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Influence of lactation stages on haematological and biochemical parameters in blood of Lacaune dairy sheep

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Abstract

The research objective was to determine haematological and biochemical parameters in blood of 30 Lacaune dairy sheep during different stages of lactation (early: 60th, medium: 120th and late: 180th days). The sheep were on average 4 years old, in 3rd lactation. Haematological parameters were determined in whole blood (white blood cell (WBC), red blood cell (RBC), haemoglobin (HGB) concentration, haematocrit (HCT) value, mean corpuscular volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC) and platelet (PLT) count), and a blood smear was used for determination of differential blood cell count. Determined biochemical parameters in blood serum were: concentration of minerals (Ca, P-inorganic, Mg and Fe), concentration of total proteins (TP), albumin (ALB), globulins (GLOB), urea, glucose (GUK), cholesterol (CHOL), high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides (TGC), beta-hydroxybutyrate (BHB) and non-esterified fatty acids (NEFA), enzyme activities (aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ -glutamyl transferase (GGT), creatine kinase (CK), activity of glutathione peroxidase (GPx) and total superoxide dismutase (SOD). With progression of lactation stages, sheep blood tests proved significant increase in WBC, RBC, HGB, HCT, monocytes, Fe, urea, TP, ALB, CHOL, HDL, LDL, BHB, AST, GGT and ALT as well as a decrease in lymphocytes, Mg, GUK and SOD. Determined changes in haematological and biochemical blood parameters of Lacaune dairy sheep during different lactation stages proved good quality nutrition of sheep and indicate the need to include this dairy breed/genotype in prospective schemes for definition of referential values for these indicators in sheep.

Key words: Lacaune dairy sheep; metabolic profile; lactation; lactation stage; adjustment

Introduction

There is a growing interest in breeding dairy sheep because of milk quality and consumer demand for sheep milk cheese. Pulina et al. (2018) pointed out that the dairy sector of small ruminants indicates potentials for significant improvement, as there is expected increase in production by 30–50 % until 2030. Four European countries (France, Greece, Italy and Spain) are leaders in the breeding of quality dairy sheep and goat breeds. Demand for the export of dairy sheep breeds is growing, and the French sheep breed Lacaune is especially popular. In 1992, it was officially confirmed that the germplasm of Lacaune sheep from France was imported into 17 countries (Barillet et al., 2001). The majority of Lacaune sheep breeding is concentrated in the areas of Roquefort, which is well-known for cheese production (full-fat, hard Roquefort cheese is especially famous). Lacaune sheep is of medium size, of stronger constitution with pronounced depth and medium developed carcass width. Careful selection for lactation traits resulted in sheep populations with high lactation potential. According to the study of Antunović et al. (2022) carried out in the Republic of Croatia, the average body weight of Lacaune sheep is 60.94 kg, and the withers height is 68.05 cm. In lactation stage from day 180 to day 200, the sheep produces around 200 litres of milk, however the best heads may give over 400 litres of milk (Mioč et al., 2007).

In recent years, the breeding of Lacaune sheep spread in the Republic of Croatia not only for the purpose of milk production, but also for pure blood breeding. Therefore, there is a need to determine the changes in haematological and biochemical parameters in the sheep blood, i.e. to define the blood metabolic profile of lactating Lacaune sheep in order to monitor the adjustment process. Lactation is one of the most demanding physiological periods in sheep life. Therefore, special attention should be given to this period in order to determine the needs of sheep during different stages of lactation, aiming to prevent major production losses and deterioration of sheep health (Antunović et al., 2021). This is especially important in highly productive animals. There is significant link between metabolic profile indicators, which include hematological and biochemical parameters in the blood, and nutritional status of sheep (Antunović et al., 2002; González-García et al., 2015). Al-Hassan (2018) pointed out that metabolic profiles should be used to evaluate animal health, to diagnose metabolic diseases and to determine nutritional status. Mineral status in blood and other body fluids depends on many factors, such as metabolic use, intestine physiology, homeostasis, inadequate ingestion or availability, excretion, etc. (Boudebza et al., 2022). The energy status assessment in the examined sheep was performed by determination of GUK, CHOL, HDL, LDL, TGC, NEFA and BHB concentrations in blood, while urea, TP and ALB concentration, as well as CK activity were used as the indicators of sheep protein supply through feed intake (Antunović et al., 2017a). Dos Santos et al. (2021) stated that the metabolic profile could be a useful tool for investigation of possible adaptation of dairy sheep to different conditions. In this sense, Nedeva et al. (2022) pointed out that determination of biochemical parameters

in Lacaune sheep blood serum was an objective indicator of their well-being.

