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Makroelementi u mlijeku lakon ovaca u ovisnosti o stadiju laktacije

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MACROELEMENTS IN THE MILK OF THE LACAUNE DAIRY SHEEP DEPENDING ON THE STAGE OF LACTATION

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SUMMARY

This research aspires to demonstrate how the stage of lactation influences the content of macroelements in the Lacaune sheep milk. The research was conducted with 30 Lacaune sheep in their fourth lactation during the early (60 days), middle (120 days), and late lactation stages (180 days). The macroelement concentration (Ca, K, Na, and Mg) of the Lacaune sheep milk was determined by the ICP. As lactation progressed, a significant increase in the concentrations of Mg and Na and a significant decrease in K were observed in the Lacaune sheep milk. A stability of Ca and P concentrations during lactation was demonstrated by the lack of significant variation in the Lacaune sheep milk, based on the stage of lactation. A significant positive correlation was detected between Ca:Mg, Ca:P, and K:P, as well as a significant negative correlation between Mg:K and K:Na. The obtained results prove a significant effect of lactation stage on the content of most macroelements in the Lacaune sheep milk, except Ca and P, which should be included in the research scheme.

Keywords: Lacaune sheep, milk, macroelements, correlation

INTRODUCTION

The demand for sheep milk is increasing, and dairy sheep breeds are becoming increasingly important (Antunović et al., 2022). Sheep milk quality is reflected throughout the prism of determination of the basic chemical indicators and the concentration of milk minerals. First of all, this refers to the determination of the macroelements, namely Ca, P, K, Na, and Mg. There are numerous functions that the macroelements mentioned fulfill in the human body. Calcium is an element that is accountable for muscle contraction, enzymes activation, and heart rhythm (Cashman, 2002). According to Zamberlin et al. (2012), sodium is a cation in extracellular fluids that is in charge of acid – base balance and osmotic pressure, whereas potassium is an intracellular cation that is vital for blood pressure, nerve impulse transmission, and bone metabolism. The Ca, P, and K requirements of young animals and children in the developmental phase are important for normal metabolic processes and appropriate growth and development (Al – Wabel 2008; Zhou et al., 2017). Calcium, P, and Mg are present in sheep milk in good amounts, and their concentration is often higher than that of other ruminant milks, particularly the cow's milk (Burrow et al., 2016; Oh and Deeth, 2017).

With an average concentration of 193.5 mg/100 g, it contains almost 36% more calcium than the cow's milk and 31% more than goat milk (Chia et al., 2017). A typical content of macroelements in sheep milk, according to Raynal – Ljutovac et al. (2008), amounts as follows: Ca 195 – 200, P 124 – 158, K 136 – 140, Na 44 – 58, and Mg 18 – 21 mg/100 g. Essential macroelements are mainly provided to the animals by feed supplements, and the amount administered with the feed, mineral blocks, or both affects their contents in milk. Nonetheless, animal variables significantly control the concentrations of several of them, such Ca and P (Gaignon et al., 2018). The concentrations of macroelements in sheep milk vary considerably and depend on numerous factors, primarily nutrition, rearing system, the sequence and stage of lactation, and the breed (Chia et al., 2017). The Lacaune sheep breed is a French breed specialized in milk production (Barillet et al., 2001), and is one of the nine specialized dairy sheep breeds mainly distributed in the

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Mediterranean countries and Europe, comprising two – thirds of the world's dairy sheep population (Pulina et al., 2018). Very few studies investigate the lactation stage concerning the macroelements content of the Lacaune sheep milk. Therefore, this study aspires to demonstrate how the stage of lactation affects the macroelement content of milk from Lacaune sheep.

MATERIALS AND METHODS

The study was authorized by the Faculty of Agrobiotechnical Sciences' Animal Welfare Commission and conducted in compliance with the Animal Welfare Act's legal provisions, as stipulated by the *Official Gazette* of the Republic of Croatia, No. 133 (2006), No. 37 (2013), and No. 125 (2013).

