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Blood metabolic profile and acid base status of lactating Travnik ewes in an extensive production system

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ABSTRACT

The research aimed to determine the blood metabolic profile and acid-base status of lactating Travnik ewes in an extensive production system. The research was carried out on 108 lactating Travnik ewes, with an average age of 3-4 years, in their 3rd lactation. The ewes were kept on pasture, and had water and animal salt *ad libitum*. Hematological indicators were determined in whole blood, biochemical parameters in serum, and acid-base status was determined in plasma. The determined average values of hematological parameters were mostly within reference values, except the lower MCHC content and higher MCV content, which indicates the instability of erythrocyte constants. Average mineral concentrations were within the reference values, except for Ca and Fe concentrations which were lower and were influenced by higher milk excretion. Most of the biochemical indicators, enzyme activity, and indicators of the acid-base balance of lactating Travnik ewes' blood were within the reference values. A high concentration of urea above the reference values was found, and concentrations of total proteins, albumins, total cholesterol, HDL and LDL cholesterol, and triglycerides at the upper limit or above the reference values were determined. Slightly lower GPx activity and higher SOD activity above reference values were determined. These indicators point to energy deficit, as well as poor grazing quality, and a lack of selenium in pasture plant species. When determining the blood metabolic profile of lactating Travnik ewes, the obtained results of the research should be considered, and they should be included in the development of reference values for the Travnik sheep breed.

Key words: Travnik ewes; hematological indicators; biochemical indicators; acid-base indicators; extensive production system

Introduction

With a significant increase in production per head in livestock production, there is an exceptional burden on animals, which makes it difficult to maintain stable physiological conditions

(homeostasis) in their body and leads to the emergence of productional diseases (ANTUNOVIĆ et al., 2013). The term "metabolic profile" refers to the analysis of blood biochemical constituents that

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are useful to evaluate and prevent metabolic and nutritional problems in dairy herds (ROSSATO et al., 2001; PUPPEL and KUCZYŃSKA, 2016). The development of laboratory equipment and computer programs that enable fast and efficient blood analysis and easier statistical processing of the obtained values of the analyzed indicators in the blood has contributed to the increasing use of metabolic profiles in animal husbandry. In addition to monitoring body weight and determining the body's condition score (BCS), it is possible to determine certain hematological and biochemical indicators, and in recent years indicators of acid-base balance in the blood more accurately determine the nutritional and health status of sheep (ANTUNOVIĆ et al., 2015). From the hematological indicators, the number of erythrocytes, leukocytes, and thrombocytes is most often determined, as well as hemoglobin concentration and hematocrit value, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). The proportion of individual white cells (lymphocytes, neutrophils, eosinophils, monocytes, and basophils) is determined from blood smears. The biochemical indicators most often determined in the blood are concentrations of minerals, glucose, urea, total protein, albumin, globulin, triglycerides, cholesterol, non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHB), and the activity of the enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), and creatine kinase (CK).

Determining the metabolic profile is especially significant in grazing ewes where it is very difficult to know and monitor the consumption and chemical composition of the ration. This often leads to various nutrient deficiencies in rations that affect the quality of sheep productivity. The case of Mediterranean countries is often mentioned where ewes graze on poor quality pastures for most of the year (ANTUNOVIĆ et al., 2015, 2019). Determination of the metabolic profile is important in physiologically stressful periods for the animal (high pregnancy, lactation, especially early lactation) which impose severe metabolic

changes that challenge the organism's maintenance of homeostatic equilibrium to compensate for the major expenditure of nutrients that lactogenesis demands (DRACKLEY, 1999; VAN SAUN, 2006, 2016, VAŽIĆ et al., 2020).

