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Reference values of blood parameters in rams of the Bosnian Pramenka breed

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A B S T R A C T

Pramenka is a breed of sheep widely distributed on the Balkan Peninsula (over 30 strains). A number of factors affect hematological and biochemical parameters in sheep blood such as breed, age, sex, region, season and health. The aim of this paper is to determine reference ranges for hematological and selected biochemical parameters in the blood of Bosnian Pramenka rams. The experiment included 362 blood samples. The rams were healthy, and were raised under farm conditions in Banat (Serbia). The blood was collected by puncture of v. jugularis, in vacuum cleaners with a purple stopper (EDTA, for hematological analysis) and with a yellow stopper (for biochemical analysis). The reference intervals of the blood parameters in Bosnian Pramenka rams were: WBC 4.34–17.0 $\times 10^9$/mL; RBC 4.34–17.0$\times 10^{12}$/mL; HGB 72.73–119.8 g/L; HTC 21.97–36.77 %; MCV 26.55–34.23 fL; MCH 8.57–11.34 pg; MCHC 311.32–345.16 g/L; PLT 118–556.45 $\times 10^9$/mL; TPROT 56.53–70.51 g/L; ALB 29.81–40.05 g/L; GLOB 22.13–35.05 g/L; UREA 2.25–9.57 mmol/L; TBIL 2.21–11.35 µmol/L; GLU 2.41–6.93 mmol/L; CHOL 0.93–1.91 mmol/L; AST 58–244 IU/L; BHB 0.18–0.54 mmol/L and LDH 172–455 IU/L. Normal frequency distribution was determined for RBC, HTC, PLT, ALB, GLOB and CHOL, while other parameters did not have a normal frequency distribution. A positive correlation was found between body weight and RBC and HGB values, and a negative correlation between body weight and WBC and BHB values. The obtained reference values will be helpful in interpreting the health and productive status of the Bosnian Pramenka population. Further studies should focus on ewes and lambs, as well as on the deviation of blood parameters in the case of various diseases.

Keywords: rams, hematology, biochemistry, Pramenka, reference value.

И З В О Д

Праменка је раса оваца која је најзаступљенија на Балканском полуострву (преко 30 сојева). Велики број фактора утиче на хематолошке и биохемијске параметре у крви оваца, као што су раса, старост, пол, регија, сезона и здравље. Циљ овог рада је да се утврде референтни опсези за хематолошке и одабране биохемијске параметре у крви овнова босанске праменке. У оглед је укључено 362 узорка крви овнова босанске праменке. Овнови су били здрави, узгајани у фармским условима на територији Баната (Србија). Крв је узимана пункцијом *v. jugularis*, у вакутајнере са љубичастим чепом (EDTA, за потребе хематолошких анализа) и са жутим чепом (за биохемијске анализе). Добијене су следеће референтне вредности параметара: WBC 4,34–17,0 $\times 10^9$/mL; RBC 4,34–17,0$\times 10^{12}$/mL; HGB 72,73–119,8 g/L; HTC 21,97–36,77 %; MCV 26,55–34,23 fL; MCH 8,57–11,34 pg; MCHC 311,32–345,16 g/L; PLT 118–556,45 $\times 10^9$/mL; TPROT 56,53–70,51 g/L; ALB 29,81–40,05 g/L; GLOB 22,13–35,05 g/L; UREA 2,25–9,57 mmol/L; TBIL 2,21–11,35 µmol/L; GLU 2,41–6,93 mmol/L; CHOL 0,93–1,91 mmol/L; AST 58–244 IU/L; BHB 0,18–0,54 mmol/L и LDH 172–455 IU/L. Нормална дистрибуција фреквенције утврђена је код RBC, HTC, PLT, ALB, GLOB и CHOL, док код осталих параметара није утврђена нормална дистрибуција фреквенције. Нађена је позитивна корелација између телесне масе и вредности RBC и HGB, а негативна корелација између телесне масе и вредности WBC и BHB. Добијене референтне вредности могу бити од користи код интерпретације здравственог и продуктивног статуса популације овнова босанске праменке. У даљим испитивањима потребно је обрадити овце и јагњад, као и одступање вредности крвних параметара код појаве различитих обољења.

Кључне речи: овнови, праменка, хематологија, биохемија, референтне вредности.

