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Hematological and blood biochemical variations in Pramenka sheep under thermal stress conditions

Husein Ohran[1*], Naris Pojskić[2], Eva Pašić-Juhas[1], Amina Hrković-Porobija[1], Edin Hrelja[3], Amina Sivac[3], Vinko Batinić[4] and Aida Hodžić[1]

[1]University of Sarajevo, Veterinary Faculty, Department of Basic Veterinary Sciences, Sarajevo, Bosnia and Herzegovina
[2]University of Sarajevo, Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina
[3]University of Sarajevo, Faculty of Science, Department of Geography, Sarajevo, Bosnia and Herzegovina
[4]University of Mostar, Faculty of Agriculture and Food Technology, Department of Zootechnics, Mostar, Bosnia and Herzegovina

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ABSTRACT

Climate change is one of the greatest global concerns regarding livestock production. The indigenous Pramenka sheep is well known for its ability to survive in difficult environmental conditions, although there is insufficient scientific evidence. This study aimed to evaluate the seasonal variations in some hematological and blood biochemical parameters in two strains of Pramenka sheep – Hercegovačka and Dubska, under natural thermal stress conditions. The calculated Temperature-Humidity Index (THI) data indicated mild to severe heat stress in the localities where the Hercegovačka Pramenka strain was being held, while cold stress was found in all the examined localities. Numerous statistically significant differences in hematological parameters were found between seasons, and within and between the two Pramenka strains. The Dubska strain exhibited significantly higher levels of red blood cells (RBC) and packed cell volume (PCV) compared to Hercegovačka, whereas the Hercegovačka strain displayed significantly higher white blood cells (WBC) and platelet (PLT) levels in comparison to Dubska. Similar patterns were noticed in the blood biochemical parameters - the Dubska strain had more seasonal variations compared to the Hercegovačka Pramenka. During the winter, the Hercegovačka strain demonstrated significantly higher levels of total protein (TP), albumin (ALB), urea (UREA), and creatine kinase (CK) compared to Dubska. Conversely, during the summer, the Hercegovačka strain exhibited significantly higher globulin (GLO) levels, whereas the Dubska strain showed higher levels of ALB and chlorine (Cl). On the basis of our results, both Pramenka strains showed a high adaptive capacity to harsh environmental conditions, the Hercegovačka Pramenka being more adapted to heat stress, and Dubska to cold stress conditions.

Key words: sheep; thermal stress; blood parameters; seasonal variations

*Corresponding author:
Husein Ohran, PhD, University of Sarajevo, Veterinary Faculty, Department of Basic Veterinary Sciences, 71000 Sarajevo, Bosnia and Herzegovina, phone: +38733729100, e-mail: husein.ohran@vfs.unsa.ba

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Introduction

Global warming is a major threat, not only to human health but also to livestock production. The Intergovernmental Panel on Climate Change (IPCC) stated that by the year 2040, the average temperature on the planet will increase by 1.5°C, while by the year 2100, it is expected to increase in some regions by almost 5°C (PÖRTNER et al., 2022). The negative impacts of climate change on animal health and production are numerous, but the magnitude of these impacts is still uncertain (GODDE et al., 2021). Climate change leads to a reduction in the quantity and quality of pastures and water resources, the emergence of new infectious agents and disease outbreaks, and long periods of drought, all of which significantly reduce the quantity and quality of animal products, and cause a financial burden for farmers (THORNTON et al., 2014). Therefore, the future of the livestock sector will be more and more challenging. Sheep farming is a very important livestock sector in Bosnia and Herzegovina (BH). Pramenka is an indigenous sheep breed raised in almost the entire area of the Balkans, and is most represented in BH, Croatia, and Serbia. It is very resistant, survives in difficult feeding and living conditions, and at the same time provides economic benefits. In their research, ĆINKULOV et al. (2008) indicated that the Pramenka is a sheep with great genetic diversity and, consequently, is a valuable source of genetic variation. A recent study on single nucleotide polymorphism (SNP) markers for thermotolerance in various sheep breeds showed that the Dubska Pramenka can be characterized as cold-adapted (ASTUTI et al., 2022). Small ruminants demonstrate greater adaptability to harsh climates compared to other livestock. The thermoneutral zone for sheep is 5-25°C (CURTIS, 1983), while RAMON et al. (2016) stated that the comfort zone is an average daily temperature of 11-21°C . However, the range of the thermoneutral zone depends on many factors, such as the species, breed, age, sex, body weight, diet, health, and physiological state of the animal. Seasonal changes, followed by variations in meteorological conditions, significantly affect the physiology of animals. When the ambient temperature exceeds the upper or lower critical values of the internal temperature of domestic animals, a special form of stress occurs, known as thermal stress – heat or cold stress, respectively (COLLIER et al., 2019). VAN WETTERE et al. (2021) state that negative effects in sheep are expressed when temperatures fall below 12°C (lower critical temperature) or rise above 25 to 31°C (upper critical temperature). There are many indicators of thermal stress in sheep. The individual interpretation of hematological and biochemical blood parameters helps in our understanding of the extent of thermal stress in sheep, enables a detailed evaluation of their ability to adapt, and facilitates genetic selection. The most effective approach to addressing climate change is to focus on breeding thermotolerant animals. Therefore, this study aimed to compare the seasonal variations in some hematological and biochemical blood parameters in two strains of the indigenous sheep breed Pramenka – Dubska and Hercegovačka, and to evaluate their adaptability to seasonal changes and thermal stress conditions.

