HEMATOLOGICAL AND BIOCHEMICAL BLOOD PARAMETERS OF PIROT PRAMENKA - ENDANGERED SHEEP POPULATION

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Abstract: Pirot breed is a highly endangered autochthonous population of sheep in Serbia, from which several important national brands originate: Pirot lamb, Pirot carpet and Pirot hard cheese. In the available literature, there are no data on hematological and biochemical parameters of the blood of this population. The aim of this paper, which represents the beginning of the research, is to determine the above indicators in the blood of Pirot Pramenka, which will contribute to the preservation of this population. Since the breed affects the value of blood parameters, the obtained results will be used to define reference intervals for this population and compare with others. The study included 30 clinically healthy sheep, 2-3 years old, from the area of Stara Planina. Blood samples were taken by puncture to c. jugularis from which, after coagulation and centrifugation, blood serum was extracted. Haematological analyzes were performed in the next 24 hours on a Siemens Advia 120 analyzer. The following parameters were determined: total number of leukocytes, erythrocytes, platelets, hemoglobin content, hematocrit value, MCV, MCH, PLT and MPV. Biochemical blood tests were performed using Olympus AU 400 analyzers. The following parameters were determined: total protein, albumin, globulin, urea, creatinine, glucose, total bilirubin, cholesterol. Also, the following minerals were determined - calcium, phosphate and magnesium, and following enzymes: AST, GGT and CK. Descriptive statistical analysis was performed using the statistical package STATISTICA (Version 8). Hematological indicators of Pirot pramenka are in reference intervals for sheep, which indicates good animal health status. Biochemical parameters of Pirot pramenka blood were within defined physiological limits, except for creatinine and phosphorus levels, which were below the lower limit, which is a consequence of dehydration and reduced alimentary intake. In accordance with the obtained results, it is necessary to correct the diet.

Key words: Pirot pramenka, hematological parameters, biochemical indicators

Introduction

Pramenka (Zapfel) belongs to the autochthonous (locally adapted) breeds of sheep that represent a unique genetic heritage created thousands of years ago. They are an important element of the regional agro-biodiversity, tradition and cultural heritage of Serbia. Different climatic and nutritional conditions, together with animal migrations, have influenced the formation of different strains of Pramenka, which differ in morphological and production characteristics: Sjenica, Svrljig, Liplje, Krivovir, Karakačan, Pirot, Vlach Vitoroga and Bardoka. They possess sets of genes responsible for good adaptability, disease resistance, longevity, and pronounced maternal instinct. However, the more productive, more intense breeds whose primary goal is profit, win. Therefore, the biological survival of autochthonous populations is endangered, which caused some strains of pramenka to acquire the status of "critical" and "endangered" population. (*Ružić-Muslić et al.*,2015).

Pirotska pramenka is the most endangered population of sheep in Serbia. According to the Domestic Animal Diversity Information System (DAD-IS), for 2021, 207 females and 25 males are bred in Serbia. Most of this population is grown in the area of the Stara Planina National Park and southeastern Serbia. The effective population size is 89, which is highly endangered and at risk of extinction. On the other hand, with good management, locally adapted genotypes could serve as a promoter of sustainable development, to reduce the pressure on regional agro-biodiversity and costs of production since these are genotypes of combined production traits, and represent a potential source of meat, wool, milk, manure and hides (Gorkhali et al., 2015; Cekić et al., 2018). Pirot Pramenka has spawned several important national brands: Pirot lamb, Pirot carpet and Pirot hard cheese, which indicates that its preservation is imperative. Its survival should be sought in increasing the economic attractiveness of breeding, which will encourage the affirmation of this sheep. Therefore, it is necessary to explore the possibility of maximum production, while monitoring the metabolism of nutrients in the body and maintaining health. The key indicators of nutritional status and animal health are the metabolic profile and hematological picture of sheep (Klinkon and Zadnik, 1997; Herdt et al., 2000; Van Saun, 2000; Antunović et al., 2002; Antunović et al. 2007; Antunović et al., 2014). Determining and monitoring the values of metabolic profile parameters show whether homeostatic mechanisms maintain blood composition within physiological limits, depending on different rearing conditions (Prodanović et al., 2012).