1. Introduction

Pramenka is an indigenous sheep breed, the most prevalent on the Balkan Peninsula. This is a very hardy breed raised under extensive conditions and it has no major requirements in the field of nutrition and care (Savić et al., 2007). The effect of different geographical areas has led to the development of more than 30 strains of sheep, including *Sjenička* (Sjenica sheep), *Svrljiška* (Svrljig sheep), *Šarplaninska* (Šar Mountain sheep), *Ovčepoljska (Makedonska)* (Ovče Pole (Macedonian) sheep), *Pirotska* (Pirot sheep), *Karakačanski-crni soj* (Karakachan Black strain), *Krivovirski soj* (Krivi Vir strain), *Kosovski soj* (Kosovo strain), *Bardoka (Bela Metohijska)* (Bardoka (White Metohija sheep)), *Lipska (Smederevska)* (Lipe (Smederevo) sheep), *Jezersko Pivska* (Lake Piva sheep), *Zetska Žuja* (Zeta Yellow sheep), *Dubska (Vlašićko-Travnički)* (Dub sheep (Vlašić Travnik strain)), *Vlaška Vitoroga* (Corkscrew-horned Wallachian sheep), *Kupreška* (Kupres sheep) and *Lička Pramenka* (Lika Pramenka sheep) (Ivanov et al., 2005; Krajinović, 2006).

The determination of reference values in blood has multiple implications in science and the profession. According to international standards, each laboratory should determine its own reference ranges on a number of healthy, strictly controlled and uniform subjects, in order to compare the results with other laboratories and world literature, thereby also checking the quality of the laboratory (CLSI, 2008; Belić and Cincović, 2015, 2019). The reference values are important for differentiation between healthy and diseased individuals, and their comparisons establish similarities and differences in different species and strains of animals. For all these reasons, the Laboratory of Pathophysiology at the Department of Veterinary Medicine in Novi Sad examined reference ranges in different animal species (Cincović et al., 2011, 2019; Belić et al., 2011, 2019 ; Cincović et al., 2020; Lakić et al., 2020; Nikolic et al., 2020).

In sheep, breed can have a significant effect on the value of blood parameters. In addition, a significant influence of age or gender on different blood parameters has been found (Oramari et al., 2014). In small ruminants, there is metabolic adaptation to the negative energy balance, and therefore knowledge of these parameters and their reference ranges is of great importance (Mohammadi et al., 2016; Miloševi-Stanković et al., 2020). The values of blood parameters in sheep can be affected by different infections (Amarante et al., 2004; Lipecka et al., 2010) as well as by heat stress (Wojtas et al., 2014). It has been shown that there is a difference between manual counting of blood cells and counting with an automatic analyzer (Ibrhim, 2014).

The aim of this study is to determine reference values for hematological and selected biochemical parameters in the blood of rams of the Bosnian Pramenka breed.

2. Material and method

Samples – The sample included 362 blood samples of rams of the Bosnian Pramenka breed. The rams were healthy, and were reared under farm conditions in Banat. They were fed a diet based on hay and a balanced concentrate portion of the meal. Water was available *ad libitum*. Rams weighed 40–80 kg.

Laboratory tests – Blood was taken by puncture of *v. jugularis* after shearing, shaving and disinfection. Blood was drawn into vacuum cleaners with a purple plug (EDTA, for hematological analysis) and a yellow plug (for biochemical analysis). The blood was transported to the Laboratory for Pathological Physiology at the Department of Veterinary Medicine of the Agricultural Faculty in Novi Sad. Analyses were performed within 3–6 hours of sampling. Hematological analyses were performed using a Nihon Kohden veterinary hematology analyzer (Japan) equipped with software for sheep. The following parameters were determined: leukocytes (WBC), erythrocytes (RBC), hemoglobin (HGB), hematocrit (HTC), erythrocyte indices (MCV, MCH, MCHC) and platelets (PLT). Blood biochemical analyses were performed using a Rayto Chemray analyzer (China). The biochemical reagents Biosystems (Spain) were used. The following parameters were determined: total proteins (TPROT), albumin (ALB), globulins (GLOB), urea, glucose (GLU), cholesterol (CHOL), aspartate aminotransferase (AST), beta hydroxybutyrate (BHB) and lactate dehydrogenase (LDH).

