show significant differences between the groups. During the whole period, eggshell thickness in all groups with supplemented diets was higher than that of the control group ($P < 0.01$), and eggshell weight in the Mix 1/2 group was greater than in the control group ($P < 0.01$). Diet additives did not have a significant effect on egg albumen height and yolk color. Although diet additives did not affect blood parameters, they decreased fecal bacteria counts, such as Escherichia coli, Klebsiella spp., Candida albicans, and Gram-negative bacteria, compared with the control group ($P < 0.01$). In conclusion, diet additives increased egg yield, egg mass, and eggshell thickness, leading to better feed conversion rates due to their antimicrobial activity, compared with the control group.

Introduction

The increase in world population and changing living standards are the main reasons for improving both the quantity and quality of animal products. Antibiotics were used in animal production as growth promoters, banned in 2006 in Europe and Türkiye, due to the resistance created by antibiotic residues. In the search for natural feed additives, herbal extracts, such as essential oils, organic acids, probiotics, prebiotics, and fatty acids, emerge as promising alternatives to improve not only animal performance and health but also product quality (Buchanan et al., 2008; Świątkiewicz et al., 2013).

Essential oils (EOs) and organic acids have shown antimicrobial and antioxidant properties, contributing to the control of some poultry diseases without the need to use antibiotics, increasing the search for natural feed alternatives to antibiotics (Brenes and Roura, 2010), able to be residue-free (Varel, 2002). Typically, EOs consider combinations of many components, making it difficult to clarify their activities (Russo et al., 1998) Among mixtures, it is possible to find terpenoids, such as geraniol, linalool, thujanol, menthol, borneol, α-terpineol, and citronellal, as well as a variety of reduced-weight molecular aliphatic hydrocarbons, such as phenols thymol, guaiacol, eugenol, carvacrol, cinnamaldehyde, cuminal, and phellandral (Dorman and Deans, 2000).

In addition to the antibacterial, antioxidant, and antifungal properties shown by EOs, they also increase feed intake and digestibility, being used to improve the productive performance in poultry (Chouhan, 2017). Thus, other ingredients originating from plants, consisting of fragrant plant extracts, as well as their purified constituents, have been tested as additives in animal production.

Humic acids (HA) correspond to natural materials with neutral or alkaline pH, capable of transferring electrons and forming chelates with many metal ions. They provide the necessary macro and micronutrients for animals (Arif et al., 2019) and they serve as growth promoters; however, the information about their effects on intestinal health and microbiota composition

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is limited. Kara et al. (2012) and Mudroňová et al. (2021) have reported that HA may positively affect the performance of animals by improving the ecosystem in the gastrointestinal tract which in turn enhances nutrient utilization and poultry health. Furthermore, Disetlhe et al. (2017) have reported that the inclusion of 1.5% HA in canola-based diets positively impacted the absorption and nutrient digestion, also improving bone and immune system advancement in poultry.

Medium-chain triglycerides (MCT) are saturated triglycerides of 4 to 12 carbons, composed mainly of caprylic (C8; 50 ± 80%) and capric fatty acids (C10; 20 ± 50%) with a small contribution of caproic (C6; 1 ± 2%) and lauric (C12; 1 ± 2%) fatty acids (Jadhav and Annapure, 2023). MCTs can be produced from bovine milk, coconut, and palm kernel oil (Jensen, 2002; Nandi et al., 2004). Medium-chain fatty acids are considered the most important type of MCT easily utilizable by non-ruminants, showing a positive impact on gut microbiota, maintaining the intestinal mucosa function, decreasing the pathogen population and inflammation rate, but increasing the useful microbial population in the gut (Jia, 2020).

However, the number of studies reporting the effects of EOs, HA, and MCT in laying hens is scarce. The hypothesis was that herbal essential oils, organic acids, and medium-chain fatty acids have a positive effect on the performance, product quality, and antimicrobial activity in laying hens, especially given in a mixture. This study aimed to determine the effects of herbal essential oils, humic acid, and medium-chain fatty acids and their mixture on the performance, egg quality characteristics, blood parameters, and fecal microbial activity in laying hens.

Materials and methods

Experimental Design and Bird Management

Animal and feeds

Procedures of this animal experiment were approved by the Local Ethics Committee of Erciyes University (Approval No. 20/120). In this study, a total of 162 commercial Hy-line W80, 38-week-old laying hens were used. Before starting the experiment, the live body weights (BW) of the laying hens and followed egg production were recorded and ranked to obtain minimum differences among the groups. The laying hens were placed in cages of 42 × 40 × 46 cm (3 hens/cage), with a total of 54 cages, in 3 floors with 9 replicates (27 hens in each group). After 21 days of adaptation to diets, laying hens were fed for 16 weeks with experimental diets, and the data were collected at 14-day intervals (14 days 8 periods). The lighting schedule was a 16-hour light and 8-hour dark cycle (lighting was obtained from 5:00 am until 9:00 pm). The poultry house had a semi-controlled environmental system.

The diet was formulated according to the nutrient requirements of Hy-line W80 in laying periods. Diet feedstuff and nutritional composition are shown in Table 1. All experimental diets were produced from a basal diet, and all were isocaloric and isonitrogenic; the differences are merely additives. This basal diet showed 89.28% of dry matter, 18.10% of crude protein, 4.70% of crude fat, 4.98% of crude fiber, 12.24% of ash, 2780 Mcal/kg, 3.80% of calcium, 0.66% of available phosphorus, 0.80% of lysine, and 0.40% of methionine. Also, the additives were mixed homogeneously as a premix and added to the experimental feeds (0.75 g/kg). Feed mixtures were prepared every month to be fresh. In determining the doses of feed additives, the values used in practice were taken into account.

Table 1. Nutritional composition of the base formulated diet (LSM + SEM)

Feeds	Ratio, %
Corn	51.62
Sunflower meal (36% CP)	18.00
Soybean meal (46% CP)	9.80
Calcium carbonate	7.95
Dried distiller grain soluble (DDGS)	4.19
Meat-bone meal	3.41
Animal fat	2.24
Molasses	2.00
Salt. NaCl	0.25
Vitamin -mineral premix[1]	0.20
Phytase enzyme 600 U/kg	0.10
DL-Methionine	0.07
L-Lysine	0.07
Sodium bicarbonate	0.05
Mycotoxin binder	0.05
Chemical composition*	
Dry matter, %	89.28
Crude protein, %	18.10
Crude fat, %	4.70
Crude fiber, %	4.98
Ash, %	12.24
Methionine, %[¥]	0.40
Lysine, %[¥]	0.80
Calcium, %[¥]	3.80
Available phosphorus, %[¥]	0.66
Metabolic energy, kcal ME/kg[¥]	2780.0

[1]Vitamin-mineral premix per kilogram of the diet, retinol acetate, 4500 mcg; cholecalciferol, 50 mg; tocopherol acetate, 40.0 mg; menadione, 5.0 mg; thiamine, 3.0 mg; riboflavin, 6.0 mg; pyridoxine, 5.0 mg; cobalamin, 0.03 mg; nicotinic acid, 30.0 mg; biotin, 0.1 mg; calcium d-pantothenate, 12 mg; folic acid, 1.0 mg, choline chloride, 400 mg, manganese, 80.0 mg; iron, 35.0 mg; zinc, 50.0 mg; copper, 5.0 mg; iodine, 2.0 mg; cobalt, 0.4 mg; selenium, 0.15 mg assured. [¥]Compositions were calculated based on NRC (1994) data of feedstuffs.

The base formulated diet for laying hens was included in all the treatments (groups) as follows: 1) control (C, 100% base formulated diet); 2) herbal essential oil (EOs) mixture supplemented diets (including thymol 100 mg/kg, gamma-terpinene 0.77 mg/kg, para-cymene 0.42 mg/kg, carvacrol 1.552 mg/kg, anethole 6.12 mg/kg, limonene 0.81 mg/kg, sabinene 0.44 mg/kg, terpinen-4-01 0.30 mg/kg): humic acid supplemented diets (0.394 mg/kg, HA); 3) medium-chain fatty acids supplementation (15 mg/kg, MCT); 4) EOs + HA + MCT mixture (EHAM); and 5) a low concentrate (Mix 1/2 of EHAM). The diets and water were offered ad libitum.

Characteristics of performance

The body weight of hens was weighed individually at the beginning and the end of the study. Feed intake was determined every 14 days and feed conversion ratio was calculated (feed consumption: egg mass for each 2, 4, and 8 periods) for each period. Diet intake was figured out by differences between the given feed and the refusals.

Chemical analyses of feed

The chemical composition (dry matter, crude protein, and crude ash) of the feed was analyzed according to established procedures AOAC (2013) of feedstuffs.

Egg production and characteristics

The egg production of hens was recorded daily. Egg yield was calculated through the formula: (number of eggs produced/ total number of eggs produced in 14 days) × 100. Egg mass (g/day) was calculated through this formula: egg weight (g) × egg yield (%).

Eggs were collected every 14 days on the last two consecutive days, and 3 eggs were randomly sampled from each cage (27 eggs from each group, a total of 162 eggs) and evaluated for egg weight, shell, and inner egg characteristics. All eggs were numbered to follow during the measurements.

The egg weight, egg yolk Roche color scale values (RSS), albumen height (Hmm), egg yolk height, and Haugh unit (HU) were measured using an Egg Analyzer® (EggAnalyzer, Orka Food Technology LLS, USA). The egg yolk color was defined as brightness (L*), redness (a*), and yellowness (b*), which were measured through a Minolta CR-400 (Minolta Co, Japan) colorimeter. Following the egg evaluation, the shells were washed and dried in an oven at 75°C for 24 hours, and eggshell weights were measured (g) using a precision scale sensitive to 0.1 g. The eggshell ratio was calculated using the formula: (shell weight / egg weight) × 100. The eggshell thickness (μm) was measured with the help of a digital micrometer sensitive to 0.01 mm in the sharp, blunt, and middle parts of the eggshells, and then the shell thickness value was determined through the arithmetic mean of these three measurements. The eggshell weight per unit area (mg/cm^2) was calculated by absolute shell weight (g) / egg surface area (cm^2), according to the formula reported by Carter (1975). The eggshell surface area was calculated using the formula: (3.9782 × egg weight $^{0.7056}$).

Fecal microbial activity

On the 84th day of the experiment, 1 g of fresh fecal samples were taken from each cage to determine fecal *Escherichia coli*, *Klebsiella* spp., *Candida albicans*, *Staphylococcus* spp., total Gram-positive and Gram-negative bacteria populations.

The samples were diluted using 1 mL of saline solution (0.85% NaCl) and were homogenized for 3 minutes. Tenfold dilutions with the sterile solution of physiological salt were prepared from the initial water samples. Then, 5 μL of the examined liquid was taken from each dilution, evenly spread on the surface of the agar media in 3 parallel repetitions and incubated at 37°C for 24 hours. The bacterial microflora concentrations in the samples were determined using 5% sheep blood agar (bioMérieux, France). The eosine methylene blue agar (EMB, bioMérieux, France) was used to determine *Escherichia coli* and total gram-negative bacteria. The chromogenic agar (CHROMagar™) was used for the identification of *C. albicans*. The microbial counts were determined as colony-forming units (CFU) per gram of samples (Ildız et al., 2018). The microorganism counts were transferred to $\log_{10}$ before statistical analyses.

Blood sampling and analysis of metabolic indicators

At the end of the experiment, 10 cc of blood were taken under the wing from randomly selected laying hens from each group, centrifuged to separate serum, and stored in Eppendorf tubes at –80°C. On the day of analysis, serum was thawed to determine glucose, total protein, triglycerides, cholesterol, high-density lipoprotein (HDL), alanine aspartate aminotransferase (AST), and alanine aminotransferase (ALT) using an autoanalyzer (AMS, VegaSys, Rome Italy) and commercial kits for each parameter.

Statistical analysis

The study was set up and carried out in a completely randomized design. The analysis of variance was performed using the one-way ANOVA procedure of the SPSS statistics program, version 22. Differences between means were analyzed by the Duncan test ($P < 0.05$).

Results and discussion

Performance traits

The initial and final BW, gain, feed intake, and FCR of laying hens with diets supplemented with essential oils, humic acid, and medium-chain triglycerides during 1, 4, 8, and overall periods are shown in Table 2. The LBW did not show any significant effect

Table 2. Effects of essential oils, humic acid, and medium-chain triglycerides on egg performance characteristics of laying hens

Parameter	Treatments						
BW (g)	C	EOs	HA	MCT	EHAM	Mix 1/2	*P*
I-LBW	1594.1 ± 22.20	1579.4 ± 35.34	1566.3 ± 20.67	1585.2 ± 17.82	1630.2 ± 22.87	1600.4 ± 24.02	0.550
F-LBW	1611.6 ± 21.16	1661.1 ± 38.75	1651.7 ± 21.86	1660.3 ± 9.35	1697.8 ± 15.72	1666.1 ± 26.63	0.270
LBW-g	17.5 ± 15.66	81.7 ± 14.64	85.4 ± 15.57	75.2 ± 17.22	67.6 ± 17.75	65.7 ± 21.57	0.090
LBW-%	1.1 ± 0.96	5.2 ± 0.91	5.5 ± 1.01	4.8 ± 1.11	4.2 ± 1.16	4.1 ± 1.39	0.910
Intake(g)							
1	93.41 ± 1.14[a]	89.54 ± 0.70[bc]	89.5 5± 0.70[c]	88.86 ± 0.6[c]	93.60 ± 0.05[a]	92.23 ± 1.30 [ab]	0.001**
4	102.02 ± 1.07	104.62 ± 1.17	101.55 ± 1.98	100.99 ± 1.08	102.93 ± 1.01	103.41 ± 1.15	0.053
8	108.22 ± 1.3[b]	112.70 ± 1.2[a]	113.60 ± 1.4[a]	107.67 ± 1.6[b]	114.93 ± 1.1[a]	113.25 ± 2.0[a]	0.005**
Total	101.68 ± 0.75[bcd]	102.40 ± 0.32[abc]	101.27 ± 0.45[d]	100.98 ± 0.40[d]	103.12 ± 0.20[a]	102.86 ± 0.23[ab]	0.004**
FCR							
1	1.56 ± 0.36	1.50 ± 0.29	1.48 ± 0.22	1.46 ± 0.23	1.50 ± 0.29	1.48 ± 0.25	0.118
4	1.76 ± 0.047	1.7 7± 0.050	1.66 ± 0.032	1.67 ± 0.032	1.77 ± 0.097	1.69 ± 0.032	0.491
8	2.12 ± 0.11	1.84 ± 0.04	1.82 ± 0.04	1.82 ± 0.05	1.88 ± 0.03	1.90 ± 0.05	0.070
Total	1.81 ± 0.06[a]	1.69 ± 0.02 [b]	1.65 ± 0.02 [b]	1.68 ± 0.02 [b]	1.70 ± 0.02 [b]	1.72 ± 0.02 [b]	0.009**

BW (g): body weight; I-LBW: initial live body weight; F-LBW: final live body weight; LBW-g: live body weight gain (g); LBW-%: live body weight gain (%); Intake (g): feed intake; FCR: Feed conversion rate (g feed/g egg mass); C: control (no additive); EOs: essential oils; HA: humic acid; MCT: medium-chain triglycerides; EHAM: a mixture containing 0.75 g/kg of EOs, HA and MCT in the diet; Mix 1/2: a mixture of EHAM at 0.375 g/kg in the diet; Total: total of the 8 periods; *P*: probability; **: $P < 0.01$. [a, b, c]: Differences between the averages are significant in the same column with different letters.

due to the treatments ($P < 0.05$). However, LBW gain almost showed a trend of greater values to the supplemented treatments than the control group ($P > 0.05$). Regarding feed intake, in period 1, it was higher in control and EHAM groups, compared with HA and MCT, but in period 8, the feed intake was greater in all treatments compared with the control group. The total feed intake was higher in EHAM and Mix 1/2 groups, compared with the groups HA and MCT ($P = 0.004$). In period 8, a trend of higher feed conversion ratio (FCR) in the control group compared with the other groups was shown ($P > 0.05$). The total FCR was significantly higher in the control group compared with the supplemented treatments ($P < 0.01$).

Antibiotics have been widely used to promote animal development in livestock production. Nowadays, the use of antibiotics in animals has been restricted only to treatments. Recently, herbal essential oils, organic acids, and some fatty acids have been used instead of antibiotics. However, each of these active substances has different actions. The following should be taken into consideration when using these substances together: firstly, if the activity of one of the substances added provides the expected effects, there may be no need to add another. Secondly, if the additives have a synergistic effect and provide extra benefits when they are added together, and thirdly, if these additives are used together and provide economical contributions by reducing the doses used, it may be beneficial to use more than one active ingredient in the same product, and the products produced in this way can be used commercially and can benefit producers. In this study, the individual and combined effects of using herbal essential oil mixtures, humic acid, and MCT, which have different actions when used as feed additives, were investigated. In the current study, the treatments EOs, HA, and MCT did not affect the final BW of the laying hens. Wang et al. (2019) have reported that 150, 300, and 450 mg/kg of Eos supply had no effect on the LBW of laying hens. Also, in the present study, BW gain was not influenced by the treatments supplemented with EOs, HA, and MCT. It has been reported that essential oil mixture (Olgun, 2016), HA (Hakan et al., 2012), and MCT supply (Wang and Kim, 2011) did not influence the LBW gain of laying hens. Laying hens are not required to receive too much BWG during the feeding period. In addition, live weight losses are not desired. Excessive live weight gain increases basal metabolism and not only consumes more feed but also may cause problems such as prolapse. Decreasing live weight may lead to decreases in egg size and survival rate of the animal. Therefore, the fact that these products do not cause a significant change in live weight can be considered positive. That could be explained as a positive effect of additives on LBW gain; although not significant, there was a trend of higher BW gain in all supplemented treatments compared with the control group.

The total feed intake was higher in EHAM, and Mix 1/2 groups, compared with the HA groups and MCT added groups. Ghanima et al. (2020) have reported that the inclusion of 300 mg/kg of essential oils enhanced the feed intake of laying hens. Also, Marume et al. (2020) have noticed that the addition of 1.0–2.0 g/kg of an essential oil increased the feed intake of laying hens. However, in contrast to others, it has been reported that the addition of 0.1% of an essential oil did not affect the feed intake of laying hens (Bölükbaşi and Erhan, 2007). It has been shown that 2.0, 4.0, and 6.0 mg/kg of HA supply to laying hens' diet (Arafat et al., 2015), and 250, 350, and 450 mL/ton of HA supply in drinking water did not affect the feed intake of laying quails (Kaplan et al., 2018). Regarding the FCR, Liu et al. (2020a) have reported that 300, 600, and 900 mg/kg of EOs supply reduced the FCR of laying hens. Also, it has been reported that 0.5 and 1.0% (Ghanima et al., 2020) or 300 mg/kg of essential oils supply (Radwan et al., 2008) reduced the FCR of laying hens. The current study showed that EO, HA, and MCT additives decreased the FCR compared with the control group. Mudroňová et al. (2021) noted that the addition of HA did not significantly affect feed consumption; however, it positively affected FCR values in laying hens. Similar positive results were observed in FCR values by Arafat et al. (2015) when giving HA by drinking water to laying hens.

Egg production and egg quality traits

The egg characteristics obtained from laying hens with diets supplemented with EOS, HA, and MCT during 1, 4, 8, and overall periods are shown in Table 3. In periods 4 and 8, the egg yield (number and %) was greater in all treatments compared with the control group, following the same trend in egg mass. Although the total egg weight did not show any significant differences between groups ($P > 0.05$), the egg yield (number and %) and the egg mass were greater in all the treatments compared with the control group ($P < 0.01$). The egg weight and the egg mass in period 8 were heavier in all treatments compared with the control group ($P < 0.01$). The

Table 3. Effects of essential oils, humic acid, and medium-chain triglycerides on egg production and egg weights of laying hens

Parameter/ Period	Treatments						
Egg production (number)	C	Eos	HA	MCT	EHAM	Mix 1/2	*P*
1	41.00 ± 0.71	41.56 ± 0.44	41.56 ± 0.34	41.56 ± 0.18	41.89 ± 0.11	41.56 ± 0.29	0.756
4	39.89 ± 0.70^{b}	41.44 ± 0.44^{a}	41.67 ± 0.17^{a}	41.56 ± 0.24^{a}	42.00 ± 0.00^{a}	41.44 ± 0.38^{a}	0.008**
8	37.89 ± 1.57^{b}	41.89 ± 0.11^{a}	41.89 ± 0.11^{a}	40.44 ± 0.44^{a}	41.22 ± 0.40^{a}	40.78 ± 0.52^{a}	0.003**
Total	39.44 ± 0.73^{b}	41.67 ± 0.17^{a}	41.56 ± 0.24^{a}	41.44 ± 0.24^{a}	41.56 ± 0.18^{a}	41.00 ± 0.17^{a}	0.001**
Egg yield (%)							
1	97.67 ± 1.65	98.89 ± 0.1.11	99.00 ± 0.78	99.11 ± 0.35	99.78 ± 0.22	98.89 ± 0.73	0.750
4	94.78 ± 1.67^{b}	98.67 ± 1.11^{a}	99.33 ± 0.33^{a}	99.00 ± 0.58^{a}	100.00 ± 0.00^{a}	98.67 ± 0.90^{a}	0.005**
8	90.22 ± 3.73^{b}	99.78 ± 0.22^{a}	99.78 ± 0.22^{a}	96.33 ± 1.05^{a}	98.11 ± 0.96^{a}	97.00 ± 1.28^{a}	0.003**
Total	94.22 ± 1.88^{b}	99.56 ± 0.34^{a}	98.89 ± 0.42^{a}	98.22 ± 0.49^{a}	98.56 ± 0.38^{a}	97.89 ± 0.35^{a}	0.001**
Egg weight (g)							
1	61.27 ± 0.79	60.34 ± 0.80	61.49 ± 0.82	61.70 ± 0.82	62.58 ± 0.97	63.12 ± 0.79	0.243
4	61.32 ± 0.58	60.23 ± 0.87	61.84 ± 0.54	61.07 ± 0.81	59.43 ± 0.67	62.12 ± 0.65	0.671
8	57.74 ± 1.00^{b}	61.62 ± 0.74^{a}	62.51 ± 0.88^{a}	61.59 ± 0.75^{a}	62.47 ± 0.80^{a}	61.43 ± 0.80^{a}	0.002**
Total	61.27 ± 0.79	61.05 ± 0.40	61.95 ± 0.62	61.31 ± 0.45	61.77 ± 0.68	61.42 ± 0.61	0.434
Egg mass (g)							
1	59.86 ± 1.52	59.72 ± 1.10	60.82 ± 0.78	61.05 ± 0.85	62.42 ± 1.05	62.48 ± 1.05	0 .318
4	58.08 ± 1.20	59.45 ± 1.21	61.35 ± 0.53	60.41 ± 0.71	59.43 ± 2.67	61.27 ± 067	0.526
8	51.95 ± 1.97^{b}	61.46 ± 0.81^{a}	62.34 ± 0.91^{a}	59.30 ± 0.84^{a}	61.31 ± 0.97^{a}	59.62 ± 0.94^{a}	0.001**
Total	56.76 ± 109^{b}	60.71 ± 0.54^{a}	61.30 ± 0.63^{a}	60.22 ± 0.52^{a}	60.88 ± 0.75^{a}	60.15 ± 0.68^{a}	0.001**

$^{a, b, c}$: Differences between the averages are significant in the same column with different letters. C: control (no additive); EOs: essential oils; HA: humic acid; MCT: medium-chain triglycerides; EHAM: a mixture containing 0.75 g/kg of EOs, HA and MCT in the diet; Mix 1/2: a mixture of EHAM at 0.375 g/kg in the diet; Total: total of the 8 periods; *P*: probability; **: $P < 0.01$.

total egg mass showed heavier values in all treatments compared with the control group ($P < 0.01$).

The diets supplemented with EOs, HA, and MCT increased the egg yield compared with the control group ($P < 0.001$). These EOs results are consistent with the ones reported by Bölükbaşi and Erhan (2007), Ding et al. (2017); Liu et al. (2020a). However, it has also been described that the EOs did not affect the daily egg production of laying hens (Arpášová et al., 2015; Olgun, 2016; Yu et al., 2018). The HA has been shown to improve the egg production of laying hens (Yörük et al., 2004; Arpášová et al., 2016; Mudroňová et al., 2021). In the present study, the egg mass increased when the diets were supplemented with EOs, HA, and MCT compared with the control ($P = 0.001$). Similar results were observed in other studies where the EO (Yu et al., 2018; Ghanima et al., 2020; Marume et al., 2020) and HA (Ozturk et al., 2009; Mudroňová et al., 2021) addition to diets increased egg mass of the laying hens.

The egg yolk characteristics obtained from laying hens with diets supplemented with essential oils, humic acid, and medium-chain triglycerides during 1, 4, 8 and overall periods are shown in Table 4. Although the egg yolk brightness showed significant differences in period 4 ($P < 0.01$). The total egg yolk brightness (L*) did not show any significant differences between the groups in the other and overall period ($P > 0.05$). The egg yolk redness was only significant in period 8 ($P > 0.05$), but the total egg yolk redness was not significant ($P > 0.05$). Although the egg yolk yellowness showed significant differences between the groups in period 1 ($P > 0.01$), , the total egg yolk yellowness was similar in periods 4, 8, and overall ($P > 0.05$). There were no significant differences between the groups in terms of albumen height (Hmm), Roche color scale (RCS), and Haugh unit (HU) in eggs.

The eggshell characteristics obtained from laying hens with diets supplemented with essential oils, humic acid, and medium-chain triglycerides are shown in Table 5. During periods 1, 4, and 8, eggshell ratio and eggshell weight UA did not show any significant differences between the groups ($P > 0.05$). The treatments showed a greater eggshell surface area in period 8 compared with the control ($P > 0.01$). The total eggshell ratio, eggshell surface area (without period 8), and eggshell unit area weight (ESUW) did not show any significant differences between the groups ($P > 0.01$).

The eggshell thickness (during periods 4 and 8) and the total eggshell thickness were higher in all treatments compared with the control group ($P < 0.01$). The eggshell weight of the MCT and Mix 1/2 addition group was significantly greater compared with the control and EOs groups; also, total eggshell weight was greater in Mix 1/2 compared with the control group ($P < 0.01$).

Diet additives did not have any significant effect on egg yolk height, brightness, redness, and yellowness ($P > 0.05$). These results are consistent with the reports showing that MCT and HA did not have any effect on egg yolk color (Arpášová et al., 2016; Bozkurt et al., 2016; Liu et al., 2020; Wang et al., 2019). It was also reported that EOs and MCT could not have any effect on egg yolk color (Wang and Kim, 2011; Klementavičiute et al., 2016). Since the EO, MCT, and HA additives do not contain colorants, egg yolk color was not influenced by these feed additives and their mixture.

The diets supplemented with EOs, HA, and MCT did not have any significant effect on the total RSS, HU, eggshell ratio, eggshell surface area, and eggshell weight UA ($P > 0.05$). Olgun (2016) has reported that the EO mixture addition to the laying hen diet did not have any effect on egg-specific gravity and shell weight. However, it has been reported that the essential oils (Özek et al., 2011; Ding et al., 2017), MCT, and HA (Hakan et al., 2012) addition increased the egg HU according to the control treatment.

The total eggshell thickness was higher in all treatments compared with the control ($P = 0.000$). Olgun (2016) and Torki et al. (2018) have reported that EOs improved the eggshell thickness. In contrast to this result, Florou-Paneri et al. (2005) have demonstrated that the dietary supplementation of rosemary, oregano, and saffron did not affect eggshell thickness. Liu et al. (2020a) reported that medium-chain α-monoglycerides increased the eggshell thickness. However, the HA addition tested no effect on eggshell thickness (Macit et al., 2021). Although it has been reported that the egg weight was increased when the diets were supplemented with essential oils, MCT, and HA addition (Özek et al., 2011; Ghanima et al., 2020; Liu et al., 2020b). In the current study, the total eggshell weight was greater only in the Mix 1/2 group compared with the control group ($P = 0.007$). As reported by Olgun (2016), in the present study, EOs did not show any significant differences in eggshell weight compared with the control (Arpášová et al., 2015; Wang et al., 2019). Also, it has been reported that EOs (Torki et al., 2018) and HA addition (Hakan et al., 2012; Macit et al., 2021) did not have any significant effect on eggshell weight. However, it has also been reported that EOs (Bayram et al., 2007) and HA (Sopoliga et al., 2016) addition to diets could decrease eggshell weight.

Fecal microbial activity

The fecal microbial activity (CFU/mL) in laying hens with diets supplemented with essential oils, humic acid, and medium-chain triglycerides are shown in Table 6. The control group showed the greatest microbial population of *E. coli, Klebsiella* spp., *C. albicans,* and total Gram-negative bacteria than other treatments ($P < 0.01$). Similarly, *Staphylococcus* spp. and the total Gram-positive bacteria were higher in the control and Mix 050 groups, compared with the other groups ($P < 0.01$). On the other hand, HA showed the lowest amounts of *E. coli, Klebsiella* spp., *Staphylococcus* spp.*, and C. albicans*.

In the current study, the diets supplemented with EOs, HA, and MCT addition positively decreased the microbial populations of fecal *E. coli, Klebsiella* spp., *Staphylococcus* spp., *C. albicans,* total Gram-positive, and total Gram-negative bacteria, compared with the control group of laying hens. It has been reported that the essential oils showed a strong antibacterial activity (Bakkali et al., 2008; Brenes and Roura, 2010; Karásková et al., 2015), which is consistent with the results obtained in the current study. Basile et al. (2006) have reported that the essential oils showed a broad antibacterial spectrum for both, Gram-positive and Gram-negative bacterial strains. Akyurek and Yel (2011) have reported that thymol and carvacrol essential oils showed some antimicrobial properties

Table 4. Effects of essential oils, humic acid, and medium-chain triglycerides on egg internal quality characteristics of laying hens

Parameter/ Period	Treatments						
Albumen height (hmm)	C	EOs	HA	MCT	EHAM	Mix 1/2	*P*
1	3.98 + 0.12	3.83 ± 0.12	3.96 ± 0.17	4.03 ± 0.17	3.69 ± 0.18	4.10 ± 0.23	0.623
4	4.55 ± 0.10	4.39 ± 0.20	4.27 ± 0.20	4.52 ± 0.19	4.24 ± 0.10	4.37 ± 0.21	0.760
8	4.17 ± 0.29	4.29 ± 0.25	4.52 ± 0.15	4.56 ± 0.22	4.39 ± 0.19	4.20 ± 0.29	0.803
Total	4.24 ± 0.10	4.38 ± 0.08	4.15 ± 0.09	4.39 ± 0.10	4.26 ± 0.33	4.30 ± 0.9	0.393
Haugh Unit, HU							
1	57.79 ± 1.21	54.41 ± 2.47	56.56 ± 189	56.76 ± 2.34	53.83 ± 2.59	57.58 ± 1.96	0.699
4	62.96 ± 1.24	60.20 ± 2.68	59.33 ± 2.32	62.41 ± 1.94	58.80 ± 1.62	60.75 ± 2.32	0.672
8	60.50 ± 3.00	58.99 ± 3.19	62.53 ± 1.61	61.66 ± 2.88	58.98 ± 2.62	57.89 ± 3.58	0.858
Total	59.45 ± 0.78	60.25 ± 0.99	57.29 ± 1.19	60.54 ± 1.29	58.56 ± 0.45	59.99 ± 0.87	0.178
Egg yolk RCS							
1	8.30 ± 0.13	7.81 ± 0.26	8.15 ± 0.28	8.15 ± 0.20	8.00 ± 0.22	8.45 ± 0.16	0.391
4	4.89 ± 0.11	5.00 ± 0.29	4.67 ± 0.17	5.11 ± 0.26	4.78 ± 0.15	4.89 ± 0.11	0.657
8	4.89 ± 0.12	4.93 ± 0.17	4.74 ± 0.09	4.71 ± 0.14	4.85 ± 0.13	4.89 ± 0.12	0.805
Total	5.38 ± 0.07	5.35 ± 0.07	5.44 ± 0.09	5.38 ± 0.07	5.36 ± 0.06	5.46 ± 0.11	0.917
Brightness (L*)							
1	65.46 ± 1.21	63.79 ± 0.92	61.98 ± 0.70	63.88 ± 1.50	63.42 ± 1.65	64.29 ± 0.32	0.437
4	64.61 ± 0.33^{a}	63.60 ± 0.41^{ab}	62.62 ± 038^{bc}	63.72 ± 0.21^{a}	62.25 ± 0.41^{c}	59.07 ± 0.34^{d}	0.001**
8	59.61 ± 0.32	60.50 ± 0.55	60.09 ± 0.53	60.67 ± 1.06	61.48 ± 0.32	61.60 ± 0.56	0.173
Total	63.12 ± 0.24	62.58 ± 0.37	62.06 ± 0.35	62.56 ± 0.22	62.10 ± 0.15	61.46 ± 0.18	0.210
Redness (a*)							
1	5.05 ± 0.17	4.83 ± 0.14	4.71 ± 0.08	4.82 ± 0.12	4.82 ± 0.12	4.80 ± 0.09	0.506
4	4.26 ± 0.07	4.45 ± 0.12	4.59 ± 0.10	4.80 ± 0.39	4.63 ± 0.09	4.40 ± 0.08	0.359
8	4.49 ± 0.15^{a}	4.83 ± 0.15^{ab}	4.95 ± 0.08^{b}	4.72 ± 0.08^{ab}	4.96 ± 0.13^{b}	4.92 ± 0.08^{b}	0.047*
Total	4.53 ± 0.04	4.59 ± 0.09	4.69 ± 0.07	4.62 ± 0.18	4.67 ± 0.08	4.53 ± 0.06	0.788
Yellowness (b*)							
1	46.36 ± 0.98^{a}	44.00 ± 0.64^{bc}	41.93 ± 0.55^{c}	45.18 ± 0.63^{ab}	43.94 ± 0.67^{bc}	45.49 ± 0.89^{ab}	0.002
4	42.32 ± 0.80	42.26 ± 0.45	42.86 ± 0.64	42.05 ± 0.62	40.58 ± 0.69	40.31 ± 0.96	0.080
8	40.34 ± 0.54	38.76 ± 0.67	39.51 ± 0.53	38.86 ± 0.86	40.65 ± 0.75	40.55 ± 0.46	0.152
Total	42.26 ± 0.34	41.98 ± 0.23	41.36 ± 0.47	42.16 ± 0.29	41.75 ± 0.36	41.95 ± 0.35	0.417

[a, b, c]: Differences between the averages are significant in the same column with different letters. L*: egg yolk brightness; Redness (a*): egg yolk redness; Yellowness (b*): egg yolk yellowness; C: control (no additive); EOs: essential oils; HA: humic acid; MCT: medium-chain triglycerides; EHAM: a mixture containing 0.75 g/kg of EOs, HA and MCT in the diet; Mix 1/2: a mixture of EHAM at 0.375 g/kg in the diet; Total: total of the 8 periods, *P*: probability; *: $P < 0.05$; **: $P < 0.01$.

after their inclusion in a diet of one-day-old broiler chicks. Essential oils supply increased beneficial microbes, such as *Lactobacillus* and yeast, while decreasing the pathogenic *Escherichia coli* amounts in the gut. Shermer et al. (1998) explained that humates could inhibit the pathogenic microbes and stimulate beneficial microbes in the gut but without any effect on anaerobic microbial amounts. Mudroňová et al. (2020) have reported that HA addition improved the stimulation and engulfing activity of phagocytes and gut health status by reducing the pathogenic microbial, such as *Enterobacteriaceae* amounts. Liu et al. (2020b) have noticed that the medium-chain α-monoglycerides are considered an efficient feed supplement to improve the production performance by modulating intestinal microflora. Medium-chain α-monoglycerides decreased the gut microbial population such as *Schlegelella and Proteobacteria*. Hermans et al. (2011) have reported that different types of MCT supply (lauric acids, caprylic acids, capric acids, and caproic acids) reduced the colonization of *Campylobacter* in the broiler intestine. In other words, essential oils could have a strong antibacterial activity, shown by reducing the pathogenic microbial, such as *E. coli, Klebsiella* spp., *Staphylococcus* spp., *C. albicans,* total Gram-positive, and total Gram-negative bacteria, improving the gut health status and, therefore, the FCR, increasing the egg production and mass and eggshell thickness, but not showing any significant effect on egg yolk characteristics.

