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Metabolički profil krvi mliječnih koza tijekom prva tri mjeseca laktacije

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BLOOD METABOLIC PROFILE OF DAIRY GOATS DURING THE FIRST THREE MONTHS OF LACTATION

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ABSTRACT

This paper investigates the changes in the blood metabolic profile of 22 Alpine goats during the first three months of lactation (15th, 30th, 60th, 90th day of lactation). The goats were on average 5 years old and in the 4th lactation. The research proved a significant influence of the time of blood sampling on the goats' blood metabolic profile indicators during the first three months of lactation. Analysis of hematological and biochemical indicators in the goats' blood confirmed a significant decrease in RBC, HGB, HCT and neutrophil content, glucose, triglyceride, HDL-cholesterol, NEFA, and Mg concentrations, as well as a decrease in GPx and GR activity and an increase in MCV, MCH, eosinophil, basophil, urea and P-inorganic content. As lactation progressed, the research confirmed a decrease and then an increase in lymphocyte and monocyte content and changes in Ca concentrations. The confirmed changes in goats' blood indicate a significant demand imposed on goats during the first three months of lactation, especially in early lactation (up to the 30th day of lactation), as there is an energy imbalance, hypocalcemia and the highest activity of antioxidant enzymes (GPx and GR) occurring in goats.

Keywords: blood metabolic profile; dairy goats; lactation; hematological indicators; biochemical indicators

INTRODUCTION

The lactation phase places high physiological demands on female animals, while also posing challenges for farmers, who must ensure the supply of sufficient, high-quality feed enriched with all essential nutrients (Antunović et al., 2021 and 2022). For a female animal, the transition period lasting up to 3 weeks after kidding is particularly important (Antunović et al., 2013; Akkaya et al., 2020), as it is also the period when the production of milk is the highest, which additionally intensifies the animal's stress and may cause weight loss due to a low-quality diet (Antunović et al., 2017 and 2024). During this period, animals have a reduced ability to consume feed, which leads to negative energy balance in the body (Matthews, 2016). Since metabolic energy derived from feed is not sufficient for increased milk production during lactation, especially in its early stages, animals mobilize their own energy reserves, which leads to weight loss (Souza et al., 2014). The Mediterranean region in Europe is known for the most regulated and well-organized market for goat products, especially goat milk and dairy products, and there is also well-structured information

collected on the goat breeding (Miller and Lu, 2019). Although only around 5% of the world's goat population is raised in Europe, dairy breeds of goats have been dominant in farming (Castro et al., 2023), so there are around 15% of the world's production of goat milk and 35% of all goat cheeses produced in Europe (Morales et al., 2019). In intensive dairy systems, together with the genetic selection of ruminants for greater milk output, the risk of metabolic disorders rises, particularly among high-yielding dairy ruminants (Celi et al., 2008; Domaćinović et al., 2023). Goats that produce more milk also have increased lipid mobilization in the body (Zamuner et al., 2020), which may cause significant weight loss during early lactation. During this period, there are various metabolic disorders and diseases occurring in goats, and these can be worsened by improper feeding of goats. The metabolic profile of goats' blood is useful in the preven-

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tion of the homeostasis disruption, as well as in regulating the goats' diet (Antunović et al., 2017; Zamuner et al., 2020). Hernández et al. (2020) highlighted the importance of determining the blood metabolic profile for better monitoring of animal production and nutrition, and for achieving efficient feed intake and animal productivity. Kayano and Kida (2015) concluded that the blood metabolic profile was a quantitative test to monitor animals' health and to diagnose or prevent many metabolic diseases. The blood metabolic profile generally involves examining hematological and biochemical parameters, along with assessing liver enzyme activity (Antunović et al., 2017). It is affected by numerous factors, primarily by nutrition and environmental conditions, and when interpreted as a whole, it serves for quantitative and qualitative monitoring of animal productivity, elimination of reproductive disorders, and prevention of numerous subclinical diseases (Akkaya et al., 2020; Van Saun, 2023). Only some available studies on lactating goats deal with a greater number of metabolic profile indicators and analyze their connection (Celi et al., 2008; Antunović et al., 2017; Manuelian et al., 2020). This research investigates the hematological and biochemical indicators in lactating goats' blood during the first three months of the lactation period.

MATERIALS AND METHODS

Experimental Design and Choice of Animals

The research into metabolic profile (hematological and biochemical indicators) was carried out on 22 lactating Alpine goats. It started on the 15th day of lactation and lasted until the 90th day. The research was carried out on a small farm near the city of Valpovo, Croatia, which has been breeding goats for many years, and currently has 40 adult goats. The criteria for selecting the goats for this research were age, order, the stage of lactation, and the number of kids in the litter. The average age of selected goats was 5 years (± 3 months), and they were in the 4th lactation with 1.5 kids in the litter (± 0.5). The goats were healthy and in good body condition. The average morning milk yield of goats was 1.51 ± 0.28 kg. The goats received a feed mixture containing 16% crude protein and 11 MJ/kg DM of metabolizable energy, provided at a level of 1.5 kg/day. Alfalfa hay was offered freely for unrestricted intake. The feed mixture was based on corn and barley, and the protein source was soybean meal. In addition, the goats had constant access to water and livestock salt (NaCl - 94% min, Mg 2000 mg/kg, Co- 18 mg/kg, Zn - 810 mg/kg, Mn -830 mg/kg, I - 100 mg/kg, Se- 10 mg/kg).