The concentrations of the studied indicators in the blood are influenced by several factors that can be divided into genetic (genotype, i.e. breed and sex of the animal) and non-genetic (nutrition, season, age of the animal, health status, reproductive status, breeding conditions, handling of samples during transport and storage, methods of determination) which have been confirmed in numerous studies with sheep (ANTUNOVIĆ et al., 2002, 2004, 2011, 2017a, 2017b; DURAK et al., 2015; CALPEREYRA et al., 2014; CARLOS et al., 2015; BRONDANI et al., 2016; OLIVEIRA et al., 2016; PESÁNTEZ-PACHECO et al., 2019). According to VAN SAUN (2000), concentrations of albumin, total protein, calcium, sodium, magnesium, phosphorus, potassium, chlorine, and NEFA have low variability and high diagnostic value, and cholesterol, urea, glucose, and BHB concentrations have medium variability and medium diagnostic significance, while liver enzymes, and creatine kinase activities have high variability and low diagnostic value. The study of the acid-base balance can provide information for the diagnosis and prognosis of various diseases. The most commonly observed type of acid-base disorder is metabolic acidosis (KANEKO et al., 2008; NOVOSELEC et al., 2013). A quality metabolic profile indicates the need to determine blood parameters distributed by region as well as genotype (breed). Namely, SHEK VUGROVEČKI et al. (2017) point out that the blood reference values of various sheep from the available literature are orientational but not adequate for the specific indigenous breeds in their natural habitat. Travnik sheep (dubška, vlašićka) are a breed of sheep originating from Bosnia and Herzegovina, and in the Republic of Croatia they are bred in the western part. Breeders of Travnik sheep, in addition to the production of lambs (meat), produce milk that is processed into cheese. Breeding of Travnik sheep is most often of extensive or semi-intensive character, where the only food is grazing. Travnik sheep belong to a larger population of sheep called

Pramenka, they are late maturing and achieve full physical development between the third and fourth year of life. The body weight of ewes ranges from 60 to 70 kg, and rams from 80 to 100 kg according to the Croatian Agricultural Agency (CAA, 2018). Sheep fertility is very good, about 130%. Travnik sheep in lactation give about 70 to 130 l of milk, which emphasizes their good potential for milk, which undoubtedly can and should be improved (NOVOSELEC et al., 2020). Lactation itself is a highly demanding physiology process that utilizes a large amount of energy. Given the fact that these sheep graze on poor quality pastures for most of the year, in lactation, especially early lactation, the sheep cannot meet the requirements imposed by the onset of lactation accompanied by reduced feed intake. Consequently, there is a negative energy balance and the highest incidence of disease in high-producing sheep. Therefore, it is necessary to notice the expected problem and to take preventive actions. In that sense, there is a need to create a metabolic profile as a quality preventive measure in preserving the quality of rations and the health of the sheep. There are no data in the available literature on the complete metabolic profile of Travnik sheep, except for the study by HRKOVIĆ-POROBIJA et al. (2017, 2019) where several indicators in the blood of this breed in Bosnia and Herzegovina were investigated. This paper aims to determine the complete metabolic profile of lactating Travnik sheep in extensive breeding through determination of hematological, biochemical, and acid-base indicators, which will help us understand their nutritional and health status.

Materials and methods

Analyses of the metabolic blood profile of Travnik ewes were undertaken on 108 lactating Travnik ewes with an average age of 4 years in an extensive production system. Ewes were selected from the flock of 1000 animals on the basis of being in the third lactation and having only one lamb in the litter. The selected ewes were healthy and in good physical condition. The average body weight of the ewes was $53.58 \text{ kg} \pm 7.11$. The body condition scores (BCS) of the ewes (1 = emaciated to 5 = obese) were evaluated by two trained

technicians according to RUSSEL (1991) and the mean was 2.69 ± 0.52 . Water and animal salt were offered to the ewes *ad libitum*. The present study was conducted in 2019 on a family farm located in Velika Peratovica, 10 km from Grubišno polje (in Croatia, $45^{\circ}45'25''\text{N}$, $17^{\circ}14'51''\text{E}$, ~211 m above sea level). The monthly mean temperature for this area from May to July in 2019 was 19°C while the mean monthly rainfall was 113 mm. Feed samples (green forage from pastures) were taken before the sheep went out to pasture, and dried and ground into a fine powder using a heavy metal free ultra-centrifugal mill (Retsch ZM 200) or knife mill (GM 200). Feed chemical composition was determined by standard methods (AOAC, 2006) and is presented in Table 1.