Blood parameters

Finally, the plasma indicators (mg/dL) of laying hens supplemented with diets with essential oils,

Table 5. Effects of essential oils, humic acid, and medium-chain triglycerides on eggshell quality characteristics of laying hens

Parameter	Treatments						
	C	EOs	HA	MCT	EHAM	Mix 1/2	*P*
ES thickness (μm)							
1	0.43 ± 0.003	0.43 ± 0.006	0.43 ± 0.002	0.43 ± 0.003	0.44 ± 0.002	0.43 ± 0.003	0.304
4	0.42 ± 0.004[b]	0.43 ± 0.003[a]	0.43 ± 0.002[a]	0.43 ± 0.002[a]	0.43 ± 0.002[a]	0.43 ± 0.003[a]	0.001**
8	0.41 ± 0.005[b]	0.43 ± 0.003[a]	0.43 ± 0.003[a]	0.43 ± 0.003[a]	0.43 ± 0.003[a]	0.43 ± 0.002[a]	0.001**
Total	0.42 ± 0.002[b]	0.43 ± 0.002[a]	0.43 ± 0.001[a]	0.43 ± 0. 001[a]	0.43 ± 0. 001[a]	0.43 ± 0. 001[a]	0.001**
ES weight (g)							
1	6.06 ± 0.11	6.00 ± 0.11	6.19 ± 0.12	6.19 ± 0.06	6.22 ± 0.08	6.28 ± 00.7	0.275
4	6.07 ± 0.08	6.13 ± 0.08	6.17 ± 0.09	6.15 ± 0.05	6.05 ± 0.05	6.12 ± 0.08	0.860
8	5.64 ± 0.09[c]	6.13 ± 0.06[bc]	6.16 ± 0.05[ab]	6.07 ± 0.07[a]	6.22 ± 0.09[a]	6.12 ± 0.08[ab]	0.001**
Total	5.94 ± 0.0 [b]	6.03 ± 0.0 [ab]	6.09 ± 0.03[ab]	6.06 ± 0.02[ab]	6.07 ± 0.02[ab]	6.11 ± 0.05[a]	0.007**
ES ratio (%)							
1	9.90 ± 0.28	9.94 ± 0.26	10.09 ± 0.45	10.07 ± 0.50	9.97 ± 0.74	9.95 ± 0.22	0.935
4	10.14 ± 0.19	9.88 ± 0.22	10.07 ± 0.20	9.96 ± 0.15	10.21 ± 0.12	10.20 ± 0.16	0.731
8	10.28 ± 0.19	10.06 ± 0.12	10.15 ± 0.15	10.18 ± 0.10	10.10 ± 0.20	10.09 ± 0.13	0.924
Total	10.28 ± 0.06	10.35 ± 0.04	10.36 ± 0.03	10.36 ± 0.05	10.22 ± 0.06	10.21 ± 0.03	0.052
ESSA (mm)							
1	72.57 ± 0.66[ab]	71.79 ± 0.67[b]	72.75 ± 0.69[ab]	72.92 ± 0.68[ab]	73.65 ± 0.81[ab]	74.11 ± 0.65[a]	0.243
4	72.61 ± 0.49	71.69 ± 0.73	73.05 ± 0.45	72.39 ± 0.68	70.89 ± 2.35	73.27 ± 0.54	0.660
8	69.58 ± 0.85[b]	72.86 ± 0.61[a]	73.59 ± 0.73[a]	72.83 ± 0.62[a]	73.56 ± 0.66[a]	72.70 ± 0.67[a]	0.002**
Total	71.78 ± 0.55	72.38 ± 0.34	73.14 ± 0.52	72.60 ± 0.38	72.99 ± 0.57	72.69 ± 0.52	0.434
ESUW (mg)							
1	8.35 ± 0.09	8.35 ± 0.09	8.51 ± 0.13	8.50 ± 0.11	8.46 ± 0.18	8.48 ± 0.56	0.842
4	8.37 ± 0.14	8.56 ± 0.16	8.45 ± 0.15	8.50 ± 0.11	8.63 ± 0.36	8.35 ± 0.11	0.891
8	8.06 ± 0.09	7.85 ± 0.11	8.00 ± 0.08	8.24 ± 0.06	8.24 ± 0.09	8.06 ± 0.12	0.051
Total	8.28 ± 0.07	8.26 ± 0.02	8.24 ± 0.05	8.31 ± 0.06	8.20 ± 0.06	8.30 ± 0.04	0.709

[a, b, c]: Differences between the averages are significant in the same column with different letters. C: control (no additive); EOs: essential oils; HA: humic acid; MCT: medium-chain triglycerides; EHAM: a mixture containing 0.75 g/kg of EOs, HA and MCT in the diet; Mix 1/2: a mixture of EHAM at 0.375 g/kg in the diet; RSS: Roche color scale values; HU: Haugh unit; ES: eggshell; ESSA: eggshell surface area, ESUW (mg): eggshell weight unit weight; Total: total of the 8 periods; *P*: probability; **: $P < 0.01$.

humic acid, and medium-chain triglycerides are shown in Table 7. The plasma glucose, triglyceride, cholesterol, HDL, and LDL were not affected by the different treatments ($P > 0.05$). Similar to this study, it was shown that the effects of essential oil mixtures (Ghanima et al., 2020), humic acids (Mudroňová et al., 2021), and medium-chain fatty acids (Liu et al., 2022) added to the rations on the blood values of laying hens were not significant. It is desired that feed additives do not cause significant changes in the blood indicators of animals. This is because a stable plasma glucose level indicates that there is no energy metabolism and metabolic diseases in animals. Similarly, triglycerides are important in terms of energy metabolism and the synthesis of new substances in the body and their change is undesirable. Cholesterol is involved in the structure of brain membranes and other cell synthesis, and high blood cholesterol levels are an undesirable blood component in terms of the emergence of cardiovascular diseases.

Conclusion

Compared with the control group, EOs, humic acid, medium-chain fatty acids and their combination and decreased ratios of the combination improved laying hens' performance traits, egg production and eggshell thickness. Also, these additives decreased bacterial loads in feces. So, the addition of EOs, humic acid, medium-chain fatty acids and their combination may act as a performance enhancer and an antibacterial agent. They may benefit the animal health status by reducing the pathogenic microbial activity in the gut. Future studies should also consider other parameters, such as diet digestibility and beneficial bacteria along the productive life of laying hens.

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Table 6. Effects of essential oils, humic acid, and medium-chain triglycerides on fecal microbial activity (CFU/mL) in laying hens

Groups	*Escherichia coli*	*Klebsiella* spp.	*Staphylococcus* spp.	*Candida albicans*	Total Gram + bacteria	Total Gram-bacteria
C	4.8 ± 0.01^{a}	4.3 ± 0.04^{a}	4.2 ± 0.03^{a}	3.9 ± 0.01^{a}	4.7 ± 0.02^{a}	5.0 ± 0.01^{a}
Eos	2.7 ± 0.05^{c}	3.3 ± 0.08^{c}	2.8 ± 0.13^{d}	2.6 ± 0.05^{d}	3.5 ± 0.09^{c}	3.5 ± 0.08^{d}
HA	2.3 ± 0.29^{d}	3.0 ± 0.10^{d}	2.1 ± 0.27^{e}	2.3 ± 0.07^{e}	3.1 ± 0.06^{d}	3.4 ± 0.04^{d}
MCT	3.5 ± 0.10^{b}	3.5 ± 0.08^{c}	3.4 ± 0.03^{c}	2.6 ± 0.10^{d}	4.1 ± 0.05^{b}	4.5 ± 0.04^{c}
EHAM	2.9 ± 0.04^{c}	3.3 ± 0.07^{c}	3.6 ± 0.06^{bc}	3.4 ± 0.04^{c}	4.4 ± 0.02^{b}	4.4 ± 0.03^{c}
Mix 1/2	3.4 ± 0.03^{b}	3.7 ± 0.03^{b}	3.9 ± 0.01^{ab}	3.6 ± 0.02^{b}	4.6 ± 0.01^{a}	4.6 ± 0.01^{b}
P	0.001**	0.001**	0.001**	0.001**	0.001**	0.001**

$^{a, b, c}$: Differences between the averages are significant in the same column with different letters. C: control (no additive); EOs: essential oils; HA: humic acid; MCT: medium-chain triglycerides; EHAM: a mixture containing 0.75 g/kg of EOs, HA and MCT in the diet; Mix 1/2: a mixture of EHAM at 0.375 g/kg in the diet; *P*: probability; **: $P < 0.01$.

Table 7. Effects of essential oils, humic acid, and medium-chain triglycerides on blood serum indicators (mg/dL) of laying hens

Groups	Glucose, mg/dL	Triglyceride, mg/dL	Cholesterol, mg/dL	HDL, mg/dL	LDL, mg/dL
C	205.4 ± 7.03	914.0 ± 217.23	128.2 ± 27.89	16.3 ± 31.61	38.3 ± 52.27
Eos	206.6 ± 7.00	1071.4 ± 187.58	116.0 ± 24.78	24.3 ± 15.38	29.7 ± 29.12
HA	201.6 ± 1.75	1155.4 ± 133.24	117.4 ± 25.04	21.9 ± 19.90	77.7 ± 41.79
MCT	211.2 ± 5.00	1009.0 ± 132.80	105.6 ± 18.84	19.3 ± 15.36	66.1 ± 28.51
EHAM	212.4 ± 3.41	1168.8 ± 124.49	105.0 ± 13.63	12.0 ± 7.97	116.8 ± 19.23
Mix1/2	206.6 ± 3.39	1140.8 ± 102.21	124.8 ± 21.47	38.1 ± 22.15	48.3 ± 35.43
P	0.680	0.833	0.976	0.960	0.573

C: control (no additive); EOs: essential oils; HA: humic acid; MCT: medium-chain triglycerides; EHAM: a mixture containing 0.75 g/kg of EOs, HA and MCT in the diet; Mix 1/2: a mixture of EHAM at 0.375 g/kg in the diet.

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The Present Situation of Guarding Dog Usage Opportunities in Livestock Production of Turkey for Wolf Damages

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***Keywords**: guarding dog, Anatolian dog, predators, sheep and goat, wolf damages.*

***Abstract.** One of the most important problems of the small ruminant sector is economic losses due to predators. The problem is mostly caused by wild predators and manifests itself, especially by the damage caused to sheep and goat flocks, while the presence of these very same predators is absolutely necessary for the ecological system. Because sheep flocks generally graze in the pasture at night, guarding them from wild predators is an important aspect of pasture-based sheep farming. For this purpose, livestock guarding dogs have been used primarily against predators such as foxes, wolves, bears, coyotes, hyenas, and big cats. In this study, a specific research survey form was developed and used to determine predator damage and the current status of shepherd dog use. Sheep and goat flocks were randomly selected from 12 different provinces where the research was implemented. Among the farms included in the research, 40.4% of them breed sheep, 52.5% breed goats, and 7.1% breed both sheep and goats. The flock size varies between 100 to 500 heads, and the breeding system is traditionally based on pasture, with some flocks being bred as nomadic herds on plateaus or pastures during summer. Among the provinces included in this research, the average number of wolf attacks and the number of animal casualties were 6.01 ± 0.704 and 12.17 ± 1.329, respectively, and the differences between provinces were statistically significant ($P < 0.05$, $P < 0.01$). The average of the total economic damage caused by wolf attacks on sheep and goat herds was determined to be 2299.7 ± 235.2 US$ and losses between provinces differed significantly ($P < 0.01$).*

Introduction

One of the most important problems of the small ruminant sector is economic losses due to predators. The problem is mostly caused by wild predators and manifests itself, especially by the damage caused to sheep and goat flocks, while the presence of these very same predators is absolutely necessary for the ecological system. For this reason, the interest in the losses caused by wild predators (wolf, bear, jackal, lynx, hyena, dingo, coyote, etc.) to pasture-based animal production is increasing day by day (Kellert et al., 1996; Mech, 1999). The level of economic loss caused by predators in small ruminant production is not trivial. In the USA alone, wild predators constitute 34% of all causes of livestock deaths (Browns et al., 1997; McNeal, 2001). In 2004, the casualties in US farms due to predator damage amounted to 155.000 heads, corresponding to 18.3 million US dollars in economic value (Jones, 2004). For this reason, controlling predator damage in small ruminant flocks is one of the most important factors that will ensure the profitability of sheep and goat farming operations.

Different methods are used to protect and control sheep, goats, and other farm animals from wild predator attacks. These approaches can be categorized as passive (non-lethal) and active (lethal) methods, although they may not be suitable for all livestock breeders (Rollins et al., 2004). Therefore, when determining the most effective livestock protection program, it is of great importance to effectively combine herd husbandry practices with an integrated approach in terms of protection from wild predator attacks. In addition, one must be careful to ensure that the protection methods chosen for predator control do not cause stress among the animals in the flock. Sizewise, sheep and goats are the easiest targets for wild predator attacks, and, among all livestock species, they are also the most exposed to attacks by predators (Frezard and Le Pape, 2003).

The most common protection methods implemented in sheep and goat flocks fall under the passive control category, which do not kill wild predators, and are more acceptable and preferred for preserving the ecosystem (Connover and Kessler, 1994; Gilsdorf et al., 2002). Passive methods are successful if the farming of sheep and goats is conducted in a problem-free and healthy environment while not directly eradicating the wild animal population in the region. Some passive methods include environmental controls such as using livestock guarding dogs (LGDs), having ewes and does giving births in shelters, keeping herds in sheltered corrals at night, scaring predators using scarecrows, traps, electric or traditional fence systems, utilizing sound-making deterrents equipment, and many more.

Apart from these methods, guarding or protective animals such as donkeys and llamas are widely used in some countries (Taşkın et al., 2011). Conversely, lethal methods for wild predator control are used when passive methods are not very effective or losses

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in sheep and goat herds continue to increase. Lethal methods include foot or leg traps, live cages or traps, firearms, and the use of chemicals such as M-44 (sodium cyanide) (Taşkın et al., 2011). The basis of the use of active (lethal) control methods is that they reduce the number of wild predators close to sheep and goat farms (Anonymous, 2005). Despite the excessive killing of wild predators such as jackals and wolves, however, these predators need to be able to reproduce to continue their species and protect their existence in the natural world. Because sheep flocks generally graze in the pasture at night, guarding them from wild predators is an important issue of pasture-based sheep farming. For this purpose, LGDs have been used primarily against predators such as foxes, wolves, bears, coyotes, hyenas, and big cats (Smith et al., 2000). Livestock guarding dogs most likely emerged in Mesopotamia and in surrounding regions, which had well-developed small sheep and goat farms (Landry, 1999).

Livestock guarding dogs, as one of the passive fighting methods, are medium to large-sized dogs kept with livestock to protect the latter from predators. They have been bred for thousands of years to protect sheep and other domesticated livestock from predators and thieves (Smith et al., 2000; Gehring et al., 2010). Livestock guarding dogs are believed to be the ancestors of today's mastiffs, which lived on the high Tibetan plateaus in prehistoric times (Guardamagna, 1995). Traditionally, LGDs have also been used to guard cattle in other parts of Europe and Asia, although this practice is less common compared with the use of LGDs to protect sheep. Coppinger et al. (1988a) reported that LGDs reduced predator losses by 60%, whereas Green and Woodruff (1988) confirmed this result with a 82% reduction, supporting the idea of LGD economic benefits to breeders. In their study with 160 breeders in the US state of Colorado, Andelt and Hopper (2000) reported that 84% of LGDs were used excellently or well, 13% were used moderately, and 3% were inadequately used. In addition, other researchers working on the subject have determined that the success rates of protecting flocks with dogs in reducing economic losses vary between 11% and 93%, with rates usually reaching 70% in short-term studies (Green and Woodruff, 1988; McGrew and Blakesley, 1982; Rust et al., 2013).

Livestock guarding dogs are also used effectively to protect goat herds, and although it is not a common practice, other social animals such as llamas, alpacas, and ostriches are also used for protection from wild predators in some countries (Landry, 2000). In an assessment made among approximately 50 LGD dog breeds from various countries for the protection of livestock animals (Landry, 2000), Pyrenees (France, Spain), Kangal-Anatolian shepherd dog (Turkey), Komondor (Hungary), Maremma (Italy) and Sharplanina (Serbia- and former Yugoslavia) were among the most common breeds (Andelt, 2004; Coppinger et al., 1988b; Green and Woodruff, 1988; Landry, 2000; Van Bommel, 2010). The effectiveness of LGDs is influenced by many features, especially their behavioural and physical features, including those body structure, body condition, ability to smell, courage, vision and hearing ability.

The aim of this study was to investigate wolf predator damage in sheep and goat flocks in Turkey and the current use of herd protection or shepherd dogs, which is one of the non-lethal (passive control) methods to protect these herds from predators, develop a solution to the problems in the field, and contribute to the very limited literature on the subject.

Materials and methods

Development of the survey form and animal material

Sheep and goat flocks were randomly selected from 12 different provinces where the research was implemented (Fig. 1). The flock size varies between 100 to 500 heads, and the breeding system is traditionally based on pasture, with some flocks being bred as nomadic herds on plateaus or pastures during summer. Usually both meat and milk are produced in these flocks. Additional feeding is provided during winter, in line with the financial resources of the farmer, and for this purpose, grains such as barley, wheat, etc. are used. Animal health services are covered by the Ministry of Agriculture District Offices and private veterinary clinics.

In this study, a specific research survey form was developed and used to determine predator damage and the current status of LGD use. The survey form consists of 11 questions and farmers were asked the following questions: to i) determine the number of wolf attacks on their flocks, ii) the number of animals killed in these attacks, and iii) the extent of damage (economic loss) done. To determine the type of measure taken by farmers against wolf attacks, they were asked to answer one of four options, which were (1) the use of shepherd dogs, (2) the use of firearms, (3) the use of scarecrows, and (4) other methods. To measure the effectiveness of these methods against wolves, farmers were asked to report the number of dogs they had, and their answers were evaluated at the province and farm levels. Turkey is known for having very famous dog breeds possessing important genetic resources in the guarding dog class. Therefore, producers were also asked to report the breed of their LGDs, such as (1) Kangal, (2) Akbaş and (3) others, to determine which breeds of dogs were used to protect their herds. A question was also included in the survey to determine the yearly economic cost of rearing and keeping LGDs. Farmers were asked whether or not they vaccinated their dogs to examine the importance they attached to the health of their dogs. The survey form also included questions about whether farmers acquired their LGDs through cash purchase, from their neighbours' farms and friends, or from their own pack of dogs, and about the number of shepherds used in their herds.

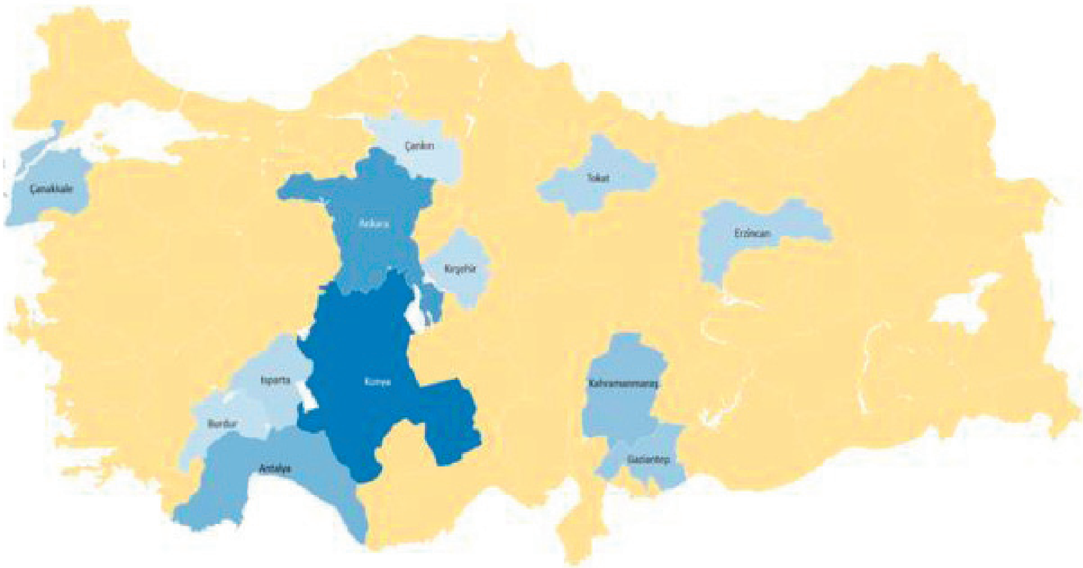

Fig. 1. Provinces where the study was conducted

Finally, to capture the producers' expectations from the government in their struggle against wolves, the survey form included (1) whether or not gun licenses should be given, (2) whether captured wolves should be released into nature, (3) whether LGDs should be provided to farmers, (4) whether livestock loss insurance should be provided free to farmers, (5) whether wolf hunting should be allowed, and (6) all the above options. Damage to the flock due to wolf attacks and annual LDG expenses were evaluated in Turkish Lira by converting them to current US dollars at the date of the study (Anonymous, 2022) using the exchange rate of the Central Bank of the Republic of Turkey.

The study was carried out in 368 sheep and goat farms included in the National Community-Based Small Ruminant Breeding Project (NCSRP) in 12 provinces (Table 1). The total number of small ruminants (sheep and goats) in the provinces studied in 2022 was 10 958 205 heads, constituting 19.48% of the total number of small ruminants in Turkey (Anonymous, 2022). Among all farms included in the research, 40.4% of them breed sheep, 52.5% breed goats, and 7.1% breed sheep and goats together. The farms where the survey was conducted were selected using a simple random sampling method, and farms with shepherd dog ownership were included in our sample, following the methodology of Güneş and Arıkan (1988). Each of the sheep and goat farms in our sample was visited separately, and survey forms were filled out during face-to-face meetings with livestock farmers and employees. Evaluations and face-to-face interviews of farms were conducted by the same person in each province and completed within the same day. The status of the different sheep and goat farms was analyzed through the use of descriptives (percentages, averages, standard error, minimum, and maximum). All statistical analyses were computed in Microsoft Office Excel 2010 for Windows and SPSS 14.01 (SPSS Inc., Chicago, IL, USA) where we used a one-way ANOVA and Duncan test to compare subsample means.

Results and discussion

The results obtained regarding the precautions taken against wolf attacks by sheep and goat farms within our scope, the dog breeds they used, methods of procuring their dogs, health and vaccination practices for shepherd dogs, the number of shepherds, and the breeders' expectation from the government are summarized in Table 2. Thereby, the precautions taken by sheep-goat farms against wolves, information about the livestock guarding dogs they used to protect their flocks from predators, the number of shepherds, and breeders' expectations from the government were determined.

Table 1. Number of animals in the province studied (Anonymous, 2022)

Number	Provinces	Sheep	Goat
1	ANKARA	1 680 217	333 259
2	ANTALYA	645 755	763 147
3	BURDUR	209 431	147 945
4	**ÇANAKKALE**	577 047	248 043
5	**ÇANKIRI**	163 848	22 743
6	ERZİNCAN	535 642	61 672
7	GAZİANTEP	620 099	231 291
8	ISPARTA	307 194	204 856
9	KIRŞEHİR	384 857	32 676
10	KONYA	2 770 980	275 489
11	KAHRAMANMARAŞ	601 216	450 933
12	TOKAT	467 040	74 722

Table 2. The precautions taken against wolves, some characteristics of livestock guarding dogs, the number of shepherds on farms and expectations from the state

Factors	Variables		Results, %
What precautions are taken against wolves?	Dog		62.6
	Firearms		28.9
	Scarecrow, etc.		2.1
	Other		6.4
What dog race/breed do you have?	Anatolian shepherd (Kangal)		32.9
	Akbaş		9.8
	Other		57.3
Are dogs vaccinated?	Yes		82.3
	No		17.7
How are dogs acquired?	Cash buying		25.4
	From neighbours		54.4
	From own pack of dogs		20.2
How many shepherds do you have?	Owner	72	19.6
	1	138	37.5
	2	103	28.0
	3	30	8.2
	4	14	3.8
	5 >	11	2.9
What is your expectation from the government in your struggle against wolves?	Gun licence		8.8
	No trapped wolf release		44.7
	Provide a free guarding dog		14.1
	Free insurance		11.1
	Wolf hunting should be legal		16.1
	All of the above		5.2

Table 3. The number of wolf attacks on sheep and goat farms

Factors	Province	N	Mean	Std. Error	Min	Max	*P*
Number of wolf attacks	Ankara	61	7.18 [abc]	2.374	0	100	*
	Antalya	29	10.34 [ab]	2.439	0	50	
	Burdur	41	5.27 [bc]	1.016	0	35	
	Çanakkale	32	13.59 [a]	5.520	0	150	
	Çankırı	18	1.17 [c]	0.345	0	6	
	Erzincan	23	6.00 [abc]	1.593	0	30	
	Gaziantep	31	1.74 [c]	0.250	1	8	
	Isparta	28	7.07 [abc]	1.739	1	40	
	Kırşehir	21	1.57 [c]	0.321	0	6	
	Konya	42	5.55 [bc]	0.790	1	25	
	Kahramanmaraş	20	1.90 [c]	0.228	0	4	
	Tokat	22	4.82 [bc]	1.033	0	18	
	Total	368	6.01	0.704	0	150	

* $P < 0.05$ a, b, c: Values within a column with different superscripts differ ($P < 0.05$).

Among the provinces included in our research, the average number of wolf attacks (Table 3) and the number of animal casualties (Table 4) were 6.01 ± 0.704 and 12.17 ± 1.329, respectively, and differed between provinces ($P < 0.05$, $P < 0.01$). The average of total economic loss caused by wolf attacks on sheep and goat herds (Table 5) was determined to be 2299.68 ± **235**.176 US dollars, and the losses differed between provinces ($P < 0.01$).

The average number of guarding dogs within a flock and their corresponding expenses (Table 6) were 4.76 ± 0.188 heads, and 256.07 ± 236.306 US dollars, respectively, and the differences in the number of guarding dogs and related expenses were different between the provinces ($P < 0.01$).

Precautions against wolves and preferred dog breeds

As a choice method for protecting their flocks against wolf attacks, 62.6% of the surveyed farmers used LGDs, 28.9% used firearms, 2.1% used scarecrows and 6.4% used other methods (Table 2, Fig. 2). These results indicate that the use of LGDs, which is among the predominant active methods used

Table 4. The number of animal casualties in flocks due to wolf attacks on sheep and goat farms

Factors	Province	N	Mean	Std. Error	Min	Max	*P*
Number of animal casualties	Ankara	61	14.26 ^{abc}	3.300	0	130	**
	Antalya	29	15.97 ^{abc}	5.187	0	150	
	Burdur	41	24.63 ^{a}	5.388	0	150	
	Çanakkale	32	19.09 ^{ab}	9.481	0	300	
	Çankırı	18	5.11 ^{bc}	1.765	0	25	
	Erzincan	23	8.04 ^{bc}	2.869	0	60	
	Gaziantep	31	7.81 ^{bc}	1.431	1	30	
	Isparta	28	18.11 ^{ab}	4.011	2	75	
	Kırşehir	21	1.00 ^{c}	.276	0	4	
	Konya	42	5.79 ^{bc}	1.022	0	30	
	Kahramanmaraş	20	4.90 ^{bc}	1.172	0	20	
	Tokat	22	6.18 ^{bc}	1.444	0	22	
	Total	368	12.17	1.329	0	300	

** $P < 0.01$ a, b, c: Values within a column with different superscripts differ ($P < 0.01$).

Table 5 Least square averages of the total economic losses caused by wolf attacks on sheep and goat flocks by province

Factors	Province	N	Mean	Std.Error	Min	Max	*P*
Total economic loss (US$)	Ankara	61	1679.24 ^{bcd}	360.755	0.00	13756.61	**
	Antalya	29	2829.78 ^{bc}	696.178	0.00	19841.27	
	Burdur	41	7468.06 ^{a}	1334.139	0.00	39682.54	
	Çanakkale	32	2523.56 ^{bcd}	1254.079	0.00	39682.54	
	Çankırı	18	787.77 ^{cd}	270.615	0.00	3968.25	
	Erzincan	23	1247.41 ^{bcd}	397.231	0.00	7936.51	
	Gaziantep	31	1279.23 ^{bcd}	237.163	158.73	4761.90	
	Isparta	28	3420.73 ^{b}	661.154	396.83	13227.51	
	Kırşehir	21	215.42 ^{d}	64.937	0.00	793.65	
	Konya	42	1058.83 ^{bcd}	185.403	0.00	5291.01	
	Kahramanmaraş	20	874.34 ^{bcd}	210.903	0.00	3174.60	
	Tokat	22	1366.04 ^{bcd}	414.747	0.00	8465.61	
	Total	368	2299.68	235.176	0.00	39682.54	

** : $P < 0.01$; a, b, c, d: Values within a column with different superscripts differ significantly ($P < 0.01$).

Table 6. Least square averages of the number of livestock guarding dogs in sheep and goat farms and expenditures on livestock guarding dogs by province

Factors	Province	N	Mean	Std.Error	Min	Max	*P*
Number of dogs	Ankara	61	6.92^{ab}	3.964	1	20	**
	Antalya	29	3.07^{de}	1.602	0	6	
	Burdur	41	5.49^{bc}	2.226	2	11	
	Çanakkale	32	5.31^{bc}	3.771	0	14	
	Çankırı	18	3.72^{cde}	1.904	0	8	
	Erzincan	23	2.30^{e}	1.295	0	5	
	Gaziantep	31	2.29^{e}	0.938	1	4	
	Isparta	28	3.86^{cde}	2.138	1	10	
	Kırşehir	21	4.29^{cd}	2.493	1	11	
	Konya	42	7.86^{a}	5.677	0	25	
	Kahramanmaraş	20	2.15^{e}	0.933	0	4	
	Tokat	22	3.77^{cde}	1.850	0	8	
	Total	368	4.76	3.605	0	25	
Dog expenses (head/year in US$)	Ankara	61	276.09^{bc}	223.886	39.68	793.65	**
	Antalya	29	245.12^{bc}	179.341	0.00	661.38	
	Burdur	41	707.83^{a}	190.383	158.73	1058.20	
	Çanakkale	32	286.67^{b}	257.549	0.00	1058.20	
	Çankırı	18	230.01^{bc}	82.934	52.91	330.69	
	Erzincan	23	181.27^{cd}	155.913	0.00	661.38	
	Gaziantep	29	122.03^{de}	83.442	0.00	476.19	
	Isparta	28	218.25^{bc}	101.347	105.82	529.10	
	Kırşehir	21	71.81^{e}	28.996	26.46	132.28	
	Konya	42	205.47^{bcd}	125.480	52.91	661.38	
	Kahramanmaraş	20	55.56^{e}	16.950	26.46	79.37	
	Tokat	22	119.95^{de}	60.900	0.00	264.55	
	Total	366	256.07	236.306	0.00	1058.20	

** $P < 0.01$; a, b, c, d, e: Values within a column with different superscripts differ significantly ($P < 0.01$).

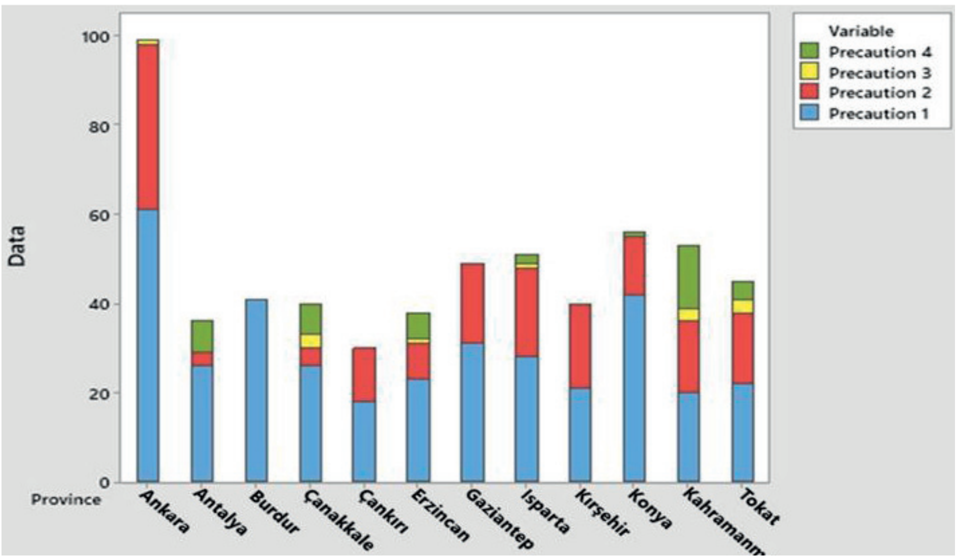

Fig. 2. Different precautions taken against predators and their distribution

Precaution 1: livestock guarding dogs; Precaution 2: firearms; Precaution 3: scarecrows; Precaution 4: other methods against predators worldwide, is also the preferred strategy adopted by farmers in Turkey. Smith et al. (2000) reported that LGDs reduce predatory damage in flocks by 11–100%. Breitenmoser et al. (2005), Gehring et al. (2010), Shivik (2006), and Smith et al. (2000) reported that the use of LGDs, along with non-lethal methods such as fencing and shepherd protection, is a prominent approach to reduce human and wildlife conflicts.

In response to predator attacks, results from our research show that the most preferred LGD breeds by the farmers were Kangal (Anatolian shepherd dog; 32.9%) and Akbaş (9.8%), but other breeds of dogs constituted 57.3% of all LGDs used in sheep and goat farms (Table 2, Fig. 3). Provinces with the highest use of Kangal and Akbaş breeds were Burdur and Ankara, respectively (Fig. 3).

The Kangal or Anatolian Shepherd is a genetic dog breed with immense popularity for small livestock farmers in Anatolia, especially in the Sivas province of Turkey, but also with global recognition. The origin of the Kangal in Anatolia can be traced to earlier than 6000 years ago, and this dog possesses an innate capacity of showing herd protection behavior independently of the shepherd. Several large "shepherd" or livestock guardian dog breeds were historically selectively bred to protect sheep and goat flocks in the Balkans, Anatolia, and the Caucasus regions (Gündemir et al., 2023). The Kangal breed herd protection ability is instinctively high and does not require any training. The Kangal breed possesses a short and dense coat and is adapted to the steppe climate of Anatolia where summers are hot and winters are cold. As a unique LGD with highly sensitive visual and olfactory features, the Kangal successfully fulfils its duty of protection against predators on most continents and countries such as South Africa and Namibia. For instance, in the Wild Cheetah Management Project (WCMP) implemented in South Africa in 2005 to reduce the damage caused by cheetahs to small ruminant flocks, Kangal dogs reduced predatory damage by 73%, with 93% of farmers recommending their use. The main reasons behind the choice of the Kangal breed in the WCMP program are the dog's large size, short hair coat, and ability to move freely and independently (Binge, 2017). The CCF (Cheetah Conservation Fund) LGD programme is centred around the breeding of LGDs in Namibia and the placement and follow-up of LGDs with farmers that were interested in participating in the programme. The selected dog breeds (i.e., Anatolian shepherd and Kangal dog) originated in Turkey and have guarded livestock from local predators, such as the brown bear (Ursus arctos), red fox (Vulpes vulpes), and grey wolf (Canis lupus), as well as other damage-causing species such as wild boar (Sus scrofa) for thousands of years (Marker et al., 2020).

The widespread purpose of Kangal breeding in Turkey is to protect livestock from predators, especially wolves in rural areas, and to use the dog as a guard or protection dog in residential areas (Akyazi et al., 2018). Due to their characteristics, they are preferred by small livestock farmers over other breeds of shepherd and guard dogs in both Turkey and abroad. Other than the Kangal dog, one of the most common LGDs in Turkey is the Akbaş shepherd dog. The Akbaş is bred in Sivrihisar, Afyon, Eskisehir

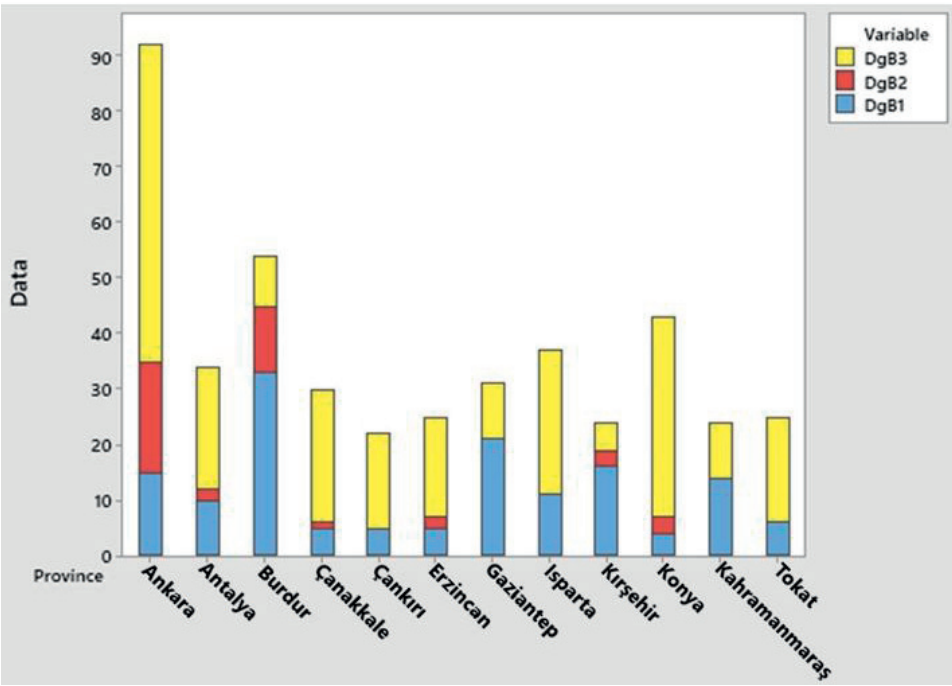

Fig. 3. Dog breeds (DgB) preferred by farmers

DgB1: Kangal, DgB2: Akbaş, DgB3: Other

and Ankara provinces, and it is similar to the Kangal in terms of instinctively protecting the herd without a need for warning (Anonymous, 2009). Compared with the Kangal, its courage and aggression against predators is high, but its adaptability and aggression towards humans are low. After the Pyrenees breed, it is considered to be one of the breeds with the least amount of aggression towards the herd, which it protects and, altogether, it is one of the breeds preferred by many animal breeders both in Turkey, Africa, and America.

Van Bommel (2010) reported that the Kangal and Akbaş breeds were traditionally used in Turkey to protect livestock from wolves, foxes, jackals, bears, wild boars and wild dogs, hence helping shepherds very effectively in herd protection. It has also been reported that when nomadic livestock farmers go to high plateaus and long journeys, LGDs are widely used, and they ensure the safety of the herds. The Kangal and the Akbaş are at the forefront among Pyrenees, Komondor Maremma, Kuvasz and other dog breeds due to their high ability to stay with the herd (Green and Woodruff, 1988).

Dog health and vaccination status

A particular goal of the research was to determine the level of awareness and behaviour regarding the care of dogs, especially vaccination (rabies vaccination, distemper and parvovirus) and health issues. Our results show that 82.3% of the farmers' dogs were vaccinated (Table 2), denoting the importance that sheep and goat farmers attach to dog health. In addition to the task they perform, shepherd dogs are both traditionally and culturally important, especially in Anatolia and, in recent years, the level of awareness about LGDs has improved. The dog vaccination status by province is shown in Fig. 4.

Dog procurement methods

Sheep and goat breeders address their need for shepherd dogs in different ways. A significant proportion of farmers, especially those who have been breeding sheep or goats for many years, generally raise their own shepherd dogs and mate their own female dogs with the most preferred males, thus providing young puppies for their own use. Our survey shows that 25.4%, 54.4% and 20.2% of farmers preferred the methods of procuring dogs through exchange of money, from friends, and their own pack of dogs, respectively (Table 2, Fig. 5). It is understood that the most common method of dog procurement among the farmers is procurement from each other and friends, and this result indicates that farmers attach more value to their social relations with other sheep and goat farmers compared with their own economic concerns. Marker et al. (2005) also suggest other measures that could be implemented to encourage farmers to use shepherd dogs, such as government subsidies to partially finance the purchasing and breeding among producers, shepherd dog fairs and exhibitions, as well as actions by non-governmental organisations.