Blood Sampling and Analysis

Blood samples were collected from the jugular vein (10 mL) of each lactating goat. Samples to be tested for biochemical indicators and for hematology analysis were collected in sterile vacuum Venoject® tubes (Sterile Terumo Europe, Leuven, Belgium) and in tubes containing ethylenediamine tetra-acetic acid (EDTA) as anticoagulant. Samples were taken on the 15th, 30th, 60th, and

90th day of the lactation period. Whole blood samples from lactating goats were analyzed to determine hematological parameters, including leukocyte (WBC) and erythrocyte (RBC) counts, content of hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Analyses were performed within two hours of collection using an automatic three-differential veterinary hematology analyzer (Sysmex PochH-100iV; Sysmex Europe GmbH, Hamburg, Germany). After that, for the purpose of determining the differential blood count, blood smears were stained according to Pappenheim. Relative relations of individual leukocytes (lymphocytes, neutrophils; eosinophils; basophils and monocytes) were calculated as a percentage of their total count. For biochemistry blood samples were centrifuged for ten minutes at $1609.92 \times g$ using a ROTOFIX 32A centrifuge (Hettich GmbH & Co. KG, Tuttlingen, Germany) after two hours of sampling. The resulting serum was analyzed on an Olympus AU400 to determine mineral concentrations (calcium - Ca, inorganic phosphorus - P, magnesium - Mg, and iron - Fe), metabolites (glucose, cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, non-esterified fatty acids - NEFA, β -hydroxybutyrate - BHB, urea, total protein, and albumin). Serum samples were further analyzed to assess enzyme activities, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), γ -glutamyl transferase (GGT), and glutathione reductase (GR), using Olympus System reagents. Serum globulin levels were calculated as the difference between total protein and albumin concentrations. The Albumin/Globulin ratio (A/G) was obtained by dividing albumin and globulin. Additionally, on an automatic Olympus AU 400 analyzer (Olympus, Japan) with the Ransel® kit (Randox, UK) at a wavelength of 240 nm, glutathione peroxidase (GPx) activity in the serum of lactating goats.

Statistical Analyses

The distribution of the collected data was assessed using the Shapiro-Wilk test (PROC UNIVARIATE). Mean values for the blood metabolic profile of goats during the first three months of lactation period were calculated with the MEANS procedure in TIBCO Statistica® 13.3.0. Data were analyzed by ANOVA, considering the time of blood sampling as a fixed factor. Mean comparisons were performed using Tukey's test, with differences considered significant at $P < 0.05$ and $P < 0.01$. Effects of blood sampling time were further evaluated using GLM repeated measures ANOVA.

RESULTS AND DISCUSSION

The research confirmed a significant decrease in RBC, the content of HGB and HCT, as well as a slight decrease in WBC in goats' blood as the lactation progressed (Table 1). Changes of hematological indicators confirm the fast and best possible adaptation of lactating goats (Attia, 2016).

Table 1. Hematological indicators of the blood of lactating goats during the first three months of the lactation period

Tablica 1. Hematološki pokazatelji u krvi koza tijekom prva tri mjeseca laktacije

Indicators / Pokazatelj	Time of measures, days (mean \pm sd) / Vrijeme uzorkovanja, dani (srednja vrijednost \pm sd)			
	15th – 15. dan	30th – 30. dan	60th – 60. dan	90th – 90. dan
WBC ($\times 10^9$ L)	11.25 \pm 3.49	10.51 \pm 3.71	9.45 \pm 2.99	10.45 \pm 3.66
RBC ($\times 10^{12}$ L)	10.24 ^a \pm 1.77	9.15 \pm 1.85	9.14 \pm 2.19	8.84 ^b \pm 2.75
HGB, g/dL	7.95 ^a \pm 1.33	7.24 \pm 1.67	7.12 ^b \pm 1.27	7.39 \pm 2.03
HCT, L/L	0.28 ^a \pm 0.04	0.25 ^b \pm 0.05	0.26 \pm 0.05	0.26 \pm 0.06
MCV, fL	27.75 ^a \pm 1.51	27.70 ^{abc} \pm 2.04	29.12 ^{abd} \pm 3.39	30.50 ^b \pm 5.24
MCH, pg	7.80 ^a \pm 0.78	7.87 ^a \pm 0.56	7.99 \pm 1.08	8.60 ^b \pm 1.36
MCHC, g/L	281.27 \pm 23.73	258.36 \pm 25.04	274.25 \pm 19.41	284.82 \pm 35.60