The aim of this research was to determine haematological and biochemical parameters in blood of Lacaune sheep during different stages of lactation.

Material and methods

Experimental design and animal selection

The research was carried out on 30 Lacaune sheep during different stages of lactation. Sheep for this research were selected from a herd of 200 heads at the Orkić Family Farm in Gundinci (latitude: 45.155; longitude: 18.492). Sheep were in the early stage of lactation (day 60) selected immediately after weaning of lambs and monitored throughout the medium (day 120) and late stages of lactation (day 180). In addition to the same stage of lactation, criteria for selection of sheep were their age, which was on average 4 years, and one lamb in the litter. The average body weight of sheep was 61 kg and body condition score (BCS) was 3.05. The milk yield during lactation (morning milking) was 1.76 ± 0.48 , 1.47 ± 0.41 , 0.91 ± 0.35 kg in early, medium and late lactation stage, respectively. The sheep included in the research were healthy and in good physical condition.

The research was carried out by obeying legal provisions determined by the Animal Protection Act (Republic of Croatia *Official Gazette* No. 133 (2006), No. 37 (2013), and No. 125 (2013)), and approved by the Committee for Animal Welfare of the Faculty of Agrobiotechnical Sciences Osijek.

Sheep nutrition and feed analysis

Sheep were fed pelleted feed with 15 % of crude protein in the amount of 1.00 kg/day, mixture of cereals (1/3 oat and 2/3 barley) in the amount of 600 g/day and alfalfa hay ad libitum. They also had animal salt and water ad libitum. Feed samples (feed mixture, cereal mixture and hay) were dried and ground into a fine powder by using a heavy metal free ultra-centrifugal mill (Retsch ZM 200) or knife mill (GM 200). Composition of feed was determined by using standard methods (AOAC, 2006). Chemical composition of feed is presented in Table 1.

Chemical composition of feed as well as determination of NDF (neutral detergent fibre), ADF (acid detergent fibre) and ADL (acid detergent lignin) and the use of methods are described in the study by Antunović et al (2021).

Blood samples and preparation for analyses

Sheep blood (10 mL) was taken in the morning from a jugular vein into two vacuum tubes (*Venoject*[®], Sterile Terumo Europe, Leuven, Belgium) containing an anticoagulant (EDTA). Sheep blood was sampled in different stages of

Table 1. Chemical composition of Lacaune sheep feed (mg/kg DM)

| Parameters (g/kg DM) | Feed | | |
|----------------------|--------------|----------------|--------|
| | Feed mixture | Cereal mixture | Hay |
| DM | 917.20 | 910.10 | 914.60 |
| Crude proteins | 149.9 | 139.1 | 136.7 |
| Crude fibre | 51.1 | 44.1 | 311.0 |
| Crude ash | 50.6 | 20.1 | 66.5 |
| Ether extract | 26.9 | 28.0 | 10.1 |
| NDF | 36.56 | 40.06 | 65.06 |
| ADF | 7.1 | 4.93 | 40.8 |
| ADL | 2.22 | 1.29 | 10.25 |

DM - dry matter; NDF - neutral detergent fibre; ADF - acid detergent fibre; ADL - acid detergent lignin