Materials and methods

Animal ethics approval. All experiments were performed according to the guidelines established by the European Community for the Care and Use of Laboratory Animals, the Ethics and Animal Welfare Committee of the International Council for Laboratory Animal Science. This study was conducted pursuant to the Law of Protection and Welfare of Animals, ("Sl. glasnik BiH", no. 25/2009 and 9/2018); and approved by the Ethical Committee of the University of Sarajevo - Veterinary Faculty (no. 01-02-18-27/20).

Animals and experimental design. The research included two strains of indigenous Pramenka sheep of a combined type of productivity - Dubska and Hercegovačka. To reduce the possibility of inbreeding, sampling was done on two different farms with two localities for each of the two examined strains. Blood samples for the Dubska strain were taken from the Vlašić and Kupres mountains, and for the Hercegovačka strain from the area Podveležje and the town of Nevesinje. Sampling was carried out on the same sheep

(according to the number on their ear tags and/or other means of identification - head color, height to the withers, etc.) in two different periods: high temperatures (July 2020) and low temperatures (February 2020). The selection of animals was two-staged - random selection of farms within the location and random selection of individuals within the farm. All the sheep were females, clinically healthy, one to six years of age, and reared under an extensive farming system. Twelve blood samples were taken from each locality and in each sampling period, i.e. 24 samples over the two periods (summer/winter) per strain - a total of 48 samples for each of the two examined strains. The total number of sheep blood samples was 96. In the winter the sheep were in the peripartum period, while in the summer they were in the final third of lactation. Throughout the summer season, the sheep predominately consumed pasture, whereas during the winter their diets consisted mainly of hay and silage. Access to water was *ad libitum* throughout both seasons.

*Geographical and meteorological data***.** During the experimental period, geographical and meteorological data were monitored. Geographical data from the examined localities were obtained using the ESRI ArcMap 10.6.1 software (ZANINOVIĆ et al., 2008). Morphological analyses were performed using the same software, more specifically the digital terrain model (DTM), which is essential to recreating terrain morphology once the external elements are removed (JIMÉNEZ-JIMÉNEZ et al., 2021). The values of air temperature and relative air humidity were obtained from the Federal Hydrometeorological Institute of Bosnia and Herzegovina (for the locations of Kupres, Vlašić, and Podveležje) and the Republic Hydrometeorological Institute of Banja Luka (for the area of town Nevesinje). Given that there are no meteorological stations in the locations where the research was conducted, to obtain accurate values, meteorological data interpolation (ŠEGOTA and FILIPČIĆ, 1996; ZANINOVIĆ et al., 2008) was performed based on data from the two nearest meteorological stations.

To assess the influence of temperature on the hematological and biochemical blood parameters, the data on air temperature and relative humidity were used to calculate the Temperature-Humidity Index (THI). Air temperature and relative humidity were measured during the entire summer and winter seasons, and also on the day when the blood was collected and 5 days before blood collection. Given the similar climatic and geographical conditions as in our research, the following formula, according to FINOCCHIARO et al. (2005), was used to calculate THI:

$$THI = T - [0.55 \times (1 - RH)/100] \times (T - 14.4),$$

where: T – air temperature (°C); RH – relative air humidity (%).

Blood Sampling and Analysis. Blood samples from the jugular vein were collected in 3 ml EDTA tubes (Ayset® Tube, EDTA 3K), 3.5 ml serum separation tubes (BD Vacutainer® SST™ II Advance), and 2 ml test tubes for glucose concentration (BD Vacutainer® NaF, Na2EDTA). Complete hematological and biochemical tests were performed on the blood samples within 24 and 48 hours, respectively. Hematological parameters were determined using an ADVIA 120 device (Siemens, USA), and the following parameters were analyzed: red blood cell count (RBC), hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), red blood cell distribution width (RDW), white blood cell count (WBC), the total number and percentage of neutrophils (Ne), eosinophils (Eo), basophils (Ba), lymphocytes (Ly) and monocytes (Mo), platelet count (PLT), and mean platelet volume (MPV). Blood biochemical parameters were determined using Olympus AU400 device (Beckman Coulter, USA), and the following parameters were analyzed: total protein (TP), albumin (ALB), globulin (GLO), total bilirubin (TB), cholesterol (CL), glucose (GLU), urea (UREA), creatinine (CRE), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), creatine kinase (CK), non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHB), calcium (Ca), phosphorus (P), sodium (Na), chlorine (Cl) and magnesium (Mg).