Table 1. Chemical composition of the ingredients in the lactating ewes' diets

| Parameters (g/kg DM) | Green forage |
|--|--------------|
| Crude proteins | 196.83 |
| Crude fiber | 168.70 |
| Crude ash | 87.03 |
| Ether extract | 26.10 |
| NDF,% | 43.67 |
| ADF,% | 24.69 |
| Digestible energy, MJ kg ⁻¹ DM | 2.28 |
| Metabolizable energy, MJ kg ⁻¹ DM | 1.98 |
| Mineral content (mg/kg DM) | |
| Ca | 7706.10 |
| Mg | 2373.61 |
| K | 44589.98 |
| P | 5568.33 |
| Na | 116.96 |
| Fe | 236.27 |
| Se | 0.06 |

DM - dry matter; NDF - neutral detergent fiber, ADF - acid detergent fiber

For determination of the Crude protein content (CP) in feed samples the Kjeldahl method was used, while ether extract (EE) was determined by using a Universal Extractions System B-811 (Buchi, Switzerland). Ash was determined by incinerating the feed samples at 550°C for 6 hours. The determination of amylase-treated NDF

was determined according to EN ISO 1647:2006 (European Committee for Standardization, 2006), while acid detergent fiber (ADF) was determined according to EN ISO 13906 (European Committee for Standardization, 2008). The digestible and metabolizable energy of feed for ewes was estimated according to DLG (1993). Feed samples were digested with 10 mL of a 5:1 mixture of HNO_3 and H_2O_2 at 180 °C for 60 min in a microwave oven (CEM Mars 6). The concentrations of minerals in solutions of digested plant samples were determined by inductively coupled plasma (ICP, PerkinElmer Optima 2100 DV). Each batch of all samples run on the ICP was analyzed with an internal pooled plasma control, and with the reference material prepared in the same way as all the other samples. All samples were analyzed in duplicate.

From each lactating ewe, before grazing, blood samples were collected from the jugular vein (10 mL) into sterile vacuum Venoject® tubes (Sterile Terumo Europe, Leuven, Belgium). For hematology analysis blood was collected in tubes containing ethylenediamine tetra-acetic acid (EDTA) as anticoagulant. The EDTA tubes were inverted several times to ensure adequate mixing of the blood with the anticoagulant. Determination of hematological parameters (number of leukocyte - WBC, erythrocytes - RBC and platelet - PLT, the content of hemoglobin - HGB, hematocrit - HCT, mean corpuscular volume - MCV, mean corpuscular hemoglobin - MCH, and mean corpuscular hemoglobin concentration - MCHC) in the whole blood of the sheep was carried out on an automatic Sysmex Poch-100iV three differential hematology analyzer (Sysmex Europe GmbH, Hamburg, Germany). A differential blood test was carried out by a microscope using the prepared blood smears colored by Pappenheim. Afterward, blood samples collected in sterile Venoject® vacuum tubes (Sterile Terumo Europe, Leuven, Belgium) were centrifuged at 1609.92 g for 10 min, and the obtained serum samples were placed into the Olympus AU400. In serum, the concentration of calcium, phosphorus-inorganic, potassium, sodium, magnesium, iron, chloride, urea, glucose, total proteins (TP), albumin, cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL)