Selection of experimental animals, feed and milk sample gathering and analysis

Thirty Lacaune sheep were involved in the research. The sheep were chosen from a flock of 200 sheep bred on the Orkić family farm in Gundinci. The results of the present research are a part of a larger experiment conducted with the Lacaune sheep (Antunović et al., 2023). The sheep were in a good physical condition, in good health, and of uniform age, lambing date, and number of lambs in the litter. The first milk sample was taken immediately after lamb weaning and refers to the early stage of lactation (60 days ± 5 days). The monitoring was then continued, with milk sampling in the middle stage of lactation (120 days ± 5 days of lactation) and finally in the late stage of lactation (180 days ± 5 days). The sheep chosen for the study were given an *ad libitum* supply of alfalfa hay, 600 g of a cereal mixture (oats and barley), and 1 kg of pelleted feed mixture (15 percent crude protein) every day. The sheep enjoyed a free access to salt and water. The milk samples were filled into the bottles (200 mL) during the morning milking. Following a vortex homogenization (VIBROMIX 10, Tehtnica, Slovenia), they were kept in a 4°C cool box and subsequently frozen (– 80°C) until microwave digestion was carried out. After being submerged in the 20% HNO_3 for a whole day, the sampling bottles were cleaned with deionized water. The procedure outlined in the research (Belete et al., 2014) was used to digest the feed and sheep milk samples from sheep. The approach was validated using the milk powder (NCSZC73015) as a reference material. Next, a 60 ml Teflon digestion container was filled with 3.0 ml of each liquid milk sample. After carefully shaking the mixture and letting it rest for 10 minutes, the optimized amounts of 6 milliliters of 70% nitric acid and 1 milliliter of 30% hydrogen peroxide were added. The container was subsequently shut. Using the following order for the microwave digestion program, the milk and feed samples were subjected to a closed microwave digestion on the Mars 6 microwave system (CEM, Matthews, NC, USA): 50 W, 165 °C (10 min); 80 W, 190 °C (20 min); and 0 W, 50 °C (10 min). A 25 ml dilution of the digest was made using deionized water. A continuous hydride generation technique was applied to evaluate the digested samples with regard to the amounts of macroelements (Ca, P, K, Na, and Mg) using an inductively coupled plasma mass spectrometer (ICP – MS, Agilent 7500a, Agilent Technologies Inc., California, USA). The samples (Optima 21000 DV, Perkin Elmer, Massachusetts, USA) were prepared for the ICP run. Table 1 lists the instrumental detection and quantification limits for figuring out what quantity of microelements was present in the sheep's milk and other feed items. Table 2 lists the macroelements that were present in the feed and water that the sheep consumed.

Table 1. The macroelement content of the Lacaune sheep feed and milk is determined using the instrumental detection and quantification limits (mg/kg)

Tablica 1. Detekcijski i kvantifikacijski limiti uređaja za utvrđivanje makroelemenata u mlijeku lakon ovce (mg/kg)

Elements/ *Elementi*	Instrumental detection limits/ *Detekcijski limit uređaja*	Instrumental quantification limits/ *Kvantifikacijski limit uređaja*
Ca	0.01306	0.043533
Mg	0.023112	0.07704
K	0.401634	1.33878
Na	0.047576	0.158587
P	0.040048	0.133493

Table 2. The concentration of macroelements in the Lacaune sheep feedstuffs and water during lactation (mg/kg DM)

Tablica 2. Koncentracija makroelemenata u hrani i vodi ovaca lakon pasmine tijekom laktacije (mg/kg suhe tvari)

Elements/	Feed/ *Hrana*			Water/
Elementi	Feed mixture/ *Krmna smjesa*	Cereal mixture/ *Smjesa* žitarica	Hay/ *Sijeno*	Voda
Ca	18876.3	562.38	6315.28	37.57
Mg	4639.53	1354.04	2018.09	65.03
K	17103.2	5154.4	16343.1	6.08
Na	7042.9	109.27	1001.3	17.04
P	10960	3356	2395	0.031

DM – dry matter / *suha tvar*

Statistical analyses

The study results obtained were analyzed using the MEANS procedure, while the effect of the lactation stages of the Lacaune sheep was evaluated using the GLM procedure applying the SAS 9.4®. The Tukey test was used to compare the mean values, and $P<0.05$ was considered to be significant for the differences between the groups (lactation stage). With the implementation of the CORR technique, Pearson's correlation was applied to assess the correlations between the macroelements of the Lacaune sheep milk. If P was less than 0.05, the correlations were deemed significant.

RESULTS AND DISCUSSION

The determined average concentrations of macroelements in the Lacaune sheep milk in lactation were within the expected limits. Namely, in the milk of the Lacaune sheep grazing on pastures in the Czech Republic, with the additions of silage, hay, cereal mixture, and mineral supplement, Michlová et al. (2016) detected the average concentrations of Ca 7.57 and 2.22 g/kg (5.72 – 9.13 and 1.79 – 3.40), Mg 0.92 and 1.15 g/kg (0.72 – 1.08 and 0.83 – 1.21), K 6.16 and 9.72 g/kg (4.17 – 8.00 and 7.87 – 11.90), and Na 1.04 and 3.11 g/kg (0.89 – 1.28 and 1.64 – 4.31). Panayotov et al. (2018) detected the following average values of macroelements (mg/100 g) in the Lacaune sheep milk in Bulgaria: Ca 218.34, P 133, Na 46.88, K 112.16, and Mg 19.36. In a research conducted in Austria, Mayer and Fiechter (2012) detected the lower average concentrations of Ca 1.846 g/L (1.590 – 2.417), P 1.454 g/L (1.316 – 1.746), and Na 0.442 g/L (0.298 – 0.753) in the milk of East Friesian dairy sheep and similar average concentrations of Mg 0.192 g/L (0.157 – 0.251) and K 1.248 g/L (0.941 – 1.621). The average concentrations of macroelements in the Lacaune sheep milk indicate the microelement richness of their milk in macroelements and the richness of macroelements in the food and water provided to the sheep (Table 2).