sd - standard deviation / standardna devijacija; WBC: leukocyte / leukociti; RBC: erythrocytes / eritrociti; HGB: hemoglobin, HCT: hematocrit; MCV: mean corpuscular volume / srednji volumen eritrocita; MCH: mean corpuscular hemoglobin / prosječni sadržaj hemoglobina u eritrocitima; MCHC: mean corpuscular hemoglobin concentration / prosječna koncentracija hemoglobina u eritrocitima; a, b, c, d - means with different superscript letters differ statistically significant: $P < 0.05$ / vrijednosti naznačene s različitim slovima u redovima statistički se značajno razlikuju: $P < 0,05$

Reduced content of HGB could be associated with increased milk production (Hassan et al., 1986). In lactating goats, the peak of milk production during lactation is from 5-10 weeks of lactation (Salama et al., 2005), which is also related to the stage of lactation in our study. Tharwat et al. (2015) reported similar results related to the lowered WBC and RBC, and HGB and HCT content in goats' blood in early lactation (weeks 2 to 3). The reduced RBC in lactating goats' blood from the 30th to 60th day of lactation was determined by Antunović et al. (2013). This research confirmed a significant increase in MCV and MCHC content in goats' blood as the lactation progressed. Similar results related to MCH and MCHC content in the blood of goats during early lactation (from the 2nd to 3rd week) were obtained by Tharwat et al. (2015).

According to Feldman et al. (2002), reference values for the content of MCV were 16-25 pg, for MCH 2,2-8 fL, for MCHC 300-360 g/L, which indicated slightly lower MCHC content and elevated MCV content. Consequently, if compared to reference values, a slightly higher MCV content could indicate a cell regeneration response, which would be expected as a response to stress caused by lactation, while a lower MCHC content might be a consequence of reticulocytosis or iron deficiency (Paape et al., 1992).

Differential blood count also revealed significant variation in most indicators depending on the time of blood sampling during the first three months of lactation (Figures 1 and 2).

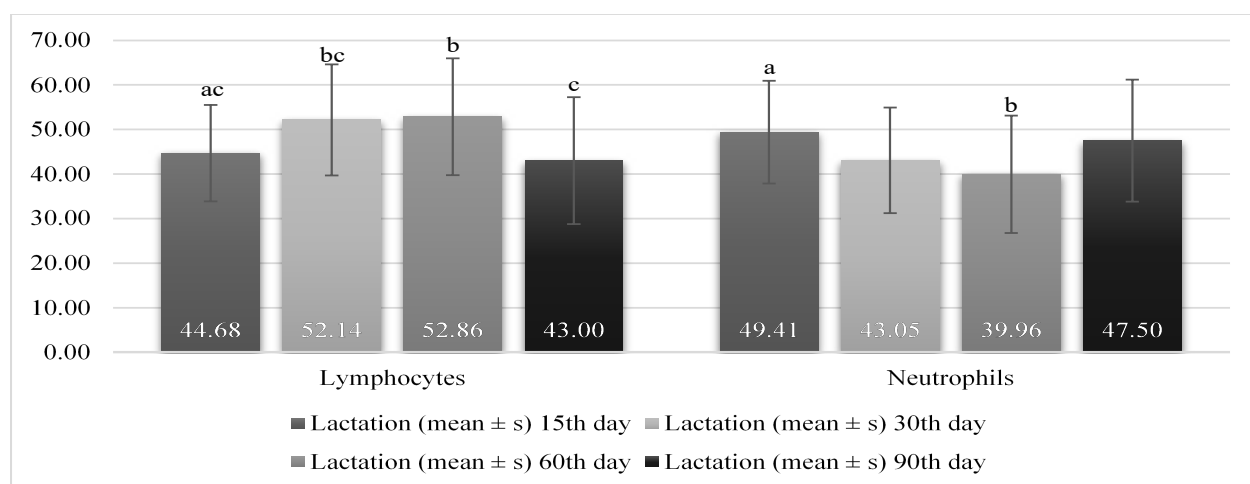


Figure 1. Change of lymphocytes and neutrophils mean \pm sd content in goats' blood (%) during the first three months of lactation period (a, b, c - means with different superscript letters differ statistically significantly: $P < 0.05$)

Grafikon 1. Promjena udjela (mean \pm sd) limfocita i neutrofila u krvi koza (%) tijekom prva tri mjeseca laktacije (a, b, c - vrijednosti naznačene s različitim slovima na stupcima se značajno razlikuju: $P < 0,05$)