In the available literature, there are no data on hematological and biochemical parameters of Pirot Pramenka. Given that breed has an impact on the value of blood parameters (*Dias et al., 2010; Addass, 2011; Vojta et al., 2011*), defining the reference range of these parameters for Pirot Pramenka will have significant implications for determining similarities and differences between different breeds, strains as well as healthy and diseased individuals.

The aim of this work, initial research, is to determine the hematological and metabolic profile in the blood of Pirot pramenka, which will contribute to the preservation of this highly endangered sheep.

Material and Method

The research was conducted on 30 sheep of Pirot Pramenka, reared in the area of Stara Planina, with an average age of 2-3 years, during the winter season. The sheep were healthy, in the last third of pregnancy. The animal diet per individual animal consisted of meadow hay (ad libitum) and 0.3 kg of maize, per head/day. Water and mineral blocks were constantly available. Sheep blood sampling was performed in the morning. Blood was taken by puncture to c. jugularis in 5 ml glass tubes, with a gray colour stopper (with anticoagulant EDTA, for glucose), yellow colour (for biochemical analysis) and purple colour, for complete blood picture. After labeling, the samples were stored at 4°C in the refrigerator, and subsequently transferred to the laboratory. Haematological analyses were performed in the next 24 hours on a Siemens Advia 120 analyzer, which is equipped with software. The following parameters were determined: total number of leukocytes (WBC), erythrocytes (RBC), platelets (PLT), hemoglobin content, hematocrit value, MCV average volume erythrocytes in a liter of blood, MCH-average amount of hemoglobin in erythrocyte, MCHC-average concentration of hemoglobin in a liter of blood, PLT-number of platelets, MPV-average volume of platelets. Since the total number of leukocytes represents the sum of neutrophilic, eosinophilic and basophilic ganulocytes, lymphocytes and monocytes, differentiation of white blood cells was performed and shown as a percentage. Biochemical analyzes of the blood were performed using an Olympus AU 400 analyzer. The blood was centrifuged for 7 minutes at 3600 rpm, during which the serum was separated, used in the analyses. The following parameters were determined: total protein, albumin, globulin, urea, creatinine, glucose, total bilirubin, cholesterol. Also, following mineral substances were determined: calcium, phosphate, magnesium, as well as following enzymes: AST (aspartate aminotransferase), GGT (glutamyl transferase) and CK (creatine kinase). Reference values of the examined biochemical parameters were taken from Baumgartner and Wittek (2017). Descriptive statistical analysis was performed using the statistical package STATISTICA (Version 8).

Results and Discussion

of Hemogram (total number leukocytes, ervthrocytes thrombocytes/platels) in the blood of Pirot Pramenka is presented in Table 1. In addition to the above parameters, the relative share of individual fractions of leukocytes (neutrophils, lymphocytes, monocytes, eosinophils, basophils), hemoglobin content, hematocrit value and erythrocyte constants: MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular Hemoglobin - average amount of hemoglobin in a single erythrocyte) and MCHC (Mean Corpuscular Hemoglobin Concentration: concentration of hemoglobin in one liter of blood). Sheep erythrocytes are among the smallest of all mammals (Kramer, 2000). Thrombocytes or platelets are discoidal cells that form in the bone marrow of mammals from the cytoplasm of megakaryocytes, do not contain a nucleus and cannot multiply (Guyton and Hall, 2006).