Statistical Analysis. The data, presented as mean (± SEM), underwent evaluation using the Kolmogorov-Smirnov test to assess normal distribution. In cases of significant deviations, the test was reiterated with the Monte Carlo (MC) permutation test. If the results differed from the comparative tests, the permutation test outcomes were prioritized. Normal distribution was assessed with IBM SPSS Statistics for Windows, version 20. To compare hematological and biochemical blood parameters between summer and winter, as well as within and between strains, the paired T-test was applied. Statistical significance was set at $P<0.05$. The paired T-test was performed using MedCalc, while the MC permutation test was performed with PAST software (HAMMER et al., 2001).

Results

Geographical and climatological data from the investigated locations are presented in Table 1. The calculated THI data was evaluated as follows: <22.2 = absence of heat stress; 22.2 to 23.3 = moderate heat stress: 23.3 to 25.6 = severe heat stress and 25.6 and above = extremely severe heat stress (MARAI et al., 2007). While the THI limit values for heat stress are fully defined, threshold values for cold stress are still a matter of debate. However, a study conducted on local sheep breeds in Spain showed that THI threshold values for cold stress are 9.8 (Latxa breed) and 10.3 (Manchenga breed) (RAMON et al., 2019; CARABAÑO et al., 2021). The average seasonal THI (THIavg) for the investigated localities did not show heat stress, except for the locality Podveležje, where mild heat stress could be noticed during the entire summer season. Since the seasonal variations in temperature and relative humidity in BH are enormous throughout the year, the mild and relatively thermoneutral values cannot be taken as reliable in terms of evaluating thermal stress in animals. Therefore, we calculated additional THI based on average and maximum temperatures, and relative humidity values on the day of sampling (THIavg-BC and THImax-BC, respectively) and 5 days before sampling (THIavg-5daysBC and THImax-5daysBC, respectively), similar to the concept of SALCES-ORTIZ et al. (2013). These THI data showed severe and extremely severe heat stress at the localities Podveležje and Nevesinje, the areas where the Hercegovačka Pramenka were being held. On the other hand, during the winter season, almost all THI data indicated cold stress in all localities, and it was extremely severe in Vlašić and Kupres, the areas where the strain Dubska Pramenka was being farmed. Additionally, the values of MiT-BC and MiT-5daysBC for the winter season and all investigated localities were below the lower critical temperature in sheep (Van WETTERE et al., 2021).

The hematological parameter values (Table 2) generally fell within the reference ranges, except for MCHC and MPV. Seasonal differences were mainly observed in the leukogram, with significant variations in total sheep samples and within the Dubska strain, particularly in RBC, PLT, and MPV. Significant differences were noted between the two strains for most parameters. During the summer, higher values of RBC and PCV were observed in Dubska compared to the Hercegovačka strain, while the Hercegovačka strain showed significantly higher WBC and PLT levels compared to Dubska.

The biochemical parameter variations are detailed in Table 3, with values generally within the reference ranges, except for decreased CRE, and increased AST, CK, and Na. Statistically significant differences in the total sheep samples between the summer and winter seasons were found for TP, ALB, GLO, TB, UREA, CRE, GGT, NEFA, BHB, and Mg. Notably, the Dubska strain exhibited more seasonal variations in biochemical parameters compared to Hercegovačka. Significant differences between the strains were found for several parameters, mainly during the winter period. The Hercegovačka strain had significantly higher values in TP, ALB, UREA, and CK during the winter period compared to Dubska. In the summer period, the Hercegovačka strain showed significantly higher GLO levels, while Dubska had higher ALB and Cl levels.