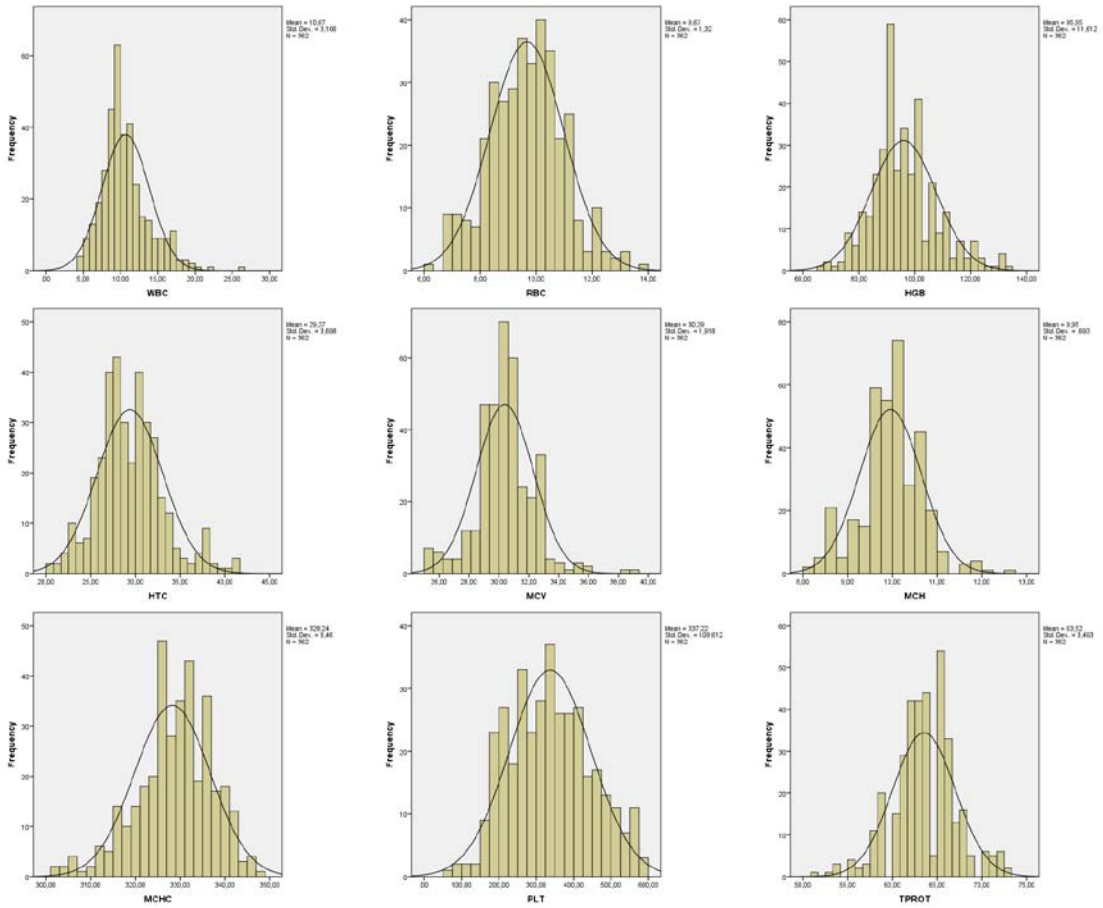
Statistical analysis – Descriptive statistical analysis involved the determination of central tendency parameters and variation of examined parameters. A 95% confidence interval was also calculated for each parameter tested, for the lower and upper reference values separately. The shape of frequency distribution was determined using skewness and kurtosis parameters, and the normality of frequency distribution was determined using the Kolmogorov-Smirnov test and was presented graphically using Q-Q plots. Frequency distribution is presented in the form of histograms and polygons, and the polygon represents an ideal normal distribution. For the parameters for which a linear trend was determined between body weight (which depends directly on age) and biochemical parameters, a regression analysis was performed and the trend was presented graphically.