Table 1. Hematological parameters of blood in Pirot Pramenka sheep population

Hematological parameters	parameters		Minimum	Maximum	Reference value*	
WBC leukocytes	10 ⁹ /L	6.93±1.30	4.24	8.45	4.0-12.0	
NE-neutrophils	$10^{9}/L$	2.13±0.99	0.76	4.27	2.0-4.0	
LY-lymphocytes	$10^{9}/L$	4.05±0.94	2.56	5.46	3.5-6.9	
MO-monocytes	$10^{9}/L$	0.16±0.07	0.07	0.27	0.0-0.4	
EO- eosinophils	$10^{9}/L$	0.40±0.18	0.18	0.74	0.0-0.6	
BA-basophils	10 ⁹ /L	0.09±0.02	0.07	0.15	0.0-0.2	
NE- neutrophils	%	30.0±10.77	18.00	54.10	10.0-50.0	
LY- lymphocytes	%	59.25±11.38	32.40	69.80	40.0-75.0	
MO- monocytes	%	2.45±1.16	1.10	3.80	0.0-6.0	
EO- eosinophils	%	5.74±1.96	2.80	9.40	0.0-10.0	
BA-basophils	%	1.42±0.21	1.10	1.70	0.0-3.0	
Erytrocytes-RBC	$10^{12}/L$	9.84±0.88	7.41	10.14	9.0-15.0	
Hemoglobin	g/L	103.11±10.99	86.00	125.00	90.0-150.0	
Hematocrit	%	30.37±3.20	26.00	35.20	27.0-45.0	
MCV	fL	34.4±2.35	29.60	37.20	28.0-40.0	
MCH	pg	11.68±0.81	10.30	13.20	8.0-12.0	
MCHC	g/L	339.0±17.58	302.00	361.00	310.0-340.0	
RDW	%	15.96±0.61	15.10	16.70	12.0-27.0	
Platelet count- PLT	10 ⁹ /L	382.72±18.94	340.50	390.54	250.0-750.0	

WBC - leukocytes, MCV - average volume of erythrocytes in a liter of blood, MCH - average amount of hemoglobin in a erythrocyte, MCHC - average concentration of hemoglobin in one liter of blood, RDW - width of red cell distribution, PLT - platelet count * W.Baumgatner and Wittek., (2017)

A complete blood count or hemogram is a basic laboratory blood test and an important diagnostic "tool" used to monitor general animal health, nutritional status, hematopoietic disorders, viral and bacterial diseases and to define a reference interval for hematological parameters in different sheep breeds, in different rearing conditions, which is especially important for the preservation of indigenous breeds. Analyzing the obtained hematological indicators of Pirot Pramenka, it is noticeable that there were no major deviations from the established reference values for sheep. Our results indicate good health status, in agreement with the results of authors who have studied other strains of Pramenka (*Šimpraga et al.*, 2013; Antunović et al., 2011; Shek Vugrovečki et al., 2017).

Table 2. Mean value and intervals of variation of hematological parameters of different strains of Pramenka from the relevant literature

Hematological parameters	Units	Dubrovnik Pramenka ^I (mean)	Zeta Žuja ² (mean)	Lika Pramenka ³	Dalmatian Pramenka ⁴
Leukocytes	10 ⁹ /L	8.11	8.05	5.6-17.0	3.02-15.64
Eritrocytes	$10^{12}/L$	9.53	8.97	6.6-9.9	7.81-12.77
Platelet	10 ⁹ /L	195.70	404.10	75-807	0-731
Hemoglobin	g/L	107.40	100.30	74.55-104.7	78.1-134.7
Hematocrit	%	0.43	0.38	0.22-0.31	0.24-0.41
MCV	fL	45.72	42.15	28.80-35.80	28.60-34.80
МСН	Pg/L	11.32	11.19	9.9-11-9	9.54-11.00
MCHC	g/L	248.00	266.30	320-353	284-362

^{1,2} Antunović et al. (2014); ³ Shek Vugrovečki et al. (2017); ⁴ Šimpraga et al. (2013)

Variations in the number of leukocytes and platelets, between individual strains of Pramenka, can be associated with pregnancy. It is known that the number of leukocytes decreases in pregnancy, which is related to the involution of the uterus (*Antunović et al., 2011*). Also, the same group of authors report a lower platelet count in highly pregnant sheep compared to non-pregnant and lactating sheep. The analysis of the differential blood picture, in terms of the relative share of lymphocytes (59.25%), shows similarity with the results of *Antunović et al.* (2011) in Dubrovnik ruda sheep (57.40%), which is an indicator of the immunomodulatory response of the organism, initiated by pregnancy, considering that Pirot pramenka and Dubrovnik sheep were in the second half of pregnancy. Eosinophils are an important indicator of parasite infestation, which correlates their high value with increased resistance to endoparasites (*Pfeffer et al., 1998; Balic et al., 2000*). Erythrocytes or red blood cells (RBCs) are responsible for oxygen transport, carbon dioxide and hydrogen ion buffering. They have a diameter of 3.2-5µm and a lifespan of 70-150 days (*Bórnez et al., 2009; Šimpraga et al., 2013*), do