Table 1. Climatological and geographical data from the investigated areas

	Hercegovačka pramenka				Dubska pramenka			
	Podveležje		Nevesinje		Vlašić		Kupres	
Climatological data								
	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter
AvT (°C)	20.59	6.76	20.10	5.34	15.08	1.00	15.77	2.11
AvRH (%)	48.73	44.20	49.47	51.72	81.60	61.60	81.53	86.90
THIavg	22.22	4.95	21.62	2.82	15.38	-3.46	16.38	-3.70
AvT-BC	21.06	5.74	19.79	2.00	13.50	5.50	12.77	2.85
MaT-BC	25.66	10.31	25.15	9.75	17.90	8.05	17.79	6.24
MiT-BC	16.91	0.86	14.55	-5.60	10.20	0.80	11.39	-0.06
AvRH-BC	23.17	65.73	58.71	85.17	84.22	97.91	84.06	96.42
RHmax-BC	100.00	62.31	91.45	100.00	100.00	99.60	100.00	98.81
THIavg-BC	21.87	2.66	21.50	-3.74	13.09	0.76	12.03	-3.21
THImax-BC	31.79	8.93	30.50	7.22	19.82	4.61	19.64	1.85
AvT-5daysBC	22.11	3.85	21.69	2.66	15.98	-2.41	16.28	-3.13
MaT-5daysBC	29.76	10.61	31.55	10.55	30.45	7.70	29.39	5.99
MiT-5daysBC	15.56	-0.49	12.00	-3.80	2.85	-10.10	5.11	-8.89
AvRH-5daysBC	54.84	45.23	58.94	65.57	70.43	86.41	70.03	85.85
RHmax-5daysBC	100.00	79.03	94.50	77.57	99.02	99.82	98.93	99.45
THIavg-5daysBC	24.39	1.25	24.01	-1.51	16.58	-10.31	16.99	-11.31
THImax-5daysBC	38.12	8.98	40.37	8.93	39.10	4.06	37.46	1.44
Geographical data								
Altitude (MAMSL)	864		877		1159		1131	
Research area (km^2)	1.383,40		1.104,00		7.043,00		15.282,00	
GPS coordinates	43.281656, 17.950202		43.256564, 18.124937		44.311932, 17.538586		43.824834, 17.365428	

Summer: June - September; Winter: November - February

AvT - average temperature during the entire summer and winter season; AvRH - average relative humidity during the entire summer and winter season; THIavg - THI calculated with average temperature and relative humidity during the entire summer and winter season; AvT-BC - average temperature on the blood collection day; MaT-BC - maximum temperature on the blood collection day; MiT-BC - minimum temperature on the blood collection day; AvRH-BC - average relative humidity on the blood collection day; RHmax-BC - maximum relative humidity on the blood collection day; THIavg-BC- THI calculated with average daily temperature and relative humidity on the blood collection day; THImax-BC - THI calculated with maximum daily temperature and relative humidity on the blood collection day; AvT-5daysBC - average temperature for 5 days before blood collection; MaT-5daysBC - maximum temperature for 5 days before blood collection; MiT-5daysBC - minimum temperature for 5 days before blood collection; AvRH-5daysBC - average relative humidity for 5 days before blood collection; RHmax-5daysBC - maximum relative humidity for 5 days before blood collection; THIavg-5daysBC - THI calculated with average temperature and relative humidity for 5 days before blood collection; THImax-5daysBC - THI calculated with maximum temperature and relative humidity for 5 days before blood collection.

MAMSL - meters above mean sea level

Table 2. Seasonal variations in hematological parameters in total samples, within and between Pramenka strains