Number of shepherds per farm

During the last decade, shepherding on farms in Turkey has become a profession that is not preferred by the younger population due to the development of the economy and socio-cultural reasons. This new reality contrasts with the traditional habits of Turkish farms filling their shepherd vacancy through

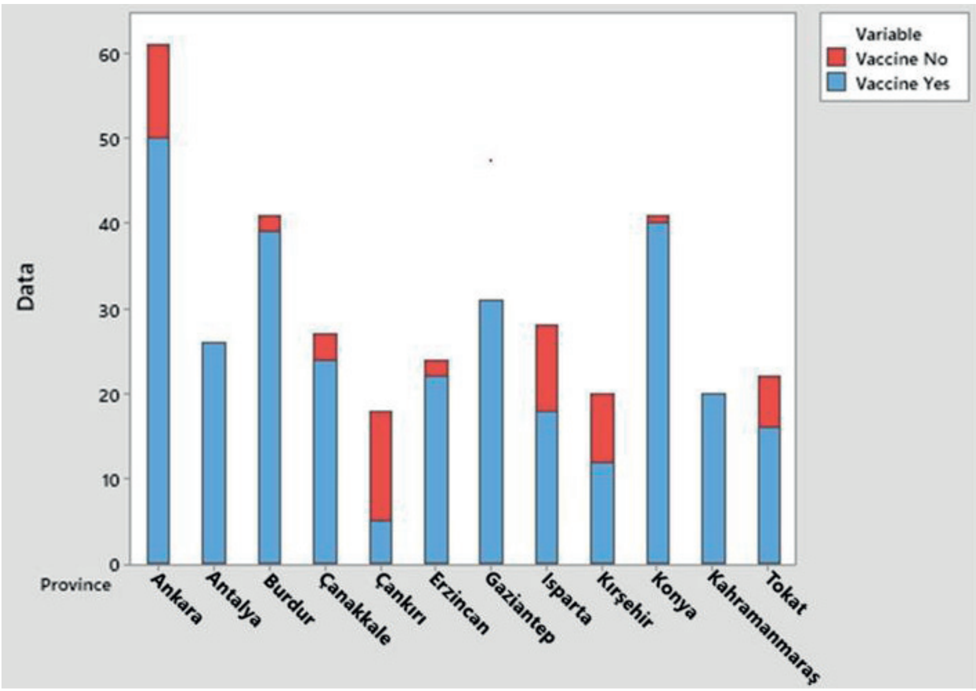

Fig. 4. Distribution of vaccination status of dogs by province

members of their own households. The problems encountered in the shepherd labour market in Europe also apply to Turkey, where the younger population (under 30 years of age) refuses to be employed as shepherds. Currently, nearly 90% of the population employed as shepherds in Turkey are refugees and asylum seekers, and they encounter a variety of problems due to legal infrastructure and working conditions. According to our study, the share of enterprises with no shepherd (self), or with 1, 2, 3, 4, 5, or more shepherds were 19.6%, 37.5%, 28.0%, 8.2%, 3.8% and 2.9%, respectively (Table 2). These results indicate that in Turkey small ruminant farmers generally prefer 1 or 2 shepherds for herd management. The number of shepherds required by businesses may vary depending on the kidding and lambing season of the animals and their feeding in summer and winter. At the same time, flock size is also an important factor affecting the number of shepherds needed. Sheep or goat owners generally allocate 1 shepherd for a herd of 250 to 300 animals. During the lambing and kidding season, a temporary assistant shepherd is employed, or the owner satisfies this need for extra labor from household members. Mosalagae and Mogotsi (2013) reported that similar problems were experienced in both developed and developing countries. It may be recommended to aggregate small flocks into larger flocks to solve this problem and employ shepherds to work in these larger herds. For example, a solution might be to protect a large flock of sheep and goats with 2 to 3 shepherds and at least 2 to 3 good LGDs.

Farmers' expectations from the government in their struggle against predators

Small livestock owners continue their production activity by protecting their sheep and goats against predators using traditional methods. In the current system, the economic consequences of compensating predator damage in the absence of animal insurance (predator incident insurance is limited to 2 attacks) are non-negligible. Sheep and goat farmers do not have a very effective and productive organizational structure, resulting in having to deal with these occurrences individually, without assistance. Our results show that the top three expectations of sheep and goat owners from the government in their fight against wolves (Table 2, Fig. 6) are that wolves should not be released to the ecosystem (44.7%), wolf hunting should be allowed (16.1%), and shepherd dogs should be given free of charge (14.1%). In Turkey, there is no specific policy that addresses the impact and damage to animal production by wildlife. For this reason, when attacked by wolves, farmers generally resort to the following measures: i) fighting methods that kill or cripple wolves, or ii) farmers may intervene in wildlife conservation areas close to their farms, or they may withdraw from livestock production activities. Graham et al. (2004) stated that these retaliatory reactions by producers are contrary to the public and political intentions of wildlife management. Moral et al. (2016) suggest implementing public policies that encourage the use of LGDs by farmers, as it is beneficial to the small livestock production system, and because the use of LGDs is the method where wild predators are least affected.

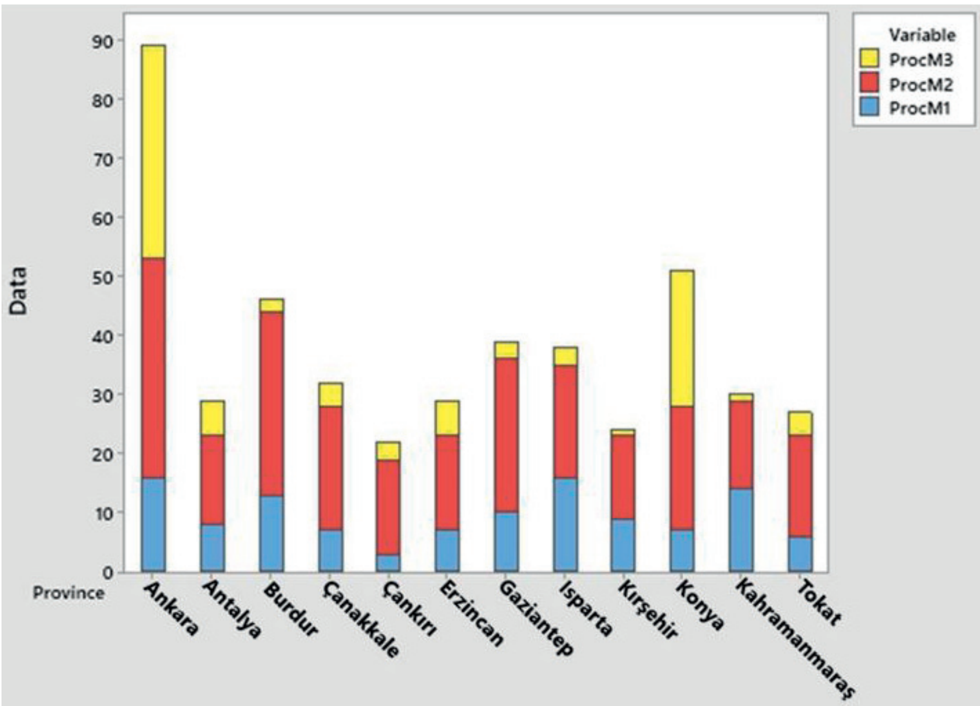

Fig. 5. Distribution of dog procurement methods (PrcM) by province

ProcM1: Cash buying, ProcM2: From friends, ProcM3: From own pack of dogs

Number of wolf attacks on sheep and goat farms

The average number of wolf attacks in sheep and goat farms (Table 3) was 6.01 ± 0.704 and differed between provinces ($P < 0.05$). The highest numbers of wolf attacks were 13.59 ± 5.520 and 10.34 ± 2.439, observed in Çanakkale and Antalya provinces, respectively, and the lowest numbers of occurrences were 1.17 ± 0.345 and 1.57 ± 1.469 in Çankırı and Kırşehir provinces, respectively. The larger number of attacks in Çanakkale and Antalya could be attributed to the presence of denser forests and mountainous terrain, relative to the other provinces surveyed. In addition to LGDs being ineffective in such geographical areas, it can be argued that LGDs might be the underlying cause of wolf attacking dogs and small livestock in mountainous areas, dense maquis and forest areas.

The average number of sheep and goats that perished due to wolf attacks (Table 4) was found to be 12.17 ± 1.329 and differed between provinces ($P < 0.01$). The highest numbers of small ruminant casualties due to wolf attacks were 24.63 ± 5.388 and 19.09 ± 9.481 heads in Burdur and Çanakkale provinces, respectively, and the lowest numbers were 1.00 ± 0.276 heads and 4.90 ± 1.172 heads in Kırşehir and Kahramanmaraş provinces, respectively. Although the preferred protection method against predators is the use of shepherds and shepherd dogs, most wolf attacks and flock casualties occurred when shepherds and dogs were accompanying small herds. Therefore, this finding supports the idea that the practices used against wolf attacks are ineffective and that simply having shepherds and guard dogs near small ruminants is not sufficient to provide the desired level of protection. Redden et al. (2015) state that domestic animals, especially small ruminants, are highly susceptible to attacks from various wild and domestic animals, hence flock protection dogs with appropriate behaviour and training can minimize predator damage to farm animals. Ogada et al. (2003) and Abade et al. (2014) reported that LGDs are trained not to chase predatory animals, but rather to warn shepherds of danger, and even in that case, utilization of LGDs can reduce predator-related losses by up to 63%.

Economic losses from predator attacks

Within the provinces of interest, the average economic loss per farm due to wolf attacks (Table 5, Fig. 7) was calculated to be 2299.68 ± **235**.176 US$, and was different between provinces ($P < 0.01$). The highest economic loss due to wolf attacks was calculated as 7468.06 ± 1334.139 US$ and 3420.73 ± 661.154 US$ in Burdur and Isparta provinces, respectively, and the least amount of economic loss was calculated as 215.42 ± 64.937 US$ and 787.77 ± 270.615 US$ in Kırşehir and Çankırı provinces, respectively.

Controlling predator damage in sheep and goat flocks is one of the most important factors for the profitability of the business. In a study conducted in the USA in 2004, the damage caused by wild predators to goat farms was estimated to be 155.000 heads, corresponding to a monetary equivalent of approximately 18.3 million US$ (Jones, 2004). Coppinger et al. (1988a), based on reports collected from producers in the United States, reported reduced feral predator attacks by 64%, and that,

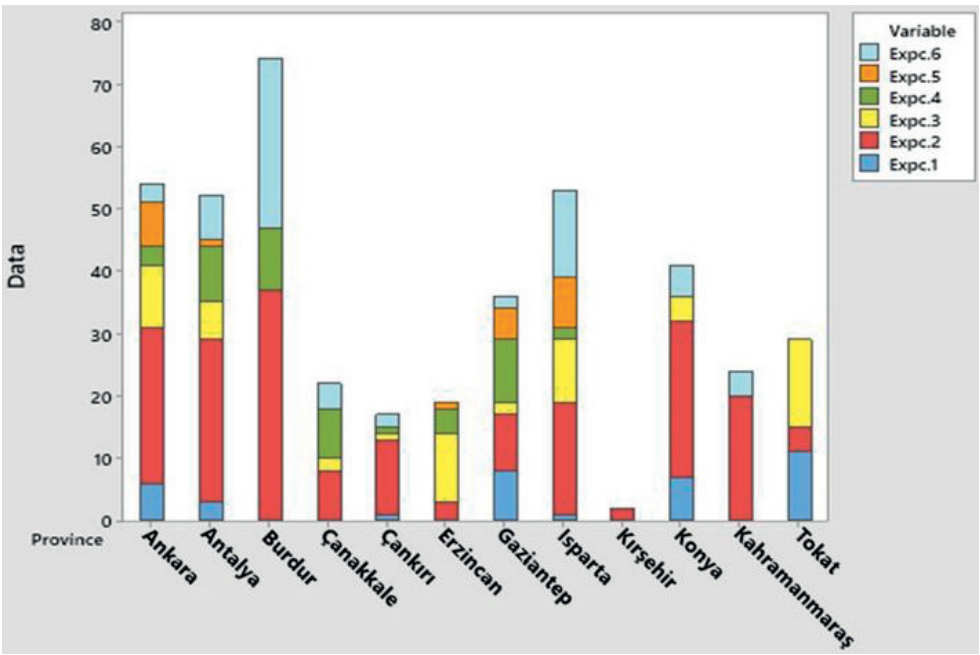

Fig. 6. Farmers' expectations (Expc) from the government in their struggle against predators

Expc.1: Gun licence; Expc. 2: No wolves released; Expc.3: Provide a free guarding dog; Expc.4: Free insurance; Expc.5: Wolf hunting should be legal; Expc.6: All of the above within one year, livestock losses fell to zero in 53% of farms implementing an LGD program. Andelt and Hopper (2000) reported a smaller number of lambs lost to predators for sheep producers with LGDs in a survey conducted in Colorado. Additionally, the same survey found that producers without LGDs lost almost six times more lambs than those with LGDs, and 84% of the 160 producers surveyed reported that LGDs were excellent or very successful in reducing predation of their sheep. Furthermore, Marker et al. (2005), in studies conducted between 1994 and 2002 to determine the effectiveness of dogs in Namibian small ruminant farms, reported LGDs being very effective in reducing livestock losses.

Number of dogs per farm and dog expenses

Although sheep and goat farmers seem to think that their LGDs are sometimes ineffective against wolves, they continue to use LGDs, believing that sheep and goat losses and even attacks on shepherds would be even higher without LGDs. The average number of dogs per farm (Table 6) was 4.76 ± 3.605, and it was different among provinces ($P < 0.01$). The provinces of Konya and Ankara have the highest number of LGDs for protection against wolf attacks on sheep herds, respectively, 7.86 ± 0.876 and 6.92 ± 3.964. Conversely, the provinces of Kahramanmaraş and Gaziantep have the least number of LGDs, respectively, 2.15 ± 0.209 and 2.29 ± 0.168.

The number of dogs required for the most effective protection against predators in small ruminant herds varies depending on the size of the pasture, the number of animals, the topography of the region, the number and species of predators, the presence or absence of fences, and the protective behaviour of LGDs. Generally, one LGD per 100 sheep is recommended. Conversely, herds of 1000 heads or larger rarely have more than 6 LGDs (Redden et al., 2015). In Turkey, the number of dogs kept in sheep and goat farms varies depending on the interest of farmers and shepherds in LGDs, as well as whether or not the land is densely forested or maquis, and the predatory potential of the region. Due to the increase in feeding and vaccination costs associated with dog ownership in recent years, however, farmers have been displaying a tendency to reduce the number of LGDs in their farms.

In the present study, the average cost spent on dogs (Table 6) used to protect sheep and goat flocks from wolf attacks was 256.07 ± 12.318 US$, and it differed between provinces ($P < 0.01$). The highest expenditure on LGDs was calculated as 707.83 ± 29.733 and 286.67 ± 45.529 US$, in Burdur and Çanakkale provinces, respectively, and the lowest expenditure was 55.56 ± 3.790 US$ and 71.81 ± 6.327 US$ in Kahramanmaraş and Kırşehir provinces, respectively. Considering that each lamb entails a cost of 80 to 110 US$ to the producer, the relative cost of an LGD is very low. These results can be explained by the fact that sheep and goat farmers generally prefer traditional methods for feeding their dogs and ignore the associated vaccination and medication costs. In the provinces where this research was conducted, there is an overall tendency of trying to reduce farm expenses by spending the least on LGDs. Redden et al. (2015) report that the estimated first-year cost for a new LGD is at least 1000 USD, with subsequent annual costs of approximately 500 US$ in Texas, USA. In addition, in Namibia, Marker et al. (2005) reported that in 2003, farmers' costs generally reached 130 US$ for both male and female LGD offspring, including the cost of neutering. In comparison, when small ruminant farmers in Turkey purchase LGDs for the first time, the costs are quite low, as they meet their needs from friends or their own pack of dogs. Conscious breeders pay 500–700 US$ for quality dogs over time or exchange their dogs for rams or sheep at this price. In Turkey, Kangal and

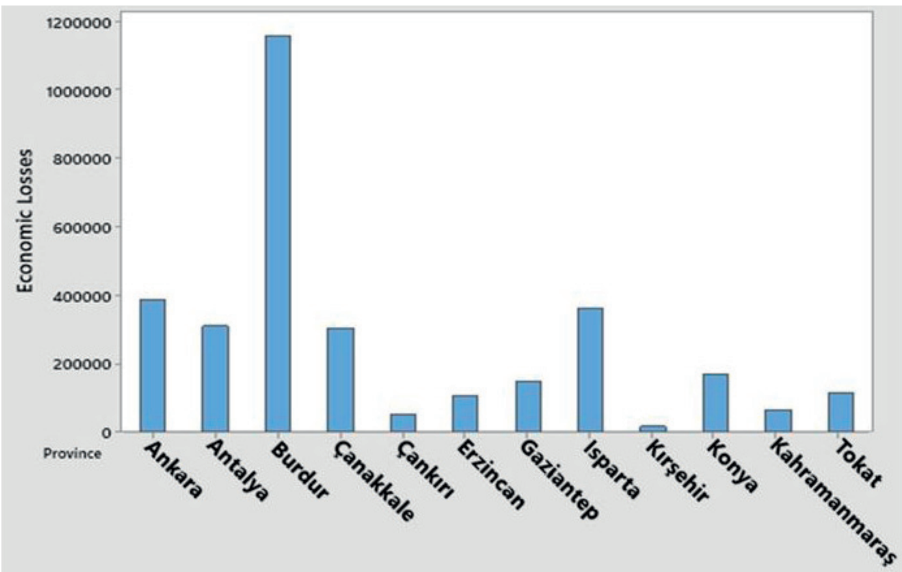

Fig. 7. Economic losses from predator attacks (US$)

Akbaş shepherd dogs are bred in public and private enterprises to supply LGDs, and although they are sold to farmers or individuals who request them for a monetary payment, these enterprises are quite inadequate to meet the aggregate demand of livestock producers in Turkey.

Conclusion

This study was carried out to alleviate the lack of information on the general situation of damage caused by wolves to small livestock farms in Turkey and on the use of livestock guarding dogs. The use of LGDs to minimize predator losses is one of the best options, and it is recommended to use local dog breeds that have adapted to Turkey's geography and livestock populations and have received a certain level of training. In this sense, motivating the use of Kangal and Akbaş LGDs will be one of the most effective solutions. In addition, the integration of herd management dogs, such as Border Collies, etc., into the flocks alongside native Turkish dog breeds that will serve as livestock guardians should be evaluated. Such a strategy will not only reduce the amount of workload and number of shepherds required, but it will also bring a new understanding and relief to the country's sheep and goat breeding.

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Conflict of interests

The authors declare no conflict of interest.

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Slaughter Performances, Body Composition and Carcass Traits of Indigenous Algerian Cattle "Brune de l'Atlas"

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Abstract. *The present study aimed to evaluate the meat production potential of the indigenous Brune de l'Atlas cattle breed as a contribution to improving local meat production in Algeria. It investigated the slaughter performance, body composition, and carcass traits of 51 Brune de l'Atlas bulls from four distinct Algerian ecotypes, aged 16.46 ± 2.94 months, and subjected to a 128.4 ± 97 days fattening period. The assessment focused on key meat production parameters, including live weight, body composition, slaughter yield, conformation, fatness, and carcass fineness. The studied bulls exhibited an average body weight of 366.56 ± 92.56 kg, with interesting true and commercial dressing percentages at 60.11 ± 11.09% and 56.1 ± 10.04%, respectively. The study revealed the production of lean carcasses with a remarkably low proportion of body fat, reflected in a remarkably low fat index of 1.48 ± 0.76 and a body fat weight rate of 5.9 ± 3.86%. While carcass conformation and compactness indices were relatively moderate, the findings highlight the promising slaughter performance of the indigenous Algerian cattle population. These results suggest that targeted genetic, nutritional, and management improvements could further elevate the value of their butchering performance, contributing to local meat supply and potentially reducing dependency on meat imports.*

Introduction

Global meat demand is experiencing significant growth, driven by rising incomes, rapid urbanization, and population expansion (Komarek et al., 2021). This trend is particularly evident in the correlation between meat consumption and gross domestic product (GDP) as reported by Food and Agriculture Organization (FAO, 2018). In Algeria, cattle fresh meat production has shown considerable progress, increasing by 63% between 1990 and 2021, reaching approximately 146 270 tons (FAO 2019). The country's current red meat consumption averages 13.5 kg per capita annually, with domestic production reaching 530 000 tons in 2019, consisting of 60% sheep, 30% cattle, 7% goat, and 3% camel meat. To meet growing demand, Algeria supplements its domestic production with imports of approximately 50 000 tons (1.3 kg/inhabitant/year), either as frozen or refrigerated meat, or as live animals for local slaughter (Ministry of Agriculture and Rural Development: MADR, 2022).

The Algerian cattle sector relies on various breeds for meat production. Dairy breeds, primarily Montbéliard and Prim'Holstein, contribute through their male offspring used for breeding or fattening, and through culled females (Haou et al., 2022). Additionally, imported suckler breeds, such as Limousin, Charolais, Aubrac, and Blonde d'Aquitaine, along with tropical cattle from Mali and Niger, are used for fattening and slaughter (Algerian Red Meat Company: ALVIAR, 2022). However, amid this diversification, the indigenous Brune de l'Atlas cattle population has declined dramatically, dropping from 80% to 34% of the national herd between 1998 and 2015 (MADR, 2015).

This decline threatens a valuable genetic resource that has evolved to thrive in tropical environments. The Brune de l'Atlas demonstrates remarkable productivity when considering its adaptation to lower-quality forage, fertility, calving interval, heat tolerance, and resistance to parasitic and infectious diseases (Hanzen et al., 2024a, b; Ferag, 2024). These adaptive traits make it especially valuable for crossbreeding programs with imported *Bos taurus*

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breeds, aiming to combine improved meat and milk production with environmental adaptation and disease resistance (Mehdid et al., 2017; Leroy et al., 2020; Rahel et al., 2020; Eulmi et al., 2023).

Currently, Algeria's national cattle population stands at 1 734 476 heads (FAO, 2023). Brune de l'Atlas, indigenous to the Atlas Mountains and spread across North Africa (Boujenane and Ouragh, 2010; Ben-Jemaa et al., 2020), comprises four distinct ecotypes: the dark gray Guelmoise adapted to forest zones, the whitish Cheurfa found in pre-forest areas, the fawn-colored Chelifian, and the blackish Sétifienne suited to more rustic conditions (National Institute for Agronomic Research of Algeria: INRAA, 2003; Rahel et al., 2017; Ben-Jemaa et al., 2018, Rahel et al., 2020). Research has revealed that these populations possess a mixed European and African ancestry, with traces of indicine genetics inherited from African ancestors (Ben-Jemaa et al., 2015).

In the modern meat market, commercial success depends on both quantitative and qualitative criteria. Markets demand well-conformed carcasses with high lean meat content, favorable muscle-to-bone ratios, and appropriate fat coverage for optimal storage and sensory qualities (Albertí et al., 2005; Blanco et al., 2020). This has led to the successful establishment of both later-maturing breeds (e.g., Charolaise, Limousine, Brahman) and early-maturing breeds (e.g., Angus, Hereford, Droughtmaster) in specific market segments with recognized quality labels (Quyen et al., 2018; Chi et al., 2023).

Currently, there is no available literature evaluating various aspects of meat production and slaughter performance of local cattle population of Brune de l'Atlas in North Africa. The primary objective of this study is to provide a comprehensive assessment of slaughter and carcass traits of beef cattle from the Algerian local population.

Fig. 1. Geographical location of Algeria and the study region (Ain Assel) within El Tarf Department (Lat: 36.767 and Lon: 8.317)

Materials and methods

Ethical statement

Studied animals were used according to the ethical principles of animal experimentation and international guidelines for animal welfare (Terrestrial Animal Health Code 2018, section 7. Art 7.5.1) and national executive decree No. 95-363 of November 11, 1995 (Algeria).

Study area

The experiment was conducted between October 2020 and December 2021 in the El Tarf department, located in the extreme northeastern region of Algeria (Lat: 36.767, Lon: 8.317) (Fig. 1). El Tarf is characterized by a semi-humid climate, with an average altitude of 287 meters above sea level. The annual rainfall averages 673 mm, ranging from a minimum of 10 mm in July to a maximum of 141 mm in November. The mean annual temperature is 23°C, with the lowest recorded temperature of 15°C in January and the highest of 32°C in August (MADR, 2021). The livestock population in the study region includes 50 408 cattle, 21 375 sheep, and 7 811 goats (MADR, 2019). This region ranks fifth in Algeria in terms of the size of its local cattle population (MADR, 2019).

Experimental animals

This study included 51 clinically normal entire male beef cattle from four ecotypes (Fig. 2 Guelmoise, Setifienne, Cheurfa, and Chélifienne) of the autochthones Atlas Brown population aged between 12 and 24 months (average of 16.46 ± 2.94 years).

The animals belonged to the same beef herd and were reared under similar breeding conditions within a semi-intensive system. They were maintained under consistent experimental conditions, accounting for factors such as age, fattening duration, husbandry conditions, livestock system, management practices, slaughter conditions, and the overall production process. To mitigate potential limitations arising from the relatively low genetic diversity within the sample population, a random sampling approach was employed to ensure the selection of unrelated experimental animals. The feeding regimen was based on the continuous distribution of a mixture consisting of barley flakes (50–65%), crushed corn (20–30%), soybean meal (10–14%), sodium bicarbonate (1.5%), appropriate vitamin supplements (5%), and natural prairie hay, all provided ad libitum, along with unrestricted water intake and preventive medical coverage. The animals belonged to four distinct coat color ecotypes (Fig. 2): black (n = 18), gray (n = 16), brown (n = 14), and white (n = 3), with respective average ages of 16.44 ± 3.38 months, 15.62 ± 2.88 months, 17.00 ± 3.42 months, and 18 ± 1.33 months. The white ecotype was particularly scarce in the region. All animals underwent a long-fed fattening period of 128.4 ± 97 days (4 to 6

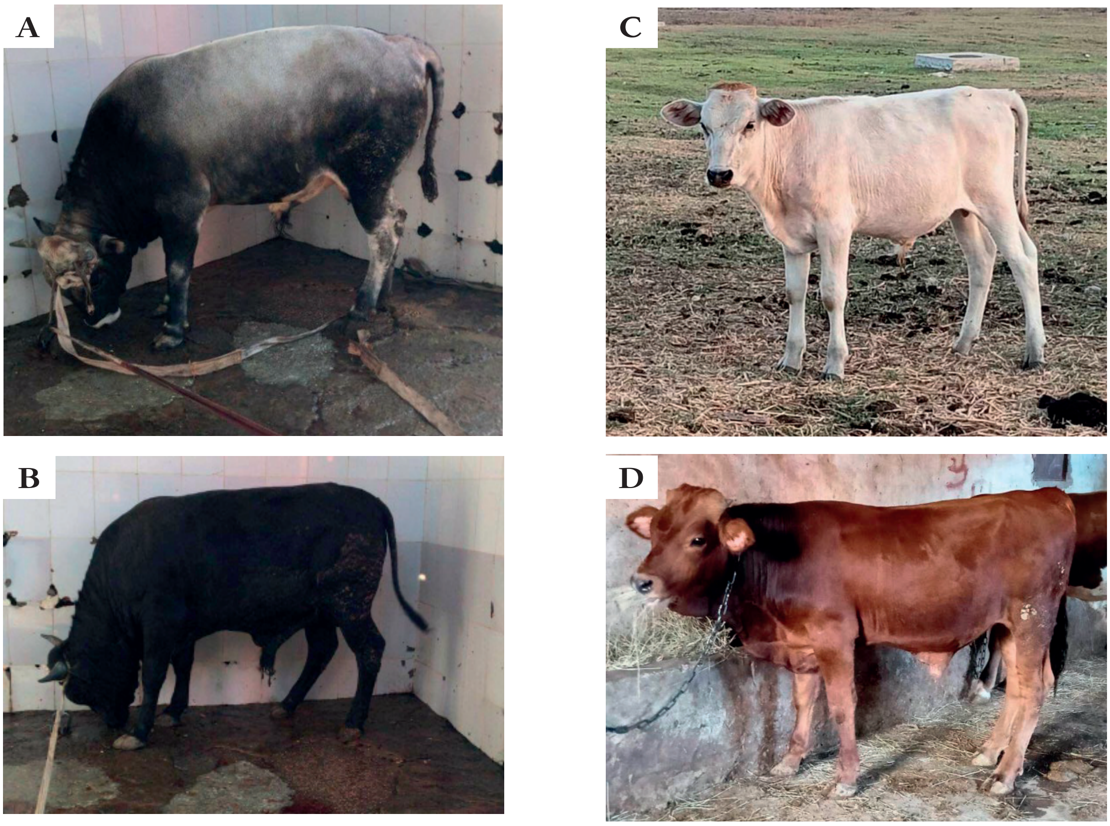

Fig. 2. Male beefs of local cattle population Atlas Brown form the Northeast Algeria (El Tarf). A: Guelmoise ecotype (gray), B: Setifienne ecotype (Black), C: Cheurfa ecotype (White), D: Chélifienne (brown).

months) (Greenwood, 2021) under a semi-intensive system. They were slaughtered for commercial meat production, with no intentional killing conducted specifically for this study. The slaughtering process took place at a licensed slaughterhouse, where the animals were given a limited resting period (1 hour) before slaughter, under the supervision of a veterinary inspector.

Methodological approach

The methodological approach adopted for this study consists of the steps outlined in Fig. 3.

The selection of the study area was based on the high concentration of local cattle, with a total population of 50 408 head, including both local and crossbred cattle (MADR, 2019). The slaughterhouse is situated within the Ain El Assel cattle market and consists of a single-block facility, which facilitated the study of the various animal components. During the identification process, data were collected regarding fattening duration, slaughter age, origin, ecotype, and sex of each animal. Prior to slaughter, the live weight of the beef cattle was recorded using an electronic scale. The animals were then bled and manually skinned. Immediately post-slaughter, the head and feet were skinned and weighed separately. The digestive tract was weighed twice: first, immediately after evisceration, including its entire contents, and second, after being emptied of its digestive contents. The carcasses were then split into two halves, with the tail positioned on the right side, and subsequently chilled at 4 ± 1°C for 24 hours. The weights of the various components of the fifth quarter were recorded. Each carcass was classified based on conformation, fatness, and fineness, following an inspection of the hot carcass and the application of beef cattle evaluation indexes.

Live/Empty Body Weight (BW)

The slaughter weight of each animal was taken within 15 minutes prior to slaughter using an electronic scale. It was considered as the live body weight (BW) with digestive tract content included. The empty body weight (EBW) was calculated by subtracting the weight of stercoral contents from the BW.

Slaughtering and fifth quarter (5^{th} Q) composition

Immediately after bleeding, the animals were skinned, and the slaughterers proceeded with evisceration. At this stage, our focus was on assessing body composition by weighing the various components of the fifth quarter (5^{th} Q). Generally, all parts of the slaughtered animal that are not classified as meat belong to the 5^{th} quarter. The red offal includes liver, lungs, spleen, kidneys, heart, tongue, snout, tail, cheeks, diaphragm, as well as white offal such as brains, sweetbreads, and gonads. These are tripe products sold raw after undergoing essential

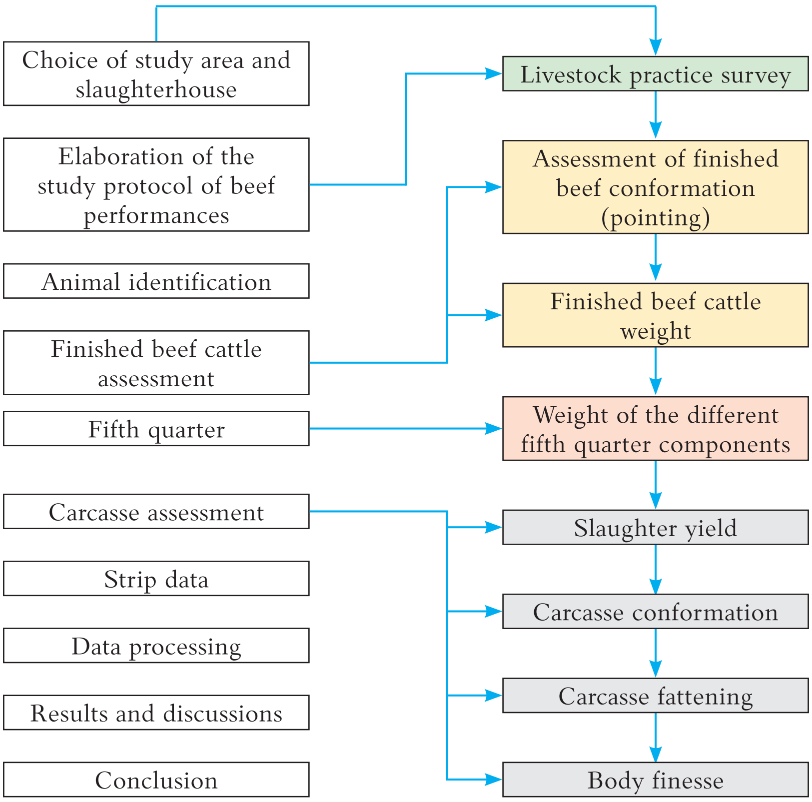

Fig. 3. Diagram showing the methodological approaches to study Atlas Brown beef cattle slaughter traits

trimming. White tripe products, such as the stomach, feet (cannons), ears, and head, require varying degrees of preparation at the slaughterhouse and are sold scalded, blanched, or even half-cooked, which gives them an ivory-white color. Blood is considered a specific co-product. Almost all skins recovered at the slaughterhouse are part of the 5th Q and are processed into leather. The weight of the 5th Q, excluding blood and stercoral contents, was determined as the sum of the weights of the following body parts: hooves, feet, head, leather, intestines, the empty digestive tract (rumen-reticulum-omasum-abomasum), red offal, glands, viscera, and tallow. The proportions of the 5th quarter components were calculated relative to body weight (BW) to allow for standardized comparison.

Carcass weight and dressing percentage

Hot/chilled carcass weight

The weight of hot carcass (HCW) was measured immediately after the end of its preparation. It was measured on scales weight bascule. The weight of chilled carcass (CCW) was obtained after 24 hours of chilling at 4°C.

Dressing percentage

The weight of digestive content was calculated in kg by difference between the full and empty weight of the digestive tract. In fact, the empty body weight (EBW) of each animal was estimated by subtracting the weight of the contents of the digestion tract of BW. The commercial dressing percentage (CDP) and the true dressing percentage (TDP) were calculated using the following formulas (Manafiazar et al., 2016): CDP (%) = (CCW/BW) × 100; TDP (%) = (CCW/EBW) × 100.

Evaluation of carcass conformation

Conformation EUROP classification

The EU system was developed in 1981, and it was called EUROP. The conformation-EUROP grid method of carcass classification represents a visual-based assessment of muscular mass development in different regions of the carcass developed by countries involved in trading in the common European Union (EU) market (EU 1994). Conformation is classified according to a 5-level grid. Each level is represented by a letter: E – excellent; U – very good; R – good; O – fair; P – poor, transformed to ordinal scoring scale from 5 for E to 1 for P for statistical analysis (Monteils and Ellies-Oury, 2018; Nogalski et al., 2019). More comprehensive data on carcass conformation indices were applied according to previous studies (Strydom, 2000; Naves, 2003; Albertí et al., 2005; Conroy et al., 2010).

Indices of carcass conformation

Each chilled half-carcass was divided into forequarters and hindquarters (HQ) by cutting between the fifth and sixth ribs, approximately 22 cm from the spine. The forequarter included the neck, shoulder, forelegs, and five ribs. The six short ribs were removed by separating them about 22 cm from the spine, along with the abdominal muscles (Rezende et al., 2019). The plate remained attached to the forequarter, with the cut passing perpendicular to the ribs at a point below the center of the rib cage. For each animal, the hindquarter mass (HM, kg), hindquarter-to-carcass compactness (HCC, %), forequarter mass (kg), and forequarter-to-carcass compactness (FCC, %) were measured on the right side of the carcass (Rezende et al., 2019). The following carcass measurements and ratios were calculated as indicators of carcass conformation:

Hip thickness index (HTI) = HT / HL, where HT is the horizontal distance between the outermost points on the medial and lateral surfaces of the leg, and HL is the hind leg length, measured as the distance from the center of the ilio-pubic fusion of the pelvis to the distal edge of the pelvic limb (Xavier, 2022).

Carcass compactness index (CCI) = CCW / CL, where CCW is the chilled carcass weight, and CL is the distance from the anterior edge of the symphysis pubis to the middle of the anterior edge of the first visible rib (Swanepoel et al., 1990).

Buttock compactness index (BCI) = HM / HL, calculated as the ratio between hindquarter mass and hind leg length (Santos et al., 2007).

Evaluation of carcass fattening

EUROP-fatness score

The carcass fattening score (CFS) was assigned to each animal through a visual assessment of the left and right chilled half-carcasses, evaluating fat distribution on both the exterior and interior sides of the ribcage. A five-point scale (1 = low, 2 = slight, 3 = average, 4 = high, 5 = very high) was employed, in accordance with European Community regulations (EU, 1994) and the criteria outlined by Monteils and Ellies-Oury (2018). Additionally, a half-point scale was used to provide greater granularity in the ratings.

Body fat weight

This parameter evaluates the proportion of body fat in the animal's carcass. It is expressed as a percentage and calculated by dividing the body fat weight (BFW) by the hot carcass weight (HCW) (Jurie et al., 2007; Bonny et al., 2016). The BFW is determined as the sum of kidney fat (KFW), digestive tract fat (DFW), mediastinal fat (MFW, also known as red tripe fat), and cover fat (CFW). The formula used is:

BFW (kg) = KFW + DFW + MFW + CFW

BFW (%) = (BFW / HCW) × 100

Fat index

The fat index (FI %) represents the ratio KFW to CCW (Naves, 2003).

FI = (KFW / CCW) × 100

Bone slimness (body fitness)

The fitness of the carcass of each studied animal was evaluated by studying the body boniness index (BBI) (Communod et al., 2013) using the following formula:

BBI = CBC / CC

where CBC is cannon bone circumference and CC is chest circumference.

Data analysis

The SPSS software (Version 26.0, IBM Corp., 2019) was used to perform statistical analysis on the data. Descriptive statistics, including mean ± standard deviation, maximum, minimum, and variance, were calculated for the quantitative variables. Ordinal dependent variables were trans-formed into quantitative variables to ensure normality. The normality of the dependent variables was assessed using the Shapiro-Wilk test and the Kolmogorov-Smirnov test ($P > 0.05$). Pearson correlation analysis was conducted to evaluate associations between the different parameters. Furthermore, the independent samples t-test, one-way analysis of variance (ANOVA), and the Duncan post-hoc test were applied to analyze differences in means between the evaluated conformation and fattening parameters, as well as to compare mean values among the four cattle ecotypes. Statistical significance was considered at $P < 0.05$.

Results

Slaughter weight, carcass weight and slaughter yield

The descriptive parameters related to live weight, carcass weight, and slaughter yield of Algerian autochthonous beef cattle are presented in Table 1. The study sample comprised 51 animals, with an average body weight of 366.56 ± 92.68 kg, exhibiting considerable variation in body weight and carcass traits. The hot carcass weight (206.51 ± 51.95 kg) and chilled carcass weight (202.12 ± 50.89 kg) indicated an approximately 2% weight loss during the chilling process. The left and right half-carcass weights (103.51 ± 26.68 kg and 103.55 ± 26.88 kg, respectively) were notably symmetrical, demonstrating uniform carcass splitting. The dressing percentages, both commercial (56.1 ± 10.04%) and true (60.11 ± 11.09%), showed a difference of approximately 4 percentage points, reflecting the impact of empty body weight calculation on yield estimation.

Weight of the fifth quarter

Table 2 presents the weights and proportions of various fifth quarter components in Algerian autochthonous cattle. The total fifth quarter weight, excluding stercoral content, averaged 95.24 ± 41.00 kg, accounting for approximately 26% of body weight. Among the components, skin contributed the highest proportion (7.11% of body weight), followed by the empty digestive tract (6.27%), red offal (5.73%), and head (5.36%). In contrast, legs represented the smallest proportion, comprising 2.92% of body weight. The considerable standard deviations, particularly in the empty digestive tract (11.12 kg) and skin (7.10 kg), highlight substantial variation among animals in these components.