not aggregate and deform as easily as erythrocytes of others species. Erythrocyte indices (MCV, MCH, MCHC) provide information on average cell size, hemoglobin content and concentration (Polizopoulou, 2010). The values for MCH and MCHC in our studies are relatively high. Higher values for MCH may indicate the presence of reticulocytes or hemolysis. MCHC is considered to be the most accurate erythrocyte constant and can be increased in the case of hemolysis (Polizopoulou, 2010). Table 3 shows the biochemical parameters of Pirot Pramenka blood. In general, biochemical analyzes are an important part of laboratory diagnostics that assess the work of internal organs, measure electrolyte levels and identify enzyme concentrations (*Čepelak and Strauss*, 2009), and above all reflect the nutritional status of the individual animal. In order to monitor the health and nutritional status of sheep, it is necessary to establish reference physiological values, which is of particular importance in the case of endangered breeds. Knowing the quality of some biochemical parameters could improve our knowledge about the specifics of sheep metabolism, but at the same time the production abilities of this endangered population. Blood biochemical parameters are the most important indicators used to determine the energy, protein, enzyme, hormone and mineral profile, as well as to assess nutritional status, milk production and animal health (Antunović et al., 2011; Milošević-Stanković, 2020).

Table 3. Biochemical parameters of blood in the population of Pirot pramenka

Biochemical parameters	Unit	Mean ± SD	Minimum	Maximum	Reference value
Total protein	g/L	72.09±2.86	68.40	77.40	59.0-78.0
Albumin	g/L	31.64±1.60	29.10	34.50	27.0-37.0
Globulin	g/L	40.45±2.22	37.40	43.00	32.0-50.0
Urea	mmol/L	4.57±0.70	3.40	5.70	3.70-9.30
Creatinine	μmol/L	59.18±8.59	47.50	73.90	75.8-174.3
Glucose	mmol/L	3.05±0.59	2.40	4.00	2.40-4.50
Total bilirubin	μmol/L	2.46±1.50	1.30	6.20	0.70-8.60
AST	IU/L	117.67±9.76	101.10	130.50	49.0-123.3
GGT	IU/L	41.19±10.01	20.50	53.90	19.60-44.10
СК	IU/L	46.16±7.03	16.3	67.50	7.70-101.0
Cholesterol	mmol/L	1.99±0.36	1.50	2.60	1.10-2.30
Calcium	mmol/L	2.49±0.11	2.40	2.70	2.30-2.90
Phosphate	mmol/L	1.28±0.22	1.00	1.60	1.30-2.40
Magnesium	mmol/L	0.86±0.06	0.80	1.00	0.8-1.10

For the analysis of sheep's protein supply, a combination of different indicators in the blood is used, which includes determining the concentration of total proteins, albumin, urea, creatinine and the activity of creatine kinase enzymes. All of these parameters, except creatinine, ranged in the physiological range. Creatinine is an indicator of kidney function. The literature states that creatinine excretion in sheep averages about 10.7 mg, with variations of 5.0-13.6 mg creatinine/kg body weight/day (Liu and McMeniman, 2006), depending on the type of diet and energy: protein ratio. At the optimal E: P ratio, lower creatinine values are most often the result of dehydration of the animal, due to reduced water intake. The established content of total proteins of 72.08 g/L is within the reference values. In the sheep's body, proteins are constantly synthesized and broken down. In healthy animals, a balance is established between the intake and synthesis of amino acids, on the one hand, and the breakdown and excretion of excess nitrogenous substances, in the form of urea, on the other hand. The obtained average urea concentrations of 4.57 mmol/L do not deviate from the given physiological values, but are lower compared to the values stated by Shek Vugrovečki et al., (2017) in the research on Lika Pramenka (6.7-10.9 mmol), which are conducted in the summer period. Of the total plasma proteins, 52-62% are albumins and represent a reserve of proteins in the body. Since albumins have the ability to reversibly bind many organic compounds, they represent an important transport system in blood plasma (Jovanović, 1983; Milošević-Stanković, 2020). Total albuminemia ranged from 29.10-34.50 g/L, which is in the reference range, but is slightly higher than the results of Kramer (2000) and Kaneko et al. (2008), who report values of 24-30g/L. The decrease in albumin concentration in the metabolic profile should always be observed together with the urea concentration. If the urea concentration is normal or elevated in hypoalbuminemia, then it is probably liver disease, while if hypoalbuminemia is associated with low blood urea concentration, then it is a protein deficiency in the diet (*Kaneko et al.*, 2008). The concentration of glucose in the serum of Pirot Pramenka ranged from 2.4-4.0 mmol/L, which does not deviate from the given reference values, but is somewhat higher in relation to the results of research by Antunović et al. (2009) and Shek Vugrovečki et al. (2017). The "mirror" of liver function are the enzymes: AST (aspartate amino transferase), GGT (gamma glutathione transferase). CK (creatine kinase) also plays an important role. Analysis of enzymes determined in the serum of the examined sheep shows that their values range within physiological values: 117.67, 41.19, 46.16 IU/L, respectively. Normal values for these enzymes, in the relevant literature, are higher compared to our results, which may be due to the chemical composition and nutritional value of nutrients from different areas. The variability of nutrient composition is related to climate, nutrient type, soil type and climatic conditions defined by different altitudes (Hrković et al., 2009). The established cholesterol content of 1.99 mmol/l is in accordance with our and reference values stated by other authors, in other strains of pramenka (Dubrovnik, Lika). Cholesterol