	Total (n=96)		P_t	Hercegovačka pramenka (n=48)		P_{Hws}	Dubska pramenka (n=48)		P_{Dws}	P_{bs}		Reference values*
	S (n=48)	W (n=48)		S (n=24)	W (n=24)		S (n=24)	W (n=24)		S	W	
RBC (10^{12}/L)	9.79±0.25	9.28±0.19	0.056	9.03±0.26	8.76±0.25	0.468	10.69±0.36	9.91±0.19	0.039	0.000	0.001	9 - 15
Hb (g/L)	105.93±4.62	99.36±2.00	0.117+	103.86±5.08	94.56±2.65	0.093+	108.42±8.29	105.16±2.52	0.418+	0.506	0.008	90.0 – 150.0
PCV (%)	30.31±0.85	28.82±0.56	0.093	28.80±0.82	28.05±0.76	0.461+	32.12±1.51	29.74±0.81	0.126	0.039	0.157	27.0 – 45.0
MCV (fL)	31.07±0.45	31.18±0.39	0.858	32.02±0.56	32.16±0.49	0.861	29.93±0.64	29.98±0.52	0.947	0.014	0.002	28.0 – 40.0
MCH (pg)	10.99±0.44	10.67±0.07	0.358+	11.53±0.50	10.77±0.08	0.098+	10.34±0.76	10.55±0.13	0.951+	0.197	0.088	8.0 – 12.0
MCHC (g/L)	355.33±14.54	345.36±3.21	0.436+	361.52±16.08	337.78±4.25	0.145+	347.84±25.99	354.53±4.07	0.907+	0.716	0.009	310.0 - 340.0
RDW (%)	15.84±0.15	16.06±0.20	0.346	15.65±0.21	15.69±0.22	0.996+	16.05±0.22	16.49±0.33	0.215	0.306	0.033	12.0 - 27.0
WBC 10^9/L)	9.75±0.58	9.10±0.33	0.254+	11.10±0.85	9.21±0.46	0.045+	8.03±0.55	8.95±0.49	0.233	0.003	0.478	4.0 – 12.0
Ne (10^9/L)	1.63±0.14	2.16±0.16	0.011+	1.67±0.22	1.77±0.16	0.746+	1.57±0.16	2.62±0.26	0.001	0.632	0.008	2.0 – 4.0
Eo (10^9/L)	1.15±0.11	0.88±0.09	0.086+	1.34±0.16	1.07±0.13	0.255	0.91±0.11	0.65±0.11	0.099	0.011	0.025	0.0 – 0.6
Ba (10^9/L)	0.32±0.03	0.17±0.01	0.001+	0.39±0.04	0.21±0.02	0.000	0.23±0.05	0.13±0.01	0.033+	0.011	0.001	0.0 – 0.2
Mo (10^9/L)	0.74±0.11	0.52±0.05	0.076+	0.94±0.18	0.58±0.08	0.068+	0.50±0.07	0.44±0.05	0.573	0.018	0.198	0.0 – 0.4
Ly (10^9/L)	5.89±0.46	5.21±0.23	0.187+	6.76±0.73	5.39±0.31	0.085+	4.86±0.38	4.98±0.34	0.814	0.031	0.211	3.5 – 6.9
Ne (%)	17.51±1.33	23.93±1.52	0.001+	15.66±1.85	19.80±1.67	0.098	19.75±1.82	28.93±2.23	0.006	0.145	0.001	10.0 – 50.0
Eo (%)	12.43±1.11	9.64±0.94	0.109+	13.39±1.78	11.52±1.30	0.382+	11.27±1.15	7.36±1.18	0.021	0.188	0.054	0.0 – 10.0
Ba (%)	3.18±0.27	1.88±0.13	0.001+	3.56±0.33	2.24±0.17	0.006+	2.73±0.42	1.46±0.13	0.004+	0.125	0.001	0.0 – 3.0
Mo (%)	7.11±0.98	5.67±0.48	0.266+	8.12±1.69	6.25±0.72	0.347+	5.89±0.67	4.97±0.59	0.369+	0.180	0.293	0.0 – 6.0
Ly (%)	59.05±1.85	55.97±1.85	0.228	58.72±2.91	55.95±2.74	0.515+	59.43±2.15	55.99±2.47	0.303	0.541	0.813	40.0 - 75.0
PLT (10^9/L)	531.29±48.75	594.36±26.91	0.348+	658.26±75.17	627.70±33.49	0.649+	377.58±34.78	554±42.67	0.002	0.001	0.102	250.0 – 750.0
MPV (fL)	14.55±0.54	12.87±0.71	0.016+	13.87±0.56	16.13±0.69	0.036	15.36±0.96	8.92±0.53	0.001+	0.148	0.000	3.5 - 6.5

Statistical significance: P<0,05; S - summer, W - Winter; P_t - P value compared in total sheep samples; P_{Hws} - P value compared within Hercegovačka strain; P_{Dws} - P value compared within Dubska strain; P_{bs} - P value compared between strains; + P value derived from exact or MC permutation test (deviation from normal distribution); * AIELLO et al. (2016);

RBC - Red blood cells; Hb - Hemoglobin; PCV - Packed cell volume; MCV - Mean corpuscular volume; MCH - Mean corpuscular haemoglobin; MCHC - Mean corpuscular hemoglobin concentration; RDW - Red cell distribution width; WBC - White blood cells; Ne - Neutrophils; Eo - Eosinophils; Ba - Basophils; Ly - Lymphocytes; Mo - Monocytes; PLT- Platelets; MPV - Mean platelet volume

Table 3. Seasonal variations in biochemical parameters in total samples, and within and between Pramenka strains