Carcass conformation

Table 3 presents the carcass conformation parameters and classification indices of Algerian Brune de l'Atlas cattle. The EUROP conformation scores (1.86 ± 1.04) indicated modest carcass conformations overall. Among the conformation indices, the carcass compactness index (CCI) and body conformation index (BCI) averaged 1.55 ± 0.37 and 0.69 ± 0.02, respectively, while the hip thickness index (HTI) was 0.34 ± 0.05. The hindquarter-to-forequarter mass ratio (HM/FM) was 1.28 ± 0.11, reflecting a significantly higher hindquarter weight ($P < 0.05$). In terms of commercial cuts, the hindquarter compactness (HCC) accounted for a larger proportion of the carcass compared to the forequarter compactness (FCC), with a difference of approximately 15 percentage points ($P < 0.05$).

Table 1. Descriptive statistics of slaughter weight, carcass weight and dressing percentage

		N	Mean	Min	Max	Variance	Std.Dev.
BW	(kg)	51	366.56	219.52	620.99	8590.91	92.68
EBW	(kg)	51	343.97	202.52	589.99	8697.66	93.26
L(1/2)CW	(kg)	51	103.51	57.00	174.00	712.01	26.68
R(1/2)CW	(kg)	51	103.55	56.00	174.00	722.61	26.88
HCW	(kg)	51	206.51	116.00	348.00	2699.13	51.95
CCW	(kg)	51	202.12	113.68	341.04	2589.71	50.89
CDP	(%)	51	56.1	29.94	72.69	100.70	10.04
TDP	(%)	51	60.11	30.86	77.73	122.88	11.09

BW: Body weight, EBW: Empty body weight, L(1/2)CW: Left chilled 1/2 carcass weight, R(1/2)CW: Right chilled 1/2 carcass weight, HCW: Hot carcass weight, CCW: Chilled carcass weight, CDP: Commercial dressing percentage, TDP: True dressing percentage.

Table 2. Descriptive statistics of the total weight of the fifth quarter and the weight of its components (Red and White tripe products)

		N	MEAN	MIN	MAX	%BW	VARIANCE	STD.DEV.
Red offal	(kg)	51	15.53	4.20	28.00	5.73	4.11	5.10
Head	(kg)	51	19.85	8.00	32.00	5.36	18.74	4.33
Legs	(kg)	51	8.45	4.00	13.00	2.92	5.21	2.28
Skin	(kg)	51	27.76	12.00	45.00	7.11	50.39	7.10
Empty digestive tract	(kg)	51	23.65	13.81	76.00	6.27	123.61	11.12
TOTAL	(kg)	51	95.24	44.00	117.00	26	25.62	41.00

Table 3. Descriptive statistics of carcass classification, carcass conformation indices

		N	MEAN	MIN	MAX	VARIANCE	STD.DEV.
Conformation-EUROP		51	1.86	1	4	1.08	1.04
HTI		51	0.34	0.26	0.48	0.002	0.05
CCI		51	1.55	0.73	2.69	0.13	0.37
BCI		51	0.69	0.62	0.74	9.85	0.02
HM/FM		51	1.28	1.13	1.34	177.65	0.11
FM	(kg)	51	47.43[a]	27	83	180.05	13.42
HM	(kg)	51	62.41[b]	37	98	244.56	15.64
FCC	(%)	51	44.54[1]	39.52	51.72	8.39	5.8
HCC	(%)	51	59.22[2]	52.3	63.16	17.75	8.42

Conformation-EUROP: carcass conformation classification. HTI: Hip thickness index, CCI: Carcass compactness index, BCI: Buttock compactness index, HM/FM: Hind/fore quarter mass ratio, FM: Forehand quarter mass, HM: Hindquarter mass, FCC: Forehand quarter to carcass mass, HCC: Hindquarter to carcass mass. Different letters between FM and HM indicate a statistically significant difference of the mean of these parameters. Different numbers between FCC and HCC indicate a statistically significant difference of the mean of these parameters.

Fattening indicators

Table 4 presents the fattening characteristics and fat distribution in Algerian autochthonous cattle. The carcass fatness score (CFS) averaged 1.85 ± 0.83, indicating a relatively lean carcass composition. This observation is further supported by the fat index (FI) of 1.48 ± 0.76%. The total body fat weight (BFW) accounted for 5.9 ± 3.86% of the chilled carcass weight, with notable variations in fat depot distribution. Among the fat depots, the digestive fat (DFW) showed the highest accumulation, followed by cavity fat (CFW) and kidney fat (KFW), with no significant difference between these three parameters ($P > 0.05$). In contrast, mesenteric fat (MFW) exhibited the lowest deposition, with a significant difference ($P < 0.05$) compared with the other fat depots.

Body fineness

The fineness level of the body and carcass of the studied beef cattle showed an average of body boniness index (BBI) of 1/8 (0.125 ± 0.02). The maximum and minimum values were 1/7 (0.14) and 1/11 (0.09).

Correlations between the different parameters

Table 5 presents the correlations between growth, slaughter, conformation, and fattening parameters in Brune de l'Atlas beef cattle. Body weight (BW) exhibited moderate positive correlations with chilled carcass weight (CCW), fifth quarter weight (5QW), and carcass compactness index (CCI) (r = 0.42–0.62; $P < 0.05$). However, BW was negatively correlated with dressing percentages (DP) and body fineness (BFI) (r = –0.45 to –0.32; $P < 0.05$), indicating that as BW increases, DP and BFI tend to decrease. Chilled carcass weight (CCW) displayed the strongest correlations among the studied parameters, with significant positive relationships observed with live weight (LW), dressing percentages (DP), fifth quarter weight (5QW), compactness index (CCI), and

Table 4. Descriptive statistics of carcass fattening score, body fat weight and fat index

		N	MEAN	MIN	MAX	VARIANCE	STD.DEV.
CFS		51	1.85	0.5	4	0.68	0.83
FI	(%)	51	1.48	0.3	6.88	1.12	0.76
KFW	(kg)	51	3.28[a]	0.5	12	6.12	2.47
DFW	(kg)	51	4.01[a]	1	12	11.94	3.46
MFW	(kg)	51	1.24[b]	0.3	11	2.22	1.49
CFW	(kg)	51	3.67[a]	1	12	9.68	3.11
BFW	(kg)	51	12.2	4	36.3	82.65	9.09
BFW	(%)	51	5.9	1.82	16.85	14.91	3.86

CFS: Carcass fattening score, FI: Fat index, KFW: Kidney fat weight, DFW: Digestive tract fat weight, MFW: Mediastinal fat weight, CFW: Cover fat weight, BFW: Body fat weight. The weights of the different CFW components were compared, and means with different superscripts are statistically different at $P < 0.05$.

Table 5. Correlations between the live weight, slaughter yield, carcass conformation, fattening level, and body fitness of the studied Brune de l'Atlas beef cattle

	CCW	BW	CDP	TDP	5thQ	HTI	CCI	KFI	BBI	CFS	EUROP
CCW	1.00	0.62*	0.47*	0.40*	0.65*	0.27	0.81*	0.19	0.14	0.45*	0.50*
BW		1.00	–0.39*	–0.45*	0.42*	–0.20	0.51*	–0.03	–0.32*	0.19	0.18
CDP			1.00	0.99*	0.25	0.56*	0.38*	0.19	0.54*	0.30	0.34*
TDP				1.00	0.24	0.60*	0.31*	0.21	0.55*	0.31	0.35*
5thQ					1.00	0.42*	0.33*	0.56*	0.12	0.62	0.68*
HTI						1.00	0.08	0.52*	0.37*	0.39	0.51*
CCI							1.00	0.02	0.06	0.32	0.35*
KFI								1.00	0.15	0.51	0.65*
BBI									1.00	–0.00	0.20
CFS										1.00	0.65*
EUROP											1.00

BW: Body weight, CCW: Chilled carcass weight, CDP: Commercial dressing percentage, TDP: True dressing percentage, 5thQ: Weight of the fifth quarter, HTI: Hip thickness indices, CCI: Compactness indices, Conformation-EUROP: carcass conformation classification, FI: Fat index, CFS: Carcass fattening score, BBI: Body boniness index. An asterisk indicates a significant correlation at $P < 0.05$.

fattening parameters (r = 0.45–0.81; $P < 0.05$). This suggests that as CCW increases, these parameters also tend to increase. Conformation parameters and fattening indices exhibited moderate to strong positive correlations (r = 0.32–0.65; $P < 0.05$), indicating parallel development during the fattening period. However, no significant correlation was found between conformation parameters and the body fineness index (BFI). These correlation patterns provide valuable insights for future genetic improvement programs in the Brune de l'Atlas population, particularly in understanding the relationships between growth, carcass characteristics, and body conformation traits.

Comparison between local cattle ecotypes

Table 6 presents a comparative analysis of the studied variables across the four ecotypes of the local cattle population. The average live weight showed no significant differences among the ecotypes ($P > 0.05$), with values ranging between 360.14 kg and 382.32 kg. Similarly, the fifth quarter weight and chilled carcass weight were not significantly influenced by ecotype ($P > 0.05$), although the Setifienne ecotype exhibited slightly higher values for both parameters compared with the others. Commercial and true slaughter yields also remained consistent across ecotypes ($P > 0.05$), with commercial yields ranging from 51.56% to 58.54% and true yields from 55.02% to 62.4%. Notably, the Setifienne ecotype demonstrated higher thigh and carcass compactness indices compared with the other groups, although these differences were not statistically significant ($P > 0.05$). The conformation-EUROP classification revealed overall low carcass conformation levels across all ecotypes, ranging between P and O categories ($P > 0.05$). However, a significant difference in fattening index (FI) was observed between the Setifienne and Cheurfa ecotypes ($P < 0.05$), with the Setifienne group exhibiting a higher fattening score. The Guelmoise and Chelifienne ecotypes showed intermediate fattening levels, whereas the fitness score remained consistently low across all groups ($P > 0.05$). Lastly, carcass fineness level (BBI) was uniform across all four ecotypes, consistently classified as 1/8 ($P > 0.05$).

Discussion

There are few reports on the slaughter and carcass characteristics of Algerian beef cattle. The fattening performance and carcass traits recorded in this study pertain specifically to young bulls of the local bovine population descended from the Algerian Brune de l'Atlas breed.

When comparing the mean slaughter weight of indigenous Brune de l'Atlas cattle (343.97 ± 93.26 kg) with that of Bos indicus indigenous African beef cattle, the weight appears to closely match that of African Zebu and Afrikaner cattle (Teye and Sunkwa, 2010). It is higher than the weights reported for Nguni, Sanga, West African Shorthorn breeds, Curraleiro Pé-Duro and Pantaneiro breeds, indigenous Ethiopian cattle, and Creole cattle, yet it is comparable to the weight of young bulls resulting from crosses with Prim'Holstein (Teye and Sunkwa, 2010; Gudeto et al., 2022; Barbosa et al., 2023; Gelaye et al., 2022), Greek Blonde indigenous cattle (Nikolaou et al., 2023), and Nelore Brazilian cattle (Barbosa et al., 2023). However, the local Algerian cattle in this study exhibit lower slaughter weights than Bonsmara from South Africa, the N'Dama breed from Congo, American Santa Gertrudis, European

Table 6. Comparison of body weight and slaughter performance and carcass traits of the four local cattle Brune de l'Atlas ecotypes

	Setifienne (n = 18)	Guelmoise (n = 16)	Chélifienne (n = 14)	Cheurfa (n = 3)
PV (kg)	370.8 ±22.48	365.09 ± 25.49	360.14 ± 23.85	382.32 ± 55.08
CCW (kg)	214.62 ± 12.08	196.49 ± 13.70	198.33 ± 12.81	173.67 ± 29.60
CDP (%)	58.54 ± 2.40	54.86 ± 2.71	55.29 ± 2.53	51.56 ± 2.85
TDP (%)	62.4 ± 2.65	59.07 ± 3.00	59.41 ± 2.81	55.02 ± 6.49
5thQ (kg)	122.86 ± 6.10	112.96 ± 6.92	118.37 ± 6.46	105.23 ± 14.96
HTI	5.37 ± 0.36	4.82 ± 0.41	4.35 ± 0.39	3.97 ± 0.90
CCI	1.61 ± 0.08	1.52 ± 0.09	1.52 ± 0.09	1.52 ± 0.21
BCI	0.72 ± 0.03	0.67 ± 0.02	0.69 ± 0.05	0.67 ± 0.03
Conformation-EUROP	2 ± 0.24	1 ± 0.27	2 ± 0.25	1 ± 0.58
FI	2.14 ± 0.23^{a}	$1.16 \pm 0.26^{a,b}$	$1.49 \pm 0.24^{a,b}$	$0.97 \pm 0.57^{b.}$
CFS	2.13 ± 0.19	1.5 ± 0.21	1.88 ± 0.20	1.67 ± 0.46
BBI	1/8	1/8	1/8	1/8

BW: Body weight, CCW: Chilled carcass weight, CDP: Commercial dressing percentage, TDP: True dressing percentage, 5thQ: Weight of the fifth quarter, HTI: Hip thickness indices, CCI: Compactness indices, BCI: Buttock compactness Index, Conformation-EUROP: carcass conformation classification, FI: Fat index, CFS: Carcass fattening score, BBI: Body boniness index. Values in the same row with different letters are statistically different ($P < 0.05$).

Pinzgauer, and Brown Swiss breeds (Strydom, 2000; Silvere et al., 2023). These differences may arise from various factors, including age, sex, type or breed of cattle used in each study, as well as differences in general management practices and the ecology of the respective regions.

In Algeria, previous studies have reported that live body weight varied between 250 and 350 kg (Aissaoui et al., 2003). However, Bouzebda Afri et al. (2007) recorded relatively higher mean live weights using barymetric formulas. These differences among Algerian studies highlight the direct and indirect effects of factors such as nutrition, age, cattle conformation, and husbandry practices. In particular, our study involved four ecotypes of the native Algerian Brune de l'Atlas cattle, all belonging to the same herd with specific husbandry and management conditions. The high variance observed in live body weight suggests strong potential for production improvement within this population, which has historically never undergone selection. Slaughter weight is a crucial indicator of calf growth, primarily dependent on dam milk production and, secondarily, on forage quality, with intake increasing significantly after three months of age (Handcock et al., 2021; Gherissi et al., 2013). In this study, measuring slaughter weight provided essential data for subsequent post-slaughter estimations, including dressing percentage, fifth-quarter proportion, carcass conformation, fattening level, and the proportion of first-class meat cuts.

The empty live weight recorded in this study ranged from 202.52 kg to 589.99 kg, emphasizing the need for selection based on this quantitative trait as part of a genetic improvement strategy for beef performance. Additionally, environmental factors such as temperature, humidity, rearing conditions, low nutrient availability, and parasitism can contribute to variations in live body weight at slaughter (Yüksel et al., 2019; Soulat et al., 2019).

The mean hot carcass weight (HCW) was 206.51 ± 51.95 kg, which was higher than that reported for indigenous African breeds such as Afrikaner and Nguni but lower than the values recorded for other African and Spanish rustic young-bull breeds (Strydom, 2000; Albertí et al., 2005). Compared with Algerian cattle from the same population and Central African N'Dama and Zebu cattle, the HCW in our study was similar to previously reported values (Bouzebda Afri et al., 2007; Silvere et al., 2023). Low meat production per cow in these systems can be attributed to several factors. Firstly, calves are primarily fed high-fiber, low-quality forages with minimal grain supplementation, leading to inadequate nutrient intake for optimal growth. Secondly, due to economic pressures, farmers often sell animals at a young age, preventing them from reaching their genetic potential in terms of live weight (Ben Salem and Khemiri, 2008). Finally, the absence of systematic selection in autochthonous breeds further contributes to the observed limitations in meat production.

The average true dressing percentage (TDP) recorded in this study was 60.11 ± 11.09%, which is notably high compared with previous findings in the same cattle population (Bouzebda Afri et al., 2007). This discrepancy may be attributed to differences in assessment techniques, such as the determination of live weight using barymetric formulas, the heterogeneity of the studied animals in terms of sex, age, and fattening period, as well as variations in the method of estimating carcass yield (true dressing vs. commercial dressing). Specifically, the commercial dressing percentage in our study was estimated at 56%, a value comparable to those reported by Bouzebda Afri et al. (2007) and Gherissi et al. (2013). When compared with other indigenous tropical cattle populations, the true dressing percentage of the studied animals closely aligns with that of Creole cattle and their crossbreeds with the Prim'Holstein breed (Naves, 1985), as well as indigenous African cattle breeds (48.6–58.7%). However, it is lower than that recorded in African suckler breeds (Strydom, 2000) but higher than values reported for Zebu and N'Dama Taurine cattle (Silvere et al., 2023). The dressing percentage for tropical local cattle breeds typically ranges between 50% and 65%, depending on factors such as breed characteristics and management practices. Some local cattle breeds have a higher bone proportion, lower muscle development, or higher fat deposition compared with specialized beef breeds, which can influence dressing percentage. The observed trend in dressing percentages supports the general pattern that heavier animals tend to exhibit higher dressing percentages.

The mean weight of the fifth quarter, excluding blood and stercoral content, was 95.24 ± 41 kg, representing approximately 26% of live weight. The individual components included red offal (heart, liver, lungs, spleen, and kidneys) at 15.53 ± 5.10 kg, the empty digestive tract at 23.65 ± 6.10 kg, the head at 19.85 ± 4.33 kg, the legs at 8.45 ± 2.28 kg, and the skin at 27.76 ± 7.10 kg. Notably, the weight of red offal in our study exceeded the values reported for tropical beef cattle breeds (4.47 to 6.49 kg), as did the weights of the skin (4.84 to 7.93 kg) and the digestive tract (4.56 to 7.41 kg) (Teye and Sunkwa, 2010). Similar weight distributions were observed for the remaining fifth quarter components. Additionally, comparable fifth quarter proportions have been reported for Bonsmara beef cattle, though they tend to be slightly lower in other African indigenous breeds (Strydom, 2000). At present, no studies have specifically examined the variability of these parameters within the indigenous North African Brune de l'Atlas cattle population, highlighting the need for further research in this area.

Carcass conformation refers to the physical shape, size, and muscling of a beef carcass, making it a key trait in determining meat quality and market value.

In this study, conformation was assessed using a combination of calculated indices and visual muscle evaluations. The mean values for hip thickness index (HTI), carcass compactness index (CCI), and buttock compactness index were 0.34 ± 0.05, 1.55 ± 0.37, and 0.69 ± 0.08, respectively. These parameters indicate the relationship between muscle mass and skeletal size. The CCI observed in the studied animals closely resembles that of male Creole indigenous cattle (1.51) as reported by Naves (1985). However, it is higher than the values recorded in indigenous African cattle breeds, which range between 0.74 and 1.02 (Strydom, 2000). A higher CCI suggests a more compact and muscular carcass, while a lower value indicates a less compact carcass with relatively more skeletal structure. The HTI of the animals studied was found to be higher than that reported for the Creole breed (0.283) by Naves (1985).

Despite these moderate values, the carcass conformation of the studied animals remains relatively underdeveloped, with an average conformation score of 1.86, corresponding to class O on the EUROP conformation grid. This conformation level is comparable to that observed in Buffalo species (Nikolaou et al., 2023) and reflects a moderate muscle development with straight to concave profiles. In contrast, higher conformation levels were reported by Alberti et al. (2010) in Spanish beef breeds, which were classified as R on the EUROP grid. Given that high-priced cuts in beef cattle are primarily derived from the hindquarters, the proportion of hindquarters in the studied carcasses was 59.22 ± 8.42% of total carcass weight, leading to a hindquarter-to-forequarter ratio of 1.28 ± 0.11. This ratio is superior to the values reported by Naves (1985) and Teye and Sunkwa (2010), which were 47.7% and 46.3%, respectively, but lower than those obtained in African Bonsmara-crossbred cattle (Slabbert et al., 1992). Naves (1985) observed that in male Creole cattle and other tropical beef breeds, the forequarter tends to be more developed than the hindquarter, leading to an imbalance in carcass distribution. This phenomenon is often linked to anatomical traits, such as an excessively developed hump on the forequarters of certain tropical breeds, affecting overall carcass balance.

The Brune de l'Atlas cattle are recognized as a rustic North African breed, primarily valued for their resilience, adaptability to harsh environmental conditions, and ability to thrive with limited nutritional resources. They have not been subjected to intensive selection for meat production or specific carcass traits, which contributes to the variability observed in carcass conformation. The differences in conformation indices and overall carcass traits can be attributed to genetic factors, breed characteristics, age, nutrition, management practices, and environmental influences. Farmers and breeders often refer to breed-specific standards when selecting animals with desirable hip thickness indices for breeding or marketing purposes, highlighting the importance of targeted selection strategies to improve carcass traits in this local cattle population.

On the other hand, the studied cattle exhibited an average low fattening status score of 1.85 ± 0.83, i.e., carcasses with a light cover of fat and muscles that were visible almost everywhere. Within the thoracic cavity, muscle tissue was clearly visible between the ribs. Compared with other cattle populations, a similar fattening state was observed in Spanish beef breeds, particularly in young bulls with low commercial weight (Alberti et al., 2010). These results were supported by the mean body fat weight, which was 12.20 ± 9.09 kg, and an average fat index (FI) of 1.48 ± 0.76. Similar results were reported for the Creole breed. In the samples studied by Guillaume (2006) on the Prim'Holstein and Charolais breeds, 22% of the carcasses were considered too fat (classified as 4) in the first breed, and only 4.3% in the second. These carcasses came from animals slaughtered at 387.5 kg for young Prim'Holstein cattle and 437 kg for Charolais bulls. Our results showed a low ability to store energy reserves, which was dependent on low slaughter and carcass weights. Moreover, the early development of body fat, which generally increases at low carcass weight, necessitates slaughter at low body weight to avoid excessive fat deposition on the carcass.

Regarding carcass quality, this low-fat deposit could be an advantage, as it is possible to seek higher slaughter weights without risking excessive and early fattening, since the local market demands light to medium carcasses. However, consideration must also be given to the complex interaction of multiple factors affecting carcass fattening variability, and their relative importance varies depending on the specific production system, breed, and environmental conditions (Yüksel et al., 2019; Soulat et al., 2019). Producers and feedlot operators often strive to manage these factors to optimize carcass quality, consistency, and marketability (Soulat et al., 2019). It is important to note that while there are breed standards and guidelines for desirable carcass conformation and fattening levels, there is inherent variability within any beef cattle population. This variation allows for genetic diversity and the potential for improvement through selective breeding programs (Barro et al., 2023; Mamede et al., 2023). Additionally, consumer preferences and market demands may influence the perceived value of different carcass conformations and fattening levels, resulting in variations in desirability depending on the specific market or final use of the meat.

The muscular development of beef cattle refers to the growth and distribution of muscle throughout the animal's body. Cattle with adequate muscling typically have a higher muscle-to-bone ratio and exhibit strong muscle definition in areas such as the hindquarters and loin. Low overall body and carcass fitness, with high variance in these parameters, was observed in the studied cattle population. Achieving optimal carcass fitness requires genetic selection, appropriate nutrition, proper management practices, and adherence to industry standards and grading systems. The specific criteria for carcass fitness may vary depending on regional preferences, breed characteristics, and market demands. Producers and processors aim to raise and select cattle that consistently yield carcasses meeting the desired fitness criteria for their target markets. Frame size is another important parameter for evaluating conformation and the fitness of beef cattle, and it has the potential to be used as a criterion in the selection of precocious beef cattle (Barro et al., 2023).

The carcass characteristics, particularly carcass weight and conformation, exhibited rather modest meat traits in the local Algerian cattle population, which has never been subjected to a structured selection program for the genetic improvement of meat production performance. Recent studies have shown that yearling weight, visual scores of body structure, muscularity, precocity, fattening, and carcass conformation traits have moderate to high heritability (Barro et al., 2023; Mamede et al., 2023), with significant genetic correlations between body weight (BW) at slaughter and carcass quality and meat traits (Albertí et al., 2005). Thus, the high variance observed in most of the studied parameters explains the heterogeneity of individuals in the local cattle population, which suggests that a selection program could be implemented to improve the economic value of bulls. It is also important to point out the established positive linear correlations between body weight and carcass weight, fifth quarter weight, and carcass conformation, as well as the negative correlation with slaughter yield and body fitness.

Conclusion

The Brune de l'Atlas cattle demonstrated heterogeneous yet promising meat production traits at a mean slaughter age of 16 months, characterized by satisfactory carcass weights, good conformation, high slaughter yields, and notably lean carcasses with low fat deposition. These findings support the potential of this local breed as a valuable source of red meat production in its pure form. To optimize the breed's meat production potential, we recommend implementing a systematic performance monitoring system for finished animals, including both pre-slaughter evaluations and detailed carcass assessments. This data-driven approach would facilitate targeted genetic improvement programs aimed at enhancing meat production traits. Additionally, strategic cross-breeding programs utilizing these local cattle could leverage heterosis effects to further improve carcass weights and conformation scores while maintaining the breed's valuable adaptation traits.

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Seasonal Population Dynamics of the Common Lice Species Infesting the Domestic Goat in Bulgaria

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Keywords: *temperature, precipitation, correlation, lice, goats, Bulgaria.*

Abstract. Background and aim. *Lice are among the most common ectoparasites in goats. They cause health problems, including weight loss, hypoproteinemia, nutritional deficiencies, reduced vitality and anemia. The present study aims to investigate the seasonal dynamics of the most common species of lice in naturally infested goats and the influence of temperature and precipitation levels on it.*

Materials and methods. *The seasonal dynamics were monitored by examining twenty goats, ten from each of two different small herds in the Stara Zagora region. At the beginning of each month, over a period of two years (from January 2018 to December 2019), the individual and average intensity of infestation were determined dynamically.*

Results. *Our findings reveal that parasitosis caused by Bovicola caprae and Linognathus stenopsis persists throughout the year, with clear seasonal patterns. Infestation intensity peaks in March, following the coldest months (December–February) when average temperatures are around 2–3°C. During the warmer months (June–August), when temperatures rise to 21–25°C, infestation intensity significantly declines. Statistical analysis confirmed a moderate negative correlation between infestation intensity and ambient temperature ($r = -0.411$, $P < 0.0001$), while no significant correlation was found with precipitation ($r = 0.023$, $P = 0.7184$)*

Conclusion. *The observed seasonal dynamics of both lice species on goats clearly demonstrate that winter provides optimal conditions for the development of their populations, confirming that phthirapterosis in goats in our region has a pronounced winter seasonality.*

Introduction

Lice are among the most common ectoparasites in goats. They are cited as one of the causes of weight loss, hypoproteinemia, nutritional deficiencies, reduced vitality, and anemia during the winter months (Chavham, 2023). Additionally, they cause health problems, behavioral changes and reproductive disorders such as the birth of offspring with reduced weight and vitality and decreased fertility (Doley et al., 2023). Temporary ectoparasites (such as ticks and fleas) are more strongly influenced by environmental abiotic factors and less by the host (Ming et al., 2023). Obligatory ectoparasites are highly host-specific and entirely dependent on the host to complete their biological cycle (Taylor et al., 2015), but they are also affected by environmental conditions (Ajith et al., 2020) like temperature and humidity (Adly et al., 2021). The region of Stara Zagora is located in the northern part of the Upper Thracian Plain, at 42°25' N latitude and 25°39' E longitude, at an altitude of 280 meters (according to data from the Regional Cadastre, Stara Zagora). The area falls within the boundaries of the Upper Thracian climatic subregion. The predominant climate in the region is transitional, between temperate continental and continental Mediterranean.

There is almost no debate among researchers that the infestation intensity (II) with lice in goats is influenced by climatic conditions across different seasons, which determines the seasonality of phthirapterosis. Only a few reports suggest that no such dependency exists (Santos et al., 2006). Most authors believe that climate and seasons affect the II with lice (Nedelchev, 1985; Yakhchali and Hosseine, 2006; Paul et al., 2012; Fomicheva, 2013; Maguini et al., 2018), with a particular emphasis on average monthly temperature and photoperiod, while attributing less importance to average monthly precipitation levels and relative humidity (Adesh et al., 2011). The main species causing phthirapterosis in Bulgaria are *Linognathus stenopsis* (Burmeister, 1838) and *Bovicola caprae* (Gurlt, 1843) (Nizamov, 2023). The last study on the seasonal dynamics of lice population numbers in domestic goats (*Capra hircus,* Linnaeus, 1758) in the country was conducted nearly 40 years ago (Nedelchev, 1985).

The purpose of this study is to examine how temperature and precipitation levels affect the seasonal dynamics of the most prevalent species of lice in naturally infested goats. These findings can help understand and predict seasonal lice infestation patterns on goats, which could be valuable for implementing targeted treatment plans in animal husbandry.

Material and methods

Material

The seasonal dynamics were studied by examining twenty goats of the Bulgarian White Dairy Goat

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breed. Ten randomly selected female goats, aged 2 to 8 years, were identified by ear tag numbers and originated from a privately owned herd of 23 animals in the village of Yazdach, Stara Zagora region. This herd was managed under a pasture-based system, and a natural infestation with *Linognathus stenopsis* was confirmed.

The second group of ten goats belonged to the herd at the training facility of Trakia University, Stara Zagora. This herd consisted of 17 animals and was maintained under a confined housing system. Experimental animals were selected from female individuals aged 2 to 8 years and were identified by their ear tag numbers. A natural infestation with *Bovicola caprae* was confirmed in this herd.

Examination methods

Goats under examination were checked for nits and adult lice. Some detected insects were collected with tweezers and stored in containers with 70° ethanol. All collected insects were transported to the laboratory. Species identification was done according to morphological features described by Price and Graham (1997). A DMi1 S/M 424790 Leica® microscope (Leica Microsystems CMS GmbH) was used for the microscopic exams.

Lice infestation was determined using the method of Brown et al. (2005) by counting all insects in a total of 7 square-shaped fields, each with an area of 10 cm². The total number of parasites found was then multiplied by 100.

At the beginning of each month, over a two-year period (January 2018 – December 2019), the individual and average intensity of infestation were dynamically assessed by counting the number of parasites on the same ten animals. During the study, the animals were not treated with insecticidal products.

Throughout this two-year period, the influence of climate-geographical factors was tracked monthly, specifically, the average monthly temperature and monthly precipitation levels (according to data from the Hydrology and Meteorology Station in Stara Zagora). This station is located 20 km from the first herd in the village of Yazdach and 5 km from the herd of Trakia University.

Statistical analysis of data

Infestation intensity (II) was calculated using the method described by Brown et al. (2005). This involved counting the number of lice in 7 designated 10 cm² areas on each goat, with the total count multiplied by 100 to obtain an estimated whole-body infestation value.

The Spearman rank correlation coefficient was used to evaluate the relationship between the studied environmental parameters and the intensity of invasion with *L. stenopsis* and *B. caprae*. Calculations were made with MedCalc 15.8 Statistical Software Package (Belgium).

Results

After identifying the insects, it was found that the first of the studied herds was infested with the species *Bovicola caprae* and the second with *Linognathus stenopsis*. Our results show that the parasitosis caused by these two species of lice persists year-round.

Table 1 presents the monthly values of the total and average infestation intensity (II) of biting lice (*Bovicola caprae*) across the 10 studied goats during the two-year experimental period.

An analysis of the data in Table 1 shows that, during the winter months, the number of lice on

Table 1. Monthly values of the total and average intensity of natural infestation with *B. caprae* in 10 goats from a farm in Stara Zagora

Month	Year 2018		Year 2019	
	Detected number	Mean II	Detected number	Mean II
January	228	22. 8	181	18. 1
February	380	38. 0	400	40. 0
March	623	62. 3	708	70. 8
April	462	46. 2	524	52. 4
May	253	25. 3	184	18. 4
June	104	10. 4	58	5. 8
July	113	11. 3	29	2. 9
August	70	7. 0	15	1. 5
September	59	5. 9	23	2. 3
October	73	7. 3	20	2. 0
November	54	5. 4	15	1. 5
December	127	12. 7	73	7. 3

II – Intensity of infestation.

all experimental animals, as well as the average II, reached their highest values. The infestation intensity peaks in March. The average II is highest after the coldest winter months (December, January, and February), when average monthly temperatures are around 2–3°C, and lowest following the warmest summer months (June, July, and August), when air temperatures range from 21 to 25°C. In spring and summer, their numbers gradually decrease, reaching a minimum in autumn, only to rise again in winter.

After statistical processing of the results, it was found (Table 2 and Fig. 1) that there is a significant ($P < 0.0001$) moderate negative correlation between II with *B. caprae* and ambient temperature (r = –0.411), which demonstrates that II increases at lower temperatures. Fig. 2 shows the variation in mean precipitation and mean infestation intensity of *B. caprae* in the Stara Zagora region between January 2018 and December 2019. Based on linear regression analysis and calculation of the Spearman coefficient (r = 0.023), there is no correlation between the two studied parameters.Table 3 presents the monthly values obtained for the total number of insects and the average infestation intensity of blood-sucking lice (*L. stenopsis*) on the 10 studied goats over the two-year period.

Data analysis in Table 3 shows that the highest II is observed in March. During the winter months, the number of lice on all experimental animals, as well as the average II, reaches its highest values. In spring and summer, these values gradually decrease, reaching a minimum in September, followed by an increase in II throughout autumn and winter. The lowest annual average monthly temperatures are followed by a peak in II.

Similar to the studies on *B. caprae*, the average II with *L. stenopsis* also showed (Table 2 and Fig. 3) a significant ($P < 0.0001$) moderate negative correlation with ambient temperature (r = –0.411). However, there is no correlation (r = 0.036) between precipitation levels and infestation intensity (Table 2 and Fig. 4).

Table 2. Correlation between environmental conditions and infestation intensity

Lice species	Environmental factor	Spearman rho (r)	95% confidence interval	*P* value
L. stenopsis	Ambient Temperature	–0.411	–0.511 to –0.300	< 0.0001
	Precipitation	0.036	–0.0914 to 0.162	0.5828
B. caprae	Ambient Temperature	–0.411	–0.511 to –0.300	< 0.0001
	Precipitation	0.023	–0.104 to 0.150	0.7184

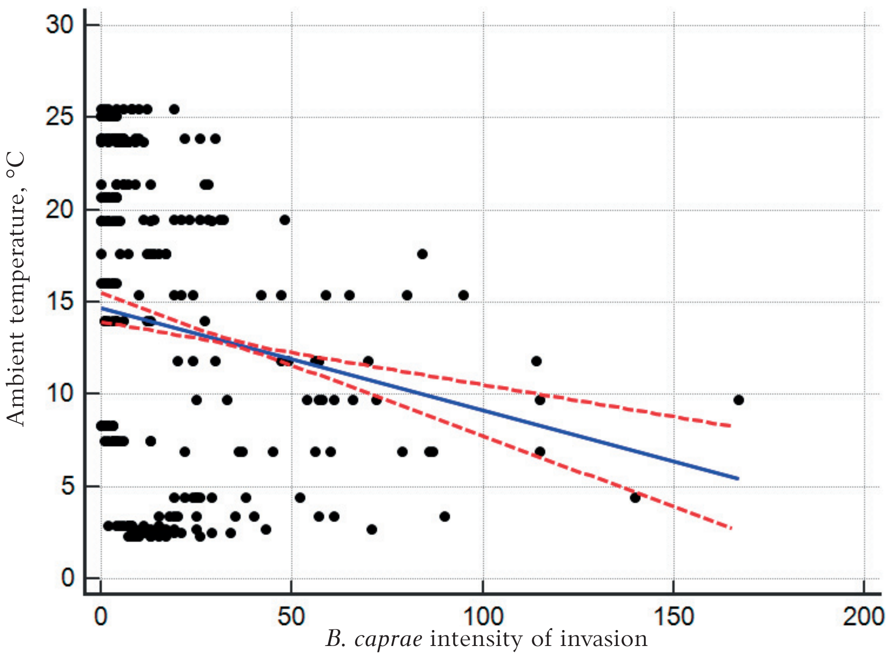

Fig. 1. Scatter plot of *Bovicola caprae* infestation intensity vs. ambient temperature

Dots: Each dot represents the monthly average infestation intensity (II) of *Bovicola caprae* during the study period (January 2018 – December 2019). Blue line: This is the regression line, showing the relationship between temperature and infestation intensity. It slopes downward, indicating a negative correlation (as temperature decreases, infestation increases). Red lines: These represent the 95% confidence intervals, indicating the range within which the true relationship likely falls.

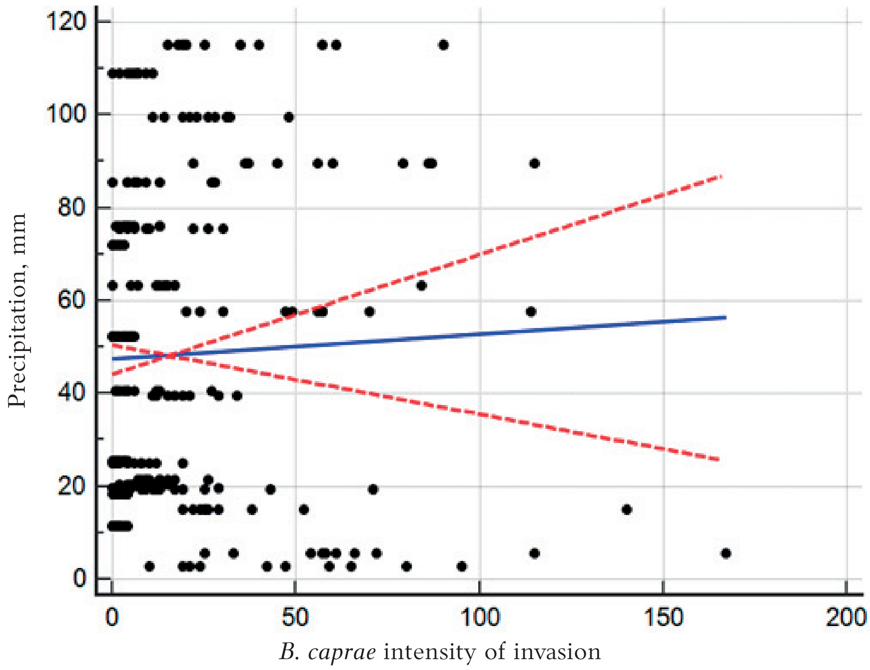

Fig. 2. Scatter plot of *Bovicola caprae* infestation intensity vs. precipitation

Dots: Each dot represents the monthly average infestation intensity (II) of *Bovicola caprae*. Blue line: This is the regression line, which is nearly flat, indicating no significant correlation between precipitation levels and infestation intensity. Red lines: The 95% confidence intervals, showing the statistical range of the relationship.