lactation. In the whole blood of the investigated Lacaune sheep were measured haematological parameters [(counts of white blood cell (WBC), red blood cell (RBC) and platelet (PLT), concentration haemoglobin (HGB) and haematocrit value (HCT), mean corpuscular volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC)]. The haematological parameters were measured by an automatic three differential veterinary haematology analyser (*Poch-100iV*, Sysmex Europe GmbH, Hamburg, Germany). Differential blood cell count was determined by microscope, using the coloured blood smears according to the Pappenheim method. Sheep blood used for determination of serum-related biochemical values was collected in the vacuum tubes (*Venoject*[®], Sterile Terumo Europe, Leuven, Belgium) and centrifuged at 1609.92 g within 10 minutes subsequent to the sampling to obtain the serum samples. The concentrations of minerals (Ca, P-inorganic, Mg and Fe), as well as the concentration of total proteins (TP), albumin (ALB), urea, glucose (GUK), cholesterol (CHOL), high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides (TGC), beta-hydroxybutyrate (BHB) and non-esterified fatty acids (NEFA) were determined in serum. In the blood serum

of Lacaune sheep were measured the enzyme activities [(aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ -glutamyl transferase (GGT) and creatine kinase (CK)] by using Olympus System reagents (Olympus Diagnostic GmbH, Lismeehan, Ireland). The globulin content was calculated from the total protein and albumin difference. After that, activity of glutathione peroxidase (GPx) in the blood serum of Lacaune sheep was determined by *Ransel*[®] kit (Randox, UK) and activity of total superoxide dismutase (SOD) was used to *Ransod*[®] kit (Randox, UK). All biochemical parameters and enzyme activities were determined on an automatic *Olympus AU 400* device (Olympus, Japan).

Statistical analyses

Obtained research results were processed by the MEANS procedure, while the influence of the lactation stages was analysed by the GLM procedure and processed by the SAS 9.4[®]. The means were compared by the Tukey's test, and the differences between the groups were declared as significant at $p < 0.05$.

Results and discussion

Miglio et al. (2018) showed that as starting point to investigated diseases, reference values for clinically healthy mid-lactating Lacaune sheep, may be used to determine the health and welfare of lactating Lacaune sheep. By analysing the data contained in the Table 2, along with the progression of lactation stages, there was a significant increase in WBC and RBC counts, as well as in the content of HGB and HCT in the blood of Lacaune sheep. It was also determined (Table 3) that lymphocytes decreased and monocytes increased, while other indicators of the haematological profile did not change significantly. When comparing the stages of lactation, the

Table 2. Effect of lactation stage on haematological parameters in blood of Lacaune dairy sheep

| Parameter | Stage of lactation | | | SEM | P-value |
|----------------------------|---------------------|---------------------|---------------------|--------|---------|
| | early | medium | late | | |
| WBC ($\times 10^9/L$) | 12.24 ^b | 12.06 ^b | 15.18 ^a | 0.491 | 0.017 |
| RBC ($\times 10^{12}/L$) | 8.93 ^b | 9.85 ^a | 10.63 ^a | 0.134 | <0.001 |
| HGB (g/L) | 105.11 ^b | 111.85 ^b | 122.60 ^a | 1.448 | <0.001 |
| HCT (L/L) | 0.38 ^b | 0.41 ^b | 0.46 ^a | 0.006 | <0.001 |
| MCV (fL) | 43.71 | 42.18 | 43.48 | 0.272 | 0.050 |
| MCH (pg) | 11.83 | 11.38 | 11.56 | 0.088 | 0.094 |
| MCHC (g/L) | 271.53 | 270.55 | 266.33 | 2.318 | 0.641 |
| PLT ($\times 10^9/L$) | 495.37 | 390.39 | 374.97 | 22.234 | 0.050 |

SEM - standard error of mean; ^{a, b} - means with different superscript letters differ significantly ($p < 0.05$); WBC - white blood cell; RBC - red blood cell; HGB - haemoglobin; HCT - haematocrit; MCV - mean corpuscular volume; MCH - mean cell haemoglobin; MCHC - mean cell haemoglobin concentration; PLT - platelet

late lactation stage exhibited significantly higher WBC count and the content of HGB and HCT than the early and middle stage of lactation, while there were no significant differences between early and middle stage of lactation. Only RBC count in blood of Lacaune sheep differed significantly between the middle and the late lactation stage.

