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The Effect of Lactation Stage on the Hematological and Serum-Related Biochemical Parameters of the Travnik Pramenka Ewes

Utjecaj stadija laktacije na hematološke i biokemijske pokazatelje krvi ovaca Travničke pramenke

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THE EFFECT OF LACTATION STAGE ON THE HEMATOLOGICAL AND SERUM-RELATED BIOCHEMICAL PARAMETERS OF THE TRAVNIK PRAMENKA EWES

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SUMMARY

The aim of the present research was to determine the hematological and biochemical parameters of the Travnik pramenka ewes during lactation. Thirty-six ewes in average age of 3-5 years were included in the research. They were monitored in three stages of lactation: early, medium, and late stage. The WBC and RBC, HGB concentration, HCT and MCH values increased significantly as lactation progressed, while significant decrease of MCV and MCHC was determined in blood. HCT was slightly higher in the early compared to late stage, and the MCH in the medium compared to the late stage of lactation. A significant increase in the concentrations of Ca, P, Mg, Na and Cl, as well as a decrease of the Fe concentration was determined. As lactation progressed, the concentrations of urea, total proteins, albumin, globulin and BHB increased, as well as ALT activity. Contrary, concentrations of glucose and NEFA decreased, as well as GGT activity. Obtained results may help in monitoring the health and nutritional status of Travnik pramenka ewes. The effect of lactation stage needs to be included in the model when preparing the reference values for the blood parameters of Travnik pramenka ewes, particularly for RBC profiles, energy, and protein metabolites as well as liver enzymes.

Keywords: hematological parameters, biochemical parameters, stage of lactation, Travnik pramenka ewes

INTRODUCTION

The knowledge about normal blood values of different animals is of an academic as well as of a practical importance for clinical and experimental interpretations (Antunović et al., 2013 and 2017b). The determination and acquisition of knowledge for interpretation of hematological and biochemical parameters is very important for monitoring the metabolism and the health status of ewes (Kramer, 2000; Antunović et al., 2017a and b). The importance of hematological parameters lies in the fact that it allows the tracking of possible anomalies of hematocrit, erythrocyte morphology, or the alterations in the total or differential blood cell count (Kramer, 2000). Piccione et al. (2009) observed that, during lactation, the mammary gland secretory cells utilized 80% of the blood circulating metabolites aimed for milk synthesis, depending on the speed of infiltration of the free amino acids, glucose, fatty acids and other constituents. A strong reduction of lipogenesis and an increased concentration

of fatty acids, which are supported by a norepinephrine and epinephrine stimulation, induce the increase in lipase activity in the mammary gland, providing the substrate for milk synthesis (Nazifi et al., 2002). In early lactation, energy intake is below the animals needs, indicating a negative energy balance, which mobilizes the body reserves (Antunović et al., 2017a). The mobilization of body reserves firstly activates the body fat reserves and induces changes in the non-esterified fatty acids (NEFA) and β -hydroxybutyrate (BHB) concentrations in the blood serum (van Knegsel et al., 2007), as well as in some other serum constituents (insulin and glucose). LeBlanc (2010) concluded that the elevated serum NEFA

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concentrations indicate a higher fat mobilisation rate, while an elevated BHB indicates the completeness of lipid oxidation.

The sheep are well adapted to broad ranges of environment and physiological factors. The Travnik (Vlašička, Dubska) pramenka has been bred in the western part of the Republic of Croatia since 1990, although the breed originates from Bosnia and Herzegovina. The Croatian breeders of the Travnik pramenka, in addition to the production of lambs (meat), produce the significant amounts of milk (around 70 to 130 L in lactation), which is most often processed into cheese. The Travnik pramenka is mostly reared extensively or semi-intensively, whereby almost the only feed for sheep is pasture (Novoselec et al., 2020). The hematological and serum-related biochemical parameters of the Travnik pramenka were previously determined in Bosnia and Herzegovina (Hrković Porobija et al., 2019). According to Al-Hassan (2018), the metabolic profiles are used to evaluate an animal's health and to diagnose the metabolic diseases and nutritional status. However, comprehensive research in hematological and biochemical parameters of the Travnik pramenka ewes during lactation, as well as the hematological and serum-related biochemical parameters included in investigation of ewes, are missing. Thus, the aim of the present research is to determine the hematological and serum-related biochemical parameters in the Travnik pramenka ewes during different stages of lactation.