Table 3. Monthly values of the total and average intensity of natural infestation with *L. stenopsis* in 10 goats from a farm in Stara Zagora

Month	Year 2018		Year 2019	
	Detected number	Mean II	Detected number	Mean II
January	188	18. 8	245	24. 5
February	391	39. 1	429	42. 9
March	792	79. 2	903	90. 3
April	455	45. 5	441	44. 1
May	273	27. 3	273	27. 3
June	148	14. 8	127	12. 7
July	168	16. 8	121	12. 1
August	102	10. 2	93	9. 3
September	88	8. 8	57	5. 7
October	102	10. 2	92	9. 2
November	122	12. 2	132	13. 2
December	237	23. 7	339	33. 9

II – Intensity of infestation

Discussion

Most publications regarding the seasonal dynamics of lice populations in goats show a marked winter seasonality, with infestations occurring when temperatures are low, humidity is high, and daylight hours are short (Yakhchali and Hosseine, 2006; Paul et al., 2012; Fomicheva, 2013; Maguini et al., 2018), which is also confirmed by the present study. However, other scientific articles about the seasonal dynamics of lice on goats show that populations, such as *Damalinia limbata* (Gervais, 1847) and *Linognathus stenopsis*, often fluctuate seasonally, with peaks in infestation rates during the warmer months. The life cycle and fecundity of lice are particularly influenced

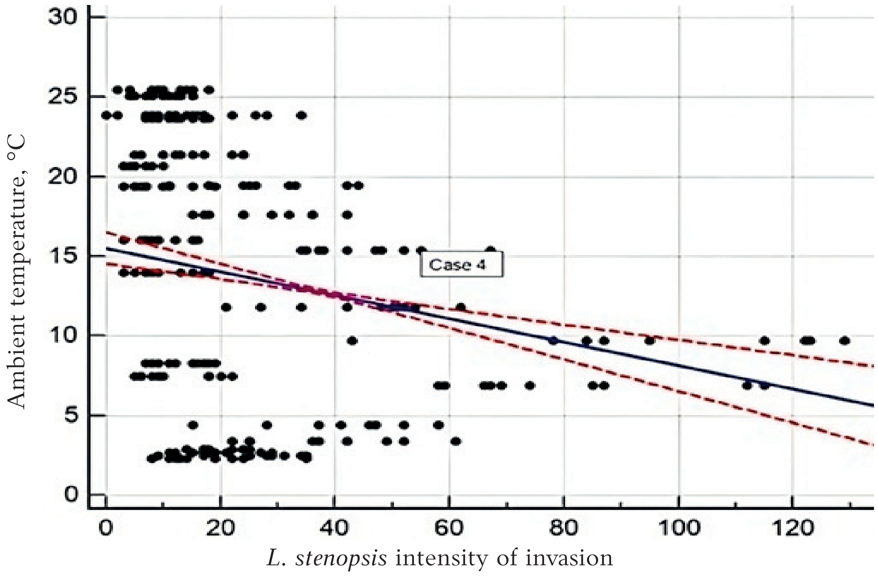

Fig. 3. Scatter plot of *Linognathus stenopsis* infestation intensity vs. ambient temperature

Dots: Each dot shows the monthly average infestation intensity (II) of *Linognathus stenopsis*. Blue line: The regression line, showing a negative correlation. Infestation increases as temperature decreases. Red lines: The 95% confidence intervals, representing the uncertainty in the relationship.

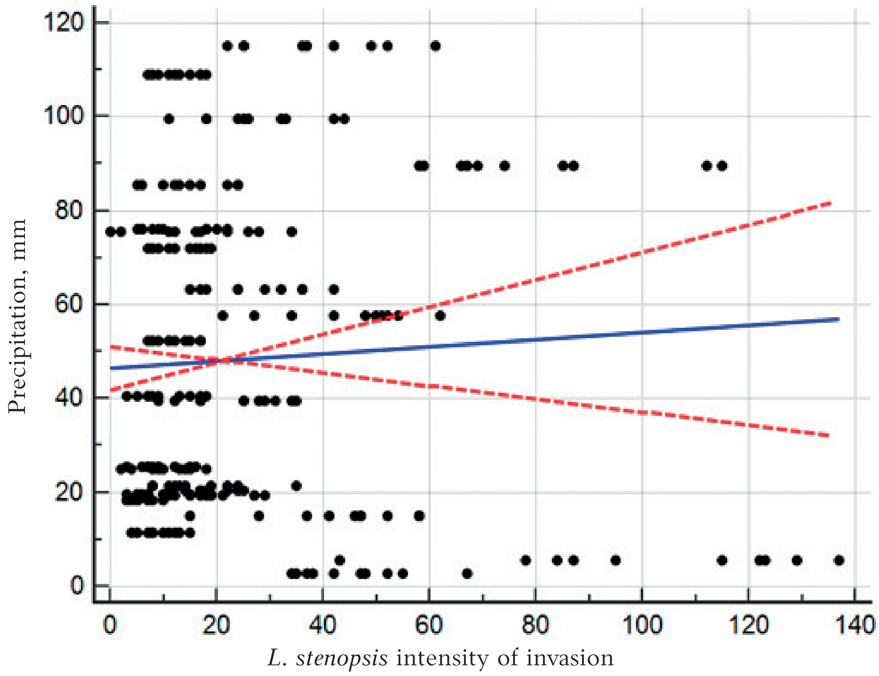

Fig. 4. Scatter plot of *Linognathus stenopsis* infestation intensity vs. precipitation

Dots: Each dot corresponds to the monthly average infestation intensity (II) of Linognathus stenopsis. Blue line: The regression line, which remains flat, indicating no significant relationship between precipitation and infestation. Red lines: The 95% confidence intervals, illustrating the variability in the data.

by ambient temperatures, as higher temperatures can accelerate their reproduction, leading to population spikes in summer (Brown et al., 2005). These contrasting results are likely due to differences in the climate zones where the studies were conducted. However, when comparing other studies on the epidemiology of phthirapterosis in goats, similar seasonal dynamics are observed across various geographic latitudes (Nedelchev, 1985; Fomicheva, 2013; Meduini et al., 2018).

A previous study on the seasonal dynamics of lice in goats in Bulgaria (Nedelchev, 1985) also reported a clear winter seasonality of parasitosis. The highest infestation intensity was recorded in January–

February, unlike the present study, which showed a later peak in March. Possible reasons could include differences in the study years (almost 40 years apart), global climate warming, as well as the influence of other factors on II, such as diet, age, sex, condition, and immunological status.

Although we selected herds representing the two main goat-rearing systems in Bulgaria – grazing and stall-based housing – we did not observe substantial differences in infestation intensity (II) between these management systems for either *Bovicola caprae* or *Linognathus stenopsis*. This is likely due to the fact that lice are stationary ectoparasites transmitted primarily through direct contact. Therefore, the management system has a minimal effect on their transmission dynamics. Future research will further explore the influence of age, sex, and body condition on infestation intensity in goats, as this study does not provide results on these factors.

Although no correlation was found between precipitation levels and lice II, it is known that temperature and humidity do not act independently; their effects are closely interconnected. We also assume that precipitation levels have some impact on lice II, as they amplify the effect of temperature. In higher humidity, low temperatures feel even lower.

Conclusion

This study demonstrates that the seasonal dynamics of lice (*Bovicola caprae* and *Linognathus stenopsis*) in goats in the Stara Zagora region are closely associated with seasonal changes, with a pronounced winter peak in infestation intensity. The results confirm a moderate negative correlation between intensity of infestation and ambient temperature, with the highest infestation rates occurring after the coldest months, specifically in March. In contrast, no significant correlation was found between intensity of infestation and precipitation levels.

Overall, this study provides valuable insights for seasonal lice management strategies in goat husbandry, indicating that preventive measures might be most effective when implemented before winter.

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The Influence of Breed on the Fatty Acid Composition of Goat Milk and the Relationship between Breed and Seasonal Temperature with Milk Yield

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Keywords: *Goat's milk, fatty acid composition, milk productivity, temperature.*

Abstract. *In order to determine the influence of temperature on the productivity of goats and effect of their breed on the content of fatty acids in milk, an experiment was conducted with 30 goats of different breeds throughout the year. The first group included 10 goats of the Saanen breed, the second group included 10 goats of the Russian white breed, and the third group included 10 Ukrainian local breed goats. Productivity of goats as well as environmental temperature fluctuations were assessed daily, and the content of fatty acids in milk was determined twice a month during the year. Breed and external temperature influenced the milk yield of goats of all groups by 81.33% and 13.00%, respectively. It should also be noted that goats of the Saanen breed have a higher content of linolenic, isocaprylic, heptadecenoic, stearic, and erucic acids compared with goats of Russian white and Ukrainian local breed goats. In Ukrainian local breed goats, the content of capric, lauric, arachinic, myristoleic, pentadecanoic, pentadecenoic, isopalmitic, palmitoleic, hexadecadienoic, linoleic, and geneicosanoic acids was higher compared with goats of the Russian white breed and Saanen breeds. Russian white goats had a higher content of undecyl, behenic and docosahexaenoic acids compared with goats of Saanen breed and Ukrainian local breed goats.*

Introduction

Today, the forecast for the growth of goat breeding in the world is positive (Liang and Paengkoum, 2019), but global climate changes associated with the variability of weather conditions and their impact on housing conditions will increasingly affect agricultural production in general and dairy cattle breeding in particular (Rojas-Downing et al., 2017; Tüfekci and Tozlu Çelik, 2021). Also, when climatic conditions change, further search for methods of adaptation of existing breeds of goats to new conditions, taking into account their characteristics, is relevant (Brahmi et al., 2012; Kyselov et al., 2022).

Previously published results indicate that seasonal factors in an extensive production system significantly influenced both the productivity of goats (Zazharska and Kostiuchenko, 2017) and the composition of goat milk in the majority of monounsaturated and branched fatty acids. However, saturated and polyun-saturated fatty acids showed only a few compositional changes in their content in goat milk under the influence of seasonal factors (Toyes-Vargas et al., 2013). Similar reports prove that there is a close dependence of the physicochemical parameters of milk of Saanen goats (Kljajevic et al., 2018) and other (Sejian et al., 2021) breeds on such a seasonal factor as heat stress. By studying the ability of goats to adapt to changes in housing conditions, scientists recommend use of local genetic resources and include them in their breeding programs. This will allow not only to improve their genetic characteristics, but also to increase the quality of milk in terms of fatty acid content (Dagong et al., 2019). At the same time, opposite reports are spreading, in which no influence of seasonal fluctuations on the differences between milk samples of six different breeds of Austrian goats in most physicochemical parameters and fatty acid composition was established (Mayer and Fiechter, 2012).

Other scientists point to a stronger influence of the genotypic factor both on milk quality indicators in contrast to other possible factors (Vacca et al., 2017; Shuvarikov et al., 2021) and on products made from it (Fresno et al., 2021). Other scientists claim that the physicochemical properties of milk samples differ significantly depending on the breed of goats, with some samples superior to others in one or more aspects (Alyaqoubi et al., 2015).

There are known repeated results emphasizing

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the more likely and stronger dependence of the quality and fatty acid composition of goat milk on the duration of lactation of goats associated with seasonal features (Ferro et al., 2017; El-Tarabany et al., 2018). Known works emphasize the significant influence on the amount of some fatty acids in milk by such factors as the age of females and the period of lactation. The effect of lactation stage on all traits was significant, except for protein percentage (Gharibi et al., 2020).

Investigating the dependence of the quality and composition of goat milk on various factors, a number of scientists found the most probable and strongest influence of the feeding method (Marcos et al., 2020) and directly of the composition of the rations (Tian at al., 2017). Moreover, there are reports of a higher influence of the goat feeding system itself than the lactation period or the age of the animals on the fatty acid composition of milk (Yakan et al., 2019). Feeding systems, especially goat grazing methods, have been shown to have a greater effect on essential fatty acid profiles than breed or a breeding method. A significantly high content of polyunsaturated fatty acids and a simultaneous low content of saturated fatty acids were found in the milk of grazing goats, compared with a higher content of saturated fatty acids and a lower content of polyunsaturated fatty acids in the milk of well-fed goats (Halyna et al., 2019; Bodnar et al., 2021).

Therefore, we can state that scientists show ambiguous views regarding the assessment of the dependence of indicators describing the influence of seasonal factors on the quality of milk in crossbred and purebred goats and the fatty acid composition of their milk. But the relevance of further research on this issue is increasing, since goat farming is in a state of positive dynamic changes in the increase of both the population and production volumes; however, the resources for further growth are quite limited (Röös et al., 2016), and the ecological situation is also gradually becoming more difficult (Jägermeyr et al., 2021; Peng and Guan, 2021).

The purpose of our experiment included a comparison of the content of fatty acids in the milk of goats of different breeds and an assessment of the dependence of their productivity on the influence of temperature.

Materials and methods

To achieve the goals of the research, an experiment was conducted to determine the indicators of fatty acid content in milk obtained from goats of three breeds and to determine the productivity of goats paying attention to the change of weather conditions during the seasons of the year (seasonal temperatures).

For the experiment, lactating goats of Saanen, Russian white breed and Ukrainian local breed were selected with 10 heads in each group formed by a separate breed. Livestock taken for the experiment was kept in a special room (vivarium) on the territory of the Sumy National Agrarian University, Sumy region, Ukraine. This special room was designed according to the principles of humane goat handling. It allowed for watering, feeding, free movement in sufficient space, and proper lactation while maintaining a healthy microclimate.

The selection of goats for the experiment was based on the principle of pairs of analogues according to animal parameters such as age, live weight and the term of the goats. The goats were kept in identical conditions during the experiment.

The experimental goats had an average live weight of 60 kg and an average milk yield per lactation of 700 kg. All manipulations with the goats in the experiment strictly followed the provisions and policies of the humane treatment of animals set out in the Council Directive 86/609/EEC (1986).

In the special room of the vivarium, the goats were kept untethered on deep litter. The floor area per goat was 1.15 m^2. Fresh water was freely available for experimental goats. The rations of the goats kept in the vivarium were complete and balanced in terms of nutrients (Table 1).

Productivity of goats was determined by the method of daily milking with subsequent summation for each month during the year. Lactation of goats was 214 days. Milk samples for evaluation of fatty acid composition were taken from goats according to ISO 707:2008 (2008). Milk samples were collected in a volume of 50 mL during each of four consecutive milkings (morning and evening for two days). Combined proportionally with each delivery, the samples were filtered, cooled to +6 ± 2ºC and analyzed within 24 hours after the last collection. Milking of goats was carried out manually in compliance with

Table 1. Feed composition

Indicator	Value
Fodder units	1.7
Exchange energy, MJ	18.0
Dry matter, kg	2.0
Crude protein, g	280.0
Digestive protein, g	170.0
Table salt, g	16.0
Calcium, g	8.5
Phosphorus, g	6.0
Magnesium, g	0.9
Iron, mg	80.0
Copper, mg	15.0
Zinc, mg	88.0
Cobalt, mg	0.87
Manganese, mg	88.0
Iodine, mg	0.68
Carotene, mg	21.0
Vitamin D, MO	900.0

sanitary and hygienic standards. Samples of milk were taken exclusively from healthy goats twice a month.

The content of fatty acids of goat milk was determined at the Institute of Animal Husbandry of the National Academy of Sciences of Ukraine, which has accreditation in accordance with the requirements of ISO/IEC 17025:2006 (2006) and the corresponding certificate No. 2T621 issued by the National Accreditation Agency of Ukraine. Homogeniser HF-0.5/25 (OHFU, China) was used for homogenization of milk samples heated to a temperature of +40°C. The homogenizer has been certified to include the requirements of ISO 9001:2008 (2008).

A modified Bligh and Dyer method was employed for the quantitative extraction of lipids (milk fat) from prepared milk samples. Milk samples (typically 1–5 mL) were accurately measured and transferred into centrifuge tubes or extraction flasks. The volumes were selected based on analytical needs and instrument sensitivity, ensuring sufficient lipid recovery for subsequent analysis. A mixture of solvents was added to each sample in specific ratios. First, methanol (CH_3OH) was added twice to the milk sample's volume (e.g., 20 mL methanol per 10 mL milk). Methanol acts as a polar solvent, facilitating protein denaturation and cell membrane disruption, thereby promoting lipid release into the solvent phase. The resulting mixture was thoroughly homogenized using a mechanical homogenizer (e.g., Polytron) or vigorously shaken for 1–2 minutes. This step ensured a uniform monophasic solution formed, maximizing contact between solvents and milk components and enhancing lipid extraction efficiency. After homogenization, an additional volume of chloroform (equal to the initially added volume, e.g., 10 mL) and distilled water (corresponding to the water content initially present in the milk) were added to the mixture. This induced the formation of a biphasic system, allowing clear separation of the organic (chloroform-rich, lipid-containing) and aqueous (methanol-water) layers upon centrifugation or gravity-assisted settling. The lower chloroform phase, containing the extracted lipids, was then collected for further analysis. . Fatty acids of milk were determined by the method of gas-liquid chromatography ISO 15885:2002 (2002). Gas chromatographic analysis of fatty acid composition was performed on a gas chromatograph with a flame ionization detector. FAME separation was performed on a capillary column (HP-5), 30 m × 0.25 mm × 0.25 μm in size with a stationary phase. Helium was used as the carrier gas with a flow rate of 1 mL/min. The injector temperature was 250°C, and the detector temperature was 280°C. Sample introduction was carried out in split mode with a separation ratio of 50:1. The volume of the introduced sample was 1 μL. The column temperature program was as follows: initial temperature of 70°C was maintained for 2 min, then heated at a rate of 10°C/min to a final temperature of 240°C with a holding time of 10 min. Identification of fatty acids was performed by comparing the retention times of the peaks with the retention times of the peaks of a standard mixture of fatty acid methyl esters. For quantitative determination, the peak area normalization method was used, using fatty acid standards at known concentrations.

The experimental data were analyzed using MS Excel 2016 and the statistical software package Statistica 12.0 (StatSoft Inc., USA). Initial data processing included the calculation of means (M), standard deviations (SD), and standard errors of the mean (SE) for each animal group, which allowed for a clear presentation of variability and reliability of the results in tabular form.

To evaluate the effect of various factors on goat milk yield, a one-way analysis of variance (ANOVA) was carried out in Statistica. This test made it possible to identify statistically significant differences between groups based on a single independent variable (e.g., breed, diet type, or season). When significant differences were observed, the Tukey's Honest Significant Difference (HSD) post hoc test was applied to determine which specific groups differed.

A two-way ANOVA with interaction effects was also performed in Statistica to analyze the combined effects of multiple factors, particularly the interaction between breed and seasonal temperature on milk yield. This allowed assessment not only of the individual influence of each factor but also of their interaction.

For a more precise evaluation of relationships and the influence of specific variables, mainly when milk yield depended on a continuous factor such as seasonal temperature, the least squares method was applied. This approach enabled the modeling of the data and estimating the effects of independent variables while controlling for potential confounding factors. Graphs and regression equations based on the least squares method were constructed using MS Excel 2016.

Differences were considered statistically significant at a probability level of $P \leqslant 0.05$. All mean values presented in the tables were calculated based on 14 independent replicates per experimental group, which ensured sufficient statistical power and robustness of the conclusions. This number of replicates helped to minimize the influence of random variation and improve the overall reliability of the experimental findings.

Experimental goats were treated with methods that allowed to alleviate their discomfort and prevent pain. The Bioethics Commission of the Sumy National Agrarian University on the care and use of animals in scientific (experimental) research (ethical approval number VT-22-0208-02) approved the methodology for conducting this experiment. Breeding and involvement of goats in the experiment in the university vivarium took place considering the requirements of the Law of Ukraine No. 3447-IV of 2006 "On the Protection of Animals from Cruelty".

Results

The analysis of the milk productivity of goats during lactation showed that it increases in spring and summer and decreases in autumn (Fig. 1).

As we can see, the absolute difference in milk yield between the Saanen breed and other breeds in July (0.50 and 0.44 L/day) is greater than in March (0.37 and 0.40 L/day). However, the relative difference between the Saanen and Russian White breeds is significantly higher in March (48.68%) compared with July (24.51%). A similar trend is observed when comparing the Saanen breed with the Ukrainian local breed (54.79% in March versus 20.95% in July). This indicates that although the Saanen breed maintains and even slightly increases its advantage in absolute terms during the peak lactation period (July), in relative terms, its superiority was more pronounced at the beginning of lactation (March), when overall milk yields were lower. In September, when the lactation period ended in most herds of the studied groups, the productivity advantage remained with the Saanen goats, and the local Ukrainian local breed goats gave the least milk. The highest average milk yield during lactation was observed in animals of all groups in July, when the temperature maximum reached 16.9–22.8°C, and the lowest milk yield was obtained in March with an average monthly temperature range of –4.8–4.4°C.

To determine the influence of breed and the average monthly temperature variation, a two-factorial analysis of variance was performed, which showed a probable influence of the breed factor on the milk productivity of goats during the year with a power of 81.33%. We can also conclude that milk yield also depended on temperature, which influenced it with a strength of 13.00% (Table 2).

The interaction of the goat breed factor and the temperature also had an effect on the milk productivity of the herd, but was only reliable at a level of 0.67%. Only 4.98% of the lactation depended on factors that were not taken into account.

The regression analysis showed a direct dependence of the milk productivity of Saanen goats on temperature fluctuations during the lactation period from March to September. Milk production increased or decreased by 2 g for every 1°C increase or decrease in temperature. Such a direct relationship between the indicators for milk productivity was found in the temperature range from –4.8°C in spring to +22.8°C in summer. The interpretation of the value of the coefficient of determination R^2 showed that 79.57% of the variance of the dependent indicator of milk productivity with a change of +/–2 g with a simultaneous change in the monthly average temperature of +/–1°C was due to the response to these temperature fluctuations and the rest of the variance was formed by unaccounted factors, including the temperature factor (Fig. 2).

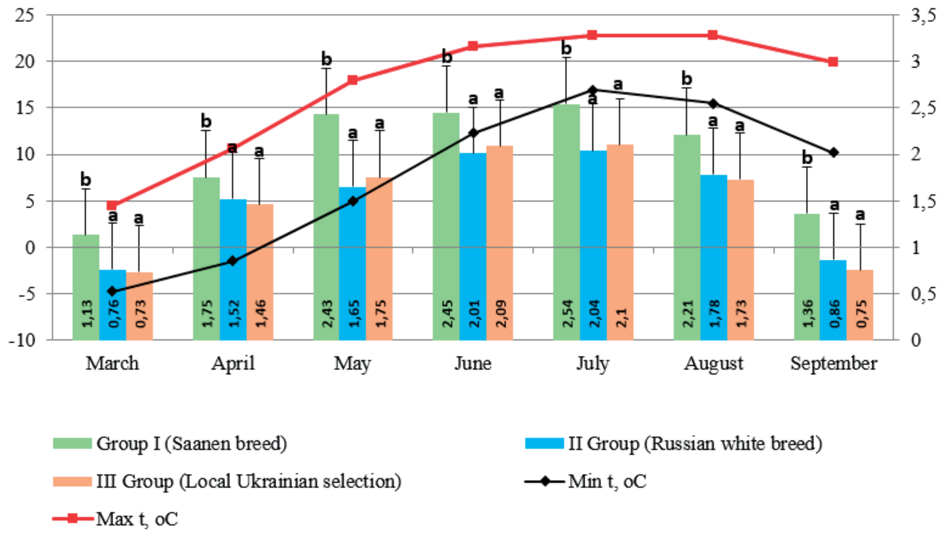

Fig. 1. Average daily productivity of goats (n = 30) during the lactation and monthly minimum and maximum outdor temperatures (different lowercase letters (a, b) indicate statistical differences between the groups at the level of $P < 0.05$)

Table 2. The influence of temperature and breed of goats on daily productivity

Source of Variation	SS	df	MS	F	*P* value	F crit	Influence
Breed	501.40	3.00	167.13	5805.04	0.00	2.61	81.33
Temperature	80.17	2.00	40.08	1392.29	2.9162E-298	3.00	13.00
Interaction factors	4.14	6.00	0.69	23.97	1.03854E-26	2.10	0.67
Unaccounted factors	30.74	1068	0.02				4.98
Total	616.46	1079					100.00

Evaluation of the dependence of changes in milk productivity of Russian White breed goats on temperature fluctuations in the range of −4.8+22.8°C during the lactation period using the regression equation showed a direct regression relationship between the indicators. When the monthly average temperature fluctuated by ±1°C, milk productivity changed by ±3 g, 77.52% of which was due to the direct influence of temperature and 22.48% due to factors not taken into account (Fig. 3).

The regression analysis showed a direct positive effect of external temperature on milk productivity of local Ukrainian local breed goats with a strength of 73.26% and the effect of unaccounted factors at the level of 26.74%, which caused an increase in milk production by 2 g when the temperature index increased by 1°C and vice versa (Fig. 4).

The milk productivity of goats from three groups thus depended on the influence of the seasonal outdoor temperature. Russian white goats were the

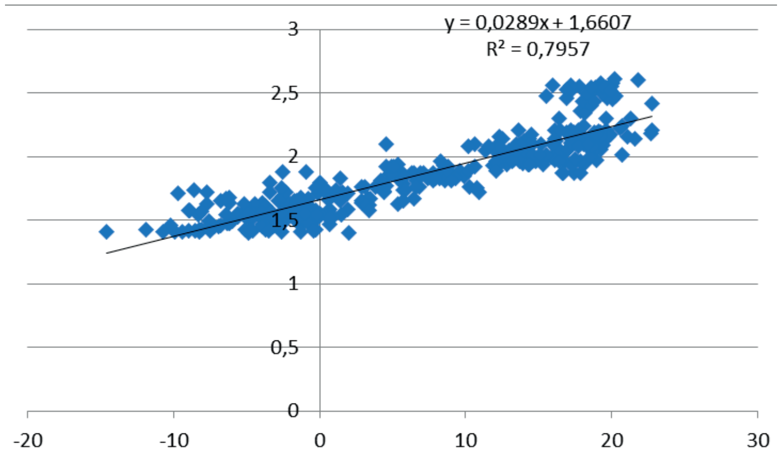

Fig. 2. Dependence of dairy productivity on changes in external temperatures during lactation in Saanen goats

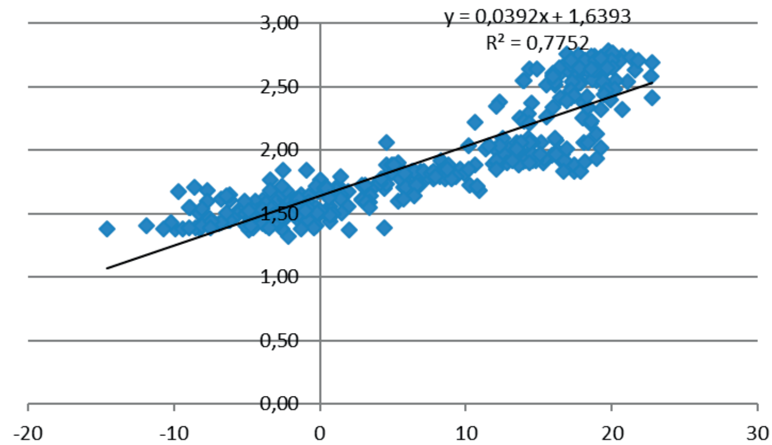

Fig. 3. Dependence of dairy productivity on changes in external temperatures during lactation in Russian white goats

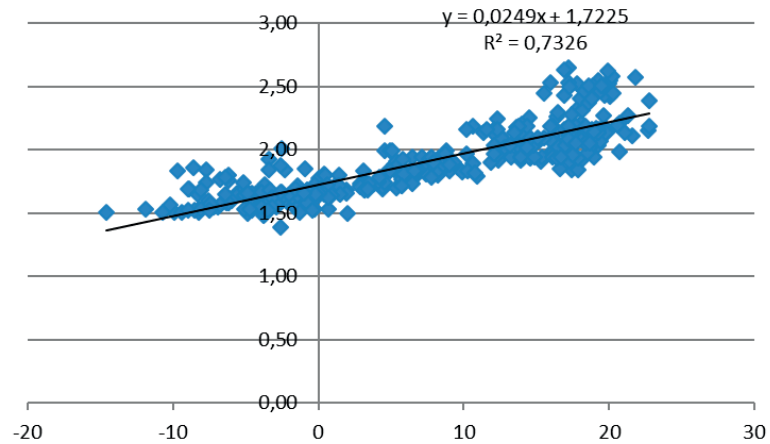

Fig. 4. Dependence of dairy productivity on changes in external temperatures during lactation in Ukrainian local breed goats

least resistant to this effect.

Analysis of medium-chain fatty acids (MCFA) in the milk of goats of different breeds revealed several statistically significant differences. In particular, it was found that goats of the Ukrainian local breed (Group 3) had a higher content of caprylic acid (C8:0) by 0.11% ($P < 0.05$) compared with the Saanen breed (Group 1), and by 0.10% ($P < 0.05$) compared with the Russian White breed (Group 2) (Table 3).

Similarly, the content of capric acid (C10:0) in the milk of Ukrainian local breed goats exceeded that of the Saanen breed by 1.01% ($P < 0.05$) and that of the Russian White breed by 0.92% ($P < 0.05$). Regarding lauric acid (C12:0), the Ukrainian local breed goats also demonstrated significantly higher levels, by 1.19% ($P < 0.05$) compared with the Saanen breed and by 1.41% ($P < 0.05$) compared with the Russian White breed. Additionally, the content of pelargonic acid (C9:0) was higher in the Ukrainian local breed by 0.01% ($P < 0.05$) compared with both the Saanen and Russian White breeds. The Ukrainian local goats also showed a higher concentration of lauroleic acid (C12:1) by 0.08% ($P < 0.05$) compared with the Russian White breed.

At the same time, the Saanen breed goats (Group 1) had a significantly higher content of isocaprylic acid (C8:0) by 0.02% ($P < 0.05$) than both the Russian White and Ukrainian local breeds. The content of lauroleic acid (C12:1) was also higher in Saanen goats by 0.05% ($P < 0.05$) compared with the Russian White breed.

On the other hand, goats of the Russian White breed (Group 2) were characterized by a significantly higher level of undecylic acid (C11:0) by 0.12% ($P < 0.05$) compared with the Saanen breed and by 0.11% ($P < 0.05$) compared with the Ukrainian local breed.

The total content of medium-chain fatty acids (MCFA) in the milk of Ukrainian local breed goats was 14.00%, exceeding that of the Saanen breed (12.25%) by 1.75 percentage points, which corresponds to a relative increase of 14.29%. Compared with the Russian White breed (12.20%), the milk of Ukrainian local goats demonstrated a higher MCFA content by 1.80 percentage points, equivalent to a relative difference of 14.75%. The difference between the Saanen and Russian White breeds was minimal, amounting to 0.05 percentage points, with the Saanen goats exhibiting a slightly higher value, representing approximately a 0.41% relative increase.

Analysis of the content of long-chain saturated fatty acids (LCFA-SFA) in the milk of goats of different breeds revealed that Saanen goats (Group 1) exhibited significantly higher levels of tridecanoic acid (C13:0) and isomyristic acid (C14:0) by 0.04% ($P < 0.05$) and 0.05% ($P < 0.05$), respectively, compared with Russian White goats (Group 2). Moreover, Saanen goats outperformed the Russian White breed in terms of stearic acid (C18:0) content by 0.72% ($P < 0.05$), and the Ukrainian local breed by 1.13% ($P < 0.05$). Their milk also contained significantly higher levels of myristic acid (C14:0) by 1.48% ($P < 0.05$) compared with the Ukrainian local breed. The concentration of pentadecanoic acid (C15:0) in the milk of Saanen goats was higher by 0.18% ($P < 0.05$) compared with the Russian White breed, while margaric acid (C17:0) was higher by 0.21% ($P < 0.05$). Regarding behenic acid (C22:0), Saanen goats showed a 0.11% ($P < 0.05$) higher content compared with the Ukrainian local goats (Table 4).

Russian White goats (Group 2) surpassed the Ukrainian local goats in stearic acid (C18:0) content by 0.41% ($P < 0.05$). The content of arachidic acid (C20:0) in the Russian White breed was higher by 0.09% ($P < 0.05$) compared with the Saanen breed. Furthermore, the behenic acid (C22:0) content in the milk of Russian White goats was higher by 0.14% ($P < 0.05$) than in the Saanen breed and by

Table 3. Medium-chain fatty acid (MCFA) content in milk of goats of different breeds (%)

Fatty acid	Group 1 (Saanen breed)	Group 2 (Russian white breed)	Group 3 (Ukrainian local breed goats)
Caproic acid (C6:0)	0.06 ± 0.006^a	0.06 ± 0.003^a	0.06 ± 0.001^a
Caprylic acid (C8:0)	1.09 ± 0.006^a	1.10 ± 0.008^a	1.20 ± 0.009^b
Isocaprylic acid (C8:0)	0.07 ± 0.003^b	0.05 ± 0.004^a	0.05 ± 0.002^a
Capric acid (C10:0)	4.49 ± 0.262^a	4.58 ± 0.131^a	5.50 ± 0.241^b
Undecyl acid (C11:0)	0.05 ± 0.006^a	0.17 ± 0.002^b	0.06 ± 0.003^a
Isolauric acid (C12:0)	0.06 ± 0.007^a	0.08 ± 0.008^a	0.07 ± 0.008^a
Lauric acid (C12:0)	5.96 ± 0.281^a	5.74 ± 0.194^a	7.15 ± 0.321^b
Lauroleic acid (C12:1)	0.45 ± 0.003^b	0.40 ± 0.004^a	0.48 ± 0.025^b
Pelargonic acid (C9:0)	0.02 ± 0.006^a	0.02 ± 0.004^a	0.03 ± 0.002^b
SUM MCFA	12.25	12.20	14.00

Note: different lowercase letters ($^{a, b, c}$) indicate statistical differences between the groups at the level of $P < 0.05$; the same letters indicate that there is no statistically significant difference between the groups.

0.25% ($P < 0.05$) than in the Ukrainian local breed. However, the level of isopalmitic acid (C16:0) was lower by 0.03% ($P < 0.05$) in the Russian White breed compared with the Saanen.

Ukrainian local goats (Group 3) exhibited the highest content of pentadecanoic acid (C15:0), exceeding that of the Saanen breed by 0.32% ($P < 0.05$) and the Russian White breed by 0.50% ($P < 0.05$). This group also had the highest level of isopalmitic acid (C16:0), which was 0.05% ($P < 0.05$) and 0.08% ($P < 0.05$) higher than in the Saanen and Russian White breeds, respectively. Additionally, the Ukrainian local goats demonstrated the highest content of margaric acid (C17:0), surpassing that of the Russian White breed by 0.44% ($P < 0.05$) and the Saanen breed by 0.23% ($P < 0.05$). The arachidic acid (C20:0) content was also highest in this group, exceeding that of the Saanen by 0.38% ($P < 0.05$) and the Russian White breed by 0.29% ($P < 0.05$). At the same time, the levels of myristic, palmitic, stearic, and behenic acids in Ukrainian local goats' milk were lower than in the other groups.

The calculation of differences in the total sums of long-chain saturated fatty acids (SUM LCFA-SFA) among the breed groups showed that the Saanen breed (Group 1) had a sum higher by 0.92 than the Russian White breed (Group 2), and by 1.74 higher than the Ukrainian local breed (Group 3). The difference between the Russian White (Group 2) and Ukrainian local (Group 3) breeds was 0.82, in favor of the Russian White.

The analysis of monounsaturated fatty acid (MUFA) content in the milk of goats of different breeds revealed that goats of the Ukrainian local breed (Group 3) had the highest content of myristoleic acid (C14:1), exceeding that of the Saanen breed (Group 1) by 0.41% ($P < 0.05$) and the Russian White breed (Group 2) by 0.56% ($P < 0.05$). This group also demonstrated the highest levels of pentadecenoic acid (C15:1), surpassing the Saanen and Russian White breeds by 0.02% ($P < 0.05$). Similarly, palmitoleic acid (C16:1) was highest in the Ukrainian local breed, with levels 0.67% ($P < 0.05$) and 0.93% ($P < 0.05$) higher than those of the Saanen and Russian White breeds, respectively. Additionally, the content of gondoic acid (C20:1) in the Ukrainian local breed was 0.02% ($P < 0.05$) higher than in the Saanen breed and 0.04% ($P < 0.05$) higher than in the Russian White breed. Regarding erucic acid (C22:1), the Ukrainian local breed showed a 0.07% ($P < 0.05$) higher concentration compared with the Russian White breed (Table 5).

In Saanen goats (Group 1), the content of myristoleic acid (C14:1) was 0.15% ($P < 0.05$) higher than in the Russian White breed. Furthermore, the Saanen breed had a higher level of heptadecenoic acid (C17:1), by 0.07% ($P < 0.05$) compared with the Russian White breed and by 0.05% ($P < 0.05$) compared with the Ukrainian local breed. The content of erucic acid (C22:1) in the Saanen breed was also 0.11% ($P < 0.05$) higher than in the Russian White breed and 0.04% ($P < 0.05$) higher than in the Ukrainian local breed. In addition, the palmitoleic acid (C16:1) level in the Saanen breed was 0.26% ($P < 0.05$) higher than in the Russian White breed.

Goats of the Russian White breed (Group 2) exhibited the highest level of oleic acid (C18:1), exceeding that of the Ukrainian local breed by 2.59% ($P < 0.05$). The corresponding value in the Saanen breed was also higher than in the Ukrainian local breed by 2.05% ($P < 0.05$).

The total content of monounsaturated fatty acids (MUFA) was highest in the Saanen breed (26.65%). This value was 0.52% higher than in the Russian White breed (26.13%) and 1.47% higher than in the Ukrainian local breed (25.18%). The difference between the Russian White and Ukrainian local breeds was 0.95%, with a higher value in the Russian White breed.

Table 4. Content of long-chain saturated fatty acids (LCFA-SFA) in milk of goats of different breeds (%)

Fatty acid	Group 1 (Saanen breed)	Group 2 (Russian white breed)	Group 3 (Ukrainian local breed goats)
Tridecanoic acid (C13:0)	0.13 ± 0.003^{b}	0.09 ± 0.004^{a}	0.13 ± 0.001^{b}
Isomyristic acid (C14:0)	0.18 ± 0.012^{b}	0.13 ± 0.011^{a}	0.17 ± 0.00^{b}
Myristic acid (C14:0)	14.86 ± 0.114^{b}	14.94 ± 0.211^{b}	13.38 ± 0.132^{a}
Pentadecanoic acid (C15:0)	1.88 ± 0.009^{b}	1.70 ± 0.018^{a}	2.20 ± 0.063^{c}
Isopalmitic acid (C16:0)	0.31 ± 0.005^{a}	0.28 ± 0.003^{b}	0.36 ± 0.002^{c}
Margaric acid (C17:0)	1.30 ± 0.100^{ab}	1.09 ± 0.092^{a}	1.53 ± 0.085^{b}
Stearic acid (C18:0)	14.43 ± 0.087^{b}	13.71 ± 0.099^{a}	13.30 ± 0.073^{c}
Arachidic acid (C20:0)	0.75 ± 0.015^{a}	0.84 ± 0.022^{b}	1.13 ± 0.093^{c}
Behenic acid (C22:0)	0.45 ± 0.011^{a}	0.59 ± 0.010^{b}	0.34 ± 0.007^{c}
SUM LCFA-SFA	34.29	33.37	32.55

Note: different lowercase letters ($^{a, b, c}$) indicate statistical differences between the groups at the level of $P < 0.05$; the same letters indicate that there is no statistically significant difference between the groups.