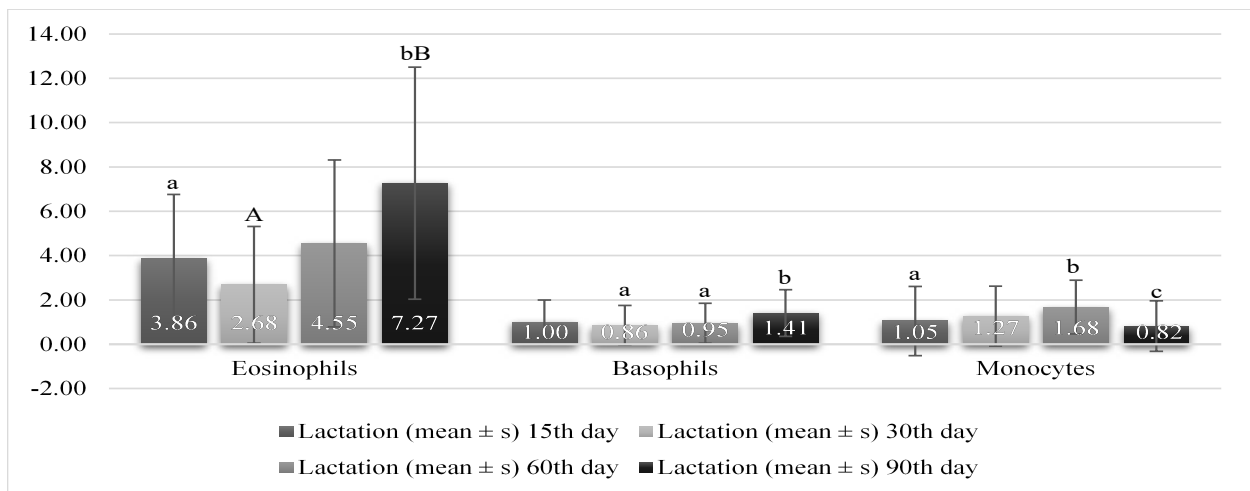


Figure 2. Change eosinophils, basophils, and monocytes mean \pm sd content in goats' blood (%) during the first three months of lactation period (A, B - means with different superscript letters differ highly significantly: $P < 0.01$; a, b, c - means with different superscript letters differ statistically significantly: $P < 0.05$)

Grafikon 2. Promjena udjela (mean \pm sd) eozinofila, bazofila i monocita u krvi koza (%) tijekom prva tri mjeseca laktacije (A, B – vrijednosti naznačene s različitim slovima na stupcima se statistički vrlo značajno razlikuju $P < 0,01$; a, b, c- vrijednosti naznačene s različitim slovima na stupcima statistički se značajno razlikuju: $P < 0,05$)

On the 30th and 60th day of lactation, goats' blood contained significantly higher content of lymphocytes and reduced content of neutrophils on the 90th day of sampling compared to the 60th day. Content of neutrophils decreased during lactation, with significantly lowest values established on the 60th day of lactation in comparison with the 15th day. Contents of eosinophils and basophils were higher on the 90th day of lactation if compared to the 30th day of lactation when those values were the lowest. When compared to the 15th day of blood sampling, the content of monocytes in goats' blood was significantly increased on the 60th day, while the significantly lowest content was recorded on the

90th day of lactation. In the study by Paapa et al. (1992) pointed out that due to the transition from blood to milk, the concentration of lymphocytes and neutrophils in the blood was consequently reduced due to more efficient phagocytosis and defense of the mammary gland against various pathogens. A significant increase in eosinophils and monocytes in the blood of lactating goats observed also in study Antunović et al. (2013). Comparison of distribution of leukocytes in goats' blood in this research with the reference ranges (Feldman et al., 2002) confirmed that most of the values were within physiological limits.

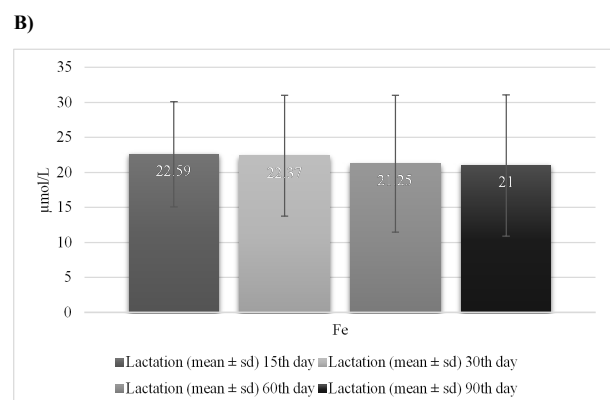
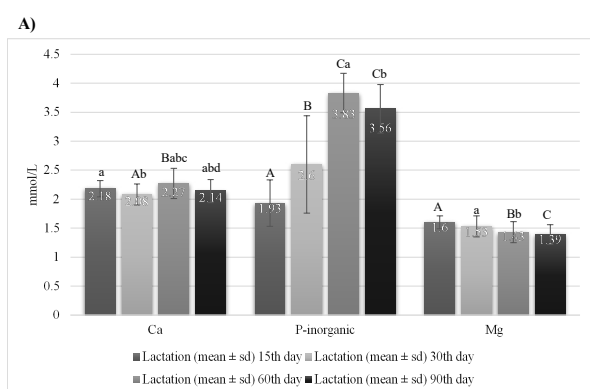