MATERIALS AND METHODS

The analyses of hematological and serum-related biochemical parameters were performed with regard to

the Travnik pramenka ewes ($n = 36$) in age from three to five years, kept in an extensive production system in a herd numbering 1,000 sheep. They were monitored in different lactation stages: early (40 ± 5 days of lactation), medium (80 ± 5 days of lactation), and late (120 ± 5 days of lactation). The ewes used in the study were healthy and in a good physical condition. The ewes were grazing extensive pastures all day and returned to a stall afterwards to be fed with hay (*ad libitum*). Salt and water were offered to all ewes *ad libitum*.

The present research was carried out in the year 2019, when the examined ewes were in the third lactation and had single lambs in the litters. The family farm was in Velika Peratovica (in Croatia, $45^{\circ}45'25''N$, $17^{\circ}14'51''E$). In this Croatian region, the mean monthly temperature from May to July 2019 amounted to $19^{\circ}C$, while the mean monthly rainfall was 113 mm.

In Table 1, the feed composition is presented, determined by the standard methods (AOAC, 2006). The Kjeldahl method was used to determine the crude protein content of feed samples, while the Universal Extractions System B-811 (Buchi, Switzerland) was used to determine the ether extract. Ash was determined by incinerating the feed samples at $550^{\circ}C$ for six hours. The amylase-treated neutral detergent fiber (NDF) was measured according to the EN ISO 1647:2006, while the acid detergent fiber (ADF) was measured according to the EN ISO 13906. Metabolizable and digestible feed energy for ewes was determined according to the DLG (1993). Mineral concentrations in the solutions of digested plant samples were determined by an inductively coupled plasma (ICP, PerkinElmer Optima 2100 DV).

Table 1. Chemical composition of pasture in diets of investigated ewes (mg/kg DM)

Tablica 1. Kemijski sastav pašnjaka na kojem su ovce napasane (mg/kg ST)

Parameters (g/kg DM) / Pokazatelj (g/kg ST)	Green forage / Biljna masa		
	1	2	3
Crude proteins / Sirove bjelančevine	155.3	193.4	241.8
Crude fiber / Sirova vlakna	178.2	182.8	150.5
Crude ash / Sirovi pepeo	89.4	87.4	84.3
Ether extract / Ekstrakt etera	25.4	27.8	26.1
NDF, %	45.58	35.94	49.50
ADF, %	25.92	24.03	24.11
DE, MJ kg ⁻¹ DM	2.29	2.41	2.13
ME, MJ kg ⁻¹ DM	2.01	2.10	1.84
Mineral content (mg/kg DM) / Sadržaj minerala (mg/kg ST)			
Ca	8870.84	7261.10	6986.36
Mg	3395.83	1869.26	2755.74
K	44769.84	39178.03	49822.06
P	5748	5857	5100
Na	104.36	40.96	205.55
Fe	226.60	207.90	274.30
Se	0.052	0.047	0.066

DM – dry matter / ST – suha tvar; NDF – neutral detergent fiber / NDF – neutralna deterđžentna vlakna; ADF – acid detergent fiber / ADF – kisela deterđžentna vlakna; DE – digestible energy / probavljiva energija; ME – metabolisable energy / metabolička energija

Each ewe sample was obtained in each of the three lactation stages, always prior to grazing. The blood samples for a hematology analysis were taken from the jugular vein (10 mL) into two vacuum tubes (Venoject®, Sterile Terumo Europe, Leuven, Belgium) containing an anticoagulant (EDTA). The determination of hematological parameters, like the white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin (HGB) concentration, hematocrit (HCT) value, mean corpuscular volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC) and platelet (PLT) count, was measured in the whole blood of the investigated ewes. The hematological parameters were measured by an automatic three differential hematology analyzer (Poch-100iV, Sysmex Europe GmbH, Hamburg, Germany). The Pappenheim method was used to determine a differential blood cell count by microscope, using the colored blood smears.