	Total (n=96)		P_t	Hercegovačka pramenka (n=48)		P_{Hws}	Dubska pramenka (n=48)		P_{Dws}	P_{bs}		Reference values*
	S (n=48)	W (n=48)		S (n=24)	W (n=24)		S (n=24)	W (n=24)		S	W	
TP (g/L)	80.64±1.13	67.18±0.85	0.001	81.52±1.76	69.12±1.22	0.001	79.68±1.38	65.07±1.01	0.001	0.421	0.034	59.0 – 78.0
ALB (g/L)	34.63±0.47	29.03±0.51	0.001	33.72±0.49	30.44±0.61	0.005	35.62±0.79	27.50±0.70	0.001	0.044	0.003	27.0 – 37.0
GLO (g/L)	46.77±1.26	38.14±0.81	0.001	49.24±2.16	38.66±1.20	0.001	44.07±0.93	37.57±1.09	0.002	0.039	0.702	32.0 – 50.0
TB (μmol/L)	2.71±0.29	2.03±0.16	0.041+	2.39±0.33	1.64±0.20	0.086+	3.05±0.47	2.44±0.23	0.264+	0.248	0.011	0.7 – 8.6
CL (mmol/L)	2.28±0.07	2.13±0.06	0.119	2.35±0.10	2.08±0.08	0.025	2.20±0.09	2.19±0.11	0.935+	0.300	0.419	1.1 – 2.3
GLU (mmol/L)	2.96±0.05	3.05±0.06	0.227	2.87±0.08	3.02±0.11	0.265	3.06±0.06	3.08±0.07	0.660	0.084	0.431	2.4 – 4.5
UREA (mmol/L)	4.56±0.27	2.94±0.25	0.001+	4.96±0.39	4.16±0.27	0.101+	4.12±0.34	1.61±0.15	0.001+	0.115	0.000	3.7 – 9.3
CRE (μmol/L)	38.03±1.11	54.03±1.79	0.001+	36.99±1.38	50.58±2.44	0.001	39.15±1.75	57.80±2.44	0.001	0.333	0.057	75.8 – 174.3
AST (U/L)	147.32±4.85	136.02±4.33	0.075+	146.55±6.89	141.70±6.99	0.635+	148.15±6.97	129.84±4.71	0.049+	0.872	0.163	49.0 – 123.3
GGT (U/L)	27.51±2.87	45.34±2.79	0.001	31.17±4.40	38.96±3.41	0.229	23.50±3.50	52.30±4.09	0.001	0.184	0.006	19.6 – 44.1
CK (U/L)	281.28±22.3	281.11±41.5	0.895+	244.79±24.28	372.51±74.09	0.112+	321.10±37.27	181.40±16.15	0.006	0.088	0.013	7.7 – 101.0
NEFA (mmol/L)	1.45±0.16	0.41±0.05	0.001+	1.02±0.18	0.50±0.08	0.013+	1.29±0.27	0.31±0.05	0.000+	0.391	0.046	0.1 – 0.9
BHB (mmol/L)	0.53±0.04	0.40±0.03	0.005+	0.57±0.06	0.43±0.05	0.113+	0.48±0.03	0.35±0.02	0.003+	0.250	0.212	0.1 – 0.4
Ca (mmol/L)	2.53±0.03	2.40±0.02	0.004+	2.52±0.03	2.43±0.03	0.072+	2.53±0.04	2.36±0.04	0.014	0.838	0.054	2.3 – 2.9
P (mmol/L)	1.61±0.06	1.66±0.07	0.561	1.60±0.05	1.67±0.12	0.603	1.61±0.10	1.64±0.07	0.797	0.907	0.620	1.3 – 2.4
Na (mmol/L)	144.74±1.06	146.66±0.57	0.112	143.32±1.36	145.84±0.86	0.093	146.30±1.61	147.57±0.72	0.521	0.162	0.118	132.0 – 145
Cl (mmol/L)	107.26±0.74	108.84±0.59	0.079+	105.28±0.73	107.75±0.75	0.024+	109.41±1.19	110.03±0.89	0.703	0.004	0.043	105 – 115
Mg (mmol/L)	0.98±0.04	1.10±0.02	0.008+	1.02±0.06	1.08±0.02	0.365+	0.93±0.06	1.11±0.02	0.006+	0.292	0.281	0.8 – 1.1

Statistical significance: P<0,05; S - summer, W - Winter; P_t - P value compared in total sheep samples; P_{Hws} - P value compared within Hercegovačka strain; P_{Dws} - P value compared within Dubska strain; P_{bs} - P value compared between strains; + P value derived from exact or MC permutation test (deviation from normal distribution); * AIELLO et al. (2016)

TP - Total protein; ALB - Albumin; GLO - Globulin; TB - Total bilirubin; CL - Cholesterol; GLU - Glucose; UREA - Ureja; CRE - Creatinine; AST - Aspartate aminotransferase; GGT - Gamma-glutamyltransferase; CK - Creatine kinase; NEFA - Non-esterified fatty acids; BHB - Beta-hydroxybutyrate; Ca - Calcium; P - Phosphorus; Na - Sodium; Cl - Chlorine; Mg - Magnesium