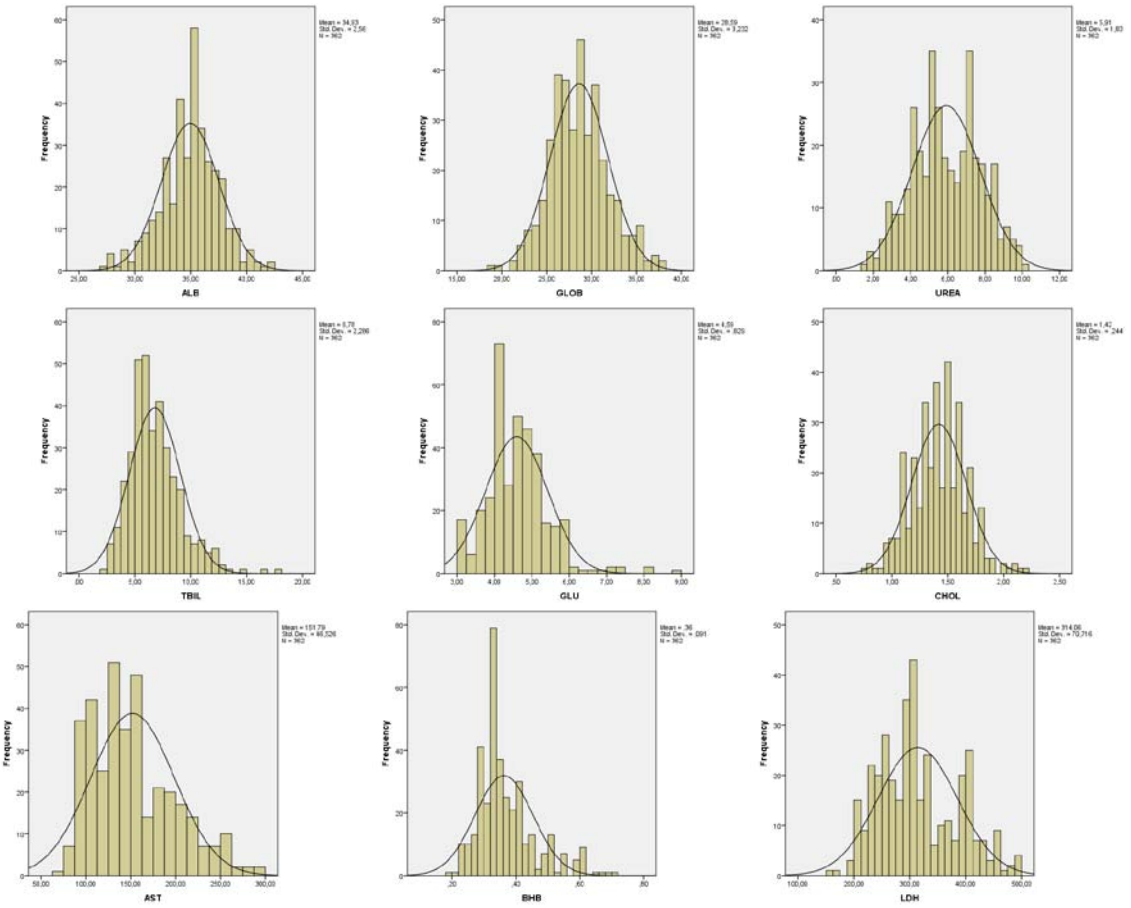
3. Results

The reference values of the blood parameters of Bosnian Pramenka rams were: WBC 4.34–17.0 $\times 10^9$/mL; RBC 4.34–17.0$\times 10^{12}$/mL; HGB 72.73–119.8 g/L; HTC 21.97–36.77 %; MCV 26.55–34.23 fL; MCH 8.57–11.34 pg; MCHC 311.32–345.16 g/L; PLT 118–556.45 $\times 10^9$/mL; TPROT 56.53–70.51 g/L; ALB 29.81–40.05 g/L; GLOB 22.13–35.05 g/L; UREA 2.25–9.57 mmol/L; TBIL 2.21–11.35 μmol/L; GLU 2.41–6.93 mmol/L; CHOL 0.93–1.91 mmol/L; AST 58–244 IU/L; BHB 0.18–0.54 mmol/L and LDH 172–455 IU/L (Table 1). Normal frequency distribution was determined for RBC, HTC, PLT, ALB, GLOB, and CHOL, while other parameters did not have a normal frequency distribution. The form of frequency distribution is shown in Figures 1–18, and the deviation of the obtained values from the normal distribution is presented for each parameter in Figures 18–36. The values of individual parameters are linearly related to the body weight (age) of the rams. A positive correlation was found between body weight and RBC and HGB values, and a negative correlation between body weight and WBC and BHB values (Figures 37–40). The 95% CI for the population average has been within the known reference value so far, but because of the range of minimum and maximum population values, there was a need to establish a reference value for this population.