The blood samples for the determination of serum-related biochemical values were collected in the vacuum tubes (Venoject®, Sterile Terumo Europe, Leuven, Belgium) and were centrifuged at 1609.92 *g* within 10 minutes subsequent to the sampling to obtain the serum samples. The concentrations of minerals (Ca, P-inorganic, K, Na, Mg, chloride, and Fe) were determined in serum. Likewise, the concentration of total proteins, albumin, urea, glucose, cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides (TGC), lactate, beta-hydroxybutyrate (BHB) and non-esterified fatty acids (NEFA). The enzyme activities were determined in the serum, like aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ -glutamyl transferase (GGT) and creatine kinase (CK), by the usage of Olympus System reagents (Olympus Diagnostic GmbH, Lismeehan, Ireland). The globulin content was calculated from the total protein and albumin difference. The *Ranse/*® kit (Randox,

UK) was used to determine the activity of glutathione peroxidase (GPx), while the activity of total superoxide dismutase (SOD) in serum was determined using the *Ransod*® kit (Randox, UK). All biochemical parameters and enzyme activities were carried out on an automatic *Olympus AU 400* (Olympus, Japan).

The present study was carried out under the legal provisions according to the Animal Protection Act (Republic of Croatia's *Official Gazette* No. 133 [2006], No. 37 [2013], and No. 125 [2013]) as approved by Committee for Animal Welfare of Faculty of Agrobiotechnical Sciences Osijek

The results were treated by the MEANS procedure, while the effect of the lactation stage was analyzed by the GLM procedure, processed by the SAS 9.4®. The means were compared by the Tukey's test, and the differences between the groups were declared significant at $P < 0.05$.

RESULTS AND DISCUSSION

The values of hematological parameters of the Travnik pramenka ewes during lactation were within the reference values (Kramer, 2000; Antunović et al., 2011). In the present study, a reduction of WBC at the earliest stage of lactation was determined (Table 2). These changes may be attributed to an increase in the cortisol level (as a result of an increased milk yield), which is probably responsible for the impairment of the cellular immune response (Caroprese et al., 2012). An opposite trend for the WBC in the ewes' blood was determined by Antunović et al. (2017b). Nozad et al. (2014) determined the statistical differences in the monthly values of hematological parameters (hemoglobin, erythrocyte, and leucocytes) in the Holstein dairy cows, which were within the normal range and could not be a consequence of infectious disorders.

Table 2. Hematological parameters and differential blood cell count of ewes in different lactation stages

Tablica 2. Hematološki pokazatelji i distribucija leukocita u ovaca tijekom različitih stadija laktacije

	Early / Rani	Medium / Srednji	Late / Kasni	SEM	P-value / P-vrijednost
WBC ($\times 10^9/L$)	6.28 ^a	9.13 ^b	8.97 ^b	0.293	< 0.001
RBC ($\times 10^{12} L$)	8.84 ^a	9.97 ^b	9.85 ^b	0.124	< 0.001
HGB (g/L)	98.15 ^a	111.95 ^b	114.61 ^b	1.525	< 0.001
HCT (L/L)	0.37 ^a	0.41 ^b	0.39 ^{ab}	0.006	0.021
MCV (fL)	41.32 ^a	40.28 ^{ab}	39.46 ^b	0.231	0.004
MCH (pg)	11.08 ^a	11.24 ^a	11.66 ^b	0.065	< 0.001
MCHC (g/L)	270.10 ^a	280.47 ^a	296.29 ^b	2.761	< 0.001
PLT ($\times 10^9/L$)	418.59	441.76	402.94	16.506	0.641
Differential blood cell count (%) / Diferencijalna krvna slika (%)					
Lymphocytes / Limfociti	61.92 ^a	53.05 ^b	53.77 ^b	1.165	0.001
Neutrophils / Neutrofili	34.49	37.68	33.39	1.118	0.270
Monocytes / Monociti	0.256	0.447	0.226	0.079	0.470
Eosinophils / Eozinofili	3.10 ^a	8.76 ^a	12.35 ^c	0.686	< 0.001
Basophils / Bazofili	0.231	0.053	0.258	0.041	0.084

SEM – standard error of mean / SEM – standardna pogreška srednje vrijednosti; a, b, c – values in rows with different letters differ significantly ($P < 0.05$) / a, b, c – vrijednosti u redovima s različitim slovima značajno se razlikuju ($P < 0,05$); WBC – number of leukocytes / WBC – broj leukocita, RBC – erythrocytes / RBC – broj eritrocita, HGB – hemoglobin / HGB – hemoglobin, HCT – hematocrit / HCT – hematokrit, MCV – mean corpuscular volume / MCV – prosječni volumen eritrocita, MCH – average hemoglobin content in erythrocytes / MCH – prosječna masa hemoglobina u eritrocitu, MCHC – mean hemoglobin concentration in erythrocytes / MCHC – prosječna koncentracija hemoglobina u krvi, PLT – platelet count / PLT – broj trombocita.