Figure 3. Change of minerals (Fig. A: Ca, P-inorganic and Mg; Fig. B: Fe) mean \pm sd concentration in goats' blood during the first three months of lactation (A, B, C - means with different superscript letters differ highly significantly: $P < 0.01$; a, b, c, d - means with different superscript letters differ statistically significantly: $P < 0.05$)

Grafikon 3. Promjena koncentracije (mean \pm sd) minerala u krvi koza tijekom prva tri mjeseca laktacije (A, B, C – vrijednosti s različitim slovima vrlo značajno se razlikuju: $P < 0,01$; a, b, c, d - vrijednosti naznačene s različitim slovima na stupcima statistički se značajno razlikuju: $P < 0,05$)

In this research, there was significant fluctuation of Ca, P-inorganic, and Mg in the blood and non-significant decrease of Fe during the first of the three months of lactation (Fig. 3). The significantly lowest concentration of Ca measured on the 30th day of lactation was expected because that was the peak of milk production and the greater Ca sequestration by the mammary gland possibly induced faster mobilization of this mineral from the bones (Horst et al., 1997). Mundim et al. (2007) reported that the greatest requirements for Ca and other nutrients in lactating goats occurred during early lactation and the peak milk production from the 40th to 60th day of lactation, which is in accordance with these research results. The observed reduced Ca concentration in comparison with reference values (Kaneko et al., 2008; 2.3-2.9 mmol/L), as well as the lowest concentration on the 30th day of lactation indicated the occurrence of hypocalcemia during the peak lactation period, and its significant increase thereafter, on the 60th and 90th day, was expected since the lowered milk production was accompanied by a decrease in Ca needs. In this research, the values of P-inorganic in goats' blood were above the normal value (Kaneko et al., 2008; 1,0-2,4 mmol/L), except on the 15th day of lactation. As Iriadam (2007) points out, the uniformity of the value of Ca in the bloodstream indicates its satisfactory reserves in the body and sufficient amounts in the goats' meal, but also the efficient metabolism of phosphorus. Compared to the

mid-lactation period, Ahmed et al. (2000) also confirmed lower P-inorganic concentration in goats' blood during early lactation. In the research of Azab and Abdel-Maksoud (1999), there was the opposite trend confirmed for concentrations of P-inorganic in lactating goats during the transitional period. In this research, the lowering of Mg concentration in goats' blood along with the lactation progress could be connected with less feed consumption during the transitional period, as well as hormonal adaptation after kidding or the postpartum hypocalcemia (Senturk, 2017). When analyzing the blood sampled on the 30th and 90th day of lactation, similar conclusions were confirmed by Antunović et al. (2025). The decrease in Mg concentrations in the blood of goats as lactation progresses may also be related to the higher content of rumen-degradable proteins in the feed mixture, which are present in the cereals used for goats. According to the investigation of sheep, Gäbel and Martens (1986) concluded that dietary protein excess may depress Mg absorbability during a few days, which is recovered by adaptation of the rumen epithelium. However, Martens et al. (2018) did not determine clear evidence for a direct relation between protein excess and Mg absorption in cows. In the blood of lactating goats, Allaoua and Mahdi (2018) found similar changes related to the lowering of Ca and Fe concentrations when compared to results of the early and middle lactation (3rd - 8th week).

Table 2. Concentration of metabolites in goats' blood during the first three months of lactation

Tablica 2. Koncentracije metabolita u krvi koza tijekom prva tri mjeseca laktacije