Discussion

Among blood parameters, the main indicators of heat stress in livestock are increases in RBC, PCV, and Hb, due to dehydration or sympathetic-driven splenic contraction. Most of the studied erythrocyte parameters stayed within the reference values, even in thermal stress conditions. Other authors reported diverse findings regarding PCV and Hb in heat stress conditions – unaffected (HAMZAOUI et al., 2013), increased (OKORUWA, 2015), or decreased (SINGH et al., 2016). The differences between studies regarding erythrocyte parameters could be species and breed-specific, production-based (dairy vs. nondairy), dependent on the lactation stage, or the extent and intensity of thermal stress (MEHABA et al., 2021). However, the only erythrocyte parameter outside the reference values was MCHC, which was increased in both seasons and strains. This finding is rather unusual, since decreases in MCH and MCHC values are common during heat stress in livestock, and could be an indication of poor thermoregulatory body fluid dynamics (HABIBU et al., 2018) or diseases, such as regenerative anemia and iron deficiency (THRALL et al., 2022). On the other hand, SHEK VUGROVEČKI et al. (2017) reported broader reference ranges for MCHC for Lika Pramenka sheep, so our finding could be an artifact or caused by other factors besides the environment. Regarding the erythrocyte parameters, our results suggest that the Hercegovačka strain may demonstrate greater resistance and adaptation to hot environments compared to the Dubska strain. This is evident in the significant increase in RBC and PCV observed in Dubska during the summer months compared to Hercegovačka. Notably, both strains appear to exhibit a robust adaptive capacity to both hot and cold climates, as indicated by the mild to severe thermal stress conditions highlighted by THIavg, THIavg-BC, and THIavg-5daysBC. In our study, we observed changes in leucocyte parameters in the Hercegovačka and Dubska Pramenka sheep strains subjected to varying thermal stress levels. Despite both strains generally maintaining leucocyte parameters within the reference values, the Hercegovačka strain exhibited significantly higher WBC values during summer, especially when compared to the Dubska strain. This elevation in WBC aligns with previous studies on heat-stressed sheep, where increased WBC counts were noted (OKORUWA, 2015). The rise in WBC during heat stress may be linked to hormonal changes, including elevated cortisone and adrenaline levels. While some reports, such as SINGH et al. (2016), have shown specific changes in leucocyte fractions under heat stress, our results suggest that these alterations may not be directly correlated with thermal stress. For instance, the increase in Ne during the winter in our study could be an immune system response, while the summer increase in Eo and Ba might be attributed to higher parasite and allergen exposure. Interestingly, PLT counts varied between strains and seasons. The Dubska Pramenka strain had a significantly lower PLT count during the summer compared to the winter, whereas the Hercegovačka Pramenka strain exhibited a higher PLT count in the summer. These findings may indicate differing adaptabilities of the two strains to distinct thermal stress conditions.

Biochemical and metabolic mechanisms have an enormous role in the response to thermal stress in animals. High temperatures can lead to increased concentrations in plasma proteins, due to dehydration. Higher values in TP, ALB, and GLO were noticed during the summer compared to the winter season in total blood samples and both Pramenka strains, emphasizing that TP was slightly above reference values. Having in mind that the Hercegovačka Pramenka sheep were in heat stress conditions, as were both strains toward the end of the lactation period, our results seem reasonable. UREA and CRE concentrations were significantly affected by season. Although still within the reference values, an increase in UREA during the summer compared to the winter period was noticed, while CRE levels were higher during the winter compared to the summer. MACÍAS-CRUZ et al. (2018) observed higher UREA levels in heat-stressed sheep. A possible reason for the higher blood UREA levels in summer/heat stress conditions is the reduction in urea waste through the kidneys, which could be due to the activation of thermoregulatory mechanisms. To