This indicated an increased load of the ewes' organism during lactation period, which is reflected in the changes of hematological parameters. A differential blood cell count is very important because the changes in individual white cell types may occur simultaneously, leaving a total WBC count unchanged. Lactation is a very stressful period for an animal and the major contributor in various physiological variations (Kim et al., 2020). In

the studies of Tsigai ewes in the first third of lactation, Antunović et al. (2011) have not detected significant differences in hematological parameters during the progress of lactation. Kim et al. (2020) suggested that a particular attention should be devoted to the hematological parameters such as the HGB, HCT, and RBC, which has demonstrated significant differences in various lactation stages of the Holstein cows.

Table 3. Blood minerals concentrations from ewes in different lactation stages

Tablica 3. Koncentracije minerala u krvi ovaca tijekom različitih stadija laktacije

Indicator, mmol/L / Indikator, mmol/L	Early / Rani	Medium / Srednji	Late / Kasni	SEM	P-value / P-vrijednost
Ca	2.25 ^a	2.72 ^b	2.62 ^b	0.027	< 0.001
P	1.18 ^a	1.81 ^a	2.08 ^b	0.038	< 0.001
Na	126.82 ^a	130.18 ^{ab}	136.93 ^b	1.36	< 0.001
K	6.00	6.27	6.58	0.41	0.201
Mg	1.01 ^a	1.08 ^b	1.19 ^c	0.104	< 0.001
Cl	106.67 ^a	112.87 ^{ab}	118.20 ^b	1.10	< 0.001
Fe (μmol/L)	28.02 ^a	23.02 ^b	21.62 ^b	0.623	< 0.001

SEM – standard error of mean / SEM – standardna pogreška srednje vrijednosti; a, b, c – values in rows with different letters differ significantly ($P < 0.05$) / a, b, c – vrijednosti u redovima s različitim slovima značajno se razlikuju ($P < 0,05$).

The lowest serum concentrations of Ca were determined in the early stage of lactation (Table 3), and the reason for this phenomenon could be an increased excretion of Ca through milk due to an increased milk production during the early lactation (Antunović et al., 2011). Calcium and phosphorus are mobilized from the bones similarly, although more calcium from the body and blood is excreted through milk (Codreanu and Calin, 2018). Liesgang et al. (2007) determined a lower concentration of Ca in the blood of ewes after lambing and in early lactation, which might be related to an increased secretion of Ca by milk and its rearrangement in bones. The same authors have detected an opposite trend for Mg concentrations. A significantly increased Ca concentration and an Mg and P concentration not significantly

increased in the blood of cows from three to six and from 22 to 38 weeks of lactation were determined by Abdel-El-Naser et al. (2014). A significantly decreased Fe concentration in the blood of ewes during the progression of lactation could be induced by an increased excretion through milk. The similar results were determined by Antunović et al. (2011) in the Tsigai ewes during the first third of lactation. An increase of serum Na and Cl concentration in the lactating ewes from the 40th to the 60th day were determined by Codreanu and Călin (2018). The reason for this phenomenon could be a decreased excretion of electrolytes through milk due to a decreased milk production during the late lactation stage.

Table 4. Biochemical parameters in blood from ewes in different lactation stages

Tablica 4. Biokemijski pokazatelji u krvi ovaca tijekom različitih stadija laktacije

Indicator, mmol/L Indikator, mmol/L	Early / Rani	Medium / Srednji	Late / Kasni	SEM	P-value / P-vrijednost
Glucose / Glukoza	3.46 ^b	3.64 ^b	4.90 ^a	0.104	< 0.001
Urea	9.69 ^a	9.39 ^a	11.02 ^b	0.199	0.002
Total proteins (g/L) / Ukupne bjelančevine (g/L)	71.40 ^a	82.43 ^b	85.51 ^c	0.773	< 0.001
Albumin (g/L)	28.69 ^a	31.42 ^b	30.69 ^b	0.215	< 0.001
Globulin (g/L)	42.71 ^a	51.01 ^b	54.82 ^c	0.679	< 0.001
CHOL	2.10	2.09	1.99	0.037	0.442
HDL	1.41	1.35	1.29	0.020	0.062
LDL	0.59	0.60	0.56	0.019	0.768
TGC	0.29	0.31	0.30	0.009	0.695
NEFA	0.38 ^a	0.14 ^b	0.13 ^b	0.027	< 0.001
BHB	0.27 ^a	0.37 ^b	0.39 ^b	0.009	< 0.001