For any animal lactation is a very stressful period and it is a major contributor in various physiological variations. The haematological profile of blood is a part of physiological variables that provide valuable information on health and welfare status of ruminants and can be therefore considered as an indicator of production performance (Bezerra et al., 2017; Antunović et al., 2017b). Most haematological parameters in the blood of lactating Lacaune sheep were within the referential values for sheep (Kramer, 2000; Antunović et al., 2021). Unlike in later lactation stages, the increase in WBC count in blood of lactating Lacaune sheep can be associated with higher milk production in the early stage of lactation (0.91:1.76 kg). Similar observations were made by Antunović et al. (2021) when analysing the blood of Travnik Pramenka sheep during lactation, as they connected significant increase in WBC count and in content of HGB, HCT, MCV and MCHC, but also a decrease in MCV, lymphocytes and eosinophils with the progression of lactation stages. This indicated an increased burden of lactation on the sheep' organism, which reflected in the changes of haematological parameters (Antunović et al., 2021). In the investigation with Holstein cows during lactation, Kim et al. (2020) concluded that particular attention should be given to haematological parameters, such as HGB, HCT and RBC, which demonstrated significant differences in various lactation stages.

The Table 4 overviews concentrations of some minerals in the blood of Lacaune sheep. As lactation progressed, the blood of Lacaune sheep exhibited significant increase in Fe concentrations and the decrease in Mg concentrations.

The research of Antunović et al. (2021) carried out on lactating Travnik sheep proved significant increase in concentrations of most minerals in blood of sheep (Ca, P, Na, Mg and Cl), as well as the decrease in concentrations of Fe as the lactation progressed. Liesgang et al. (2007) determined similar changes for magnesium concentration in blood of lactating sheep.

Boudebza et al. (2022) tested sheep blood and determined concentrations of Mg were higher in the middle lactation stage than in the early lactation stage, which is opposed to the results obtained in this research. An increase in Fe in the blood of Lacaune sheep is associated with a decrease in milk production with the approaching end of lactation (from 1.76 to 0.91 kg), which led to a excretion of Fe with milk. Miglio et al. (2018) determined that changes in levels of iron in blood serum differed from those found in other sheep breeds, which indicated considerable variations depending on age, physiological status and breed/genotype of the sheep.

This research proved significant increase in the concentrations of urea, TP, ALB, and CHOL, HDL, LDL and BHB, and the decrease in GUK in blood of Lacaune sheep as lactation stages progressed (Table 5). Concentrations of other biochemical parameters in blood of Lacaune sheep (TGC and NEFA) did not change much in different stages of lactation.

Table 3. Effect of lactation stage on differential blood cell count of Lacaune dairy sheep

| Parameter (%) | Stage of lactation | | | SEM | P-value |
|-----------------------|--------------------|---------------------|--------------------|-------|---------|
| | early | medium | late | | |
| Lymphocytes | 66.32 ^a | 65.00 ^{ab} | 60.80 ^b | 0.920 | 0.042 |
| Segmented neutrophils | 25.34 | 24.94 | 29.07 | 0.503 | 0.095 |
| Band cells | 1.45 | 1.88 | 1.07 | 0.152 | 0.107 |
| Basophils | 0.18 | 0.18 | 0.43 | 0.068 | 0.250 |
| Eosinophils | 6.55 | 7.64 | 7.60 | 0.503 | 0.594 |
| Monocytes | 0.16 ^b | 0.36 ^b | 1.03 ^a | 0.097 | <0.001 |

^{ab} - means with different superscript letters differ significantly ($p < 0.05$)

Table 4. Effect of lactation stage on blood mineral concentration in Lacaune dairy sheep

| Parameter (mmol/L) | Stage of lactation | | | SEM | P-value |
|--------------------|--------------------|--------------------|--------------------|-------|---------|
| | early | medium | late | | |
| Ca | 2.40 | 2.49 | 2.44 | 0.022 | 0.210 |
| P | 1.83 | 1.81 | 1.95 | 0.053 | 0.550 |
| Mg | 1.53 ^a | 1.32 ^b | 1.40 ^a | 0.026 | 0.002 |
| Fe (μ mol/L) | 17.57 ^b | 17.59 ^b | 25.16 ^a | 1.013 | 0.003 |

SEM - standard error of mean; ^{a, b} - means with different superscript letters differ significantly ($p < 0.05$)