maintain homeothermy, the blood is redistributed to peripheral tissues to dissipate the heat through the skin, and more water is reabsorbed into the kidneys to avoid dehydration. Since only in the Hercegovačka strain were no significant differences noticed in UREA between seasons , this could be another indicator that this strain is well adapted to heat stress. CRE levels were remarkably decreased below the reference values, with significantly higher values during the winter compared to the summer season. Similar results were also found by AUTUKAITE et al. (2021), which implies that THI above or below threshold values is associated with decreased CRE. Both strains exhibited elevated AST levels above the reference values in both seasons, but only statistically significantly in Dubska in the summer compared to the winter season. Similar results were reported in sheep (KARTHIK et al., 2021), and goats in both heat and cold stress conditions (BANERJEE et al., 2014). Elevated AST levels during thermal stress are due to increased stimulation of gluconeogenesis by corticosteroids (KAMAL et al., 1989), and are a common finding in thermal-stressed sheep. NAZIFI et al. (2003) found a positive correlation between T4 and ALT/AST concentrations, suggesting that increased thyroid activity in cold conditions could be the reason for higher AST levels. On the other hand, HRKOVIĆ-POROBIJA et al. (2017) reported significant differences in AST concentrations between different locations and sampling periods, indicating that these results could be due to changes in the chemical and nutritive values of the sheep's feed. Higher temperatures and humidity cause an increase in GGT levels through the degradation of glutathione (AUTAKAITE et al., 2021). In our study, it was the opposite, as significantly higher values of GGT were found during the winter period in the Dubska strain, probably due to the intensification of metabolic processes and the response to negative energy balance (STEVANOVIĆ et al., 2015). Furthermore, we found extremely increased CK levels during both seasons, which were significantly higher in the summer compared to the winter in the Dubska strain. Such results could be attributed to greater activity during the summer months since the sheep were being reared under an extensive farming system. NEFA is considered to be a reliable indicator of metabolic adaptation to high heat load in small ruminants (SEJIAN et al., 2019). ABDALLA et al. (1993) demonstrated higher NEFA levels in crossbred ewes exposed to temperatures up to 35°C. An increase in circulating NEFA levels is considered a common finding in animals with restricted feeding (BAUMGARD and RHOADS, 2013), and the increase in heat stress conditions can be explained as the effort by the animal to maintain energy balance. Considering the severe THImax-BC, THIavg-5daysBC, and THImax-5daysBC values, we believe that elevated NEFA values during summer are caused by heat stress, considering also the physiological state of the sheep. BHB serves as an important metabolic regulator in a changing climate (ALEENA et al., 2016). AUTUKAITE et al. (2020) reported increased levels of BHB in Merino sheep when the THI was above 20, which is similar to our results, while MEHABA et al. (2021) observed no differences in BHB concentrations under heat stress conditions in sheep. Electrolyte concentrations can also be altered due to thermal stress. Increased Na levels may result from dehydration and/or increased aldosterone secretion in the summer, while in the winter, these findings could be attributed to greater feed intake. NAZIFI et al. (2003) reported elevated Mg levels under cold stress conditions compared to heat stress, aligning with our results, especially for the Dubska strain ($P<0.05$), which experienced severe cold stress.

Conclusions

Despite challenging conditions, nearly all the examined blood parameters stayed within the reference ranges, although statistically significant variations were observed between strains and seasons. On the basis of our findings, it seems that both Pramenka strains have a high adaptive capacity to harsh environmental conditions. The Hercegovačka Pramenka is more resistant to heat stress, while Dubska copes better with cold stress conditions. This is highlighted by the statistically significant differences observed in most examined blood parameters between strains and by season. Although Pramenka may not be a highly productive

sheep breed, it could still be utilized for farming in harsh environmental conditions.

However, additional experimental studies with thermoneutral groups are necessary. The confirmation of these adaptability traits should be sought on the genetic level, which will be the subject of our future research.

Ethics approval

This study was conducted pursuant to the Law of Protection and Welfare of Animals, ("Sl. glasnik BiH", no. 25/2009 and 9/2018); and approved by the Ethical Committee of the University of Sarajevo - Veterinary Faculty (no. 01-02-18-27/20).

Declaration of competing interest

No potential conflict of interest was reported by the authors.

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SAŽETAK

Klimatske promjene jedan su od najvećih globalnih izazova za animalnu proizvodnju. Autohtona ovca pramenka poznata je po sposobnosti preživljavanja u teškim uvjetima okoliša, no za to nedostaje znanstvenih dokaza. Cilj istraživanja bio je, u uvjetima prirodnog temperaturnog stresa, procijeniti sezonske varijacije određenih hematoloških i biokemijskih pokazatelja u krvi kod dva soja pramenke - hercegovačke i dubske. Na lokalitetima na kojima se uzgaja hercegovački soj pramenke izračunate su vrijednosti indeksa temperature i vlage (THI) s obzirom na blagi do jaki stres visokom temperaturom okoliša (toplotni stres). Isto je učinjeno i za stres niskom temperaturom okoliša (stres hladnoćom)na svim istraživanim lokalitetima. Većina analiziranih hematoloških i biokemijskih pokazatelja u krvi bila je unutar referentnih vrijednosti. Brojne su statistički znakovite razlike ($P<0,05$) u hematološkim pokazateljima utvrđene između godišnjih doba te unutar i među sojevima pramenke, osim za MCH, Eo%, Mo% i Ly%. Sličan je obrazac primijećen i za biokemijske pokazatelje u krvi. Dubski soj imao je više sezonskih varijacija u odnosu na hercegovački. Među sojevima utvrđene su statistički znakovite razlike za ukupne proteine, albumin, globulin, ureju, GGT, CK i NEFA, uglavnom u zimskom periodu. Na temelju hematoloških i biokemijskih pokazatelja u krvi oba su soja pramenke pokazala visoku sposobnost prilagodbe na teške uvjete okoliša, pri čemu je hercegovačka pramenka prilagođenija uvjetima toplotnog, a dubska stresa hladnoćom.

Ključne riječi: ovce; temperaturni stres; pokazatelji u krvi; sezonske varijacije