SEM – standard error of mean / SEM – standardna pogreška srednje vrijednosti; a, b, c – values in rows with different letters differ significantly ($P < 0.05$) / a, b, c – vrijednosti u redovima s različitim slovima značajno se razlikuju ($P < 0,05$); CHOL – cholesterol / CHOL – kolesterol, HDL – high-density lipoprotein / HDL – lipoproteini visoke gustoće, LDL – low-density lipoprotein / LDL – lipoproteini male gustoće, TGC – triglycerides / TGC – trigliceridi, NEFA – non-esterified fatty acids / NEFA – neesterificirane masne kiseline, BHB – β-hydroxybutyrate / BHB – β-hidroksibutirat.

The energy status assessment in the examined ewes was performed by the determination of glucose, cholesterol, HDL, LDL, triglycerides, NEFA and BHB concentrations in blood, while urea, total protein, and albumin concentration, as well as a CK activity, were used as the indicators of ewes' protein supply by feed (Antunović et al., 2017a). Significantly lower concentrations of NEFA and glucose and a higher BHB in blood of early lactating sheep in the present study indicate an energy deficit. In a pronounced negative energy balance, the intensive lipomobilization mostly leads to a decreased extend of gluconeogenesis, followed by hypoglycemia at the beginning of lactation (Doepel et al., 2002). A lowered concentration of glucose in the blood of early lactating dairy cows was determined by Abd-El Naser (2014). After lambing, a decrease in the NEFA and an increase in the BHB concentrations have indicated a negative energy balance when lipomobilization stops (Douglas et al., 2006;). Pesántez-Pacheco et al. (2019) have determined the highest levels of BHB and the smallest levels of NEFA in a postpartum period

of high-yielding ewes, which means that these changes had occurred earlier than in the low-yielding ewes. In the present study, a blood NEFA concentration (0.38 mmol/L) in early lactation shows evidence of a high lipomobilization. The increase of NEFA and BHB concentrations in the blood of ewes in early lactation, along with a decreased glucose in blood, could also reflect a low energy intake by the ewes. An increase in both the NEFA and glucose concentrations, along with a decrease in BHB in the blood of ewes during a medium and late stage of lactation, indicate a satisfactory energy supply by sheep at the later stages of lactation. During the dairy cows' medium lactation stage, the serum NEFA concentration were relatively low because an energy balance becomes positive when the cows supply a tissue reserve (Walters et al., 2002). A significant increase of glucose concentration in blood, as well as the total proteins, urea, and triglycerids in the ewes' blood, affected by a lactation stage, was also determined by Codreanu and Calin (2018).

Table 5. Blood enzymes activities from ewes in different lactation stages

Tablica 5. Aktivnosti enzima u krvi ovaca tijekom različitih stadija laktacije

Enzyme, U/L / Enzim, U/L	Early / Rani	Medium / Srednji	Late / Kasni	SEM	P-value / P-vrijednost
AST	138.62	154.64	181.37	10.195	0.245
ALT	21.05 ^a	26.12 ^b	24.22 ^b	0.531	< 0.001
ALP	163.54	206.34	173.92	10.810	0.225
GGT	60.16 ^a	50.02 ^b	52.85 ^b	1.457	< 0.001
CK	278.31	181.05	119.55	25.386	0.125
SOD, U/mL	0.31	0.29	0.34	0.013	0.386
GPx	251.20	217.35	257.57	25.160	0.792

SEM – standard error of mean / SEM – standardna pogreška srednje vrijednosti; a, b, c – values in rows with different letters differ significantly ($P < 0.05$) / a, b, c – vrijednosti u redovima s različitim slovima značajno se razlikuju ($P < 0.05$); AST – aspartate aminotransferase / AST – aspartat aminotransferaza, ALT – alanine aminotransferase / ALT – alanin aminotransferaza, ALP – alkaline phosphatase / ALP – alkalna fosfataza, GGT – γ -glutamyl transferase / GGT – γ -glutamil transferaza, CK – creatine kinase / CK – kreatin kinaza, SOD – superoxide dismutase / SOD – superoksid dismutaza, GPx – glutathione peroxidase / GPx – glutation peroksidaza.