cholesterol, triglycerides (TGC), β -hydroxybutyrate (BHB) and non-esterified fatty acids (NEFA), as well as activities of enzymes: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatine kinase (CK) and γ -glutamyl transferase (GGT) were measured using Olympus System reagents (Olympus Diagnostic GmbH, Lismeehan, Ireland). Globulin content was calculated as the difference between total protein and albumin. The activity of glutathione peroxidase (GPx) in the serum was determined using a Ransel® kit (Randox, UK) on an automatic Olympus AU 400 (Olympus, Japan) analyzer on a wavelength of 240 nm. The activity of total superoxide dismutase (SOD) in the serum was determined with a Ransod® kit (Randox, UK) on an automatic analyzer (Olympus AU 400, Olympus, Japan) at a wavelength of 510 nm. Samples of plasma were obtained from sterile vacuum tubes containing Li-heparin and analyzed by a Rapid Lab 348 automatic analyzer, which works on the basis of ion-selective electrodes. The following parameters were determined: pH, the partial pressure of carbon dioxide (pCO_2), the partial pressure of oxygen (pO_2), the total pressure of carbon dioxide (tCO_2), actual base excess-Cbase(B), standard base excess-Cbase(Ecf), oxygen saturation (sO_2) and bicarbonate (HCO_3^-). The Committee for Animal Welfare of the Faculty of Agrobiotechnical Sciences, Osijek, established that the research was being carried out under the legal provisions according to the Animal Protection Act (Official Gazette No. 133 of 2006, No. 37 of 2013 and No. 125 of 2013). Animal care and the conditions of the research followed the recommendations of European Union directive 2010/63/EU (European Commission, 2010). The distribution of data was verified by the Shapiro-Wilk test (PROC UNIVARIATE). The results of sheep's blood in a concentration of hematological, biochemical, and acid-base parameters were performed by the MEANS procedure. The results were presented as mean, min and max values, standard deviation, and standard error of mean. All data were analyzed with the statistical software SAS 9.4®.

Results

The established average values of hematological parameters were mostly in an accordance with the reference values for sheep. However, lower mean values were found for MCHC content and higher for MCV content.

By analyzing the data from Table 3 it is visible that the mineral concentrations obtained were within the reference values for sheep, except for the concentrations of Ca, Na, and Fe which were lower, and Cl was higher.

Table 2. Hematological parameters and leukocyte distribution from lactating Travnik ewes

| | Mean | Min | Max | SD | SEM | Reference values* |
|----------------------------|--------|--------|--------|--------|-------|--------------------|
| WBC ($\times 10^9/L$) | 8.06 | 3.20 | 18.70 | 3.05 | 0.29 | 4-12 |
| RBC ($\times 10^{12} L$) | 9.53 | 7.01 | 13.03 | 1.29 | 0.12 | 9-15 |
| HGB (g/L) | 107.73 | 77.00 | 170.00 | 15.85 | 1.53 | 90-150 |
| HCT (L/L) | 0.39 | 0.26 | 0.57 | 0.06 | 0.006 | 0.27-0.45 |
| MCV (fL) | 40.24 | 35.40 | 48.50 | 2.40 | 0.23 | 28-40 |
| MCH (pg) | 11.30 | 10.20 | 13.80 | 0.68 | 0.07 | 8-12 |
| MCHC (g/L) | 281.27 | 225.00 | 386.00 | 28.69 | 2.76 | 310-340 |
| PLT ($\times 10^9/L$) | 422.25 | 77.00 | 809.00 | 171.54 | 16.51 | 250-750 |
| Leukocyte distribution (%) | | | | | | |
| Lymphocytes | 56.46 | 31.00 | 87.00 | 12.10 | 1.16 | 40-75 ¹ |
| Neutrophils | 35.30 | 11.00 | 65.00 | 11.62 | 1.12 | 10-50 ¹ |
| Monocytes | 0.32 | 0.00 | 7.00 | 0.83 | 0.08 | 0-6 ¹ |
| Eosinophils | 7.75 | 0.00 | 28.00 | 7.13 | 0.69 | 0-10 ¹ |
| Basophils | 0.18 | 0.00 | 2.00 | 0.43 | 0.04 | 0-3 ¹ |

*Smith (2002); ¹Kramer (2000); SD- standard deviation; SEM- standard error of mean; WBC-number of leukocytes, RBC-erythrocytes, HGB-hemoglobin, HCT-hematocrit, MCV-mean corpuscular volume, MCH- mean corpuscular hemoglobin, MCHC-mean corpuscular hemoglobin concentration; PLT - platelet count