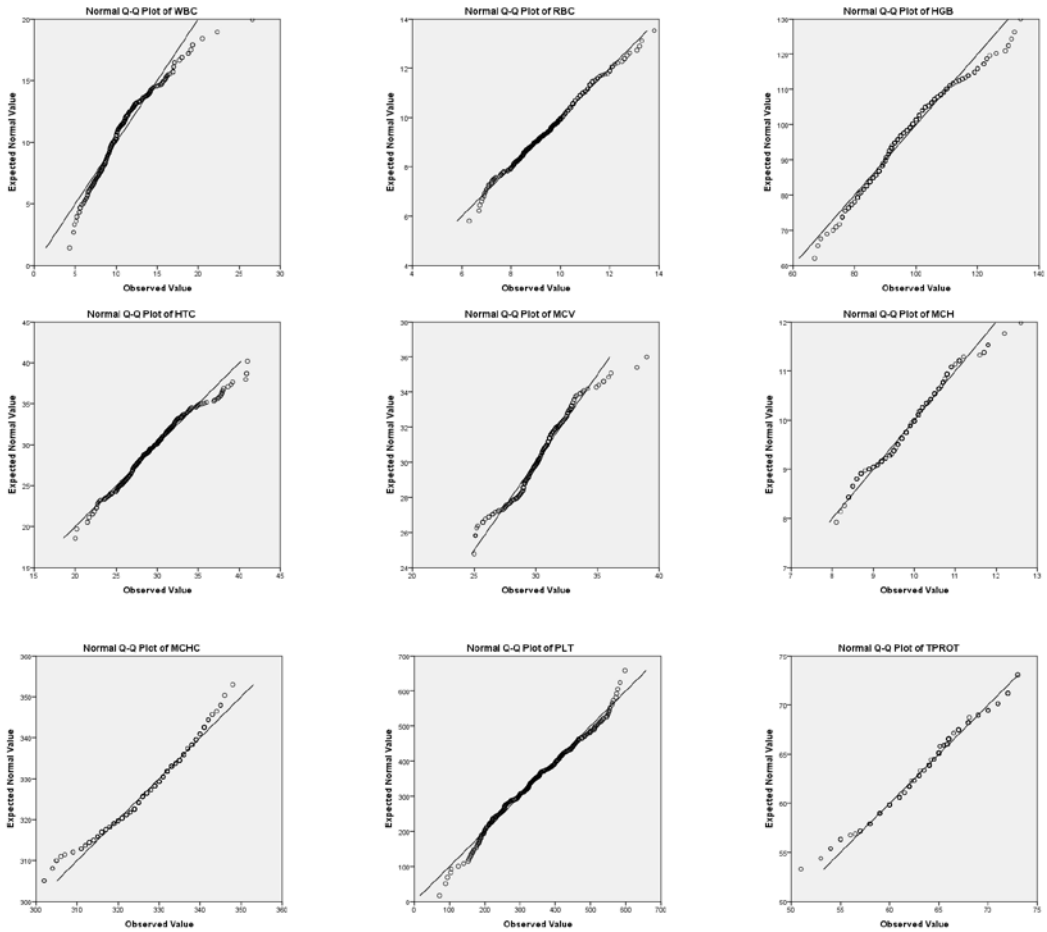
Table 1
Descriptive statistics and 95%CI for lower and uper reference values of blood parameters