Table 5. Effect of lactation stage on biochemical parameters in blood of Lacaune dairy sheep

| Parameter (mmol/L) | Stage of lactation | | | SEM | P-value |
|--------------------|--------------------|---------------------|--------------------|-------|---------|
| | early | medium | late | | |
| GUK | 3.62 ^a | 2.74 ^b | 2.88 ^b | 0.063 | <0.001 |
| UREA | 8.37 ^b | 10.80 ^a | 9.85 ^a | 0.230 | <0.001 |
| TP (g/L) | 81.26 ^b | 86.02 ^{ab} | 89.83 ^a | 0.992 | 0.001 |
| ALB (g/L) | 32.81 ^b | 34.48 ^{ab} | 35.91 ^a | 0.441 | 0.014 |
| GLOB (g/L) | 48.45 | 51.54 | 53.91 | 1.061 | 0.106 |
| CHOL | 1.94 ^b | 2.91 ^a | 2.96 ^a | 0.079 | <0.001 |
| TGC | 0.36 | 0.40 | 0.40 | 0.007 | 0.050 |
| HDL | 1.13 ^b | 1.57 ^a | 1.58 ^a | 0.036 | <0.001 |
| LDL | 0.65 ^b | 1.16 ^a | 1.20 ^a | 0.045 | <0.001 |
| BHB | 0.20 ^b | 0.32 ^a | 0.33 ^a | 0.012 | <0.001 |
| NEFA | 0.10 | 0.09 | 0.10 | 0.013 | 0.929 |

SEM - standard error of mean; ^{a, b} - means with different superscript letters differ significantly ($p < 0.05$); TP - total proteins; ALB - albumin; GUK - glucose; CHOL - cholesterol; HDL - high-density lipoprotein; LDL - low-density lipoprotein; TGC - triglycerides; BHB - beta-hydroxybutyrate; NEFA - non-esterified fatty acids

By monitoring the progression of lactation stages, Antunović et al. (2021) determined the increase of concentrations of GUK, urea, TP, ALB, GLOB, BHB and the decrease in NEFA concentrations in blood of Travnik Pramenka sheep. The research of Gonzales-Garcia et al. (2015) carried out in France confirmed the decrease of NEFA and significant decrease of GUK in blood of Lacaune sheep in lactation stage from day 35 to day 103, as well as until day 133. Concentration of BHB was also increasing with progression of lactation stages.

Decreased blood GUK concentrations in lactating sheep have to be considered in light of constant energy loss because of milk production. Decreased blood serum TGC

and CHOL concentrations in early lactation Lacaune sheep are consistent with an increased energy requirement during this period of lactation. Similar conclusion was also reached by Antunović et al. (2011).

If compared to the early lactation stage, urea concentration in the blood of sheep during medium and late lactation stages was significantly increased. Similar result was obtained by Antunović et al. (2017a) in their research on Merinolandschaf sheep during lactation.

The number of authors (Araujo et al., 2015; Kelly et al., 2000) showed that blood proteins are taken as indicators of gut metabolic function and health status. When monitoring progression of lactation stages, Brito et al. (2006) determined significant increase of urea, CHOL, and P concentration, significant decrease of GUK, as well as non-significant ALB in blood of lactating Lacaune sheep. Blood ALB concentrations show long-term protein status of animals, while TP concentrations are the best indicator of protein reserves in an organism. When compared with reference values for TP and ALB, defined by Kaneko et al. (2008) as 60-79 and 24-30 g/L, respectively, this research measured higher concentrations for both indicators, which is connected with good quality nutrition rich in protein. High-protein feed is also reflected in the increased urea concentration in blood of lactating sheep, which have been increased significantly if compared with the reference values of 2.86-7.14 mmol/L, according to Kaneko et al. (2008). It is known that urea N concentrations in milk or blood can be used as indicators of protein intake and metabolism of lactating sheep (Cannas et al., 1998), and that they relate to more intensive nutrition provided to herds with higher milk yields. Positive effects of high-protein diet on TP and ALB levels were repeatedly reported in earlier study on cows (Raggio et al., 2007). The research of Slavov et al. (2018) carried out in Bulgaria confirmed expected variations of TP, ALB and GLOB concentrations in blood of Lacaune sheep being in the first and second lactation stage, yet all values were within the normal physiological range. The influence of lactation stage on significant increase of TP, urea, BHB and TGC in sheep' blood was also determined by Codreanu and Calin (2018).