Indicators / Pokazatelji (mmol/L)	Time of measures, days (mean \pm sd) / Vrijeme uzorkovanja, dani (srednja vrijednost \pm sd)			
	15th day – 15. dan	30th day – 30. dan	60th day – 60. dan	90th day – 90. dan
Glucose	4.59 ^{Aa} \pm 0.45	4.22 ^B \pm 0.34	4.48 \pm 0.39	4.18 ^b \pm 0.63
Cholesterol	2.98 \pm 0.69	2.78 \pm 0.71	2.83 \pm 0.59	2.80 \pm 0.66
HDL-cholesterol	1.64 ^a \pm 0.35	1.55 \pm 0.38	1.41 ^b \pm 0.25	1.54 \pm 0.28
LDL-cholesterol	1.27 \pm 0.39	1.25 \pm 0.55	1.32 \pm 0.54	1.29 \pm 0.63
Triglycerides	0.22 ^A \pm 0.10	0.15 ^{Ba} \pm 0.05	0.15 ^{Ba} \pm 0.06	0.21 ^b \pm 0.11
NEFA	0.24 ^A \pm 0.12	0.24 ^B \pm 0.07	0.10 ^{Ca} \pm 0.04	0.20 ^{Cb} \pm 0.11
BHB	0.45 \pm 0.14	0.49 \pm 0.11	0.50 \pm 0.12	0.52 \pm 0.13
Urea	6.07 ^A \pm 1.78	9.28 ^B \pm 1.30	9.72 ^B \pm 1.90	9.23 ^B \pm 1.92
Total protein, g/L	73.96 \pm 9.47	70.52 \pm 9.69	74.03 \pm 9.62	73.37 \pm 13.17
Albumin, g/L	27.43 \pm 3.99	26.14 \pm 4.09	27.10 \pm 3.85	26.34 \pm 6.03
Globulin, g/L	46.54 \pm 6.86	44.38 \pm 6.91	46.93 \pm 6.99	47.03 \pm 7.98
Albumin/Globulin	0.60 \pm 0.09	0.60 \pm 0.10	0.58 \pm 0.08	0.56 \pm 0.09

sd - standard deviation / standardna devijacija; NEFA: non-esterified fatty acids / nezasićene masne kiseline; BHB: β -hydroxybutyrate / β -hidroksibutirat; A, B, C - means with different superscript letters differ high statistically significant: $P < 0.01$ / vrijednosti naznačene s različitim slovima u redovima statistički se visoko značajno razlikuju: $P < 0,01$; a, b, c, d - means with different superscript letters differ significant: $P < 0.05$ / vrijednosti naznačene s različitim slovima u redovima statistički se značajno razlikuju: $P < 0,05$

In the present research, the authors determined a significant reduction of glucose concentration in goats' blood during the first month of the lactation period (Table 2). Similar results with glucose changes in the blood of dairy goats during the transition period were determined by Akkaya et al. (2020). Zamuner et al. (2020) also confirmed that after kidding, the goats' blood plasma

glucose rapidly decreased, and the concentration of urea gradually increased. Similar results were also reported by Manuelian et al. (2020). It is known that the concentration of glucose is associated with the metabolic status of the animals, and some papers confirmed a decrease in the blood of lactating goats during negative energy balance (Di Trana et al., 2006). Leibova and Pozovnikova (2024)

reported that highly productive lactating ruminants had a greater need for glucose because of significant milk production. Glucose is the dominant substrate for lactose synthesis, and during lactation, milk production causes a decreased level of glucose in the blood. In this research, a significant increase in urea concentration in the blood of lactating goats is in line with results published in other studies on lactating goats (Radin et al., 2015; Soares et al., 2018). The changes in blood urea in lactating goats were most likely a reflection of enhanced catabolism of amino acids to support milk production during NEB (Zamuner et al., 2020), but Van Saun (2009) argued that these changes might be connected with the changes in DMI. In the present study, a significant increase in urea in the serum of goats was found as lactation progressed. This was also confirmed in the research by Sadjadian et al. (2013), who associated an increase in dry matter consumption in goats after kidding. In the study conducted on dairy goats, Tosto et al. (2021) determined similar changes in urea contained in blood with the highest concentration after peak lactation, between the 5th and 8th week, which is also in line with our results. In a study on lactating goats during the transition period, Cajueiro et al. (2021) also reported a significant rise in blood urea concentrations as lactation progressed, with values measured on days 0, 10, 20, 30, 40, 50, and 60 postpartum being 47.9, 56.0, 69.8, 63.8, 70.9, 75.5, and 77.7 mg/dL, respectively. Higher blood urea concentrations compared to reference values as lactation progressed in the present study indicate a diet high in proteins (especially rumen-degradable protein), but also indicate an energy imbalance. It is known that proteins from cereal grains are considered highly degradable in the rumen (Kopceková et al., 2008), which were the base of the feed mixture in the present study. Concentrations of total proteins, albumin, globulin, and the A/G ratio did not differ significantly depending on the time of blood measurement in goats during lactation. A smaller decrease in the A/G ratio was observed as lactation progressed, which is in agreement with studies conducted with goats (Bhoite et al., 2019) and cows in lactation (Rajora and Pachauri, 1994). The lactating goats are reportedly less prone to pregnancy toxemia and lactational ketosis than the lactating ewes and cows (Marutsova and Binev, 2017). In this research, the changes in blood BHB concentration suggested the differences in ketone body metabolism between goats and cows, which could be related to inherent interspecies differences in the intermediary metabolism of lipids (Delavaud et al., 2019) and mammary lipogenesis (Chilliard et al., 2014). In a study by Marutsova and Binev (2017), lactating goats have demonstrated that plasma BHB ≥ 0.8 mmol/L was associated with subclinical ketosis, and BHB > 1.6 mmol/L was associated with clinical ketosis. In this research, BHB concentrations increased as lactation progressed. In the study into lactating goats, Cajueiro et al. (2021) did not determine changes in BHB concentrations in goats' blood. In this research, the observed changes in BHB concentrations (Table 2) during the first of three months of lactation period in dairy goats were below the level of satisfactory energy balance during the 15th and 30th