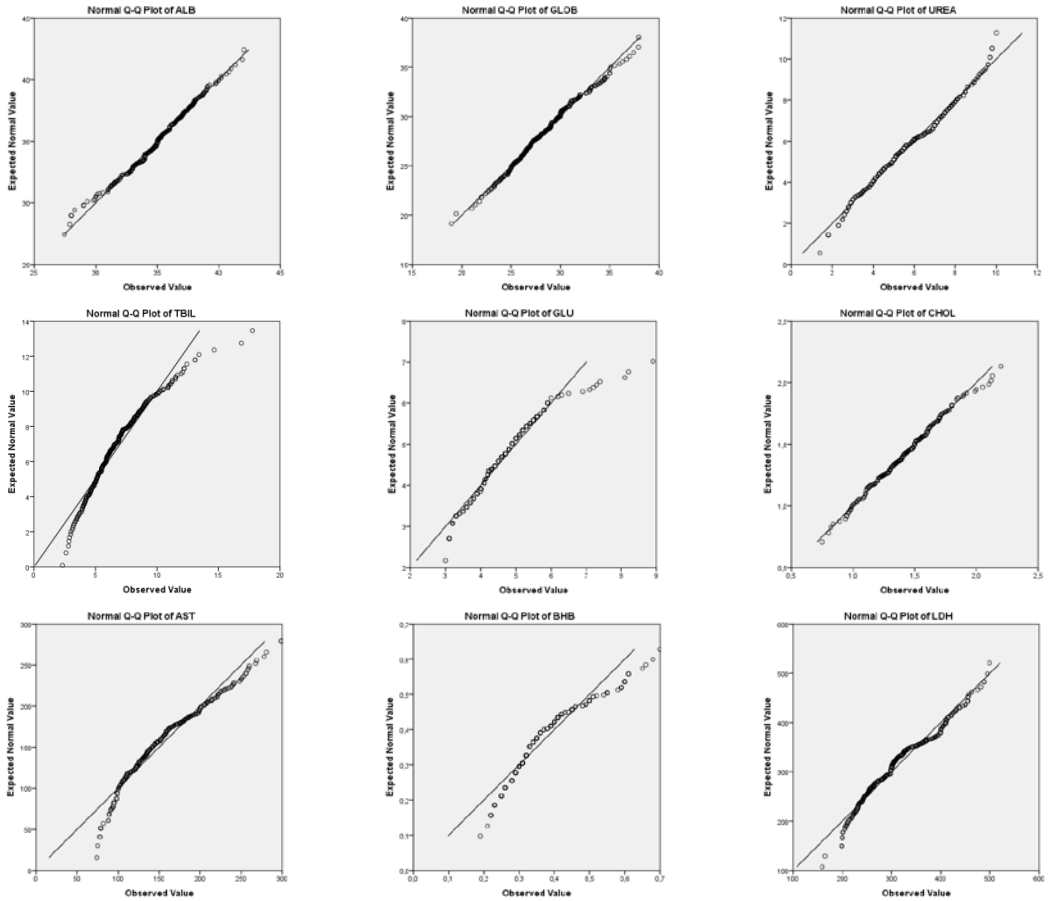
	Lower ref. value	95%CI for lower ref.value	Higher ref. value	95%CI for higher ref. value	Mean	SD	Skew ness	Kurtosis	Normality
WBC	4.34	4.01–4.67	17.00	16.7–17.3	10.67	3.17	1.07	2.04	No
RBC	7.03	6.89–7.17	12.31	12.1–12.4	9.67	1.32	0.07	0.03	Yes
HGB	72.73	71.4–73.8	119.18	119–121	95.95	11.61	0.61	0.71	No
HTC	21.97	21.6–22.4	36.77	36.4–37.2	29.37	3.70	0.50	0.72	Yes
MCV	26.55	26.4–26.7	34.23	34.0–34.3	30.39	1.92	0.17	2.33	No
MCH	8.57	8.5–8.64	11.34	11.3–11.4	9.95	0.69	0.03	0.92	No
MCHC	311.32	310–312	345.16	344–346	328.24	8.46	-0.54	0.35	No
PLT	118.00	107–129	556.45	545–567	337.22	109.6	0.18	-0.59	Yes
TPROT	56.53	56.2–56.9	70.51	70.2–70.9	63.52	3.49	-0.14	0.83	No
ALB	29.81	29.5–30.1	40.05	39.8–40.3	34.93	2.56	-0.22	0.37	Yes
GLOB	22.13	21.8–22.5	35.05	34.7–35.4	28.59	3.23	0.23	0.22	Yes
UREA	2.25	2.06–2.44	9.57	9.38–9.76	5.91	1.83	0.00	-0.67	No
TBIL	2.21	1.97–2.47	11.35	11.1–11.6	6.78	2.29	1.11	2.33	No
GLU	2.41	2.32–2.5	6.93	6.84–7.02	4.59	0.83	1.16	3.59	No
CHOL	0.93	0.90–0.95	1.91	1.89–1.93	1.42	0.24	0.10	0.15	Yes
AST	58.73	53.9–63.5	244.84	239–249	151.79	46.53	0.74	0.01	No
BHB	0.18	0.17–0.19	0.54	0.53–0.55	0.36	0.09	1.21	1.47	No
LDH	172.63	165–179	455.49	448–462	314.06	70.72	0.42	-0.56	No