In this research, differences in influence of different lactation stages on the blood CHOL were significant. CHOL is a component of serum lipoproteins. Its concentration in serum indicates overall lipoprotein concentrations. CHOL concentrations in cows were at the lowest level in their early lactation (Jozwik et al., 2012). Blood CHOL level increased over time after calving, which is a physiological effect necessary to ensure sufficient VLDL synthesis to avoid accumulation of TGC in the liver (Gross et al., 2013). In their research on lactating sheep, Nazifi et al. (2002) confirmed the increase in concentrations of HDL and LDL cholesterol with the progression of lactation. The increase of cholesterol levels with peak values lasting 8 weeks in lactating cows was reported by Machal et al. (1996), which suggested that that animals showed no clear symptoms of lipid metabolism disturbances.

When the glucose metabolism is deficient NEFA blood concentrations, as a marker of lipid catabolism, increase. In this research, BHB and NEFA levels were within physiological

Table 6. Effect of lactation stage on blood enzyme activities in Lacaune dairy sheep

| Parameter (U/L) | Stage of lactation | | | SEM | P-value |
|-----------------|---------------------|---------------------|---------------------|--------|---------|
| | early | medium | late | | |
| AST | 216.26 ^b | 330.91 ^a | 344.95 ^a | 13.252 | <0.001 |
| ALP | 397.37 | 338.10 | 351.82 | 17.704 | 0.340 |
| GGT | 86.72 ^b | 111.13 ^a | 121.16 ^a | 3.330 | <0.001 |
| CK | 138.74 | 139.73 | 140.55 | 4.492 | 0.987 |
| ALT | 17.74 ^b | 21.45 ^a | 22.05 ^a | 0.511 | <0.001 |
| GPx | 833.87 | 917.85 | 1119.97 | 53.150 | 0.083 |
| SOD (U/mL) | 0.43 ^a | 0.29 ^b | 0.45 ^a | 0.022 | 0.008 |

SEM - standard error of mean; ^{a, b} - means with different superscript letters differ significantly ($p < 0.05$); AST - aspartate aminotransferase; ALT - alanine aminotransferase; ALP - alkaline phosphatase; GGT - γ -glutamyl transferase, CK - creatine kinase; GPx - glutathione peroxidase; SOD - superoxide dismutase.

ranges, as set by Reintke et al. (2021) to <0.85 and 0.1-0.5 mmol/L, respectively, which confirmed the absence of severe metabolic stress. Pesántez-Pacheco et al. (2019) found the highest BHB concentrations and the lowest NEFA in the postpartum period of highly productive Lacaune dairy sheep. Beta-hydroxybutyrate is one of the major ketone bodies, which is increased in the serum when NEFA level exceeds the oxidative capacity of the liver (Drackley, 1999). Their research results suggested good adaptation of sheep, as the intensity of the negative energy balance was connected with the intensity of NEFA mobilisation, and consequently, with BHB production, which remained within the referential range throughout the whole research period (Drackley, 1999). González-García et al. (2015), and Pesántez-Pacheco et al. (2019) found higher BHB concentrations in Lacaune sheep in later lactation stage than in the first-time lambing sheep. Significantly higher concentrations of GUK and lower BHB in blood of early lactating sheep in this research indicated an optimal energy supply of sheep. Within this research, concentration of BHB in blood of Lacaune sheep was lower in early lactation stage than in later lactation stages. Marutsova and Marutsov (2018) reviewed the literature with respect to lipid mobilisation especially important in dairy animals and reported that diagnostic markers for subclinical and clinically-induced ketosis for BHB in sheep range from 0.5-1.6 mmol/L, and from 1.6 to 7 mmol/L. These authors concluded that the BHB concentrations from 1.10-1.20 mmol/L and 2.15-3.10 mmol/L were taken as diagnostic markers for subclinical and clinically-induced ketosis in research on lactating Lacaune sheep. Doepel et al. (2002) reported that the increase in BHB concentration could be connected with the incomplete oxidation of NEFA in the citric acid cycle at the time of negative energy balance. Brito et al. (2006) also did not confirm the influence of lactation stage on concentrations of NEFA in blood of Lacaune sheep. In these investigation determined low concentrations of NEFA in blood of lactating Lacaune sheep prove quality energy supply. Therefore, when trying to understand energy metabolism and adaptability

to environmental conditions, determined concentrations of NEFA in sheep blood can be taken as one of the quality indicators (Snoj et al., 2014).