lactation day (BHB: 0.45 and 0.49 mmol/L, respectively), while the concentrations of BHB in blood on the 60th and 90th lactation day (0.50 and 0.52 mmol/L, respectively) indicated satisfactory energy balance in lactating goats. Radin et al. (2015) concluded that blood serum BHB concentrations ranging from 0.50 to 0.76 mmol/L did not cause any metabolic disturbances in the first 4 weeks of lactation in multiparous goats. Three main factors significantly contribute to the rise in NEFA concentrations in animals' blood: heightened energy demands from increased milk production, stress following parturition, and decreased feed intake (Bell, 1995). The BHB and NEFA concentrations found in the present research confirmed the findings on lactating goats of Sadjadian et al. (2013). A similar conclusion was reached by Akkaya et al. (2020) in their research on goats during the transition period of lactation in Turkey. The study of Manuelian et al. (2020) completed on the Mediterranean breed and Saanen goats in Italy during lactation, determined a similar trend in blood NEFA concentrations (significantly decreasing concentrations as lactation proceeded), which is in line with our research results. Cajueiro et al. (2021) observed a decrease in NEFA concentrations in the blood of lactating goats as lactation progressed, with values of 0.32, 0.18, 0.19, and 0.17 mmol/L on days 0, 10, 20, and 30 postpartum, followed by an increase on days 40, 50, and 60, reaching 0.27, 0.25, and 0.30 mmol/L, respectively. These findings align with the results of our study. Namely, found significantly higher concentrations of triglycerides in the blood of goats in early lactation indicated increased body fat mobilization. Since the liver is not able to oxidize all NEFA either partially (by forming ketone bodies) or fully, it is converted into triglycerides. Li et al. (2024) also determined higher triglyceride concentrations in the blood of goats after kidding, which was associated with accelerated metabolism of fat to maintain basic life activities. Ríos et al. (2006) argued that this could be connected with increased feed consumption and energy-rich diets. The reduction of blood triglycerides in postpartum and during lactation, as determined in this research, was confirmed in the study on lactating goats carried out by Iriadam (2007). Determined concentrations of albumin in the blood can be an indicator of liver function and nutritional status (Caldeira, 2005), which sufficiently emphasizes the importance of the labile reserve of proteins that animals reach in the event of a nutritional deficit. In the present research, the authors did not determine changes of albumin concentration in the blood of goats during the first of the three months of lactation.

Furthermore, this research resulted in the non-significant changes in most blood enzymes in dairy goats during the first of the three months of lactation, except for the significant reduction in the GR and GPx activity as the lactation progressed (Table 3). Similar results in the blood of lactating goats were obtained by Allaoua and Mahdi (2018). ALT and AST are key catabolic enzymes that play a crucial role in maintaining proper liver function in animals. Schmitz et al. (2021) reported that the activities of the antioxidant enzymes GPx and SOD

fluctuate throughout lactation, serving to counteract the heightened oxidative stress experienced during this period. As pointed out by Xu et al. (2014) and Schönfeld and Wojtczak (2008), during the lactation period, especially during the early stage of lactation, there is a mobilization of body fat, which is related to the release of NEFA and increased metabolic efforts due to ensuring high milk production. The mentioned authors conclude that because of this, antioxidant enzymes play an important role in the elimination of oxygen radicals from the body. Sayiner et al. (2020) tested the cows' blood to determine a significant increase in the GPx levels in the early postpartum period, and those could be a messenger of metabolic diseases if GPx activities increased up to the highest levels 8 days after calving ($P < 0.0001$), and then decreased again after 30 days post calving. Those authors stated that GPx was essential as a metabolic indicator in the pre- and postpartum period. The increase in the activity of GPx and GR in the blood of goats can be associated with an increase in the amount of intracellular hydrogen peroxide, as reported by Skotnicka et al.

(2010). The conclusions of the research by Janiak et al. (2010) indicate that the GR enzyme is responsible for the regeneration of reduced glutathione levels, which consequently affects GPx activity. Pilarczyk et al. (2012) reported the highest serum GPx activity in the first two months of the lactation period, when the lowest activity was determined in dry cows. GPx activity increases during early lactation when milk production is at its highest, and when the animal is under severe stress, however, it decreases in the middle stage of lactation. There is also the factor of physiological adjustment and adaptation of the animal body to less production of milk during mid-lactation and supply of nutrients (Moolchandani and Sareen, 2018). In their research into oxidative status in dairy goats in the periparturient period, Festila et al. (2012) argued that such a condition might be due to the loss of homeostatic control in the postpartum period. However, Huang et al. (2021) determined increased GPx activity in the subclinical hypocalcemia of goats during early lactation. Such findings are in accordance with our research results.