Table 3. Descriptive statistics of macroelements in the Lacaune sheep milk in lactation

Tablica 3. Opisna statistika makroelemenata u mlijeku lakon ovaca tijekom laktacije

Elements / *Elementi* mg/kg	Mean / *Prosjek*	Sd	Minimum / *Minimum*	Maximum / *Maksimum*
Ca	2372.96	296.94	1630.47	3061.88
Mg	182.50	29.17	110.39	263.71
K	1330.16	354.73	475.25	2100.89
Na	662.37	362.71	280.37	2003.03
P	1633.97	182.55	1170.00	2045.00

Sd – stan. dev. / *standardna devijacija*

Table 4. Lactation stage effect on macroelement concentration in the Lacaune sheep milk

Tablica 4. Utjecaj stadija laktacije na koncentracije makroelemenata u mlijeku lakon ovce

Elements / *Elementi* mg/kg	Lactation stage / *Stadij laktacije*			SEM	P – value / *P – vrijednost*
	Early – *Rani*	Medium – *Srednji*	Late – *Kasni*		
Ca	2322.10	2385.90	2413.50	31.128	0.475
Mg	164.92^{b}	174.61^{b}	209.72^{a}	3.057	<0.001
K	1633.49^{a}	1381.85^{b}	950.64^{c}	37.186	<0.001
Na	450.16^{b}	500.76^{b}	1061.96^{a}	38.023	<0.001
P	1594.26	1630.55	1676.87	19.136	0.204

SEM – stand. err. of mean / *SEM – stand. pogr. sred. vrijed.*; $^{a, b, c}$ / significantly differ in the rows ($P<0.05$) / $^{a, b, c}$ / *značajna razlika u redovima ($P<0.05$)*

Significant differences were detected in the effects of the lactation phase on the concentrations of macroelements in the Lacaune sheep milk. Namely, as lactation progressed, a significant ($P<0.05$) increase in the concentrations of Mg and Na and a significant ($P<0.05$) decrease in K in the Lacaune sheep milk were detected. The stability of Ca and P concentrations during lactation was indicated by the lack of significant variation ($P<0.05$) between the Lacaune sheep milk concentrations based on the lactation stage. In the Lacaune sheep milk during lactation, the smallest fluctuation was detected for the Ca and P concentrations (+3.94% and +5.18%), a higher fluctuation was observed for the concentration of Mg and K (+27.2% and –41.80%), and the highest fluctuation was detected for the Na concentration (+135.91%). The revealed changes can be linked with a lower milk

production and a higher protein content of milk in the late phase of ewe lactation, which is also physiologically supported. Bijl et al. (2013) detected that the levels of Mg, Ca, and P in cows during lactation demonstrated a significant positive correlation with the protein content, largely due to their association with the casein micelles. According to Holt et al. (2011), magnesium has the ability to attach to milk casein micelles, whereas Na and K are involved in maintaining the osmotic equilibrium between blood and milk, signifying that the variations in this protein are related to casein. The studies conducted with the Lacaune sheep in Estonia (Tatar et al., 2022) also came to the similar results as the present study. The authors also detected insignificant ($P>0.05$) changes in the Ca and P concentrations in milk from the 2nd to the 6th month of lactation, as well as a significant ($P<0.05$) increase in the Na and Mg concentrations and a significant ($P<0.05$) decrease in K concentrations in milk from the 2nd to the 7th month of lactation. The same authors also determined the stability of Ca concentration in the Lacaune sheep milk during lactation, which is in agreement with the results of the current study. Variable concentration of Na in the Lacaune sheep milk during lactation may be associated with their intake of an excess amount of minerals if they have an *ad libitum* access to a saltlick (NRC, 2007). Stocco et al. (2019) detected that, among the macrominerals in the milk, the concentrations of Na were mainly increased in the middle phase of lactation, while the concentration of K tended to decrease in the second lactation phase in the milk of cows in different phases of lactation. The abovementioned authors detected an increase in Ca and P in milk during lactation at 6% and 10%. A significant increase in Mg concentration in milk during lactation indicates dynamic changes in Mg metabolism. Similar results in goat milk during lactation were obtained by Pan et al. (2023). As lactation progressed, Panayotov et al. (2018) also detected an increase in Mg concentrations in the Lacaune sheep milk in Bulgaria, but they have also detected a significant increase in Ca concentrations, in contrast to the present research. Kuchtik et al. (2017) observed a considerable increase in Ca and P concentrations (1365.7 to 1515.7 and 1199.5 to 1419.5 mg/L) as lactation progressed (from 57 to 197 days) in the milk of the Lacaune sheep in the Czech Republic. In a research pertaining to the Travnik pramenka, Antunović et al. (2020) observed a noteworthy reduction in the levels of Ca, P, K, and Mg, along with a negligible rise in Na concentrations as lactation drew to its conclusion. Consistent with the current study, Antunović et al. (2016) similarly observed a considerable rise in Mg and Na concentrations and a minor increase in Ca and P concentrations in the milk of the Dubrovnik sheep as lactation advanced.