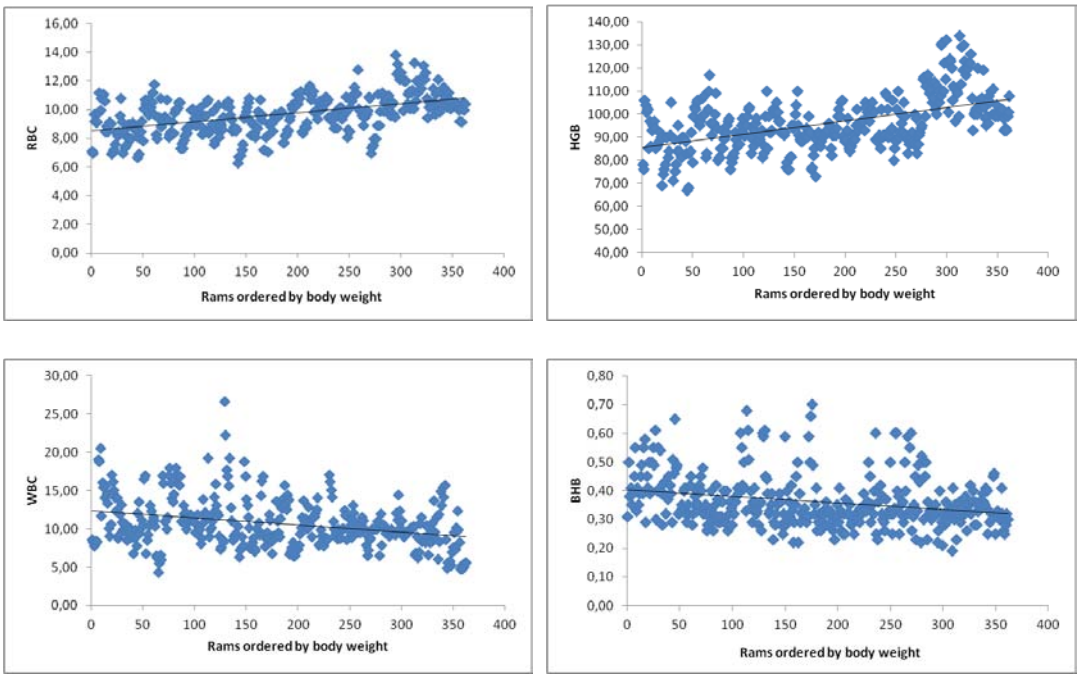


Figures 1–18: Frequency distribution of ram blood parameters (the histogram shows the obtained values and the polygon shows normal frequency distribution)





Figures 19–36: Q-Q plot of the deviation of the obtained values of the tested parameters from the normal distribution



Figures 37–40: Relationship between body weight and blood parameters in rams (only significant relations are shown)

4. Discussion

Determining the form of frequency distribution helps in the appropriate statistical interpretation of the data obtained. If the distribution is not normal, logarithmic transformation may be used or non-parametric statistics may be used when using the median as a positional mean in the population estimate. Shek Vugrovečki et al. (2017) in the Lika Pramenka sheep determined normal distributions for RBC, HGB, MCV, MCH, UREA and TPROT and log normal or non-normal distributions for other hematology and biochemistry parameters. Our results show that a large number of parameters in the tested rams do not show a normal distribution. The use of smaller groups of sheep, in an appropriate statistical analysis, allows the determination of the reference interval without the transformation of raw data (Vojta et al., 2011), and the method was applied to the Dalmatian sheep.