Table 3. Enzyme activity in goats' blood during the first three months of lactation

Tablica 3. Aktivnosti enzima u krvi koza tijekom prva tri mjeseca laktacije

Enzymes / Enzimi U/L	Time of measures, days (mean \pm sd) / Vrijeme uzorkovanja, dani (srednja vrijednost \pm sd)			
	15th day – 15. dan	30th day – 30. dan	60th day – 60. dan	90th day – 90. dan
AST	131.12 \pm 23.71	126.02 \pm 25.70	121.04 \pm 29.67	121.06 \pm 25.03
ALT	28.42 \pm 7.39	25.16 \pm 7.20	27.10 \pm 6.95	27.41 \pm 8.05
CK	179.95 \pm 38.07	166.55 \pm 41.54	158.59 \pm 34.13	166.14 \pm 41.58
GGT	48.70 \pm 11.53	48.23 \pm 10.09	48.69 \pm 9.55	48.13 \pm 8.74
GR	100.44 ^A \pm 23.05	87.90 \pm 20.39	81.38 ^B \pm 19.73	87.53 ^B \pm 20.43
GPx	1108.40 ^{Aa} \pm 274.10	897.80 ^{Ab} \pm 185.58	714.70 ^{Bb} \pm 178.45	832.80 ^{Bb} \pm 163.53

sd-standard deviation / standardna devijacija; AST: aspartate aminotransferase / aspartate aminotransferaza; ALT: alanine aminotransferase / alanin aminotransferaza; GGT: γ -glutamyl transferase / γ -glutamyl transferaza; CK- creatine kinase / kreatin kinaza; GPx: glutathione peroxidase / glutathione peroksidaza; GR: glutathione reductase / glutathion reduktaza; A, B, C - means with different superscript letters differ high statistically significant: $P < 0.01$ / vrijednosti naznačene s različitim slovima u redovima statistički se visoko značajno razlikuju: $P < 0,01$; a, b, c, d - means with different superscript letters differ significant: $P < 0.05$ / vrijednosti naznačene s različitim slovima u redovima statistički se značajno razlikuju: $P < 0,05$

CONCLUSION

This research confirmed the significant influence of the time of blood sampling on the indicators of the blood metabolic profile of goats during the first three months of lactation. As lactation progressed, analysis of hematological indicators confirmed a significant decrease in RBC, HGB, HCT, and neutrophils, and an increase in MCV, MCH, eosinophils, and basophils. There was also a decrease and then an increase in lymphocyte and monocyte content. With the advance of lactation, biochemical indicators in the goats' blood showed a significant decrease in the concentrations of glucose, triglycerides, HDL-cholesterol, NEFA, and Mg, as well as a decrease in the activity of GPx and GR, an increase in the concentrations of urea and P-inorganic, and the initial increase and then a decrease in the concentration of Ca. The mentioned changes in blood metabolic profile indicate a significant load put on goats during the first three months of lactation, especially in early lactation (up to the 30th day of lactation), when lactating goats are

prone to energy imbalance, hypocalcemia and the highest activity of antioxidant enzymes (GPx and GR).

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METABOLIČKI PROFIL KRVI MLIJEČNIH KOZA TIJEKOM PRVA TRI MJESECA LAKTACIJE

SAŽETAK

Cilj rada bio je istražiti promjene metaboličkoga profila 22 mliječne koze alpske pasmine tijekom prva tri mjeseca laktacije (15., 30., 60. i 90 dana laktacije). Koze su bile prosječne dobi 5 godina, u 4. laktaciji. Na temelju provedenih istraživanja utvrđen je značajan utjecaj vremena uzorkovanja krvi na pokazatelje metaboličkoga profila u koza u prva tri mjeseca laktacije. Analizom hematoloških i biokemijskih pokazatelja u krvi utvrđeno je značajno smanjenje RBC-a, sadržaja HGB-a, HCT-a i neutrofila, koncentracija glukoze, triglicerida, HDL-kolesterola, NEFA-e i Mg, kao i smanjenje aktivnosti GPx-a i GR-a te povećanje sadržaja MCV-a, MCH-a, eozinofila, bazofila, koncentracija uree i P-inorganski kako je laktacija odmicala. S odmicanjem laktacije utvrđeno je smanjenje pa povećanje sadržaja limfocita i monocita te suprotne promjene za koncentracije Ca. Navedene promjene u krvi koza ukazuju na značajnu opterećenost u prva tri mjeseca laktacije, osobito u ranoj laktaciji, kada je utvrđen energetski disbalans, hipokalcemija i najviše aktivnosti antioksidacijskih enzima (GPx i GR).

Ključne riječi: metabolički profil krvi, mliječne koze, laktacija, hematološki pokazatelji, biokemijski pokazatelji

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