The concentration of urea in the blood of ewes in the medium and late lactation stages have significantly increased, as compared to an early stage. A similar phenomenon was observed by Antunović et al. (2017a) and Roubies et al. (2006), who reported that this could either be due to an increased deamination or due to an increased protein intake. Abdelatif et al. (2009) determined a gradual increase of urea concentration in the lactating ewes along with the lactation, which was possibly due to an increase in the feed intake, related to the higher nutrient demands.

In the present study, the differences between lactation concerning the blood cholesterol and triglycerides were not significant, although a decrease is evident. A similar conclusion was derived by Kim et al. (2020) in the Holstein cows during various lactation stages, suggesting that a particular attention should be devoted to the biochemical parameters and enzyme

activity determination in blood, such as the concentrations of urea, glucose, NEFA and BHBA, as well as to the activities of ALT and GGT that have manifested significant differences in various lactation stages. In the present study, the GGT was within the reference values for sheep (15-60 U/L; Kaneko et al., 2008). A significantly higher activity of ALT, as well as a non-significant activity of AST in the ewes' blood during the lactation progress, could be related to the quality of diet (pasture). Radkowska and Herbut (2014) found that a high concentration of nitrogen compounds in pasture may influence the nitrogen metabolism in liver, which could be manifested by the higher serum activities of ALT and AST. Roubies et al. (2006) concluded that a more intense liver function of lactating ewes was due to the higher requirements for energy and proteins, necessary for the maintenance and milk production. Similar results with the Merinolandschaft ewes during lactation were found by Antunović et al. (2017a).

CONCLUSION

In the present study, the differences of the most of hematological and serum biochemicals were determined in the Travnik pramenka ewes during lactation. The effect of the lactation stage needs to be included in the model when preparing the reference values for the blood parameters of the Travnik pramenka ewes, particularly for the RBC profiles, energy, and protein metabolites, as well as for the liver enzymes. The obtained results may be helpful in monitoring the health and nutritional status, as well as in the ewe management improvement.

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UTJECAJ STADIJA LAKTACIJE NA HEMATOLOŠKE I BIOKEMIJSKE POKAZATELJE KRVI OVACA TRAVNIČKE PRAMENKE

SAŽETAK

Cilj ovoga istraživanja bio je utvrditi hematološke i biokemijske pokazatelje u krvi ovaca travničke pramenke tijekom laktacije. Istraživanje je provedeno na 36 ovaca prosječne dobi od tri do pet godina tijekom triju stadija laktacije: ranoga, srednjeg i kasnog. WBC i RBC, koncentracija HGB-a te vrijednost HCT-a i MCH-a značajno su se povećavali tijekom laktacije, dok je utvrđeno i značajno smanjenje vrijednosti MCV-a i MCHC-a u krvi. Sadržaj HCT-a bio je viši u ranome stadiju u odnosu na kasni stadij laktacije, a sadržaj MCH-a u srednjem stadiju u odnosu na kasni stadij laktacije. Utvrđeno je značajno povećanje koncentracije Ca, P, Mg, Na i Cl tijekom laktacije, kao i smanjenje koncentracije Fe. Kako je laktacija napredovala, utvrđeno je povećanje koncentracije ureje, ukupnih proteina, albumina, globulina i BHB-a te aktivnosti ALT-a. Suprotno tome, koncentracije glukoze i NEFA-e su se smanjile, kao i aktivnost GGT-a. Dobiveni rezultati mogu pomoći u praćenju zdravstvenoga i hranidbenoga statusa travničke pramenke. Stoga je u model za utvrđivanje referentnih vrijednosti krvi ovaca travničke pramenke potrebno uključiti utjecaj stadija laktacije, osobito za RBC, energetske i proteinske metabolite te jetrene enzime.

Ključne riječi: hematološki pokazatelji, biokemijski pokazatelji, stadij laktacije, travnička pramenka

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