When compared to the early lactation stage, analysis of data overviewed in the Table 6 confirmed significant enzyme activity (AST, GGT, ALT) and the decrease in SOD activity in blood of Lacaune sheep in the late lactation stage. In this investigation determined the activity of most enzymes was within the referential values (Kaneko et al., 2008). When increase in activities of ALT, AST and GGT in the blood of sheep during lactation indicated an increase in hepatic metabolism. This is consistent with previously conducted researches on lactating Merinolandschaf sheep (Antunović et al., 2011).

In blood of lactating Travnik Pramenka sheep, Antunović et al. (2021) determined significant increase of ALT activity and the decreased GGT activity. Progression of lactation stages showed significantly higher ALT and AST activities in the sheep' blood, which could be related to the quality of diet. Roubies et al. (2006) concluded that more intense liver function of lactating sheep happens because of higher requirements for energy and proteins, which are necessary for maintenance and milk production. Similar results with Merinolandschaf sheep during lactation were found by

Antunović et al. (2017a). Cozzi et al. (2010) confirmed the increased activity of AST, GGT and CK in blood of Holstein cows from early lactation stage (from 10 to 89 days) to mid lactation (90 to 215 days).

Conclusion

Determined changes in haematological and biochemical blood parameters of Lacaune dairy sheep during different lactation stages proved good quality nutrition of sheep and indicate the need to include this dairy breed/genotype in prospective schemes for definition of referential values for these indicators in sheep.

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Utjecaj stadija laktacije na hematološke i biokemijske pokazatelje u krvi lakon ovce

Sažetak

Cilj je ovoga rada utvrditi hematološke i biokemijske pokazatelje u krvi 30 lakon ovaca tijekom različitih stadija laktacije (rani: 60., srednji: 120. i kasni: 180. dan). Ovce su bile prosječne dobi 4 godine u 3. laktaciji. U punoj krvi su utvrđeni hematološki pokazatelji (broj leukocita: (WBC), eritrocita (RBC), hemoglobin (HGB), hematokrit (HCT), prosječni volumen eritrocita (MCV), prosječni hemoglobin u eritrocitu (MCH), prosječna koncentracija hemoglobina u eritrocitu (MCHC) i trombociti (PLT), a iz krvnih razmaza i diferencijalna krvna slika. Od biokemijskih pokazatelja u serumu utvrđeni su: koncentracije minerala (Ca, P-anorganski, Mg i Fe), ukupnih protein (TP), albumin (ALB), globulina (GLOB), uree, glukoze (GUK), kolesterola (CHOL), HDL-kolesterola (HDL), LDL-kolesterola (LDL), triglicerida (TGC), beta-hidroksibutirata (BHB) i neesterificiranih masnih kiseline (NEFA) te aktivnost enzima (aspartat aminotransferaze (AST), alanin aminotransferaze (ALT), alkalne fosfataze (ALP), γ -glutamil transferaze (GGT), kreatin kinaze (CK), glutation peroksidaze (GPx) i superoksid dismutaze (SOD). Utvrđeno je značajno povećanje WBC, RBC, HGB, HCT, Fe, urea, TP, ALB, CHOL, HDL, LDL, BHB, AST, GGT i ALT kao i smanjenje limfocita, Mg, GUK i SOD u krvi kako je laktacija odmicala. Utvrđene promjene u hematološkim i biokemijskim pokazateljima u krvi lakon ovaca tijekom laktacije ukazuju na kvalitetan obrok kojim su hranjene ovce, ali i potrebu uključivanja pasmine/genotipa osobito mliječnoga predznaka u buduće sheme izrade referentnih vrijednosti za navedene pokazatelje u ovaca.

Ključne riječi: lakon ovca; metabolički profil; laktacija; stadij laktacije; prilagodba

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