Hematological parameters were compared with the results of the authors dealing with Pramenka or other local breeds, as well as with data from the internationally recognized literature. Shek Vugrovečki et al. (2017) found the following reference values for Lika Pramenka sheep: WBC 5.6–17 $\times10^9$/mL; RBC 6.6–9.9 $\times10^{12}$/mL; HGB 74–104 g/L; HTC 22–31 %; MCV 28–35.8 fL; MCH 9.9–11.9 pg; MCHC 320–354 g/L; and PLT 72–807 $\times10^9$/mL. Šimpraga et al. (2013) found the following values for organically reared sheep: WBC 3–15.6 $\times10^9$/mL; RBC 78–12.77 $\times10^{12}$/mL; HGB 78–134.7 g/L; HTC 24–41 %; MCV 28–34 fL; MCH 9.54–11.0 pg; MCHC 284–362 g/L; and PLT 0–731 $\times10^9$/mL. Antunović et al. (2014) examined hematologic parameters in the *Dubrovačka Ruda* (Dubrovnik Ruda) and *Zetska Žuja* (Zeta Yellow) and obtained the following mean values: WBC 8.08 $\times10^9$/mL; RBC 9.2$\times10^{12}$/mL; HGB 103 g/L; HTC 41 %; MCV 43.5 fL; MCH 11.6 pg; MCHC 250 g/L; and PLT 300 $\times10^9$/mL. The hematological parameters in the present study are consistent with the results of authors in Serbian region who have addressed other strains. When it comes to the Tsigai breed, Antunović et al. (2009) found the following averages of hematological parameters in a population of organically grown sheep: WBC 11.97 $\times10^9$/mL; RBC 11.02 $\times10^{12}$/mL; HGB 120.44 g/L; HTC 46 %; MCV 42.2 fL; MCH 10.97 pg; MCHC 260 g/L; and PLT 429 $\times10^9$/mL. The following reference ranges are reported worldwide (Kramer, 2000): WBC 4–8 $\times10^9$/mL; RBC 9–15 $\times10^{12}$/mL; HGB 90–150 g/L; HTC 27–45 %; MCV 28–40 fL; MCH 8–12 pg; MCHC 310–340 g/L; and PLT 800–1100 $\times10^9$/mL. The comparison with the results from the internationally recognized literature shows that Bosnian Pramenka sheep have twice the upper reference value for leukocytes, and lower values for hemoglobin, erythrocytes, MCV and platelets. The erythrocytes of the Pramenka breed ram are smaller than the erythrocytes of other breeds and species. Due to the clinical significance of anemia in sheep, it is also necessary to be aware of the morphometric properties of erythrocytes, which have been described in detail in Lika Pramenka (Žura Žaja et al., 2019).

Blood biochemical parameters were compared with the values obtained from geographically close regions as well as from the globally recognized literature. Shek Vugrovečki et al. (2017) found the following benchmarks for blood biochemical parameters in Lika Pramenka sheep: TPROT 66.7–91 g/L; ALB 35.4–47.5 g/L; UREA 6–10.9 mmol/L; TBIL 5–11 µmol/L; GLU 1.4–3.7 mmol/L; CHOL 0.74–2.7 mmol/L; and AST 110–241 IU/L. Šimpraga et al (2013) reported the following reference values for the biochemical parameters: TPROT 66.8–87.4 g/L; ALB 28.5–44.7 g/L; UREA 3.5–7.8 mmol/L; GLU 2.9–4.3 mmol/L; AST 66.2–129.3 IU/L. Antunović et al. (2009) obtained the following results: TPROT 68.8–81 g / L; ALB 28.5–32.6 g / L; UREA 8.3–11.5 mmol / L; TBIL 2–3 µmol / L; GLU 3.5–4.6 mmol / L; CHOL 1.2–2.0 mmol / L; AST 85–138 IU / L and LDH 373–509 IU / L. In the world literature (Kaneko et al., 2008), the following reference values for sheep were reported: TPROT 60–79 g/L; ALB 24–30 g/L; UREA 2.86–7.14 mmol/L; TBIL 1.71–8.55 µmol/L; GLU 2.78–4.44 mmol/L; CHOL 1.35–1.97 mmol/L; AST 60–280 IU/L. Stevanović et al. (2015) examined different biochemical parameters in Karakachan sheep blood. The average values for the parameters were as follows: TPROT 61.92 g/L; ALB 31.03 g/L and AST 97.66 IU/L. Liver enzymes can be useful in assessing sheep infestation with fluke (Hodžić et al., 2013). Various factors can affect the value of blood biochemical parameters such as lactation, pregnancy, grazing or nutrient mineral composition (Antunović et al., 2014). BHB concentration is very significant in examining the metabolic status of sheep in the lambing period, and a concentration above 0.7 mmol/L in the pre-lambing period indicates poor metabolic adaptation (Ramin et al., 2007; Panousis et al., 2018).

The reference values obtained in our experiment can be compared with those obtained for other strains of Pramenka, but they cannot be compared with the results used in the world literature, which confirms the importance of determining reference ranges in our geographical area. The resulting reference values may be useful in interpreting the health and productive status of the Bosnian Pramenka population. Further studies should focus on ewes and lambs, as well as on the deviation of blood parameters in the case of various diseases.

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