concentration is a direct reflection of the physiological status of animals, so in the late phase of pregnancy, its content may be higher compared to the lactation stage (Antunović et al., 2002). Cholesterol is a precursor of vitamin D, steroid hormones and bile acids, and is an integral part of cell membranes. It enters the body through food or is synthesized in the body itself, mainly in the liver. Cholesterol metabolism is physiologically in a state of equilibrium. Depending on whether the body receives a higher or lower amount of cholesterol (exogenous) through food. more or less endogenous cholesterol will be synthesized in the liver. Synthesis in the liver is stimulated by its reduced level in the blood and insulin, and is inhibited by elevated plasma cholesterol, glucagon and glucocorticoids (Bruss, 1997). The concentration of calcium in sheep serum averaged 2.49 mmol/L and was within the reference values, while phosphorus levels (1.28 mmol/L) were below the physiological range. Hypophosphatemia in the serum of Pirot Pramenka is most likely the result of reduced alimentary intake, which is in accordance with the results of Stefanović et al. (2015) who conducted research on Karakačan Pramenka. Similar results are stated by Antunović et al. (2009) who in research on Dubrovnik pramenka, record the concentration of inorganic phosphorus in the blood from 1.05-1.75 mmol/L, which can be linked to phosphorus deficiency of pastures. Also, the reduced level of mineral elements in the blood may be a consequence of the inability of animals to fully utilize the mineral substances from the diet, which results in a milder form of deficiency, which is not manifested as a major metabolic disorder (Hrković et al., 2009). The concentration of magnesium in the blood plasma ranged from 0.80 to 1.10 mmol/l, and the obtained values in the serum of the examined sheep were from 0.80-1.0 mmol/l.

Table 4. Intervals of variations of biochemical parameters of different strains of Pramenka as reported in the relevant literature

Hematological parameters	Units	Dubrovnik pramenka ¹	Karakačan ²	Lika pramenka ³	Dalmatian pramenka ⁴	Textbooks ⁵
Total protein	g/L	72.70-86.70	49.00-76.00	66.7-91.0	66.8-87.4	60-79
Albumin	g/L	31.70-32.80	26.50-47.30	35.4-47.5	28.5-44.7	24-30
Urea	mmol/L	4.30-5.60	-	6.70-10.9	3.50-7.80	2.86-7.14
Creatinine	μmol/L	86.0-97.0	-	74.5-103.2	98-144	106-168
Glucosa	mmol/L	2.60-3.30	-	1.40-3.70	2.90-4.30	2.78-4.44
Total bilirubin	μmol/L	0.01-4.00	-	5.00-11.00	-	1.71-8.55
AST	IU/L	-	65.00-172.00	110.7-241	66.2-129.3	60-280
GGT	IU/L	9.00-69.00	25.60-86.90	14.3-80.0	31.7-71.7	20-52
Cholesterol	mmol/L	1.90-2.70	-	0.74-2.47	-	1.35-1.97
Calcium	mmol/L	2.92-3.14	2.15-3.22	2.15-2.76	-	-
Phosphate	mmol/L	1.05-1.75	0.89-2.18	1.31-2.39	- 4×.	-