Table 3. Blood minerals concentrations of lactating Travnik ewes (mmol/L)

| | Mean | Min | Max | SD | SEM | Reference values* |
|--------------------------|--------|--------|--------|-------|------|-------------------|
| Ca | 2.52 | 1.72 | 3.07 | 0.28 | 0.03 | 2.88-3.20 |
| P | 1.85 | 1.14 | 3.30 | 0.39 | 0.04 | 1.62-2.36 |
| Na | 131.22 | 107.00 | 146.00 | 11.02 | 1.33 | 139-152 |
| K | 5.35 | 3.05 | 8.50 | 1.91 | 0.41 | 3.90-5.40 |
| Mg | 1.09 | 0.77 | 1.54 | 0.14 | 0.02 | 0.90-1.31 |
| Cl | 111.68 | 100.00 | 139.00 | 9.16 | 1.10 | 95.0-103.0 |
| Fe ($\mu\text{mol/L}$) | 24.42 | 7.70 | 41.00 | 6.48 | 0.62 | 29.70-39.7 |

*Kaneko et al (2008); SD- standard deviation; SEM-standard error of mean

Table 4. Blood biochemical parameters of lactating Travnik ewes (mmol/L)

| | Mean | Min | Max | SD | SEM | Reference values* |
|----------------------|-------|-------|-------|------|------|------------------------|
| Glucose | 4.05 | 2.32 | 8.96 | 1.09 | 0.10 | 2.78-4.44 |
| Urea | 9.97 | 1.17 | 16.21 | 2.07 | 0.20 | 2.86-7.14 |
| Total proteins (g/L) | 79.33 | 62.20 | 99.40 | 8.04 | 0.77 | 60.0-79.0 |
| Albumin (g/L) | 30.22 | 23.30 | 36.20 | 2.24 | 0.22 | 24-30 |
| Globulin (g/L) | 49.11 | 34.70 | 68.40 | 7.06 | 0.68 | 35-57 |
| CHOL | 2.01 | 1.02 | 3.03 | 0.38 | 0.04 | 1.35-1.97 |
| HDL | 1.36 | 0.96 | 1.94 | 0.21 | 0.02 | 1.09-1.18 ¹ |
| LDL | 0.58 | 0.23 | 1.15 | 0.20 | 0.02 | 0.36-0.40 ¹ |
| TGC | 0.25 | 0.12 | 0.47 | 0.09 | 0.01 | 0.0-0.2 |
| NEFA | 0.23 | 0.02 | 1.46 | 0.28 | 0.03 | < 0.4 ² |
| BHB | 0.34 | 0.16 | 0.55 | 0.09 | 0.01 | < 0.8 ³ |

*Kaneko et al (2008); ¹Antunovic et al. (2011); ²Oetzel (2004); ³Russel (1984); SD - standard deviation; SEM-standard error of mean; CHOL - cholesterol, TGC - triglycerides, BIL - bilirubin; NEFA - non-esterified fatty acids, BHB - β -hydroxybutyrate

Table 5. Blood enzymes activities of lactating Travnik ewes (U/L)

| | Mean | Min | Max | SD | SEM | Reference values* |
|-----------|--------|-------|---------|--------|-------|--------------------|
| AST | 156.70 | 13.20 | 1188.80 | 105.46 | 10.19 | 60-280 |
| ALT | 23.74 | 12.30 | 38.00 | 5.51 | 0.53 | 22-38 ¹ |
| ALP | 181.56 | 54.90 | 508.90 | 112.35 | 10.81 | 50-300 |
| GGT | 56.66 | 19.80 | 118.40 | 15.14 | 1.46 | 40-94 |
| CK | 187.69 | 63.00 | 2251.00 | 263.82 | 25.39 | 35-280 |
| SOD, U/mL | 0.31 | 0.14 | 1.28 | 0.14 | 0.01 | 0.184 ² |
| GPx | 241.63 | 4.73 | 961.26 | 230.60 | 25.16 | > 600 ³ |

*Smith (2002); ¹Jackson and Cockcroft (2002); ²Maan et al. (2013); ³Pavlati et al. (2012) - whole blood; SD - standard deviation; SEM - standard error of mean; AST - aspartate aminotransferase, ALT - alanine aminotransferase, ALP - alkaline phosphatase, GGT - γ -glutamyl transferase, CK - creatine kinase, SOD - superoxide dismutase, GPx - glutathione peroxidase