Table 5. Content of monounsaturated fatty acids (MUFA) in milk of goats of different breeds (%)

Fatty acid	Group 1 (Saanen breed)	Group 2 (Russian white breed)	Group 3 (Ukrainian local breed goats)
Myristoleic acid (C14:1)	2.09 ± 0.032[b]	1.94 ± 0.018[a]	2.50 ± 0.039[c]
Pentadecenoic acid (C15:1)	0.11 ± 0.002[a]	0.11 ± 0.001[a]	0.13 ± 0.001[b]
Palmitoleic acid (C16:1)	3.46 ± 0.052[a]	3.20 ± 0.069[b]	4.13 ± 0.021[c]
Heptadecenic acid (C17:1)	0.47 ± 0.005[b]	0.40 ± 0.007[a]	0.42 ± 0.008[a]
Oleic acid (C18:1)	19.60 ± 0.181[b]	20.14 ± 0.162[b]	17.55 ± 0.167[a]
Heneicosanoic acid (C20:1)	0.10 ± 0.002[a]	0.080 ± 0.001[b]	0.12 ± 0.003[c]
Erucic acid (C22:1)	0.37 ± 0.001[a]	0.26 ± 0.001[b]	0.33 ± 0.003[c]
SUM MUFA	26.65	26.13	25.18

Note: different lowercase letters ([a, b, c]) indicate statistical differences between the groups at the level of $P < 0.05$; the same letters indicate that there is no statistically significant difference between the groups.

The analysis of polyunsaturated fatty acid (PUFA) content in the milk of goats with different breeds showed that goats of the Ukrainian local breed (Group 3) had a significantly higher content of hexadecadienoic acid (C16:2), exceeding that of the Saanen breed (Group 1) by 0.08% ($P < 0.05$) and that of the Russian White breed (Group 2) by 0.25% ($P < 0.05$). The same group (Ukrainian local) also exhibited the highest concentration of linoleic acid (C18:2), which was 0.80% ($P < 0.05$) higher than in the Saanen breed and 1.08% ($P < 0.05$) higher than in the Russian White breed. Similarly, arachidonic acid (C20:4) was most abundant in the milk of Ukrainian Local goats, surpassing the Saanen breed by 0.05% ($P < 0.05$) and the Russian White breed by 0.10% ($P < 0.05$). The content of docosatrienoic acid (C22:3) was also higher in the Ukrainian local breed by 0.01% ($P < 0.05$) compared with both the Saanen and Russian White breeds (Table 6).

At the same time, the milk of Saanen goats (Group 1) contained a higher proportion of linolenic acid (C18:3) by 0.13% ($P < 0.05$) and docosadienoic acid (C22:2) by 0.06% ($P < 0.05$), both compared with the Russian White breed.

Goats of the Russian White breed (Group 2) were characterized by a significantly higher content of docosahexaenoic acid (C22:6), which exceeded that of the Saanen breed by 0.27% ($P < 0.05$) and the Ukrainian local breed by 0.18% ($P < 0.05$). However, the level of docosatetraenoic acid (C22:4) in the Russian White breed was lower by 0.01% ($P < 0.05$) compared with the Saanen breed.

The total amount of polyunsaturated fatty acids (SUM PUFA) was highest in the milk of Ukrainian local goats (7.79%), exceeding the values observed in the Saanen breed (6.20%) by 1.59% and in the Russian White breed (6.15%) by 1.64%. No statistically significant difference was observed in total PUFA content between the Saanen and Russian White breeds, amounting to only 0.05%.

The comparative analysis of total saturated (SFA) and unsaturated fatty acids (UFA) in the milk of goats from different genotypic groups (Table 5) did not reveal statistically significant differences ($P > 0.05$) among the examined breeds. Nevertheless, distinct tendencies were observed in both absolute and relative concentrations (Table 7).

Specifically, the milk of goats belonging to the Ukrainian local breed (Group 3) showed the highest proportion of total saturated fatty acids, reaching 69.61%. This value tended to exceed those observed in the Russian White breed (Group 2, 66.08%) and the Saanen breed (Group 1, 65.69%). In contrast, the total content of unsaturated fatty acids was slightly higher in the milk of Saanen goats (Group 1, 33.17%), showing a consistent trend toward elevated UFA levels compared with the Russian White (Group 2, 32.60%) and Ukrainian local breeds (Group 3, 32.52%).

While not statistically significant, these findings may reflect breed-specific differences in lipid metbolism and warrant further investigation with larger sample sizes and additional biochemical profiling.

Discussion

It is known that the percentage of milk yield significantly depends on the season (Midau et al., 2010; Silva et al., 2021). According to reports, in dairy goats, climate change in the form of an increase in ambient temperature causes heat stress and negatively affects milk productivity (Yamani and Koluman, 2020; Sunagawa et al., 2015). However, according to the results of our research, milk productivity did not decrease during spring and summer temperature extremes, but was more sensitive to its decrease in the autumn, which did not quite coincide with the conclusions of other authors (Midau et al., 2010; Silva et al., 2021; Sunagawa et al., 2015; Yamani and Koluman, 2020).

Beyond seasonal influences on milk yield, evaluating the impact of breed on milk quality composition is crucially important for breeding programs and enhancing the nutritional value of dairy products. Modern research emphasizes the predominant influence of breed on milk quality parameters and

Table 6. Content of polyunsaturated fatty acids (PUFA) in milk of goats of different breeds (%)

Fatty acid	Group 1 (Saanen breed)	Group 2 (Russian white breed)	Group 3 (Ukrainian local breed goats)
Tetradecadienoic acid (C14:2)	0.82 ± 0.053[a]	0.80 ± 0.067[a]	0.97 ± 0.043[a]
Hexadecadienoic acid (C16:2)	0.87 ± 0.014[a]	0.70 ± 0.031[b]	0.95 ± 0.026[c]
Linoleic acid (C18:2)	3.07 ± 0.025[a]	2.79 ± 0.038[b]	3.87 ± 0.019[c]
Linolenic acid (C18:3)	1.28 ± 0.010[b]	1.15 ± 0.009[a]	1.29 ± 0.011[b]
Arachidonic acid (C20:4)	0.10 ± 0.006[b]	0.05 ± 0.004[a]	0.15 ± 0.028[c]
Docosadienoic acid (C22:2)	0.15 ± 0.005[b]	0.09 ± 0.003[a]	0.14 ± 0.002[b]
Docosatrienoic acid (C22:3)	0.09 ± 0.004[a]	0.09 ± 0.006[a]	0.10 ± 0.001[b]
Docosatetraenoic acid (C22:4)	0.05 ± 0.002[a]	0.04 ± 0.001[b]	0.06 ± 0.005[a]
Docosahexaenoic acid (C22:6)	0.17 ± 0.008[a]	0.44 ± 0.015[b]	0.26 ± 0.004[c]
SUM PUFA	6.20	6.15	7.79

Note: different lowercase letters ([a, b, c]) indicate statistical differences between the groups at the level of $P < 0.05$; the same letters indicate that there is no statistically significant difference between the groups.

Table 7. Total amounts of saturated and unsaturated fatty acids in milk of goats of different breeds (%)

Fatty acid category	Group 1 (Saanen breed)	Group 2 (Russian white breed)	Group 3 (Ukrainian local breed goats)
Sum of saturated fatty acids (SFA)	65.69 ± 2.164[a]	66.08 ± 2.008[a]	69.61 ± 2.018[a]
Sum of unsaturated fatty acids (UFA)	33.17 ± 0.987[a]	32.60 ± 1.271[a]	32.52 ± 1.215[a]

Note: different lowercase letters ([a, b, c]) indicate statistical differences between the groups at the level of $P < 0.05$; the same letters indicate that there is no statistically significant difference between the groups.

derived products (Vacca et al., 2017; Shuvarikov et al., 2021; Fresno et al., 2021), as well as on the physicochemical properties of milk from various goat breeds (Alyaqoubi et al., 2015). Our results, based on a comparative analysis of fatty acid content in the milk of Saanen, Russian White, and Ukrainian local goat breeds, corroborate these findings, demonstrating significant breed-specific differences in fatty acid profiles. This supports the notion that the genetic factor is one of the strongest influences on milk quality (Vacca et al., 2017; Shuvarikov et al., 2021).

Analysis of medium-chain fatty acid (MCFA) content revealed substantial breed differences, underscoring the influence of breed on the milk fat profile. Our study showed that the Ukrainian local goat breed had statistically significantly higher levels of several important MCFA compared with the the Russian White breed. These findings, demonstrating notable excesses (ranging from 0.01% to 1.41% in absolute terms for individual acids), may indicate unique metabolic peculiarities of the Ukrainian local breed. For instance, the higher content of caprylic and capric acids, known for their antimicrobial properties (Žan et al., 2016), could enhance the functional attributes of milk from this breed. These data partially concur with studies by Halyna et al. (2019) and Bodnar et al. (2021), who also noted variations in the content of caprylic, capric, and lauric acids among breeds, although their specific values for Alpine goats differed from our findings for the Saanen breed.

Concurrently, Saanen goats exhibited statistically significantly higher levels of isocaprylic acid (C8:0) and lauroleic acid (C12:1) compared with the Russian White breed, which may reflect breed-specific characteristics in the metabolism of branched and unsaturated fatty acids. Russian White goats (Group 2), in turn, displayed a substantially higher level of undecylic acid (C11:0). The total sum of MCFA was highest in the milk of Ukrainian local goats (14.00%), surpassing the Saanen and Russian White breeds by 1.75% and 1.80%, respectively. We believe this is linked to the adaptation of local breeds to specific husbandry and feeding conditions, influencing the synthetic activity of the mammary gland, or to a genetic predisposition for more efficient de novo synthesis of fatty acids from precursors. Such significant differences in the MCFA profile highlight the potential of local breeds for selection and production of milk with improved characteristics, which is important in the context of the growing role of goat farming and environmental challenges, as noted by Röös et al. (2016).

In the profile of long-chain saturated fatty acids (LCFA-SFA), significant breed differences were observed, indicating distinct lipid metabolic pathways in the studied goat groups. Analyzing the obtained data, we see that the highest content of unsaturated fatty acids was in goats of the Saanen breed compared with goats of other breeds (Salari et al., 2016). Saanen goats (Group 1) demonstrated statistically significantly higher levels of several LCFA-SFA compared with the Russian White breed. Importantly, the content of myristic (C14:0) acid in Saanen goats was also substantially higher than in the Ukrainian local breed. Such data coincide with the data of other researchers (Žan et al., 2016), who proved that goats of the Saanen breed that grazed in highland conditions had a large amount of similar fatty acids, C16:0, C18:1, C14:0 in their milk, and C10:0. Under the conditions of our experiment, the highest content of saturated fatty acids in Saanen goats was stearic acid (C18:0). These data align with some previous studies indicating specific characteristics of the saturated fatty acid profile in Saanen goats. However, some of our results contradict the general assertion by Kyselov et al. (2022) that Saanen goats were "inferior to other breeds" in saturated acid content, as we observed higher levels of specific SFA in Saanen goats. This study also confirms with data (Kyselov et al., 2022) in terms of the amount of saturated acids in the milk of Saanen goats, which were inferior to other breeds. Other publications (Galina et al., 2019; Bodnár et al., 2021) have noted a similar trend, namely that only three saturated fatty acids – caprylic (C8:0), capric (C10:0) and lauric (C12:0) – were present in significantly higher amounts in Alpine goat milk than in goats of the Saanen breed, while the average content of saturated fatty acids in milk was 74.52 and 73.05%. These parameters may indicate particular adaptive mechanisms or genetic characteristics influencing lipid metabolism.

In contrast to the Saanen and Russian White breeds, Ukrainian local goats exhibited the highest content of specific LCFA-SFA, despite having the lowest total sum of LCFA-SFA (50.31%). This indicates a unique fatty acid profile characteristic of the locally adapted breed. For instance, the increased content of branched-chain fatty acids (isopalmitic) is believed to be associated with the peculiarities of the rumen microbiome and its influence on fatty acid synthesis. The difference in the total LCFA-SFA content between breeds (Saanen and Russian White having higher values) may be attributed to genetic factors affecting the efficiency of de novo fatty acid synthesis in the mammary gland or the utilization of circulating fatty acids. These differences hold significant implications for the nutritional value of milk, as various saturated fatty acids affect human health differently, and understanding their concentrations in milk from different breeds can aid in developing dietary recommendations and breeding strategies.

Analysis of the monounsaturated fatty acid (MUFA) profile in the milk of different goat breeds revealed significant breed-specific characteristics, which may indicate varying efficiency in MUFA synthesis or their incorporation into milk fat. Ukrainian local breed goats demonstrated statistically significantly higher levels of several valuable MUFA, compared with both Saanen and Russian White breeds. This suggests a potentially higher dietary value of milk from local breeds, as MUFA are known for their role in reducing the risk of cardiovascular diseases and improving blood lipid profiles. Concurrently, Saanen goats also had higher levels of some key MUFA acids compared with the Russian White breed, and palmitoleic (C16:1) acid compared with the Russian White. These data are consistent with numerous studies that consistently indicate a higher content of unsaturated fatty acids in Saanen goat milk (Salari et al., 2016; Yurchenko et al., 2018; Mykhalko et al., 2022). This may be attributed to the genetic characteristics of the Saanen breed that promote more active synthesis or accumulation of unsaturated fatty acids. Other researchers (Yangilar, 2013) found indicators of the content of stearic (C18:0) and oleic acid (C18:1) in the milk of Saanen goats to be identical to our results. Russian White goats (Group 2) distinguished themselves with the highest level of oleic acid (C18:1), surpassing the Ukrainian local breed. This parameter was also high in Saanen goats. A high content of oleic acid is nutritionally important as it is the main monounsaturated acid in milk, positively impacting health. Our data regarding the significant content of oleic acid aligns with the results of Yangilar (2013), who also reported substantial levels of this acid in Saanen goat milk. The total sum of MUFA was highest in the Saanen breed (26.65%), which is consistent with previous observations regarding their propensity for synthesizing a larger quantity of unsaturated fatty acids. Such inter-breed differences in the MUFA profile may be attributed to the complex interplay of genetic factors influencing the activity of desaturases, which are responsible for the formation of unsaturated bonds in fatty acids, as well as differences in lipid metabolism characteristic of each breed. Understanding these breed-specific features is crucial for optimizing selection and producing dairy products with desired nutritional characteristics.

In the context of polyunsaturated fatty acids (PUFA), significant inter-breed differences were observed, emphasizing the varying contribution of breed to the formation of this important class of fatty acids. Ukrainian local goats stood out with significantly higher levels of several PUFA compared with both Saanen and Russian White breeds. This has important nutritional implications, as linoleic and arachidonic acids are essential fatty acids that play a key role in preventing cardiovascular diseases, possess anti-inflammatory properties, and influence brain development. The observed advantage of local goats in the content of these acids indicates their potential as a source of milk with a high biological value. The total sum of PUFA was also highest in the milk of Ukrainian local goats (7.79%), significantly (by 1.59%

and 1.64%, respectively) exceeding the values for Saanen and Russian White breeds.

Our results demonstrate that the PUFA profile is breed-specific. While previous discussions might have suggested a superiority of Saanen goats in linoleic acid, our study clearly indicates the dominance of the Ukrainian local breed for linoleic acid (C18:2). According to our results, linolenic acid (C18:2) was the highest content of unsaturated fatty acids in Saanen goats compared with goats of other breeds. Concurrently, Saanen goats had a higher content of linolenic acid (C18:3) and docosadienoic acid (C22:2) compared with the Russian White breed. This confirms that different breeds may have varying propensities for synthesizing or incorporating specific PUFA into milk fat, which could be related to unique enzymatic systems (e.g., desaturases) or genetic markers controlling the metabolism of these acids. Russian White goats distinguished themselves with a significantly higher content of docosahexaenoic acid (C22:6), surpassing both other breeds. This is a valuable indicator, as C22:6 is an important omega-3 fatty acid associated with numerous health benefits. Such significant variations in PUFA content among breeds can be explained by the complex influence of breed on fatty acid metabolism, and potentially by breed-environment interaction. Although this study did not detail feeding specifics, it is known that diet (especially grazing) has a significant impact on the PUFA profile, often even greater than breed (Halyna et al., 2019; Bodnar et al., 2021). However, the observed differences likely reflect inherent breed-specific genetic characteristics since all animals were kept under the same conditions. Understanding these differences allows for a more targeted use of genetic resources from various breeds to produce milk with an optimal PUFA profile.

Our results regarding the higher content of unsaturated fatty acids in the milk of Saanen goats compared with goats from local selection and goats of other breeds are consistent with our previous data (Mykhalko et al., 2022). Similar to the claims of Tur et al. (2015), in our study, the content of certain unsaturated fatty acids in the milk of Saanen goats was higher compared with saturated fatty acids. A comparative analysis of the total sums of saturated (SFA) and unsaturated fatty acids (UFA) in the milk of goats from different genotypic groups did not reveal statistically significant differences. But the total amount of saturated and unsaturated fatty acids did not differ. Nevertheless, clear tendencies were observed in both absolute and relative concentrations. This aligns with our previous data (Mykhalko et al., 2022) and other studies (Tur et al., 2015), which also found no significant differences in the total quantity of SFA and UFA but noted breed-specific characteristics in their ratio. The consistency of our results with previous studies (Mykhalko et al., 2022; Tur et al., 2015) demonstrating a higher UFA content in Saanen goat milk across various management conditions confirms the stability of this breed-specific characteristic. The observation that the total amounts of SFAs and UFAs did not differ suggests that genetic and environmental factors primarily influence the ratio between these fatty acid groups rather than their overall concentration.

Discrepancies with some previous studies concerning genetic correlations (Maroteau et al., 2014) may be explained by complex genetic architecture and gene-environment interactions. Also, our assumptions are confirmed by research (Yurchenko et al., 2018), which in its conclusions states that goats of the Saanen breed have high levels of unsaturated fatty acids such as C16:0, C16:1 and C18:1 in their profile. However, our studies are somewhat inconsistent with the findings of other scientists who claim that in Saanen goats no significant genetic correlation was found between fat content and the presence of C16:0 fatty acids, while correlations between fat content and specific fatty acids of goats (C6:0–C10:0) were positive (0.17–0.59). In addition, the genetic correlation between fat content and C14:0 was negative (−0.17 to −0.35) (Maroteau et al., 2014). Such indicators of the content of fatty acids, in our opinion, are undoubtedly related not only to the breed factor and the peculiarities of feeding, but also to the significant influence of external factors of a natural nature. The influence of natural factors, such as seasonality and diet, on the fatty acid profile must be undeniable (Marcos et al., 2020; Tian et al., 2017; Yakan et al., 2019), and this can modify the expression of genetic characteristics. For example, studies show that feeding systems, especially grazing, have a greater influence on the essential fatty acid profile than breed, which may explain some differences compared with the literature, where housing conditions might have varied (Halyna et al., 2019; Bodnar et al., 2021). The variation in mammary gland absorptive and synthetic capacities, driven by breed, constitutes a fundamental cause of inter-breed differences in milk fatty acid composition. Distinct breeds exhibit varying efficiencies in the uptake of fatty acids from the bloodstream and possess differential activities of enzymes involved in de novo fatty acid synthesis within the mammary gland. This ultimately leads to the formation of a characteristic fatty acid profile for each breed.

The consistently higher content of unsaturated fatty acids in the milk of Saanen goats, corroborated by our findings and prior research (Salari et al., 2016; Žan et al., 2016; Yurchenko et al., 2018), holds significant implications for the nutritional value of dairy products. Milk with an elevated UFA content is considered more beneficial for human health, as these acids play a crucial role in the prevention of cardiovascular diseases, exhibit anti-inflammatory properties, and influence brain development. The genetic predisposition of the Saanen breed towards the synthesis of a greater quantity of UFAs represents a valuable trait that can be considered in goat selection and breeding programs. The detection of specific fatty acids – oleic acid (C18:1), myristic acid (C14:0), stearic acid (C18:0), and linoleic acid (C18:2) – at elevated concentrations in Saanen goat milk underscores the unique biochemical profile of this breed. These individual fatty acids exert diverse effects on milk flavor, texture, and health-promoting attributes. For instance, oleic acid is a monounsaturated fatty acid known for its positive impact on the cardiovascular system. Linoleic acid is a polyunsaturated omega-6 fatty acid essential for numerous physiological processes. Our results indicate that local goat breeds, such as the Ukrainian local, can be a valuable resource for producing milk with an elevated content of specific beneficial fatty acids, opening new opportunities for selection and improving the nutritional value of dairy products. Further research with larger sample sizes and additional biochemical profiling can provide a deeper understanding of these interrelationships.

Conclusions

During the lactation period from March to September, goats of the Saanen breed have a higher milk productivity than their Russian white counterparts and Ukrainian local breed goats. The milk productivity of the experimental goat depended 13.00% on the external temperature and 81.33% on the breed. During lactation, at an average monthly variation of outdoor temperatures in the range of –4.4°C to 22.8°C, the milk volume of Saanen breed goats and Ukrainian local breed goats increased by 2 g and that of Russian White goats by 3 g when the temperature increased by 1°C.

The study revealed statistically significant differences in the content of medium-chain (MCFA), long-chain saturated (LCFA-SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA) among goats of the Saanen, Russian White, and Ukrainian local breeds. This underscores the predominant influence of the genetic factor on milk quality.

The milk from Ukrainian local and Saanen breeds demonstrated high nutritional potential. Goats of the Ukrainian local breed showed a statistically higher content of a number of important MCFA (e.g., caprylic, capric, lauric acids) and PUFA (particularly, linoleic and arachidonic acids), indicating their potential as a source of milk with improved functional and biologically valuable characteristics. The Saanen breed, in turn, stands out with a higher content of certain unsaturated fatty acids, including the total sum of MUFA, which has positive implications for human health.

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Conflict of Interest

The authors have not declared any conflict of interests.

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Application of Effective Technological Methods for the Production of Environmentally Safe Cow's Milk

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Abstract. *The production of crop and livestock products in agro-ecosystems requires not only systematic monitoring of quality and environmental safety, but also the development of effective technological methods for the production of environmentally safe cow's milk. The purpose of the research is to analyze the effectiveness of the applied technological methods in feeding productive cows for the production of environmentally safe milk and increasing animal productivity. Experiments were conducted on dairy cows of the Ukrainian black and red-motley dairy breeds with different types of feeding: silage-root, silage-hay, silage-haylage, and silage-hay-concentrate. The main diet of cows was supplemented with a specially developed mineral-vitamin premix, taking into account the antagonistic effect of its components on the ecotoxicants lead (Pb) and cadmium (Cd). The animals of the control group were on the main diet, while the second and third groups additionally ate the premix. The average daily milk yield was 14.0–14.8 kg. Laboratory chemical analysis of average feed samples for the concentration of macro- and microelements, including Pb and Cd, was carried out by the atomic absorption method. The STATISTICA version 10.0 program was used for statistical processing of the results obtained. Chemical analysis of feed showed an increased content of heavy metals lead, cadmium, copper, and zinc. Analysis of diets showed a deficiency of macro- and microelements in cobalt (64.5%), iodine (59.8%), phosphorus (43.1%), sulfur (33.5%), calcium (7.6%), and magnesium (7.2%) in cows with silage-root type of feeding; deficiency of cobalt (54.1%), phosphorus (47.4%), iodine (40.1%), sulfur (28.0%), manganese (2.3%) in silage-hay feeding; deficiency of cobalt (80.7%), iodine (65.2%), phosphorus (56.7%), sulfur (5.5%) in silage-hay feeding; and deficiency of cobalt (79.83%), iodine (20.15%), phosphorus (50.91%), sulfur (25.15%), manganese (20.15%), magnesium (2.7%) in silage-hay-concentrate feeding. Cobalt (54.1–80.7% average percentage of deficiency), phosphorus (43.1–56.7%), iodine (20.15–65.2%), sulfur (5.5–33.5%), manganese (2.3–20.15%), magnesium (2.7–7.2%) and calcium (7.6%) were the most deficient in all diets. The deficiency of these essential elements increases the toxic effects of Pb and Cd. Feeding a mineral-vitamin premix contributed to a lower incorporation of heavy metals in the body of animals of the experimental groups, which positively affected their physiological state and productivity, which increased on average by 1.3–1.7 times compared with animals of the control groups ($P < 0.001$). The silage-hay type of feeding was the most effective in relation to feeding the premix in the second experimental group. The milk productivity of cows increased by 1.6 times compared with the control group ($P < 0.001$). In cows with a silage-root crop type of feeding, productivity increased by 1.3 times, in cows with silage-hay-concentrate by 1.3 times, in cows with silage-haylage by 1.4 times, and in cows with silage-hay by 1.6 times, respectively. Balancing the main diet with an antitoxic premix contributed to a slight increase in milk production from 3477–4426 kg per lactation in the first control groups to 5444–5999 kg per lactation in the second experimental groups. Subcutaneous injection of a plant biopreparation enhanced the antitoxic effect of the premix, which also had a positive effect on the productivity of cows in the third experimental groups, where productivity increased by an average of 1.3–1.7 times. Among cows in the third experimental groups, the silage-hay type of feeding proved to be the best, milk productivity increased by 1.7 times compared with the control group ($P < 0.001$). The production of environmentally safe milk in areas of local contamination with heavy metals of technogenic, military origin requires the use of new proven technological techniques with feeding in rations of various types of special premixes, which allows for maximum balance of feeding, restoration of homeostasis of the organism in which heavy metals are incorporated, and increased cow productivity and milk production.*

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Introduction

Russia's aggression against Ukraine has significantly complicated the environmental security situation not only in agriculture, but also in the country as a whole. Shelling of the Ukrainian infrastructure by "Shaheds", mining, rocket attacks, undermining of the power plant dam, shelling of the Chernobyl nuclear power plant zone, etc. are turning into a global problem, due to the emergence of a humanitarian catastrophe from hostilities. There is a threat of biological, chemical, nuclear disasters, etc. (Kovaleva, 2025; Nayd́onova, 2023).

Hazardous pollutants and xenobiotics, including heavy metals such as cadmium and lead, cause local pollution around developed industrial centers. The accumulation of ecotoxicants in soil, plants (animal feed), even in small concentrations, is primarily caused by man-made sources. Highways with a large number of mobile vehicles, the vast majority of which have gasoline engines, pollute the soil with lead. Heavy metals enter the environment in significant quantities with emissions from stationary sources like enterprises. A significant anthropogenic and man-made impact is exerted by enterprises of the oil and gas industry complex, especially if they suffer man-made accidents or are hit during shelling by an aggressor. In war conditions, the combustion of rocket fuel during shelling of the territory, the flight of drones with internal combustion engines, etc. also causes atmospheric air pollution with toxic heavy metals, oxides of nitrogen, sulfur and carbon, including the impact on the development of the greenhouse effect. Pollutants settle on the soil surface and migrate in trophic chains, entering the body of dairy cows with feed. Intensive use of pesticides and mineral fertilizers increases the accumulation of heavy metals in the soil, their transfer to plants, disrupting the micro- and macroelement composition of animal feed in various soil and climatic zones of Ukraine, including Forest-Steppe, Polissya.

Analysis of the environmental situation in Ukraine shows that environmental pollution by heavy metals has increased over the past decades. The situation was especially complicated during the war. According to forecasts, the state of the environment and agroecosystems will be difficult in the post-war period (Kushnir and Onipko, 2018). Soil contamination with heavy metals can cause potential environmental risks, crop losses and human health risks (Mingtao Xiang et al., 2021).

The adverse impact on agrobiogeocenoses located around developed industrial centers, including the effect of intensive traditional farming, is increasing in different countries of the world. Phosphate mineral fertilizers, which are applied to the soil to increase crop yields, contain trace elements, including heavy metals, which are potentially dangerous to humans and the environment (Kratz et al., 2016). Providing crops with the necessary macro- and micronutrients is the main goal of any type of fertilizer. However, micronutrients can become harmful when present in high concentrations. In Germany, the content of undesirable elements in fertilizers is regulated by the German Fertilizer Ordinance, which sets limit values for cadmium, arsenic, mercury, chromium, and lead. Copper and zinc are considered essential elements and are therefore no longer regulated (Kratz et al., 2016). Frequent use of phosphate fertilizers can lead to the accumulation of heavy metals and undesirable concentrations in agricultural soils and subsequently in plants. There is particular concern about the accumulation of cadmium and uranium, as these metals are toxic and can threaten soil fertility, leach into groundwater and be absorbed by crops (Bigalke et al., 2017).

Once in the soil, heavy metals accumulate to harmful concentrations undesirable for agricultural plants, especially those used as feed in animal diets of any type of feeding. Soil fertility can also be negatively affected. The transfer of toxic heavy metals such as cadmium and lead from the soil to plants, and then to the body of productive animals can complicate the production of high-quality environmentally safe cow's milk (Vardhan et al., 2019; Portiannyk and Mamenko, 2021).

The entry of cadmium and lead into the body of animals causes cumulative toxicity, negative effects on internal organs and systems (Hashemi, 2018). Animal productivity decreases (Goff, 2018). The accumulation of pollutants in soil and feed significantly increases the risk of incorporation into the body of dairy cows, which threatens the health of animals and, through contaminated milk, humans themselves (Hashemi, 2018; Rezza et al., 2018). Chronic cadmium intoxication leads to nephrotoxicity, hepatotoxicity, immunotoxicity, osteotoxicity, oxidative stress of liver and kidney cells, damage to deoxyribonucleic acid, carcinogenesis, and cancer (Liu et al., 2009).

Today there is a need to develop new technological methods in feeding and production of environmentally safe livestock products if the rations contain toxic heavy metals. Monitoring the intake of mineral elements with feed into the body of animals and their accumulation in livestock products is of great importance (Hejna et al., 2018). Research into the relationship between the concentration of trace elements in feed and milk is an important basis for effective environmentally safe dairy farming, which contributes to increasing animal productivity, improving the quality and environmental safety of the milk produced (Ren-ju et al., 2015).

The purpose of the research is to analyze the effectiveness of technological methods used in feeding high-yielding cows to produce environmentally safe milk and increase animal productivity.

Materials and methods

Scientific and economic experiments were conducted in the farms of the Forest-Steppe zone of Ukraine. The experiment was conducted on dairy cows of Ukrainian black and red-motley dairy breeds in the third lactation period. Rations with different types of feeding were used: silage-root, silage-hay, silage-haylage, silage-hay-concentrate. Animals of the first control group were fed the main ration. The second and the third groups, in addition to the main ration, a specially developed mineral and vitamin premix MP-A, were fed with the content of elements of toxicant antagonists and elements that, under conditions of intoxication, compensate for the lack of essential elements in the body: selenium, sulfur, magnesium, manganese, phosphorus, cobalt, iron, iodine, as well as methionine – an amino acid containing a ligand sulfhydryl group, vitamins A, D, E, PP, C, H, B2, etc. In the third experimental group of cows with different types of feeding, a subcutaneous injection of a biological preparation from the extract of medicinal plants BP-9 was used to enhance the antitoxic effect of the premix.

The average live weight of the cows was 500–545 kg. The average daily productivity was 14.0–14.8 kg. The milk production of animals was recorded based on ten-year control milk yields. The comparative period was 42 days, and the experimental period was one 120 days (Table 1). All manipulations with animals were carried out in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes (Strasbourg, 1986).

Feeding the premix at the rate of 1% of the ration

Table 1. Scheme of scientific and economic experiments

Animal groups	Number of goals in the group	Average live weight at the beginning of the experiment, kg, ±5–10%	Age, months, at the beginning of the study	Duration of the experiment, days	Feeding type	Feeding characteristics
Comparative period						
Control group 1	12 – 1 65 – 2 21 – 3 42 – 4	500	64	42	Silage-root crops – 1 Silage-hay – 2 Silage-haylage – 3 Silage-haylage-concentrate – 4	The main diet balanced in terms of feed units, digestible protein, etc. indicators. The composition includes the following feeds: alfalfa hay (2, 3, 4), cereal-legume (1, 3, 4), wheat straw (1, 2), corn silage (1–4), alfalfa haylage (1–4), fodder beet (1, 2), corn grits (1, 4), barley (2), oat (3) and pea (3, 4), which exceed the maximum permissible concentration for Cd, Pb, Cu, Zn.
Experi-mental group 2	the same	500	64	42		the same
Experi-mental group 3	the same	500	64	42		the same
Experimental period						
Control group 1	the same	500	65	120	same	the same
Experi-mental group 2	the same	500	65	120		Basic ration + developed premix. MP-A-1, MP-A-2, MP-A-3, MP-A-4
Experi-mental group 3	the same	500	65	120		Basic diet + developed premix + drug BP-9

Note:
1 – experiment with Ukrainian black-mottled dairy breed of cows with silage-root crops type of feeding;
2 – experiment with Ukrainian black-mottled dairy breed of cows with silage-hay type of feeding;
3 – experiment with Ukrainian red-mottled dairy breed of cows with silage-haylage type of feeding;
4 – experiment with Ukrainian red-mottled dairy breed of cows with silage-haylage-concentrate type of feeding.

was as follows: cows with silage-root and silage-hay feeding type received 250 g per head per day; cows with silage-hay-concentrate feeding received 290 g; cows with silage-haylage feeding received 255 g. The recipe (formula) of the premix MP-A and the plant biopreparation BP-9, the methodology for developing the mineral-vitamin premix MP-A adapted to the actual daily feeding rations belongs to the authors of this publication Mamenko and Portiannyk (Ukraine).

Agricultural lands of farms are located near environmentally harmful man-made objects of influence on agrobiogeocenoses, highways with increased traffic of vehicles, such as Kyiv, Kharkiv, Dovzhansky routes, main oil and gas pipelines, etc. Average feed samples (n = 6) were selected according to the generally accepted method in zootechnical practice from feed storage areas, reinforced concrete pits with silos, haylage, storage facilities for storing hay, straw, haystacks with fodder beets, warehouses for storing concentrated feed, mixed feeds, etc.

Laboratory chemical analysis of plant samples for the content of macroelements Ca, P, Mg, K, S, and microelements Fe, Cu, Zn, Mn, J, Pb, Cd was carried out by atomic absorption spectrophotometry (AAS-30 spectrophotometer). The deficiency of macro- and microelements in feed was established relative to the average nutritional value of feed specified in detailed standards (Kalashnikov et al., 1985).

The material for analyzing the relevance of the problem and discussing it included scientific review and research articles from international scientific sources that are in open access on Scopus and Web of Science Core Collection databases.

Ecological monitoring of the Forest-Steppe zone of Ukraine is carried out by scientists regularly in accordance with the stages of R&D implementation. State registration number: 0121U113933 dated November 18, 2021 (Fig. 1).

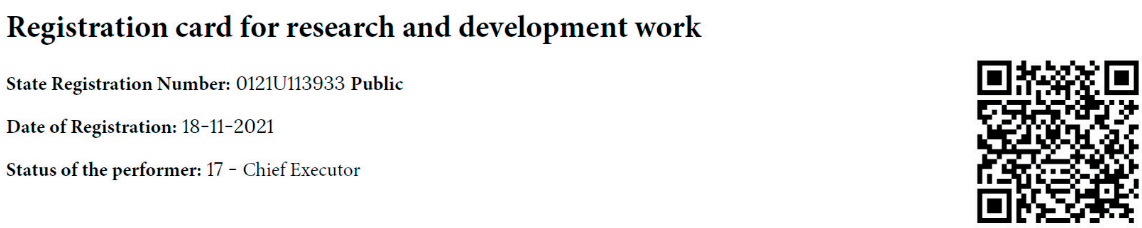
For each sample, the mean value of the trait in the sample (M) and the standard deviation (SD) were calculated. The estimate is given in the form of M ± SD. Differences between the mean values were considered statistically significant at $P < 0.05$. The calculation was performed in the STATISTICA software package version 10.0.

Results

The determining role belongs to the factor of feeding and mineral-organic nutrition of dairy cows in reducing the load of their body with heavy metals, especially cadmium and lead. To develop a special mineral-vitamin premix of the MP-A type, which was used in feeding cows of the second and third experimental groups, a deep analysis of one of the main factors in such ecological situations, i.e., feeding, was carried out.

The quality of feed is determined by the type of plants, their botanical composition, the vegetation phase, climatic conditions, including the influence of abiotic and anthropogenic factors, agricultural techniques for crop cultivation and storage conditions. For laboratory analysis, we selected average samples of feed that were used to feed the experimental groups of cows. In addition, we selected average samples of those feeds that could be used in feeding when creating optimal types of feeding in terms of heavy metal contamination. The feed that was part of the rations and fed to the cows was of good quality. Hay and straw had a pleasant smell and their own color. In silage, crushed plant particles retained their structure and consistency, the leaves were elastic and easily separated from each other, the smell was pleasant, specific. Hay also had a pleasant smell, a characteristic consistency and was of good quality. Concentrated feed had a glossy surface, a characteristic shine, color and sweetish-fresh taste. Silage and hay were prepared in above-ground reinforced concrete silage-hay trenches, hay and straw in slats and bales, and concentrated feed was stored in a warehouse.

Against the background of good quality feed, the content of heavy metals in them, in particular cadmium, lead, copper and zinc in concentrations exceeding the maximum permissible norms, indicates their high hidden danger, since it is impossible to visually determine the content of xenobiotics, as well as radioactive substances, in feed. The amount of cadmium in the diet of animals with silage-root type of feeding exceeded the norm by an average of 2.1–3.2 times, lead exceeded the norm by 2.4–5.7 times, copper by 1.4–2.3 and zinc by 1.2–2.4 times (Figs. 2–5). A comparison of established concentrations of heavy metals in feed was carried out following the mandatory minimum list of studies of raw materials, products of animal and plant origin, feed raw materials, feed, vitamin preparations, etc. that should be conducted in state laboratories of


Registration card for research and development work

State Registration Number: 0121U113933 **Public**

Date of Registration: 18-11-2021

Status of the performer: 17 - Chief Executor

Fig. 1. Registration card for research and development work
Source: Ukrainian Institute of Scientific and Technical Expertise and Information (n.d.)