¹Antunović et al. (2009); ²Stevanović et al. (2015); ³Shek Vugrovečki et al. (2017); ⁴Šimpraga et al. (2013); ⁵Kramer (2000) and Kaneko et al. (2008)

Deviations of some parameters in relation to the reference values, from the relevant literature, are most likely a consequence of the feeding conditions of the animals. Therefore, we believe that in order to better understand the characteristics of the metabolic profile of Pramenka, it is necessary to expand the research to the examination of the floristic composition of the soil at the places of cultivation of individual strains.

Conclusion

Pramenka (Zapfel) belongs to the autochthonous (locally adapted) breeds of sheep that represent a unique genetic heritage created thousands of years ago and an important element of the regional agro-biodiversity, tradition and cultural heritage of Serbia. Pirot Pramenka is the most endangered population of sheep in Serbia. It has produced several important national brands: Pirot lamb, Pirot carpet and Pirot hard cheese, which indicates that its preservation is imperative. Therefore, it is necessary to explore the possibility of maximum production, while monitoring the metabolism of nutrients in the body and maintaining health. The key indicators of the nutritional status as well as the health condition of the individual animal are the metabolic profile and the hematological picture of the sheep. In the available literature, there are no data on hematological and biochemical parameters of Pirot pramenka. Hematological indicators of Pirot Pramenka are in reference intervals for sheep, which indicates good animal health status. Biochemical parameters of Pirot pramenka blood were within defined physiological limits, except for creatinine and phosphorus levels, which were below the lower limit, which is a consequence of dehydration and reduced alimentary intake.

Hematološki i biohemijski parametri krvi pirotske pramenke - ugrožene populacije ovaca

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Rezime

Pirotska ovca je visoko ugrožena autohtona populacija ovaca u Srbiji, od koje potiče nekoliko važnih nacionalnih brendova: pirotsko jagnje, pirotski ćilim i pirotski kačkavalj. U dostupnoj literaturi, nema podataka o hematološkim i biohemijskim parametrima krvi ove populacije.

Cilj ovog rada, koji predstavlja početak istraživanja, je utvrđivanje navedenih pokazatelja u krvi pirotske pramenke, što će doprineti očuvanju ove populacije. Obzirom da rasa utiče na vrednost parametara krvi, dobijeni rezultati će poslužiti za definisanje referentnih intervala za ovu populaciju i upoređivanje sa drugim. Ispitivanjem je obuhvaćeno 30 klinički zdravih ovaca starosti 2-3 godine, sa područja Stare planine. Uzorci krvi uzimani su punkcijom v. jugularis iz kojih je, nakon koagulacije i centrifugovanja, izdvojen krvni serum. Hematološke analize su izvršene u naredna 24 sata na analizatoru Siemens Advia 120.Određeni su parametric:ukupan broi leukocita. eritrocita. trombocita. hemoglobin, vrednost hematokrita, MCV, MCH, PLT i MPV. Biohemijske analize krvi su izvršene korišćenjem Olympus AU 400 analizatora. Utvrđeni su sledeći parametri:ukupan protein,albumin, globulin, urea, kreatinin, glukoza, ukupan bilirubin, holesterol. Od mineralnih materija su određeni: kalcijum,fosfat i magnezijum, a od enzima:AST,GGT i CK.Deskriptivna statistička analiza je urađena korišćenjem statističkog paketa STATISTICA(Version 8). Hematološki pokazatelji pirotske pramenke su u referentnim intervalima za ovce što ukazuje na dobar zdravstveni status grla. Biohemijski parametri krvi pirotske pramenke su se kretali u definisanim fiziološkim granicama, osim nivoa kreatinina i fosfora, koji su bili ispod donje granice, što je posledica dehidratacije i smanjenog alimentarnog unosa. U skladu sa dobijenim rezultatima, neophodno je izvršiti korekciju obroka.

Ključne reči: pirotska pramenka, hematološki parametri, biohemijski pokazatelji

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