Table 6. Blood acid-base balance of lactating Travnik ewes

| | Mean | Min | Max | SD | SEM | Reference values* |
|----------------------------|-------|-------|-------|-------|------|--------------------|
| pH | 7.33 | 7.25 | 7.37 | 0.06 | 0.01 | 7-32-7.54 |
| pCO ₂ , kPa | 7.71 | 5.80 | 12.80 | 1.35 | 0.22 | 6.19 ¹ |
| pO ₂ , kPa | 8.81 | 5.40 | 20.20 | 2.79 | 0.45 | 6.12 ¹ |
| HCO ₃ , mmol/L | 22.79 | 15.90 | 28.80 | 2.99 | 0.48 | 20-25 |
| ctCO ₂ , mmol/L | 24.63 | 17.20 | 30.80 | 3.13 | 0.50 | 21-28 |
| Cbase(B), mmol/L | -4.73 | -9.30 | 1.40 | 3.07 | 0.49 | -1.15 ¹ |
| Cbase(Ecf), mmol/L | -3.84 | -8.70 | 2.70 | 3.07 | 0.50 | - |
| sO ₂ % | 80.95 | 76.0 | 99.10 | 10.77 | 1.30 | 69.78 ¹ |

*Smith (2002); ¹Sobiech et al. (2005); SEM - standard error of mean; pCO₂ - partial pressure of carbon dioxide, pO₂ - partial pressure of oxygen, HCO₃ - bicarbonate, ctCO₂ - total pressure of carbon dioxide, Cbase(B) - actual base excess, Cbase(Ecf) - standard base excess

Average urea concentrations were notably higher, and albumin, total protein, total, HDL, and LDL cholesterol and triglyceride concentrations were at the upper limit or only slightly higher compared to their reference values for sheep (Table 4). Other biochemical parameters measured in the blood of Travnik ewes were within the reference values.

The activity of most enzymes in the blood of lactating Travnik ewes was within the reference values shown in Table 5. Significantly lower GPx activity and slightly higher SOD activity were found compared to the given reference limits of these enzymes for sheep.

The analysis of acid-base balance indicators in the blood did not reveal major deviations compared to the reference values.

Discussion

The basic rule that applies when determining the metabolic profile is that the mean value of the established indicators in the blood should preferably be in the middle of the reference values for sheep (VAN SAUN, 2000). The results of hematological and biochemical parameters of lactating Travnik ewes were within the reference values for sheep according to SMITH, 2002 and KANEKO et al., 2008. However, lower mean values were found for MCHC content and higher for MCV content. This indicates the instability of erythrocyte constants. VOJTA et al. (2011) found a similar number of RBCs and HGB and MCH content in the blood of Dalmatian sheep, as well as lower values of MCV and MCHC. Energy balance is a highly important nutritional factor that significantly affects the expression of productivity, reproduction, and animal health. For energy status analysis of lactating ewes, biochemical indicators such as NEFA, BHB, glucose cholesterol, and triglycerides in the blood were determined. Concentrations of glucose, NEFA, and BHB in the blood of lactating Travnik ewes were within the recommended range of values, but the concentrations of total, HDL and LDL cholesterol, and triglycerides were at the upper limit or slightly higher than the reference values for sheep (Table 3). Namely, triglyceride concentration uptake by the mammary gland for milk synthesis

increased lipolysis, and decreased lipogenesis or limited liver capability to complete lipoproteins as the transportable form of triglycerides (MCART et al., 2013). The high blood cholesterol concentration during lactation arises because there is a strong reduction in lipogenesis, esterification, and an increase in the release of free fatty acids stimulated by catecholamine (epinephrine norepinephrine) (ZUMBO et al., 2007). The increase of triglycerides in the blood of lactating ewes was probably influenced by the negative energy balance that induces fat mobilization from adipose tissue.