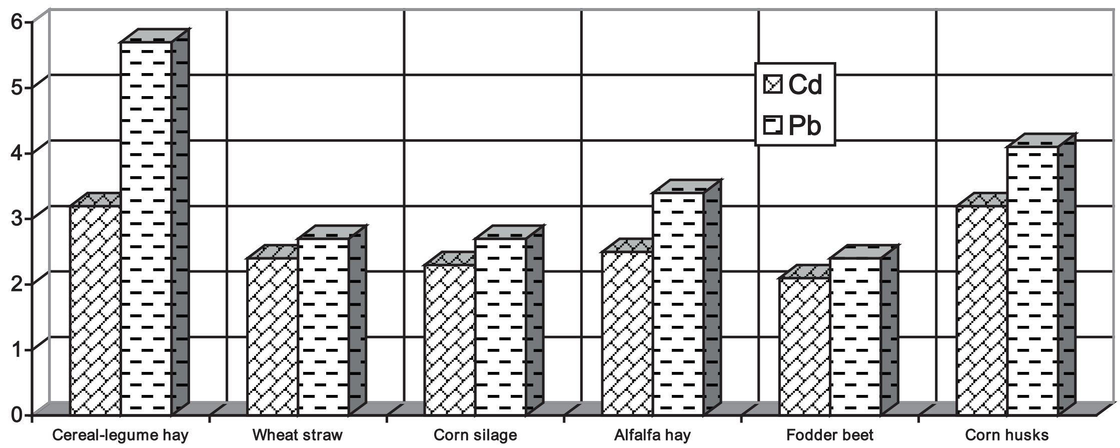


Fig. 2. Cd and Pb content in silage-root crops type feed of the main ration, mg/kg (source: developed by the authors).

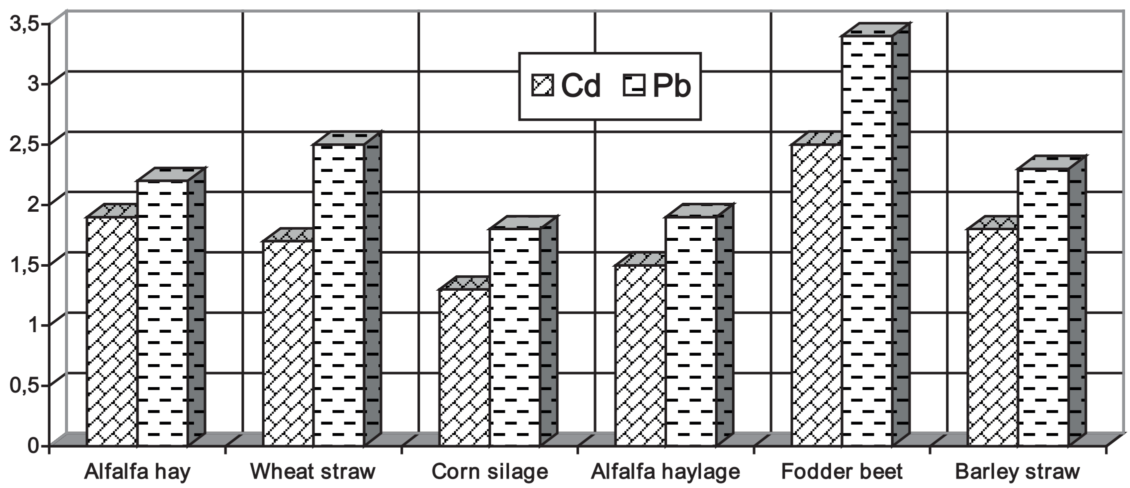


Fig. 3 Cd and Pb content in the main silage-hay type feed, mg/kg (source: developed by the authors).

veterinary medicine and based on the results of which a veterinary certificate (F-2) is issued (State Department of Veterinary Medicine, 1998).

Cereal-legume hay had the highest excess of the permissible concentration for cadmium and lead exceeding the norm by 3.2 and 5.7 times, corn stalks had the highest concentration of copper exceeding the norm by 2.3 times, and wheat straw had the highest zinc content, which exceeded the permissible norm by 2.4 times. The concentration of heavy metals in the diets of dairy cows with other types of feeding fluctuated. There are many reasons for that. Different farms used different amounts of mineral fertilizers and pesticides, which affected the different concentration of mobile forms of pollutants in the soil, the distance of agricultural lands where fodder crops were grown from sources of technogenic influence, etc., but in the feed of dairy cows with silage-hay type of feeding, the highest concentration of cadmium, lead, copper and zinc was found in fodder beets exceeding the norm by 2.5 times, 3.4 times, 3.8 times, and 4.1 times, respectively. Fodder beets had the highest level of all studied elements compared with other feed types. Where cows were fed a silage-hay diet, compared with other experiments, a high concentration of zinc in oat grain and peas was found exceeding the norm on average 6.3–6.8 times, while an excess of the permissible norm of cadmium, lead, cuprum and zinc was also observed.

Pea grits among other feeds differed in the highest concentration of Cd, Pb, and Cu in cereal-legume hay, where the level recorded exceeded the norm by 3.9 times. Feed of the diet of animals with a silage-hay-concentrate type of feeding demonstrated the highest excess of the norm of Pb by 7.3 times, Zn by 7.8 times, and Cu by 4.1 times. In terms of the content of Cd in feeds, this diet ranks last together with the diet of a silage-hay type of feeding of dairy cows. Among other feed types in the diet, cereal-legume hay accumulated the most cadmium, lead, and copper. Corn grain accumulated the most zinc.

The main diet of the experimental groups of cows for different types of feeding was compiled according to the nutritional value of the feed, which was established after laboratory analysis, taking into account the content of the studied Cd, Pb, Cu, and Zn. In the main diet of experimental cows with silage-root, silage-hay, silage-haylage and silage-hay-concentrate feeding, the number of feed units and metabolizable energy was maximally balanced. Crude

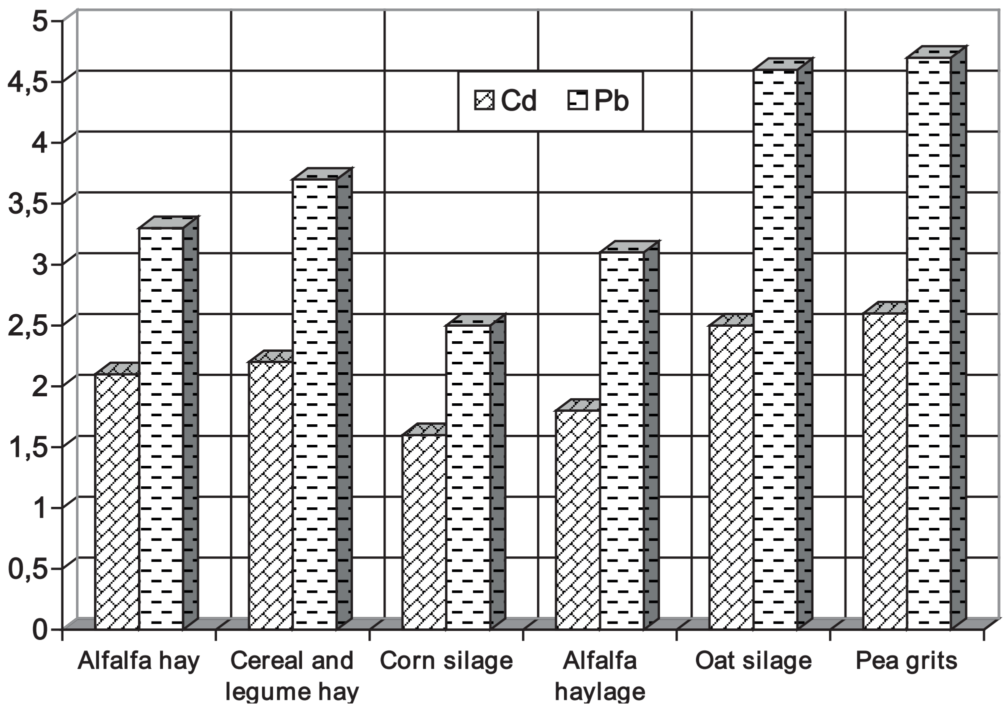


Fig. 4. Cd and Pb content in the main silage-haylage feed ration, mg/kg (source: developed by the authors).

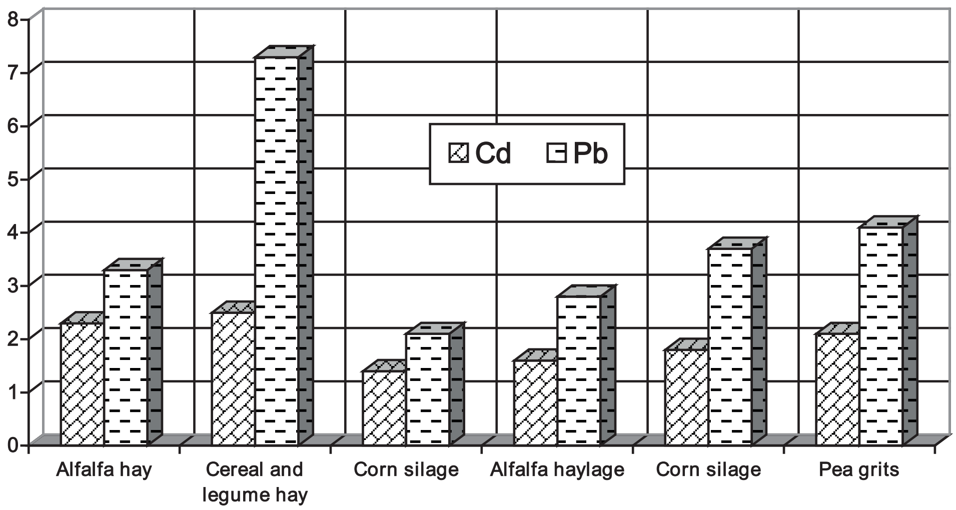


Fig. 5. Cd and Pb content in the main ration of silage-haylage-concentrate type feed, mg/kg (source: developed by the authors).

fiber, crude and digestible protein were balanced in the diet of cows with silage-root crops, silage-hay and silage-haylage feeding type.

In the diet of cows with silage-hay-concentrate feeding, it was not possible to balance the rations due to the set of feeds; therefore, the deficit of crude protein was 7.03% and that of crude fiber was 12.66%. It is important that the content of digestible protein was within normal limits. The sugar-protein ratio was within normal limits in all diets. The content of crude fat was also within normal limits.

At the same time, a low dry matter content was observed in the diet of animals with silage-root crop feeding (15.1%), silage-hay (17.9%) and silage-hay-concentrate (3.62%). Only in the diet of cows with silage-hay type of feeding this deficiency was not observed, which is to some extent due to the type of feeding, and only the silage-hay type diet did not have a dry matter deficiency. In addition, in the diet of cows with silage-root crop feeding, silage-hay and silage-haylage, a starch deficiency of 26.5%, 32.9%, and 17.4% was observed, respectively. In the diet of animals with silage-hay-concentrate type of feeding, the starch content was within normal limits. Among macro- and microelements in the diet of cows with silage-root type of feeding, the greatest deficiency was in cobalt (64.5%), iodine (59.8%), phosphorus (43.1%), sulfur (33.5%), calcium (7.6%), and magnesium (7.2%); in the diet of cows with silage-hay type of feeding, the deficiency was in cobalt (54.1%), phosphorus (47.4%), iodine (40.1%), sulfur (28.0%), and manganese (2.3%); in the diet of cows with silage-haylage, the deficiency was in cobalt (80.7%), iodine (65.2%), phosphorus (56.7%), and sulfur (5.5%); and in the diet of cows with silage-hay-concentrate, it was in cobalt (79.83%), iodine (20.15%), phosphorus (50.91%), sulfur (25.15%), manganese (20.15%), and magnesium (2.7%). As we can see, the most deficient elements in all diets were as follows (in descending order of %): cobalt (54.1–80.7% average percentage of deficiency), phosphorus (43.1–56.7%), iodine (20.15–65.2%), sulfur (5.5–33.5%), manganese (2.3–20.15%), magnesium (2.7–7.2%) and calcium (7.6%).

The basic diet of dairy cows in all four experimental farms can be considered balanced in terms of basic indicators such as feed units, metabolizable energy, crude and digestible protein, crude fiber, crude fat, sugar, as well as macro- and microelements of potassium, iron, and, in some diets, calcium, magnesium, and manganese. The diets had an optimal sugar–protein ratio of 0.8–1.1, a sugar–starch ratio of 0.6–1.2, and a calcium–phosphorus ratio of 2.31:1 in animals fed silage-root crops, 4.7:1 in silage-hay, 7.4:1 in silage-haylage, and 5.6:1 in silage-hay-concentrate, which is due to the low phosphorus content in the feed, since cadmium and lead can reduce its entry into the plant from the soil.

Thus, the developed basic diets for dairy cows in all experimental farms, while being balanced in the main most important indicators, did not contribute to a decrease in the absorption of heavy metals into the blood and an increase in milk productivity of animals in the first control groups (Table 2).

Tables 3–6 show the descriptive statistics. The calculation includes the arithmetic mean, median with lower and upper quartiles, maximum and minimum average daily milk yield for each experimental group. At the same time, a slight difference was found between the arithmetic mean and median. The STATISTICA program allowed for the most accurate data analysis and description of the average daily animal productivity obtained as a result of the research.

As can be seen from Tables 2–6, the productivity of cows in the first control group fed the main diet had lower productivity compared with animals in the second and third experimental groups fed a specially developed mineral-vitamin premix ($P < 0.001$). The lowest milk productivity among cows in the control group was in animals fed silage-hay type of feeding at 11.40 ± 0.61 kg, followed by a slightly higher productivity in animals in the control group fed silage-hay type of feeding (14.04 ± 0.61 kg), in cows fed silage-root (14.31 ± 0.74 kg) and in cows fed silage-hay-concentrate (14.51 ± 0.67 kg).

The use of a technological method in the experiment, which involved feeding animals of the second and third experimental groups a special premix MP-A developed according to the method by Portiannyk and Mamenko, improved the situation

Table 2. Milk production of cows (M ± SD)

Indicators	Animal feeding type											
	Silage-root crops			Silage-hay			Silage-haylage			Silage-haylage-concentrate		
	Control group 1	Experimental group 2	Experimental group 3	Control group 1	Experimental group 2	Experimental group 3	Control group 1	Experimental group 2	Experimental group 3	Control group 1	Experimental group 2	Experimental group 3
Average daily milk yield, kg	14.31 ± 0.74	17.85 ± 0.89	18.68 ± 1.03	11.40 ± 0.61	18.41 ± 0.70	19.62 ± 1.11	14.04 ± 0.61	19.67 ± 0.84	21.65 ± 0.73	14.51 ± 0.67	19.33 ± 0.92	22.62 ± 0.70

Notes: degree of probability compared with the data of the control group $P < 0.001$; n = 12.
Source: developed by the authors

Table 3. Descriptive statistics of average daily milk yield of experimental cows with silage-root crops type of feeding, n = 12

Animal groups	% Valid obs.	Mean	Median	Sum	Minimum	Maximum	Lower Quartile	Upper Quartile	Standard Deviation	Standard Error
Control group 1	33.33333	14.31083	14.27000	171.7300	13.52000	15.38000	13.60000	15.14500	0.739367	0.213437
Experimental group 2	33.33333	17.85250	17.61000	214.2300	16.79000	19.57000	17.28000	18.49000	0.886414	0.255886
Experimental group 3	33.33333	18.68333	18.39500	224.2000	17.41000	21.02000	18.13500	19.07500	1.027675	0.296664

Source: developed by the author

Table 4. Descriptive statistics of average daily milk yield of experimental cows with silage-hay feeding, n = 12

Animal groups	% Valid obs.	Mean	Median	Sum	Minimum	Maximum	Lower Quartile	Upper Quartile	Standard Deviation	Standard Error
Control group 1	33.33333	11.40083	11.40500	136.8100	10.42000	12.42000	10.88000	11.72000	0.611116	0.176414
Experimental group 2	33.33333	18.41167	18.12500	220.9400	17.75000	20.21000	18.06000	18.61500	0.701218	0.202424
Experimental group 3	33.33333	19.62083	19.22000	235.4500	18.18000	22.02000	19.14500	20.18000	1.109344	0.320240

Source: developed by the authors

Table 5. Descriptive statistics of average daily milk yield of experimental cows with silage-haylage feeding type, n = 12

Animal groups	% Valid obs.	Mean	Median	Sum	Minimum	Maximum	Lower Quartile	Upper Quartile	Standard Deviation	Standard Error
Control group 1	33.33333	14.04417	13.91000	168.5300	13.27000	15.59000	13.69500	14.29500	0.609835	0.176044
Experimental group 2	33.33333	19.67417	19.38000	236.0900	18.31000	21.14000	19.20500	20.18500	0.840146	0.242529
Experimental group 3	33.33333	21.65000	21.52000	259.8000	20.36000	23.06000	21.40000	22.13000	0.730790	0.210961

Source: developed by the authors.

Table 6. Descriptive statistics of average daily milk yield of experimental cows with silage-haylage-concentrate feeding type, n = 12

Animal groups	% Valid obs.	Mean	Median	Sum	Minimum	Maximum	Lower Quartile	Upper Quartile	Standard Deviation	Standard Error
Control group 1	33.33333	14.51417	14.49500	174.1700	12.92000	15.23000	14.30500	15.15000	0.668192	0.192891
Experimental group 2	33.33333	19.33250	19.32500	231.9900	17.82000	21.71000	18.94000	19.53500	0.916070	0.264447
Experimental group 3	33.33333	22.62250	22.52500	271.4700	21.36000	24.17000	22.30500	22.84500	0.695494	0.200772

Source: developed by the authors.

with the quality and environmental safety of milk. The milk productivity of animals under different types of feeding slightly increased compared with animals of the control groups ($P < 0.001$). The premix recipe ensured the normalization of the content of heavy metals in the diets of dairy cows with different types of feeding, taking into account the antagonistic, synergistic and adaptive effects of its components. The production of the premix is not complicated and is available to any farm if necessary.

Therefore, thanks to the proven technological method of balancing rations with a special mineral-vitamin premix, we managed not only to reduce the burden of heavy metals on the body of productive animals, to support the healthy functioning of important organs and systems of the body due to the essential, biogenically important mineral elements and vitamins introduced into the premix, but also to slightly increase milk productivity up to 6 thousand kilograms per lactation, which makes this method economically effective and environmentally appropriate, since the milk produced was environmentally safe and met both domestic and international quality standards.

Discussion

We completely agree with other researchers (Renju et al., 2015) that feed consumption plays a decisive role in influencing the milk production of cows as well as the quality and environmental safety of the milk produced. Ren-ju et al. (2015) also investigated the content of trace elements, including heavy metals, in cow's milk under the influence of various feeds. The researchers determined the concentration of lead, cadmium, arsenic, copper, magnesium, calcium, iron and zinc in various feeds and milk using a method similar to ours – the method of atomic absorption spectrophotometry. The determination of the content of lead, cadmium and arsenic was carried out using AAS in a graphite furnace, while the content of copper, magnesium, calcium, iron and zinc was determined using flame atomic absorption spectrometry. The results showed that lead, cadmium, arsenic and copper were present in feeds, but lead, cadmium and arsenic were weakly detected in milk samples, and copper was not detected at all in milk samples. The content of magnesium in concentrated feeds was lower than in other types of feeds (Song Ren-ju et al., 2015). Magnesium deficiency in feed under the conditions of our experiment caused a deficiency of the element in the diet of cows with silage-root type at the level of 7.2%, and with silage-hay-concentrate type at 2.7%. In general, in all diets, magnesium was the most deficient (2.7–7.2%), which is to some extent consistent with other studies (Ren-ju et al., 2015). It has also been found that there was more magnesium in the milk of animals that consumed more concentrated feeds than forage (Ren-ju et al., 2015). This indicates that the use of magnesium in concentrated feeds was higher than in forage. The content of calcium and zinc was the opposite of magnesium, and the use of calcium and zinc in forage feeds was higher than in concentrated feeds. The researchers did not establish patterns of changes in iron in different feeds and milk samples. We agree with the opinion of Ren-ju et al. (2015) that the ratio between the content of trace elements, including metals, in milk and feed will become the theoretical basis for dairy farming, which is very important for increasing milk yields, quality and environmental safety of milk in dairy production.

The deficiency of these essential elements only enhances the toxic effects of Cd and Pb. Since some elements, such as cobalt and sulfur, are antagonists of heavy metals, other elements – calcium, phosphorus, iodine, manganese, magnesium – support the normal functioning of organs and systems important for the body of productive cows, which are affected by heavy metals and perform a protective function of the body. The lack of these rather important biochemically active elements in the diet, in our opinion, is due not to different feeding styles of cows, but to the low intake of these elements from the soil into animal feed with an increased content in the soil of such dangerous toxicants as Cd, Pb, Cu, Zn. These xenobiotics affected the reduction of the transition of vital biogenic essential elements from the soil to plants. Due to their high biological activity in the form of various salts of sulfates, nitrates, chlorides, etc. in high concentrations they migrated to plants that were included in the feeding rations as feed. This is the main reason that should beware zootechnical specialists who are directly involved in the development of diets for dairy cows and milk production in conditions of local contamination of agroecosystems with heavy metals Cd, Pb, Cu, and Zn near developed industrial centers. The content of such biogenic elements as potassium, iron, and in some diets, manganese, magnesium and calcium was in sufficient quantities, but against this background, the content of heavy metals Cd, Pb, Cu, Zn exceeded the physiological norm. The intake of carotene corresponded to the norm in the diets of all farms, as well as the content of vitamins D and E.

Other scientists (Tao et al., 2020) emphasize that environmental pollution with heavy metals threa-tens the health and life of animals and humans due to their migration through the food chain, and we also agree with this. Chinese scientists took feed samples in the same way as we did in our study, analyzing them for the content of cadmium, mercury, chromium, and arsenic using the method of atomic absorption spectrophotometry or atomic fluorescence spectrometry for analysis. Researchers found high levels of contamination with feed pollutants in Hubei province. They emphasize the importance of monitoring the content of heavy metals and point to the implementation of a feed management strategy, conducting boron mediation to reduce the impact of pollutants.

If the feed contains trace elements of heavy metals, especially such as lead and cadmium, in high concentrations this causes their transfer to milk. Milk formed in the mammary gland contains Cd, Pb, Cu, and Zn and accumulates in the alveoli, ducts and cistern. The capacity of the udder plays an important role in this. It depends on these cavities. As a result of the contraction and relaxation of muscle fibers, milk from the upper alveolar sections passes into the cistern. The nervous system plays a key role here because it controls this process.

From the upper alveolar sections, milk passes into the cistern due to the contraction or relaxation of muscle fibers. This process is controlled by the nervous system. Chronic exposure of dairy cows to heavy metals affects the process of milk production. The most dangerous ecotoxicants in this case are Pb and Cd (Portiannyk and Mamenko, 2021). Lead and cadmium disrupt the nervous system of farm animals. The accumulation of toxicants in muscle tissue and internal organs negatively affects the processes of milk formation and milk productivity, which in all cows of the control groups was lower for all types of feeding compared with the experimental groups and averaged 11.4–14.5 kg ($P < 0.001$).

Feeding the mineral-vitamin premix MP-A contributed to a lower incorporation of heavy metals, especially toxic ones such as cadmium and lead, from the gastrointestinal tract, which positively affected the general physiological condition of the animals, and at the same time the productivity, which increased on average by 1.3–1.7 times compared with the animals of the control groups ($P < 0.001$). For cows of the second experimental group with silage-hay feeding type, feeding the premix was the most effective. The milk productivity of the animals increased by 1.6 times compared with the control group ($P < 0.001$). Taking into account the environmental conditions of the experiment, this technological method in feeding cows contributed to an increase in the milk productivity of the animals in the following order. In cows with silage-root crop feeding, milk production increased by 1.3 times, with silage-hay-concentrate by 1.3 times, with silage-hay by 1.4 times and with silage-hay by 1.6 times, respectively. The main rations of dairy cows were calculated at 4500 kg per lactation, and the average daily yield was 14 kg. Milk productivity of animals less than 4500 kg is not profitable for all farms. Balancing the main ration with the developed mineral-vitamin premix contributed to a slight increase in milk production from 3477–4426 kg per lactation in the first control groups to 5444–5999 kg per lactation in the second experimental groups. A subcutaneous injection of the herbal biopreparation BP-9 from nine medicinal herbs enhanced the antitoxic effect of the premix, which also had a positive effect on the productivity of cows in the third experimental groups. Their milk productivity increased by an average of 1.3–1.7 times. Among cows in the third experimental groups, the silage-hay type of feeding proved to be the best, the milk productivity of animals increased by 1.7 times compared with the control group ($P < 0.001$).

At the end of the experiment, we managed to produce milk from animals mainly from the third experimental groups with silage-root (0.018 ± 0.002 mg/kg, cadmium; 0.014 ± 0.003, lead) and silage-hay (0.012 ± 0.002 mg/kg, cadmium; 0.014 ± 0.004 mg/kg, lead) type of feeding, which met both domestic quality and environmental safety standards, and the requirements of Regulations (EC) No. 853/2004 and No. 2023/915. In animals from the second experimental group, where the mineral-vitamin premix itself was used in the technology, the lead content decreased to 0.016 ± 0.004 mg/kg in cows with a silage-haylage diet, which also meets the domestic quality standard and the Regulation (EC). In our opinion, longer feeding of the premix contributed to a decrease in the lead content in the milk of cows of the second experimental groups and other types of feeding in this order, first with silage-hay-concentrate type diets, then in silage-hay and silage-root crops. The degree of probability compared with the data of the control group is $P < 0.01$. We tested one complex technological method of using a premix and a phytopreparation from an extract of nine medicinal herbs, which allows reducing the load on the body of productive cows with toxic heavy metals cadmium and lead, to produce environmentally safe milk.

The feeding diet plays a key role in the formation of milk in the mammary gland. A balanced diet with a special mineral and vitamin premix improves the process of milk formation, especially in animals with a larger amount of juicy feed according to the silage-root type of feeding. Intensive accumulation of milk in the mammary gland has a reflex effect on the cavities of the alveoli, ducts, and cisterns. When the walls of the udder cavities relax, their capacity increases, and the mammary gland can hold more milk. Transferring animals to triple milking under experimental conditions contributed to the improvement of the process of milk formation. In the first hours after machine milking, the intensity of milk synthesis is higher. The more time passes since the last milking, the more the process of milk formation slows down. For cows of the black-and-white dairy breed, the process of milk formation is more intense in the first six hours after the last milking. Experience of scientific research by Ukrainian and foreign scientists (Kovaleva, 2025, Gutyj et al., 2016, Savchuk et al., 2021, Portiannyk and Mamenko, 2021, Ren-ju et al., 2015) indicates the negative impact of heavy metal ecotoxicants on the internal organs liek the liver, kidneys, spleen, lymphatic and nervous systems, enzymatic and hormonal, circulatory, etc., which leads to a disruption in the production of the hormones prolactin and oxytocin, which are responsible for the process of milk formation and excretion. Toxicants block the process of oxytocin entering the mammary gland from the blood. Morphobiochemical analysis of the blood showed an increased content of pollutants. This system fails. The alveoli are squeezed weakly, the tubules contract slowly, and their cavity does not increase. There are no favorable conditions for the release of milk into the ducts of the glands. In the experiment, we selected cows with a productivity of approximately the same level of 14–15 kg per day, 4362–4636 kg per lactation. All animals were with the third lactation period.

The diets of dairy cows were balanced in terms of macro- and microelement composition, taking into account the mechanism of absorption of elements, the dynamics of movement of heavy metals – lead and cadmium – in the body of cows. We included heavy metal antagonist elements in the premixes in an amount sufficient to reduce their transfer into the blood from the digestive tract. The introduction of vitamins into the premix contributed to the restoration of homeostasis of the animal's body, improving the functioning of its organs and systems. As a result, we managed to achieve a positive effect, reducing the concentration of heavy metals in the milk of cows under different types of feeding and ensuring optimal quality, environmental safety of milk and productivity of cows ($P < 0.01$ and $P < 0.001$).

Thus, the technological method for the

production of environmentally safe milk by feeding the premix MP-A, which contains elements that are antagonists of toxic elements lead and cadmium in the second and third experimental groups of cows, limited the entry of heavy metals into the blood due to the displacement of elements from metabolic processes. A subcutaneous injection of the biological preparation BP-9 in the third experimental groups of animals under different types of feeding contributed to the improvement of the milk formation process, the secretory activity of the mammary gland, and an increase in animal productivity by an average of 1.6 times. As a result of the experiment, milk production increased to 5697–6899 kg per lactation ($P < 0.001$).

Conclusions

The production of environmentally safe milk in areas of local contamination with heavy metals of anthropogenic-technogenic, military origin requires the use of new experimentally proven technological techniques using special mineral and vitamin premixes containing heavy metal antagonist elements, biologically active essential elements selenium, iron, iodine, calcium, phosphorus, vital for animals exposed to toxic effects, vitamins, amino acid methionine, etc., which allows for maximum balance in feeding of dairy cows and restoration of homeostasis of the organism in which pollutants are incorporated. Reducing the toxic effect of heavy metals ensured the production of high-quality environmentally safe cow's milk and contributed to an increase in the average daily productivity of cows from 11–14 kg to 18–23 kg per lactation, which is one of the indicators of the effectiveness of the developed zootechnical methods. Animals fed silage-haylage and silage-haylage-concentrate diets had better lactation productivity.

Further research is aimed at monitoring the concentration of pollutants in animal feed in different soil and climatic zones of Ukraine.

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VETERINARY TODAY: HEALTH, WELFARE, AND REPRODUCTION MANAGEMENT IN DAIRY COW HERDS

Abstracts

ORGANISERS:
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ASSESSMENT OF HEALTH OF DAIRY COW HERDS

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Optimal herd health is fundamental to productivity, sustainability, and animal welfare in modern dairy systems. A comprehensive assessment strategy must address the multifactorial nature of bovine health, incorporating nutrition, disease prevention, reproductive efficiency, and welfare monitoring. Effective herd health management relies on the integration of veterinary expertise, farmer decision-making, and the use of data and technology for continuous monitoring and improvement [1].

Implementation of structured herd health programmes on commercial dairy farms has demonstrated the value of regular health assessments, early disease detection, and proactive interventions. Practices such as routine locomotion and body condition scoring, reproductive and udder health monitoring, and performance benchmarking are essential tools in identifying health trends and informing management decisions [2].

Particular emphasis is placed on the transition period, a critical phase in the production cycle associated with high risk of metabolic and infectious diseases. Transition cow management, including nutritional planning, metabolic profiling, and monitoring of key performance indicators, plays a pivotal role in determining overall herd health outcomes [3, 4].

Integrating these components into a cohesive herd health plan supports improved fertility, higher milk yields, and enhanced animal welfare, contributing to the long-term success and resilience of dairy operations [5].

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DIGIT AMPUTATION IN CATTLE

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Digit amputation is usually the recommended surgical therapy for complicated claw disorders in cattle where deeply located structures distal to the fetlock joint are severely infected or injured [1]. An unaffected and load-bearing partner claw is required [2]. Removal of the infected or necrotic tissue (by digit amputation) results in a rapid reduction in pain and lameness, and faster procedures than in the case of resections [2]. In addition, proper drainage of exudate is achieved [2]. After a thorough clinical examination, especially of distal limbs, the prognosis of each case, possible complications and necessary aftercare should be discussed with the owner before making a therapeutic decision. Economic parameters should also be considered [1, 3]. Perioperative pain management, including the application of non-steroidal anti-inflammatory drugs and local anaesthesia, is necessary to improve animal welfare and ensure the safety of the veterinarian during surgery [3]. Intravenous regional anaesthesia is recommended [3, 4]. Multiple methods of digit amputation in cattle have been described [1, 3]. Disarticulation at the proximal interphalangeal joint is our preferred method. The skin is circularly incised proximal to the coronary band. After removing the affected claw, the deep flexor tendon is shortened as proximal as possible within the tendon sheath [1] and the articular cartilage of the distal surface of the first phalanx should be scraped with a bone curette [4]. The amputation wound is left unclosed to heal by secondary intention [2], and a pressure bandage, usually with antibiotic, is applied. Systemic antibiotics are recommended in most cases (at least until the first bandage change) due to local signs of inflammation [5]. The first change of the bandage should usually be made after 3–5 days, depending on the initial lesions. The interval between further bandage changes depends on the progress of healing and the local findings [5]. To avoid direct weight bearing on the amputation stump, which could disturb the healing process, the operated animal should be housed in a box stall with a firm, dry and non-slip floor next to a comfortable bedded resting area [1]. Deep bedded pack with straw is not recommended after surgery [1]. With good surgical therapy, appropriate aftercare and close lameness monitoring, the operated animal could remain in the herd as long as the other animals [1, 2].

Keywords: claw, surgery, cow, lameness, deep sepsis.

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ASSESSING TRANSITION COW MANAGEMENT IN DAIRY COWS

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The last decade or so has seen an increasing focus placed on the management of the transition cow largely due to the increasing recognition of the drastic negative consequences of poor transition management on milking cow welfare and performance, not only in terms of cow health and welfare [1,3], but also in terms of lost milk production [4] and reproduction. Understanding of the social environment of the cow has the potential to greatly benefit dairy cow welfare and production and a better understanding of how prepartum management factors influence postpartum health and milk production [4] can help farms to plan facilities and organize the day-to-day management of cows and will assist in improving cow welfare and productivity [5]. This paper will argue that this approach should be extended to cover the entire dry period and suggests possible key parameters for monitoring the processes and outcomes during this critical period [2].

Keywords: transition, health, production.

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CHALLENGES OF INTRODUCING ANIMAL HEALTH VISITS IN LATVIA

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In 2016, the European Union adopted the 'Animal Health Law', and Article 25 laid down requirements for all productive and wild animal establishments to receive regular animal health visits (AHV) from a veterinarian. The goal of AHV is to strengthen animal health by improving disease prevention, through enhanced biosecurity, and disease detection. Regular AHV will improve animal health and welfare, reduce the use of antibiotics, and enhance sustainability and economic efficiency [1].

Currently, in Latvia, the monitoring of infectious diseases (which must be controlled under the EU regulations), control of biosecurity, animal welfare, and the responsible use of medicines in livestock are carried out by official Food and Veterinary Service inspectors. Management of other infectious and non-infectious diseases (diagnosis, prevention, and treatment) and health services in general are provided by private practicing veterinarians. In Latvia, about 250 veterinarians are engaged in the practice of productive or mixed animals. Of these, about 90 veterinarians are hired by herd owners, and about 20 veterinarians are independent and have contracts with several herds for veterinary services. Based on this fact, in 2023, a research project led by the Ministry of Agriculture of Latvia "Development of guidelines for organizing animal health visits and implementation of a pilot project for evaluating the effectiveness of the developed guidelines" was launched. The project is developing guidelines for the implementation of AHV in herds of cows, sheep/goats, poultry, and fisheries. From the perspective of 'Animal Health Law', the AHV must be mandatory, realized by independent private practicing veterinarians, and applied for all herds of productive animals. Due to the small number of independently practicing veterinarians in Latvia, AHV will be introduced gradually, starting with the largest herds. From July 2024, Latvia will have a unified electronic system for accounting for the use of veterinary medicines, with a focus on the use of antibiotics in farm animals (e-VETIS). It will help AHV-performing veterinarians detect health problems at the herd level earlier. The primary challenges for the implementation of AHV in Latvia are multifaceted and demand strategic interventions: 1) to increase the number of independently working farm animals' veterinarians; 2) further education is required in the implementation of AHV; 3) herd health visits (which reduces other herd health problems) need more emphasis in Latvia; 4) there is a lack of public, animal husbandry specialists' understanding of the need for the mandatory AHV; 5) the possibility of duplication of welfare visits by official inspectors and veterinarians should be eliminated; 6) AHV is planned as a paid service for owners of herds, but visits by official inspectors are for free (sponsored by the State).

Keywords: animal health visits, productive animals, infectious diseases.

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BOVINE PAIN: IMPORTANCE, IDENTIFICATION, AND CONTROL IN MODERN CATTLE FARMS – META-ANALYSIS

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Pain in cattle leads to changes in behaviour, autonomic function, and neuroendocrine activity, resulting in negative emotional responses and compromised welfare [1,4]. Chronic pain can reduce food intake and weight gain while increasing heart rate and blood pressure and decreasing body temperature [1].

Assessing pain in farm animals typically involves monitoring physiological and behavioural changes [1,3,4]. However, pain detection in cattle remains challenging due to their natural stoicism. Cattle have evolved to conceal pain to avoid appearing vulnerable to predators, making it difficult for veterinarians and farmers to recognize signs of discomfort [3,4].

A scientifically developed pain scale helps assess cattle pain by evaluating seven signals, each scored from 0 to 2, with a total score above 5 indicating severe pain. This scale is a valuable tool for both veterinarians and farmers to detect subtle signs of pain [3].

Pain management involves addressing inflammation and systemic processes while minimizing tissue damage. NSAIDs are commonly used to control pain, fever, and inflammation [1,4]. Proper pain relief is crucial during surgical procedures and conditions such as mastitis, metritis, and lameness [1,4]. Studies have shown that administering analgesics after calving improves recovery, appetite, and milk production [1,2]. Pain is also a significant concern during difficult calving for both the cow and the calf [1,4].

Recent advancements in pain assessment have improved our understanding of bovine pain indicators and led to the development of approved analgesics, optimized dosing strategies, and better pain management protocols [1].

Key words: pain, welfare, behaviour, therapy.

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MODERN REPRODUCTIVE MANAGEMENT IN DAIRY COWS: A COMBINATION OF CURRENT TECHNOLOGIES AND PROVEN CLINICAL EXAMINATION TECHNIQUES

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The reproductive performance of a dairy herd influences the timing of pregnancy during lactation, milk production efficiency, herd demographics and herd replacement dynamics [1]. There has been considerable concern about the decline in reproductive performance of Holstein cows from the late 1950s to 2000 [2]. The significant improvement in reproductive performance after 2000 coincided with the introduction and implementation of synchronisation programmes, the development of automated activity monitoring systems, the modernisation of facilities and many improvements in other aspects of dairy cattle management [3–4]. It is well known that peripartal diseases in particular have a negative impact on the fertility of dairy cows. As our understanding of the underlying biology of subfertility in cows with these diseases is poor, methods to alleviate depression in pregnancy must be holistic and aim to minimise the risk factors that predispose cows to disease [5]. The most commonly used data types in the context of individual animal management were cow activity, rumination, milk yield and milk conductivity. Farmers see benefits in the involvement of the vet and many want to be proactively asked about the data available [6]. Targeted reproductive management aims to identify subgroups of cows that require a specific reproductive management strategy to optimise reproductive performance. Sensor-based technologies for real-time monitoring of individual cow behaviour offer unprecedented opportunities to develop predictive tools for use in targeted reproductive management [7]. Of particular importance appear to be the intensity of an estrus event and the expression of estrus within the voluntary waiting period. Both traits appear to be robust predictors of individual cow fertility and could be used as selection criteria for targeted reproductive management. Targeted reproductive management may also provide an opportunity to reduce the use of reproductive hormones without compromising reproductive performance [8, 9]. This is because the number of cows per production unit continues to increase for economic reasons [10], resulting in increased workload for farmers and vets in aspects such as reproductive assessment, particularly pregnancy diagnosis. Veterinarians are exposed to various occupational hazards, and increased workload is a major contributor to mental instability, leading to mental illness and physical disorders, which disproportionately affect large animal veterinarians. One way to reduce veterinarians' workload and free up time for other useful veterinary examinations is to combine point-of-care pregnancy tests or laboratory tests for bovine pregnancy-associated glycoprotein in plasma or milk with transrectal ultrasonography [11].