Table 5. Correlation coefficients between the concentrations of macroelements in the milk of the Lacaune ewes during lactation

Tablica 5. Korelacijski koeficijenti između koncentracija makroelemenata u mlijeku lakon ovce tijekom laktacije

Elements / *Elementi*	Ca	Mg	K	Na	P
Ca	1.00				
Mg	0.45 <0.001	1.00			
K	0.06 0.55	– 0.43 <0.001	1.00		
Na	0.02 0.821	0.51 <0.001	– 0.82 <0.001	1.00	
P	0.67 <0.001	0.29 0.006	0.45 <0.001	– 0.20 0.056	1.00

The analysis of the correlation coefficients of macroelement concentrations in the milk of Lacaune sheep during lactation revealed significant ($P<0.05$) variations. A significantly ($P<0.05$) strong positive correlation was found between Ca:Mg, Ca:P, and K:P, but a significantly negative correlation between Mg:K and K:Na was also detected. Other associations between the macroelements in Lacaune sheep milk were not significant. Significant correlations detected between the macroelements in milk indicate their strong interdependence. Tatar et al. (2022) also a significant positive correlation between the Ca:P (0.534**) and Ca:Mg (0.197**) in the milk of Lacaune sheep during lactation, as well as a significant positive correlation between the Mg:P (0.330***) and Mg:Na (0.637***) and a significant negative correlation between the Mg:K (– 0.432**) and K:Na (– 0.677***). In the Lacaune sheep milk in the Czech Republic, Kuchtik et al. (2017) also detected a significant positive correlation between the Ca:P (0.67**). Antunović et al. (2020) came to similar conclusions in their research of the Dubrovnik sheep when they also detected a significant positive correlation between the Ca:P, but also Mg:P and a significant negative correlation between the K:Na in milk. Likewise, in the early and medium lactation phases (0.920** and 0.933**), Pšenkova et al. (2022) discovered a substantial positive connection between the Ca:Mg in the Tsigai sheep milk; however, they also discovered a significant negative correlation in the late lactation stage (– 0.956**).

CONCLUSION

The obtained results demonstrate a significant effect of lactation stage on the content of most macroelements in the Lacaune sheep milk, except Ca and P. The significant correlations detected between the macroelements in milk indicate their strong interdependence. Therefore, the age of the sheep and the stage of lactation should be included in the analysis scheme when determining the macroelements in milk. The Lacaune sheep milk is rich in macroelements, which offers a good perspective for the breeding of this breed through the prism of an increased consumption thereof in human diet.

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MAKROELEMENTI U MLIJEKU LAKON OVACA U OVISNOSTI O STADIJU LAKTACIJE

SAŽETAK

Cilj je ovoga istraživanja prikazati kako stadij laktacije utječe na sadržaj makroelemenata u mlijeku lakon ovce. Istraživanje je provedeno na 30 lakon ovaca u 4. laktaciji tijekom ranoga (60. dan), srednjeg (120. dan) i kasnog stadija laktacije (180. dan). U mlijeku lakon ovaca utvrđeno je ICP metodom koncentracije sljedećih makroelemenata: Ca, P, K, Na i Mg. Utvrđeno je značajno povećanje koncentracija Mg i Na te značajno smanjenje K u mlijeku lakon ovce odmicanjem laktacije. Koncentracije Ca i P nisu značajno varirale ovisno o stadiju laktacije u mlijeku lakon ovce, što ukazuje na njihovu stabilnost tijekom laktacije. Utvrđena je značajno jaka pozitivna korelacija između Ca:Mg, Ca:P i K:P, ali i značajno negativna korelacija između Mg:K i K:Na. Na temelju dobivenih rezultata može se zaključiti da je stadij laktacije značajno utjecao na sadržaj većine makroelemenata (osim Ca i P) u mlijeku lakon ovce, te ga se treba uključiti u shemu istraživanja.

Ključne riječi: lakon ovca, mlijeko, makrolementi, koncentracija, korelacija

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