Assessing the protein supply of animals is a more demanding and difficult procedure compared to assessing energy status. Therefore, a combination of different indicators in the blood is used, which includes determining the concentration of urea, creatinine, total protein, albumin, and creatine kinase activity. Liver function can be assessed by determining the activity of various enzymes (GGT, AST, ALT, SDH) and the concentration of total cholesterol, NEFA, BHB, and total bilirubin (VAN SAUN, 2000). Concentrations of urea above the reference value, and at the upper limit or slightly higher concentrations of total proteins and albumin indicate a ration rich in proteins. The blood parameters used to evaluate the protein status are total protein, albumins, and globulins, and this provides background information about protein biosynthesis, utilization, and excretion, as well as renal failure, liver damage, and nutritional health (ŠTOLCOVA et al., 2020). ALADROVIĆ et al. (2018) point out that the concentrations of albumin in the blood can be a quality indicator of the supply of feed proteins to animals. Blood urea concentrations are an indicator of the current supply of protein to animals through feed, while albumin concentrations are a good indicator over a longer period of time (more than a month). KOHN et al. (2005) presented that the concentration of urea can be considered as a good indicator of the amount of nitrogen consumed through feed. Also, the concentration of urea in the blood is an indicator of the proper energy supply to the animals. Similar changes of blood urea concentrations in Dubrovnik sheep on pasture during early lactation were observed by ANTUNOVIĆ et al. (2019). The

finding that urea levels were notably above the reference values in the present study might indicate that these values should be considered in the future as a reference for this breed of sheep. Increased urea concentration in the blood of lactating ewes may be due to a lack of fermentable energy in the ration, so rumen microorganisms use ammonia to synthesize their proteins which can be further absorbed through the epithelium of the rumen into the blood and transported to the liver, where they are synthesized in urea. Higher concentrations of urea can be the result of pathological conditions but also other factors (lactation, season, heat stress, etc.). A high serum urea concentration may indicate increased ammonium detoxication and can be considered a risk factor for lipomobilization (PARK et al., 2010). VOJTA et al. (2011) found similar TP concentrations and GGT activity in Dalmatian sheep blood, as well as lower AST activity and cholesterol and urea concentrations. SHEK VUGROVEČKI et al. (2017), in a study on Lika sheep on pasture, also determined higher urea concentrations (8.62 mmol/L) in comparison with the reference values. Estimation of animal mineral supply is highly variable because most minerals in the animal organism are tightly regulated by the homeostatic mechanism. Therefore, the most common concentrations of minerals (especially macronutrients) in the blood are not taken as a replica of nutrition when the homeostatic system is functioning well (VAN SAUN, 2000). Lower concentrations of Ca, Fe, and Na in the blood of Travnik sheep in lactation might indicate increased excretion of Ca and Fe through milk, due to the increased milk production during early lactation (ANTUNOVIĆ et al., 2011). Namely, LIESEGANG et al. (2007) determined a lower concentration of Ca in ewes' blood after lambing and in early lactation, which might be associated with the increased secretion of Ca through the milk and its rearrangement in the bones. Rather, hypocalcemia may be symptomatic of inadequate pre- or postpartum feed intake (SEIFI et al., 2011). Significantly decreased Fe concentrations in the blood of Merinolandschaf sheep and Dubrovnik sheep in lactation were determined by ANTUNOVIĆ et al. (2017a, 2019). Markedly lower