Keywords: targeted reproductive management, precision livestock farming, pregnancy diagnostics.

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HERD HEALTH MANAGEMENT OF DAIRY COWS IN LITHUANIA: WHERE ARE WE?

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The study explores innovative approaches to herd health management in Lithuanian dairy farms, focusing on the use of real-time monitoring systems to enhance disease prevention, improve reproductive management, and optimize overall farm efficiency. Key advancements include automated milk analyzers, in-line sensors, and real-time biomarker monitoring for early detection of subclinical ketosis (SCK) and subclinical acidosis (SCA).

A central hypothesis tested was the relationship between milk fat-to-protein (F/P) ratio and metabolic health status. Data from 320 cows during early lactation revealed that cows with SCK exhibited a 36% higher F/P ratio and elevated NEFA levels compared with healthy cows, while SCA cows had a 23.77% lower F/P ratio. These findings establish the F/P ratio as a robust non-invasive biomarker for early metabolic disorder detection.

The study also highlights the potential of milk composition analysis for monitoring the energy balance, calving ease, and susceptibility to mastitis. Automated systems like the BROLIS HerdLine Milk Analyzer demonstrated high accuracy in measuring fat, protein, and lactose concentrations, offering actionable insights into cow health.

Incorporating these technologies allows for better energy management, reduced greenhouse gas emissions, and enhanced farm sustainability. The findings emphasize the integration of sensor technology in dairy farming as a vital tool for advancing herd health management and addressing challenges related to metabolic disorders and environmental impacts.

Keywords: dairy cow health, milk fat-to-protein ratio, subclinical ketosis, innovative technologies, biomarkers, sustainable dairy farming.

MANAGING SUBACUTE RUMINAL ACIDOSIS IN DAIRY CATTLE

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The feed composition for high-yielding cows under intensive production conditions contributes to subacute rumen acidosis (SARA), which leads to pathologies such as rumenitis, laminitis, reproductive disorders, and decreased productivity and longevity. The first part of the study aimed to investigate the interrelations between reticulo-ruminal (RR) pH, productivity (P), milk fat (MF), milk protein (MP) level and somatic cell count (SCC) in milk. RR pH was established using intra-ruminal boluses *SmaXtec*. The second part of this work investigated whether the rumen wall thickness (RWT) is correlated with the cow chewing activity (ChA) induced by feed composition changes. Initially, four dairy cows (10 to 34 after parturition) were monitored using intra-ruminal boluses over a 79-day period. The dynamic of RR pH was analyzed in context with P, MF, MP, and SCC. Then, RWT in 44 cows (16 first-lactation and 28 older cows) was analyzed relating to data obtained from the neck ties sensors (*Allflex Livestock Intelligence system*). Despite the similar feeding, an individual RR pH fluctuating range in each cow was detected, and it was statistically different in all test cows 6.0 ± 0.05, 6.2 ± 0.04, 6.3 ± 0.06 and 6.5 ± 0.05, respectively, especially between the first and the fouth cow ($P < 0.05$). MF and MP levels were not statistically significantly different just between the 2second and the third cows, but these parameters were higher in the first and fourth cows ($P < 0.05$). The *P* was the lowest in the first cow (26.1 ± 0.32 kg/day), which had the lowest RR pH, and it was significantly lower than in the fourth cow (29.4 ± 0.34 kg/day), which had the highest RR pH. Higher milk yield was in the second and third cows (39.2 ± 0.29 and 31.2 ± 0.31 kg/day). A weak correlation between RR pH and energetically corrected milk (r = 0.19; $P < 0.01$) and MP level (r = 0.35; $P < 0.01$) were detected in all cows together. In our study, MF, MP, and SCC were not altered statistically significantly due to feed composition changes $P > 0.05$. A negative statistically significant correlation was established regarding ChA and RWT dynamic 10 days after the changes in feeding composition (r = 0.45; $P < 0.01$). Cows thad had RWT increased diminished *P* (35.3 ± 6.94 and 40.0 ± 8.14 kg/day, respectively, $P < 0.05$). Diminished ChA and increased RWT tend to diminish cow *P*. The longer lifespan (2760 days) was for the first cow with the higher RR pH, and the second was for the fourth cow (2336 days) with the lowest RR pH. For cows with medium RR pH, it was 2021 and 1797 days, respectively. In conclusion, despite the same feeding composition, the reticulo-ruminal pH dynamic could be in individual ranges for particular cows. Rumen wall thickening is a sign that demonstrates the effect of the feed consumed on the cow organism and productivity.

Keywords: SARA, cow chewing activity, rumen wall thickness.

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EFFECT OF THERMAL ENVIRONMENT ON DAIRY COWS

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Due to global warming, heat-induced stress is becoming an increasingly important problem for dairy cows in Lithuania. A temperature-humidity index (THI) is a single value representing the combined effects of air temperature and humidity associated with the level of heat stress.

The aim of this study was to evaluate the effects of THI on the level of stress hormone cortisol in cows and productivity of dairy cows in climatic conditions of two dairy farms in Lithuania.

The study was conducted in 2019–2020 in two selected dairy farms in southern Lithuania. Necessary data on environmental conditions and productivity of cows on farms were collected, averages of temperature-humidity index were calculated. 20 urine samples were tested to determine the level of stress hormone in cows. Statistics were processed using statistical data analysis.

The largest differences between the average air temperatures (^{0}C), relative humidity (%), air velocity (m/s) and temperature-humidity index values in the farm regions were observed in the winter season.

A statistically significant dependence of cortisol concentration in the body on THI was also found ($r = 0.902$, $P < 0.05$).

In both farms, the highest temperatures-humidity index averages were recorded in 2019 June (74.2 and 73.9). The statistical analysis demonstrated that the temperature-humidity index had a negative effect on the milk yield, fat content and protein content of cows ($P < 0.05$).

Keywords: dairy cows, temperature-humidity index, cortisol, bulk milk characteristics.

SHEEP ANAESTHESIA PROTOCOL EVALUATION FOR TRAUMATOLOGY SURGERIES

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In recent years, approximately 20 000 sheep have been used every year in biomedical research across Europe. Especially orthopaedic studies frequently rely on sheep because of the weight and size of the animal, as well as the regeneration time of healing like in humans. Here, surgical interventions are commonly used, and researchers are obliged to minimize any kind of suffering according to the 3R principle (reduce, refine, replace) and animal's welfare legislation. There is responsibility to ensure appropriate anaesthesia and/or analgesia for all ruminants undergoing painful procedures. The aim of this study is to evaluate how the heart rate and non-invasive arterial pressure of sheep change during traumatological operations.

Twenty female Lithuania black-headed sheep at the age of 13–14 months and 30–35 kg of body weight were obtained and handled at the Biological Research Centre of the Lithuanian University of Health Sciences. Permission for the study was obtained from national regulatory bodies. After arrival, the animals were given 2 weeks of habituation to the staff members and regular handfeeding. Prior to surgery, the sheep were not fasted. The sheep underwent general anaesthesia. The sheep were pre-treated with xylazine (0.05 mL/kg, i.m.) and, after 5 min, with fentanyl (1 mL i.m.) before general anaesthesia was induced using propofol CRI (20 mg/kg/h, i.v.). Following orotracheal intubation, anaesthesia was maintained with propofol (20 mg/kg/h), fentanyl (3 mL/h), 21% of oxygen, and 1 L/min of ambient air by inhalation. Oxygen saturation, temperature, heart rate (HR), respiratory rate (RR) and non-invasive blood pressure were monitored continuously. For local anaesthesia, 2% lidocaine (max. 4 mg/kg,) was administered along the incision sites. As soon as the animals breathed spontaneously, they were ex-tubated and the rumen tube was removed. Regarding pain management, ketoprofen (2 mg/kg, i.v.) was administered following 3 days post-surgery.

In this study, it was observed that only after premedication the heart rate was lower than after starting the procedure after 15 min, 95.8 ± 27.63 and 104.4 ± 31.1 beats per minute, respectively. This can be influenced by the intubation of the animal, because approximately after 10–15 min from laying the animal on the table, the animal was intubated. The heart rate remained within normal range for young sheep throughout the operation. The average duration of surgery was 52 ± 22.5 min. Vital parameters were monitored every 15 min during surgery. During data processing, the dynamics of mean non-invasive arterial pressure were observed, MAP was highest at the beginning, 127 mmHg ± 27.3 mmHg, and slowly decreased during the entire surgery. This is possibly the influence of anaesthetics, as all drugs used for anaesthesia dilate blood vessels and thus reduce arterial pressure. Blood pressure remained higher than normal range during the entire procedure.

The study found that the blood pressure of the sheep remained high even though signs of pain such as the pinch test, reflexes and pupillary movement were negative, ensuring adequate analgesia. An anaesthesia protocol, ensuring analgesia and adequate anaesthesia were used, but closer monitoring is needed in future studies.

STANDING ROMANOV VERSUS LITHUANIAN BLACKHEAD SHEEP HEART ULTRASOUND METHODOLOGY

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Baseline echocardiographic measurements are essential for accurate and reliable diagnostics of cardiovascular diseases. To avoid misinterpretation of echocardiographic images and measurements, establishing standardized reference values is necessary. Several studies have highlighted the suitability of sheep as models for cardiovascular research due to their morphological similarities to humans, including comparable adult heart size, cardiomyocyte nuclei count, venous drainage patterns, and physiological responses to cardiovascular disease induction [1]. Consequently, sheep are widely used and considered reliable in biomedical imaging studies [2, 3]. Additionally, the increasing popularity of sheep as companion animals, appreciated for their gentle nature and ease of care, has elevated the need for precise cardiovascular diagnostics in veterinary medicine [4]. The aim of this study was to establish cardiac ultrasound methodology of two Lithuanian sheep breads to asses baseline cardiac ultrasound measurements.

Physically mature Lithuanian blackhead sheep (LBS) (N = 12) aged 300 to 310 days weighing 33.1 ± 1.47 kg and 20 Romanov sheep (RS) (N = 20) aged 180 to 200 days weighing 22.6 ± 1.80 kg were analyzed in this study. Two-dimensional, M-mode and pulsed wave Doppler echocardiographic studies were accomplished while measuring normal cardiac dimensions, time indices and blood volumes. A 4–5 MHz phased-array transducer was used to acquire the images. The transducer was pressed to the skin approximately 2–3 cm dorsal to the fourth and fifth right intercostal spaces. All the echocardiographic measurements were significantly correlated with body weight and breed.

This study demonstrated that it is possible to perform a cardiac ultrasound examination on standing sheep with ease, without disrupting physiology or causing stress to the animal. The sheep remain calm while standing, able to ruminate and breathe without interruption. No anesthesia was used in this study, meaning the physiological parameters of the heart were not affected. This study results showed that it is possible to measure the left ventricle ejection fraction, which was significantly higher in LBS. The left ventricular posterior wall index (LVPi) and the intraventricular septum index were measured, and LVPi was significantly higher in Romanov sheep. Using ultrasound examination on standing sheep makes it possible to calculate early and late diastolic pressure ratio, which was higher in Romanov sheep. The human cardiac ultrasound protocol was adapted for sheep ultrasound measures, and aortic flow acceleration time as well as velocity time integral were observed in both sheep breeds. Depending on the protocol, the left ventricular diastolic and systolic volume as well as the stroke volume were measured. The observed measurements were higher in LBS.

This study concluded that echocardiography could be used as a tool in diagnosing and further researching cardiac diseases and disorders of Lithuanian blackhead and Romanov sheep.

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ANALYSIS OF FACIAL EXPRESSION IN COWS AS ONE OF ANIMAL WELFARE INDICATORS

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Presently, a key component of animal welfare is the animal's affective (emotional) state and its assessment. Such studies attract great scientific interest focused primarily on negative experiences such as pain, fear, and suffering, which farm animals experience at different stages of their lives. Increased numbers of studies highlight that cows exhibit specific pain behaviours and facial expression as a new and reliable technique which could be developed to recognize and assess pain. Pain in farm animals can be caused by disease, injuries, poor hygiene and housing or inadequate management practice. However, disease (such as mastitis, lameness, peritonitis, etc.) is a major cause of pain in dairy cows, negatively affects welfare, and decreases productivity; therefore, analysis of facial expression can be a valuable early pain detection tool [1–4]. Thus, we aimed to determine parameters of dairy cows (that were affected with subclinical or clinical mastitis) on a facial expresion scale. A total of 30 cows were allocated into equal (N = 10) three groups: 1 (control, healthy cows), 2 (subclinical mastitis) and 3 (clinical mastitis); and photo images (N = 150) based on facial expressions were evaluated. Pain assessment relied on the evaluation of potential pain-related facial expression performances in four regions of the face (each region was scored on a 0–2 scale). Eye and ear position, nostril and facial expression were measured as described in scientific literature [1, 3]. The condition of the changed by 50% ($P = 0.07$) and 37.50% ($P = 0.01$), the ear by 42.85% ($P = 0.06$) and 42.85% ($P = 0.04$), the nostril by 62.50% ($P = 0.18$) and 50.00% ($P = 0.05$), and the facial expression by 33.33% ($P = 0.01$) and 22.22% ($P = 0.001$) in groups 2 and 3 of cows, respectively, compared with the group 1. Early detection (changes in a cow's normal facial expression suggest the presence of pain) of any health problem will ensure that cows can get proper health care as soon as possible, reducing the impact on welfare, productivity and dairy farm economy. We extend our study by developing an automated system (utilizing the power of artificial intelligence) for the detection and analysis of facial expressions.

Keywords: cow, welfare, pain, facial expression, mastitis.

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EVALUATION OF THE TRANSITION PERIOD OF COWS: PRINCIPLES OF STANDARD OPERATING PROCEDURES (SOP)

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The transition period for dairy cows, which spans 3–4 weeks before and after calving, is a critical phase influencing animal health, milk production, and farm efficiency. During this period, cows undergo significant metabolic and physiological changes, making effective management through standard operating procedures (SOP) crucial. Proper nutrition is key during the transition period to prevent metabolic disorders like ketosis and hypocalcemia, which affect milk yield and reproductive performance. A balanced diet with adequate protein, energy, and micronutrients helps facilitate smooth lactation. Studies indicate that feeding strategies with a proper energy-to-protein ratio reduce ketosis risk and enhance milk production [1].

The transition period carries an increased risk of metabolic and infectious diseases, such as subclinical ketosis and mastitis. Regular veterinary check-ups and blood parameter monitoring (e.g., non-esterified fatty acids, beta-hydroxybutyrate) help detect health risks early, enabling timely interventions. Continuous health monitoring during this time reduces disease incidence and improves milk production [2]. Housing conditions significantly impact cow comfort and productivity. Factors such as heat stress, poor ventilation, and inadequate bedding can decrease feed intake and increase disease susceptibility. Research shows that well-ventilated barns and comfortable resting areas improve cow welfare and milk production efficiency, while clean bedding reduces mastitis cases [2, 3] Behavioral changes during the transition period, such as fluctuations in blood calcium levels, can indicate physiological stress and metabolic imbalances. Monitoring cow behavior, including feed intake and lying time, allows for early detection of health issues, enabling preventive actions that support health and productivity [2]. Farm management practices and human factors play a crucial role in SOP implementation. Variations in management practices, herd genetics, and seasonal factors influence cows' responses to transition protocols. Studies show that training farm personnel in SOP principles reduces disease incidence and improves herd performance. Educating staff on proper feeding and health monitoring can reduce metabolic disease rates and boost milk production [2, 3].

Managing the transition period effectively using SOP principles is vital for optimizing cow health and productivity. Balanced nutrition, continuous health monitoring, and appropriate housing conditions are essential. By following evidence-based SOP guidelines, farmers can reduce disease risks, increase milk yield, and improve farm efficiency. Future research should explore genetic differences, seasonal variations, and farm-specific adaptations to enhance SOP effectiveness.

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CATTLE C-SECTION: ADVANTAGES AND DISADVANTAGES

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The cesarean section is among the oldest surgical procedures performed in veterinary medicine [1]. The indications for a cesarean operation and the rationale behind making an appropriate decision have been extensively reviewed [2]. Analysis of published cases indicates that the following six major indications collectively account for 90% of all cesarean operations: fetomaternal or fetopelvic disproportion, incomplete dilation or induration of the cervix, uterine torsion, fetal anomalies, abnormal fetal disposition, fetal emphysema [3]. However, among cattle veterinarians, there is a diversity of opinions regarding cesarean sections: some advocate for the procedure, citing its benefits and favorable outcomes, while others contend that it has a negative economic impact and poor long-term prognosis [2]. The main C-section techniques and their advantages are the following [2]:

- Left paralumbar: prevents intestinal prolapse but may cause rumen prolapse and difficulty extracting the calf;
- Right paralumbar: harder to keep intestines inside; used for large calves in the right uterine horn;
- Ventral midline/paramedian: preferred when the cow cannot stand; better for fetal emphysema but challenging suturing and udder interference;
- Ventrolateral (lying): hidden incision, easier uterus access, but high infection and herniation risk;
- Left-sided oblique (standing): commonly used, allows better uterine access and calf extraction but requires physical strength.

The primary determinant of a successful cesarean section in bovines is the timing of the intervention. Early surgical intervention significantly increases the probability of survival for both the cow and the calf. Current veterinary guidelines recommend performing a cesarean section within a maximum of six hours to optimize outcomes [1]. If legs and/or the head cannot be manually repositioned into the birth canal, an immediate decision to perform a C-section should be made [2]. Caution should be taken when predicting the outcome in cases of emphysematous fetuses: fetotomy is not always an option, and in such cases, the survival rate after surgery is also quite low. Intensive post-operative care and high doses of medication are essential for the cow's recovery [4].

Keywords: cesarean section, cattle, pros and cons.

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EFFECT OF LOW FREQUENCY OSCILLATIONS ON CASEIN MICELLE SIZE IN RAW MILK

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The application of vibration therapy of blood flow improvement in the medical field has been explored intensively during recent decades. However, compelling evidence about the health effects of vibration (especially treatment effectiveness) is still unclear. The treatment capabilities of the low power acoustic pulse therapy (APT) have been widely reported. It is known to produce various responses in biological tissues [1, 2]. APT pressure (10 ± 15 megapascal) has been shown to produce new blood vessels and improve tissue function with long term exposure [1]. In our previous investigation [3], an unbalanced vibratory motor was applied to the DeLaval Harmony model milking unit. During in vivo experiments, while milking, the vibrator induced mechanical milking-similar vibrations in the udder. The vibrations were spreading to the entire udder and caused physiotherapeutic effects such as activated physiological processes and increased udder base temperature by 0.57℃, thus increasing the blood flow in the udder. The application of low-frequency vibrations did not alter observable changes in milk yield and quality parameters oranimal welfare indicators. Moreover, casein micelle size and milk fat globules can vary depending on farming factors (seasonal variation, stage of lactation), and cow genetics [4]. Here, we aimed to estimate the effect of vibration on casein micelle size in raw milk under in vitro conditions. Casein micelle size analysis was performed using Beckman Coulter - Delsa™Nano Series analyzer. Raw milk samples were exposed to the 25-Hz vertical and 41-Hz horizontal vibrations for 15 s (A sample), 1 min (B sample), 2 min (C sample) and 7 min (D sample). K sample (no vibration) served as control. Milk measurement temperature was 24.5–25.00°C, refractive index was 1.3328, viscosity was 0.8980 (cP), scattering intensity was 30617 (cps), and attenuator was 0.3 (%). The average size of casein micelle size in raw milk distributed as follows: 646.1 nm, 642.3 nm, 640.5 nm, 621.5 and 617.6 nm in K, A, B, C, and D samples, respectively. To sum up, we can state that vibration had no significant influence on casein micelle size in raw milk.

Keywords: low-frequency vibrations, cow's raw milk, casein micelles size.

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ULTRASOUND SCANNING FOR QUALITY ASSESSMENT OF LONGISSIMUS DORSI MUSCLE IN CATTLE: MEASURING MEAT QUALITY IN LIVE ANIMALS

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The meat industry is observing an increase in the population of purebred animals from the finest meat breeds globally, due to the introduction of innovative solutions in animal husbandry, feeding practices, breeding techniques, and overall management [1]. Ultrasound can be added in breeding programs to select animals with the potential to pass on superior meat qualities [2]. Key indicators of good quality include fat thickness, the area of the longissimus dorsi muscle, hide thickness, the percentage of intramuscular fat, and the marbling score [3]. The aim of this study was to determine the relationship between daily body weight and indicators of quality meat using ultrasound imaging. During the study, a total of 603 cattle scans were performed. Daily weight gain was calculated by deriving the average of age and weight. Ultrasound scan was performed using MyLabOneVET ultrasound equipment (Esaote, Germany). During the scan, the sensor was pressed deep into the muscle for a few seconds until the image on the screen was smooth and consistent. Different structures are visible during the examination: subcutaneous fat, transverse section of the long back muscle, and fat layer near the rib cage. After the examination, the created images are saved. After the examination, the stored images are evaluated and adipose tissue measurements are performed in the scanner, then the muscle area and the fat layer near the ribs are evaluated in this way. The data were calculated using installed formulas, according to which it is possible to accurately calculate the yield percentage while the animal is still alive. The measurement data were coded and compiled using an Excel (MsOffice, USA) calculator. To calculate the relationship between the outcome variables, the Pearson correlation test was performed, and the correlation coefficient R was calculated, which does not depend on the measurement units of the variables. This study was conducted in six different farms in Lithuania. Before each ultrasound examination, the cattle were weighed, and their heads were fixed accurate scanning. After analyzing the collected data, it was found that the daily weight of the animals varied from 0.51 kg/d up to 2.7 kg/d, on average 1.41 kg/d. A weak positive correlation ($r = 0.372$) was found in correlation tests between daily body weight and long back muscle area, $P < 0.01$. The study also analyzed the relationship between yield grade and daily weight gain. It was found that there was no significant correlation between these two parameters. A similar trend was observed when analyzing the relationship between daily body weight and intermuscular fat percentage (IMF): the correlation was very weak, but positive. Analysis of the data on fat thickness and daily weight demonstrated that there was no correlation between these parameters. This shows that the daily weight does not have a significant effect on the thickness of subcutaneous fat. However, a weak positive correlation ($r = 0.383$) was found between daily body weight and the thickness of the longus back muscle, $P < 0.01$.

The results of this study do not prove the benefits of ultrasound scanning. The study found only a weak relationship between the measured parameters. The study has several methodological limitations, so further research is needed to accurately assess the benefits of ultrasound scanning.

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RELATIONSHIP BETWEEN SOMATIC CELL COUNT AND REPRODUCTIVE PARAMETERS IN DAIRY COWS

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Milk yield, somatic cell count and reproductive performance are the main determinants of dairy enterprise profit, and udder health status is a factor that has a detrimental effect on both traits [1]. Rearte et al. [2] have reported the effect of somatic cells counts (SCC) on different indicators of reproductive performance, such as longer days to first service, lower conception risk and higher risk of pregnancy loss. The aim of this work was to analyze the influence of the count somatic cells in cow's milk on reproduction in dairy cows. The study was carried out on 300 Holstein lactating dairy cows, in accordance with the Law on the Care, Keeping and Use of Animals of the Republic of Lithuania. According to the count of somatic cells in milk, cows were selected and divided into three groups: Group 1 – SCC up to 200 thousand/mL (N = 100); Group 2 – SCC 201–400 thousand/mL (N = 100); Group 3 – SCC > 401 thousand/mL (N = 100). Information on cow herd reproduction (service period in days, insemination index, duration of calving in days, first heat after calving in days) was taken from the herd management system. Arithmetic means, their errors and statistical reliability of the data were calculated for each evaluated trait. Statistically reliable data were considered when $P < 0.05$. The study demonstrated a significant relationship between somatic cell count in milk and reproductive performance in dairy cows. Higher SCC levels (> 401 thousand/mL) were associated with a 40.76% increase in the service period, a 1.9-fold increase in the insemination index ($P < 0.05$), and a two-day delay in the first oestrus after calving. Additionally, gestation duration slightly decreased by 0.82% compared with cows with SCC below 200 thousand/mL. These findings highlight the negative impact of elevated SCC on reproductive efficiency, emphasizing the importance of effective udder health management in dairy farming.

Keywords: dairy cows, somatic cell count, reproduction parameters.

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INFLUENCE OF ESTRUS ON MILKING PARAMETERS IN DAIRY COWS

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Accurate estrus detection is a crucial component of reproductive management in dairy cows, influencing successful fertilization and pregnancy [1]. Effective reproductive management directly impacts milk production and the economic performance of dairy farms. In recent years, automated technologies have become increasingly common in the dairy industry [2]. One of the most significant advancements is the implementation of automatic milking systems (AMS). AMS allows cows to enter milking stalls voluntarily, individually, and without guidance from farm staff [3]. Consequently, understanding how cows interact with their environment and how this influences their behavior and movement through the AMS is essential for the system's success [4, 5].

The objective of this study was to assess the influence of estrus on various milking parameters, including milk yield (kg), milk flow rate (kg/min), and electrical conductivity of milk (μS). The study was conducted in Lithuania using data from 25 Holstein cows in their second lactation and fresh milk cows. Data were collected through the GEA "DairyPlan C21" (Germany) herd management system and the GEA Dairy Robot R9500 automated milking system, which milks the cows 2–4 times per day, based on need. Estrus detection was performed using the GEA CowScout system.

The results showed that during the estrus period, the average daily milk yield per cow was 42.8 ± 1.38 kg, which was 9.95% lower ($P < 0.05$) than the period before estrus and 7.41% lower than after estrus. A 2.32% decrease in milk production was observed after estrus. On the day before estrus, the average electrical conductivity peaked at 477.68 ± 5.18 μS, 8.6% higher ($P > 0.05$) than the estrus day. The average milk flow rate during the study was 2.76 ± 0.14 kg/min, which was 10.9% lower ($P > 0.05$) than the day before estrus. No direct correlation between the milk flow rate and estrus was found. However, estrus had a direct influence on the average milk yield. Comparison of the average milk production on the day of estrus to the day before estrus showed a decrease of 4.78 kg ($P < 0.05$).

Keywords: cow, estrus, milking parameters.

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POSSIBILITIES TO USE YEAST CELL WALLS POLYSACCHARIDES AND WALNUT NUTSHELLS FOR MYCOTOXIN DECONTAMINATION

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Mycotoxins are toxic secondary metabolites that are naturally produced by specific filamentous fungi [1]. Currently, more than 400 potentially toxic mycotoxins have been identified as dangerous contaminants in food and agriculture that cause significant harm to humans and animals through their carcinogenic, immunosuppressive, teratogenic and mutagenic effects [2]. Dairy cattle are often exposed to multiple mycotoxins that can occur before, during or after harvesting due to the high levels of maize and grass silage in their daily ration. Thus, contamination of feed with mycotoxins in the dairy sector can cause serious feed safety and security issues and significant losses for the ruminant industry.

The purpose of the current study was to assess the effectiveness of *Geotrichum fermentants*, *Rhodotorula rubra* and *Kluyveromyce marxiamus* yeast cell walls polysaccharides and walnut nutshells in reducing mycotoxin levels.

A static in vitro model of the gastrointestinal tract was used as a first assessment tool to determine the effectiveness of yeast cell walls polysaccharides and walnut nutshells in reducing levels of the mycotoxins aflatoxin B1 (AFB1), zearalenone (ZEA), deoxynivalenol (DON) and T-2 toxin concentrations. Mycotoxin concentrations were established using high-performance liquid chromatography (HPLC) with fluorescence (FLD) and ultraviolet (UV) detectors.

It was found that the greatest impact on the reduction of AFB1 and ZEA concentrations was determined with inserted *G. fermentants* yeast cell walls polysaccharides ($P < 0.05$), DON concentration with *R. rubra* yeast cell walls polysaccharides ($P < 0.05$) and T-2 toxin concentration with *G. fermentants, R. rubra* and *K. marxiamus* yeast cell walls polysaccharides ($P < 0.05$). Although the highest mycotoxin reduction effect while using walnut nutshells was established for AFB1 and DON.

The research findings suggest that yeast cell walls polysaccharides and walnut nutshells may be highly effective in reducing mycotoxin concentrations, thereby improving feed safety and quality for dairy cattle.

Key words: mycotoxins, yeast cell walls polysaccharides, walnut nutshells.

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THE INFLUENCE OF CALVES' AGE ON THE COUNT OF RUMEN BACTERIA AND PROTOZOA

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The anatomy and physiology of the digestive system of a newborn calf and a mature ruminant are distinct. The digestive tract of a newborn calf is considered sterile, but colonization by a variety of microorganisms commences within 20 minutes and intensifies with each day of life [1]. The complex microbiota of the ruminant gastrointestinal tract affects not only the digestion of feed, but also the overall immune system, nutrient absorption, and general metabolism [2]. The present study aimed to investigate the influence of calves' age on the count of rumen bacteria and protozoa.

The eight-month study included 30 clinically healthy Lithuanian Black-and-White Holsteinized calves, aged from three to eight months. They were housed in compliance with the Calf Welfare Requirements. The calves were divided into three groups based on their age: group 1-G included 3-month-old calves, group 2-G included 3.5-month-old calves, and group 3-G included 8-month-old calves. All calves were provided with a balanced diet and had access to water ad libitum. Samples of rumen fluid were obtained from the calves in all groups using a probe. The count of Protozoa was determined in a Fuchs-Rosenthal counting chamber (Blaubrand, Wertheim, Germany) using an Olympus U-TV1X-2 microscope (Tokyo, Japan). The genera of protozoa were identified according to the method of Dehority [3]. Bacterial counts were determined in accordance with the methodologies outlined in the relevant standards: the Aerobic and Facultative Anaerobic Bacteria count according to LST ISO 4833-1:2013; the Lactic Acid Bacteria – LST ISO 15214:2009; the Enterobacteriaceae – LST ISO 21528-2:2009.

In 8-month-old calves, the total protozoa count was on average 18.6% higher if compared with those at 3 and 3.5 months of age. Among all age groups, *Entodinium* was the most dominant genus found in the rumen fluid. The population of aerobic and facultative anaerobic bacteria was on average 45.1% higher in 8-month-old calves than in the groups of younger animals. Lactic acid bacteria and anterobacteriaceae were first detected at 3.5 months of age, with their levels being 46.8% lower and 10.6% higher, respectively, compared to 8-month-old calves. The total count of Protozoa was found to be influenced by calf age to the extent of 68.4%. Furthermore, the count of five protozoa genera *Diplodinium*, *Enoploplastron*, *Entodinium*, *Isotricha* and *Opisthotrichum* were found to be influenced by calf age to the extent of 52.14%. Furthermore, the number of aerobic and facultative anaerobic bacteria was found to be influenced by calf age to the extent of 21.6%. Finally, the number of lactic acid bacteria was found to be influenced by calf age to the extent of 42.3%.

Keywords: bacteria, protozoa, rumen, calves, age.

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INTERPRETATION OF BLOOD BIOCHEMICAL TEST RESULTS IN CATTLE

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Biochemical testing of cow blood is essential for several reasons, critical to both animal health and the agricultural sector. Routine biochemical blood tests enable the early identification of various diseases, such as metabolic disorders. Early diagnosis facilitates timely intervention, reducing the spread of diseases and enhancing animal welfare. On farms, it is useful to study cows at different stages of lactation and also the cows in the transit period.

Blood tests can identify deficiencies or imbalances in essential nutrients, including minerals and vitamins (magnesium, calcium, potassium, phosphorus). This data is crucial for adjusting feed formulations to maintain optimal health and productivity [1].

Biochemical markers such as glucose, ketone bodies, and liver enzymes (GGT, GOT, GPT, ALP) provide valuable insights into the metabolic health of cows, which is especially important for high-yielding dairy cows prone to metabolic stress that can impact milk production and overall health [2].

Blood tests also evaluate the hormonal status of cows, helping in the management and improvement of reproductive performance. Identifying issues like subclinical ketosis or mineral imbalances can prevent reproductive failures and boost fertility rates.

Early disease detection and treatment lower mortality rates and production losses, yielding significant economic benefits for farmers. Regular biochemical testing ensures cow health, directly influencing milk production efficiency and profitability.

Keywords: cows, blood tests, biochemical markers.

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PRECISION LIVESTOCK FARMING: CHALLENGES, OPPORTUNITIES, PROSPECTS AND ENVIRONMENTAL IMPACTS

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The effects of digitalization are most extensively recorded in the field of dairy cattle husbandry [1]. Precision livestock farming (PLF) is a noteworthy emerging technology that possesses the capacity to significantly transform the livestock farming sector, set apart from several other intriguing developments [2]. The primary objective of PLF is to enhance the efficiency and effectiveness of animal production, as well as improve the overall health and well-being of animals [3]. Smart farming, also known as PLF, has the potential to enhance animal welfare on farms, reduce greenhouse gas emissions and improve environmental performance, facilitate product segmentation and marketing of livestock products, deter illegal livestock trade, and bolster the economic stability of rural regions when implemented effectively [2]. The technologies encompassed in this list are automatic weighing systems, radio frequency identification sensors for detecting and monitoring individual animal behaviour, body temperature monitoring, geographic information systems for evaluating and optimizing pastures, unmanned aerial vehicles for managing herds, and virtual fencing for managing herds and grazing [4]with increased consumption of animal products predominately due to the advancing economies of South America and Asia. As a result, livestock production systems have expanded in size, with considerable changes to the animals' management. As grazing animals are commonly grown in herds, economic and labour constraints limit the ability of the producer to individually assess every animal. Precision Livestock Farming refers to the real-time continuous monitoring and control systems using sensors and computer algorithms for early problem detection, while simultaneously increasing producer awareness concerning individual animal needs. These technologies include automatic weighing systems, Radio Frequency Identification (RFID. The objective is to automate the management of animals on large farms through the use of algorithms and networked smart devices that continuously monitor individual animals, compare this data to expected norms, and make decisions regarding climate, nutrition, and reproduction, among other things [2].

Various techniques were employed to ascertain PLF technology. These methods encompass queries conducted on scientific literature databases, attendance at technological exhibits, and input received from peers.

Consequently, it is anticipated that PLF will enhance the efficiency of animal production, as well as improve animal health and welfare. The implementation of PLF has the potential to facilitate advancements in animal health, production, and welfare. Nevertheless, there is currently little scientific evidence to support the notion that the use of PLF has any discernible effects. It is anticipated that PLF will modify the interaction between animals and humans and will significantly influence veterinary treatment [3].

Keywords: dairy cattle; herd health; precision livestock farming.

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ENVIRONMENTAL IMPACT REDUCTION: STRATEGIES FOR MITIGATING ENTERIC METHANE IN DAIRY FARMING

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Rising concerns about greenhouse gas emissions from dairy farming emphasize the need for strategies to reduce methane (CH_4) emissions from cattle. Enteric CH_4, generated by rumen microbial fermentation, accounts for 35–55% of on-farm greenhouse gas emissions, with dairy production responsible for over 70% of agricultural outputs [1]. This study evaluates nutritional strategies to mitigate enteric CH_4, focusing on natural feed additives to improve nutrient utilization and lower emissions.

Certain studies propose that substituting grass silage with maize silage enhances rumen fermentation of propionate over acetate, thereby diminishing CH_4 emissions in dairy cows. When maize silage entirely supplanted grass silage in the diet of dairy cows, CH_4 emissions diminished by 8–11% [2]. Feeding cows the red seaweed *Chondrus crispus*, comprising 6% of the dry matter, resulted in a 13.9% reduction in CH_4 [3] large-scale adoption depends on technical and financial factors, as well as validation from pilot studies.</p></sec><sec><title>Methods</title><p>A survey was developed to identify barriers and drivers towards the adoption of CH₄-reducing algal-based feeds. Concurrently, a randomized complete block design study was conducted to investigate the effect of <italic>C. crispus</italic> on enteric CH₄ emissions and milk production in a typical Maine organic dairy farm. Twenty-two organically certified Holstein and Jersey cows averaging 29 ± 6.8 kg of milk/d and 150 ± 69 days in milk, were blocked and randomly assigned to a control diet without <italic>C. crispus</italic> (0CC. Cows fed daily with a 1 g blend of essential oils exhaled less CH_4 (444 ± 12.5 L/d) than cows fed a feed without the mixture (479 ± 12.5 L/d) [4]mainly in the form of methane. Essential oils are a group of plant secondary metabolites obtained from volatile fractions of plants that have been shown to exert changes in the rumen fermentation and may alter feed efficiency and to reduce methane production. The objective of this study was to investigate the effect on rumen microbial population, CH4 emissions and milking performance of a mixture of essential oils (Agolin Ruminant, Switzerland. The utilization of tannin or saponin-rich flora has become increasingly favoured for mitigating or eradicating protozoa in the rumen. Another study has shown that a commercial citrus extract diminished methane production while enhancing propionate concentration and the concentration of propionate-producing bacteria *Megasphaera elsdenii* [5]. The incorporation of 41 g of oilseed rape oil per kg of dry matter in the diet resulted in a 22.5% reduction in daily CH_4 emissions from dairy cows [6].

Incorporating specific compounds into cattle diets can reduce methane emissions, improve feed efficiency, and support animal health, offering a sustainable approach to mitigating agriculture's climate impact while maintaining farm profitability.

Keywords: methane emission, cattle, health.

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RISK FACTORS DURING PREPARTUM AND THEIR EFFECT ON CALF HEALTH

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Prepartum period has a lot of influence on the health and production of both the cow and the calf. A number of environmental and nutritional factors have a part to play in the metabolic status of the dam, its immune system response, and colostrum quality, which in turn will influence health parameters of the offspring.

One of the main environmental factors, increasing in importance and severity nowadays, would be heat stress that a preparturient dam experiences. A number of scientific papers show that this factor not only has a sudden and severe effect on the cow and reduces the newborns weight and health status but might also have a long-term effect on the future productivity of the offspring.

Nutrition and the metabolic status of the cow during prepartum influences the metabolism of the cow for the upcoming lactation and the health status of the calf. Fatty acid synthesis, quality and quantity modifies certain pathways responsible for the immune response, biosynthesis in the liver and on the mammary gland, especially for the production of the colostrum. A dam, with a compromised metabolism, usually will have an imbalance of fatty acids, which will also be mirrored in the colostrum quality. Not only might the colostrum quality have an impact on the health status of the calf, but the offspring's metabolism is already at risk of being affected in the uterus during development.

Therefore, the prepartum period should be further closely studied and better prophylaxis and wellbeing for the dams should be provided in order to ensure a better health status not only for the cow, but for the ofspring as well. In doing so, future generations of cattle have a better chance of reaching their predisposed potential and the animals have a better chance of surviving for more lactations.

Keywords: offspring, prepartum, metabolism, risk factors, colostrum, heat stress.

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