GPx and slightly higher SOD activity was found in the present study compared to the reference values for sheep. This indicates a lack of selenium in the sheep's grazing, given the lack of this trace element in the soil in this area (POPIJAČ and PRPIĆ-MAJIĆ, 2002; ANTUNOVIĆ et al., 2010, 2020). Similar results for blood SOD (0.234 U/mL) were determined in Dubrovnik sheep by ANTUNOVIĆ et al. (2015). There is very little research on indicators of acid-base status in sheep. Generally, metabolic activity modifies the acid-base balance, although it is difficult to estimate the degree of its contribution to the metabolic component of acid-base balance (CASTILLO et al., 1998). In the present study, there was no deviation in the acid-base status indicators in the blood of sheep compared to the reference values for sheep. SOBIECH et al. (2005) determined similar pH values and pCO₂, lower sO₂, and higher content Cbase(B) in the venous blood of sheep. Animal nutrition is one of the important factors, and especially the nutritional cationic-anion balance, on which the acid-base balance in animal blood depends. Acid-base balance is an important homeostatic mechanism of the body because cellular enzymes act within narrow pH limits (NOVOSELEC et al., 2013). Therefore, the pH value in the blood of lactating ewes can be taken as a valuable indicator of acid-base balance, and it was within the reference values in the present research. CASTILLO et al. (1996) in a study on gravid sheep found a significant association between blood acid-base balance and energy metabolism. The authors concluded that lipid mobilization is a source of the production of strong metabolic acids, which leads the organism to change the acid-base balance towards metabolic acidosis. At the same time, the respiratory system responds to this by hyperventilation to excrete excess acids involved in this metabolism, thereby reducing pCO₂ which was not the case in the present research. The concentration of bicarbonate ions (HCO₃⁻) in plasma depends on the saturation of hemoglobin with oxygen. The decrease in the concentration of hemoglobin in the tissues is accompanied by an increase in the concentration of HCO₃⁻ in erythrocytes, which consequently passes through the cell membranes into the plasma (ADAMS et al., 1991). After that, the erythrocytes,

together with the blood, travel to the lungs, where the reverse process takes place. Hemoglobin undergoes an oxygen enrichment process which increases its acidity, and as a result, a hydrogen ion is released which is neutralized by the HCO_3^- anion, creating CO_2 and H_2O_2 , which are secreted by the alveolar air due to the increase in partial pressure. None of these processes were present in our case because the above indicators of acid-base balance were within the reference values.

Conclusion

On the basis of the results of the present study, it is evident that the extensive production system of Travnik sheep during the lactation period is satisfactory as all animals were healthy, but there is much room for improvement. When determining the metabolic profile of Travnik sheep, the fact should be considered that higher concentrations were determined of urea and lower concentrations of Ca, Fe, as well as lower activity of GPx in the blood compared to the reference values. This indicates the need to improve nutrition and include the changes in the concentrations of blood parameters identified in the development of reference values for the Travnik sheep breed.

Conflict of interest

The authors declare that they have no conflict of interest.

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SAŽETAK

Cilj istraživanja bio je utvrditi metabolički profil krvi i acido-bazni status ovaca travničke pramenke u ekstenzivnom proizvodnom sustavu. Istraživanje je provedeno na 108 ovaca travničke pramenke prosječne dobi 3 – 4 godine, u trećoj laktaciji. Ovce su boravile na pašnjaku, a vodu i stočnu sol imale su ad libitum. Hematološki pokazatelji određeni su u punoj krvi, biokemijski pokazatelji u serumu, a acido-bazni status u plazmi. Utvrđene prosječne vrijednosti hematoloških pokazatelja bile su u najvećoj mjeri u fiziološkim granicama, osim manje prosječne vrijednosti MCHC-a te veće vrijednosti MCV-a, što upućuje na nestabilnost eritrocitnih konstanti. Prosječne koncentracije minerala bile su u granicama referentnih vrijednosti, osim koncentracija kalcija i željeza koje su bile manje, a pod utjecajem su većeg izlučivanja mlijekom. Većina biokemijskih pokazatelja, aktivnosti enzima i pokazatelja acido-bazne ravnoteže krvi ovaca travničke pramenke bila je u granicama referentnih vrijednosti. Treba naglasiti utvrđenu veću koncentraciju uree te na gornjoj granici ili veće od referentnih vrijednosti koncentracije albumina, ukupnih proteina, ukupnog kolesterola, HDL i LDL-kolesterola i triglicerida, kao i manju aktivnost GPx te nešto veću SOD-a. To upućuje na energetske deficit te kvalitetu paše i nedostatak selena. Pri utvrđivanju metaboličkog profila travničke pramenke u obzir treba uzeti rezultate dobivenih istraživanja te ih uvažavati pri izradi referentnih vrijednosti za ovu pasminu.

Ključne riječi: travnička pramenka; hematološki pokazatelji; biokemijski pokazatelji; acido-bazni pokazatelji; ekstenzivni proizvodni sustav
