RESEARCH ARTICLE

EVALUATING THE IMPACT OF SEASON AND BREEDING AREA ON BLOOD PARAMETERS IN PRAMENKA SHEEP EXPOSED TO THERMAL STRESS CONDITIONS

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ABSTRACT

This study aimed to investigate seasonal variations in blood parameters of two Pramenka sheep strains, Dubska and Hercegovacka, across and within four areas in Bosnia and Herzegovina under thermal stress conditions. It included 48 sheep from Vlasic and Kupres (breeding areas of the Dubska strain), and Nevesinje and Podvelezje (breeding areas of the Hercegovacka strain). The investigation was conducted across both summer and winter seasons, encompassing a total of eight herds (two per area). Hematological parameters included erythrocyte, leukocyte, and platelet counts, mean platelet volume, the total count of granulocytic and agranulocytic leukocytes, hemoglobin concentration, red blood cell distribution width and erythrocyte parameters. Biochemical analyses covered total bilirubin, total cholesterol, glucose, urea, creatinine, creatine kinase, non-esterified fatty acids, beta-hydroxybutyrate, proteins, liver enzymes and electrolytes. Results revealed significantly higher values of red blood cell parameters during the summer period in the area of Nevesinje ($P<0.05$) compared to the Podvelezje area. Additionally, it's important to emphasize significantly increased levels of basophils during the summer season across all areas, except for Vlasic, when compared to the winter season. Furthermore, the amounts of total proteins, albumin and globulin were significantly increased in all areas during the summer compared to winter. The study findings underscore the substantial influence of the breeding area, encompassing its geological composition, spatial distribution, and land cover, on specific blood parameters investigated in sheep during periods characterized by adverse temperature conditions.

Keywords: Blood parameters, sheep, Pramenka, thermal stress, season

INTRODUCTION

Animal husbandry serves as the primary source of animal protein, including eggs, meat, and milk, crucial for the sustenance and nutrition of impoverished households in low- and middle-income countries. Additionally, it provides a primary or supplementary source of income for farmers (Leroy et al., 2022). Domestic sheep, particularly autochthonous breeds, are widely distributed species around the world. Due to their distinct biological traits, such as short gestation periods, high fertility, quick development rates, high feed conversion efficiency, and high disease resistance, certain animal types are commercially of particular importance in this context (Adams and Ohene-Yankyera, 2014). In Bosnia and Herzegovina (BiH), the importance of sheep breeding is constantly rising. The autochthonous Pramenka breed is predominantly represented in BiH and Serbia, though it is also reared across the Balkans. Historically, the Pramenka breed was widespread throughout Europe, however, it has been largely replaced by breeds with more pronounced productive performance. Originating from the mouflon (*Ovis musimon*), as indicated by Cinkulov et al. (2008), this breed offers significant economic advantage and exhibits remarkable resilience, thriving even in demanding conditions related to feeding and housing, as highlighted by Katica et al. (2004). According to data sourced from the Breeding Program for Sheep in the Federation of BiH, there are four distinct autochthonous strains of Pramenka sheep in BiH, namely: Dubski, Privorski, Kupreski, and Hercegovacki (Breeding Program for Sheep in the Federation of Bosnia and Herzegovina, 2018). However, Adilovic and Andrijanic proposed a different classification, identifying two additional strains, i.e., the Podveleski and Varcarski strains (Adilović and Andrijanić, 2005). Animal husbandry, especially the breeding of resilient autochthonous Pramenka breed, plays a crucial role in supporting the livelihoods of farmers in BiH, since these sheep are renowned for their genetic and phenotypic traits. However, while these traits are well-documented and have been pivotal in their widespread use, the effects of climate change on these valuable characteristics remain largely unknown.

The increasingly pronounced effects of climate change present a significant threat globally, affecting both human well-being and livestock productivity. The Intergovernmental Panel on Climate Change (IPCC) reports a steady increase in Earth's temperature by 0.2℃ per decade, with projections indicating a potential rise in the global average surface temperature to between 1.4℃ and 5.8℃ by 2100 (IPCC, 2023). Climate change impacts animal husbandry through direct effects like heat stress, extreme weather events, and reduced productivity, especially in "water-stressed regions", as well as indirect effects such as increased water requirements, the emergence of new diseases, and economic disruptions (Godde et al., 2021). BiH follows this global trend with projected temperature increases. Climatological analyses suggest a trajectory of temperature elevation until 2030 compared to the baseline period of 1961-1990, with an anticipated annual increase of up to 1.0°C across the entire state territory (Radusin et al., 2022). These changes pose challenges to animal husbandry, including sheep farming, altering climate patterns and affecting ruminants through physiological changes. Thermal stress, a prominent consequence of climate change, affects livestock when ambient temperatures exceed upper or lower critical thresholds for the internal temperature of domestic animals, leading to heat or cold stress (Collier et al., 2019). Understanding how climate change influences the favorable Pramenka traits is essential, as rising temperatures, altered precipitation patterns, and increased frequency of extreme weather events could significantly impact their health and productivity, as previously demonstrated (Cwynar et al., 2014; Rana et al., 2014). Inspired by similar study designs, we focused on evaluating how seasonal variations affect the hematological and biochemical parameters of two Pramenka strains, Dubska and Hercegovacka, across different breeding areas of BiH under thermal stress conditions.

MATERIAL AND METHODS

Ethics Committee Approval

This study is under 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).

Study area and sample collection

This study was conducted in two distinct regions of BiH, focusing on Dubska and Hercegovacka strains of Pramenka sheep. Dubska strain was examined in the central region, including Vlasic mountain (Mudrike village - 44.306747°N,17.536004°E) and Kupres plateau (Zvirnjaca village - 43.824834°N, 17.365428°E), involving four distinct flocks. Figure 1 illustrates the spatial representation and distribution of the agricultural zones, forested regions and semi-natural habitats of Mudrike and Zvrinjaca villages. Conversely, the Hercegovacka strain was examined in the southern region, covering Podvelezje (Kruzanj village - 43.281656°N, 7.950202°E) and Nevesinje areas (Ziljevo village - 43.256564°N, 18.124937°E), with two flocks in each area. Figure 2 provides a spatial distribution of urban, agricultural and forested land cover categories in the areas of Kruzanj and Ziljevo villages. The geographical selection criteria included altitude (Kupres and Vlasic), above-average summer and winter temperatures, and the presence and abundance of the Pramenka sheep strains in these areas.

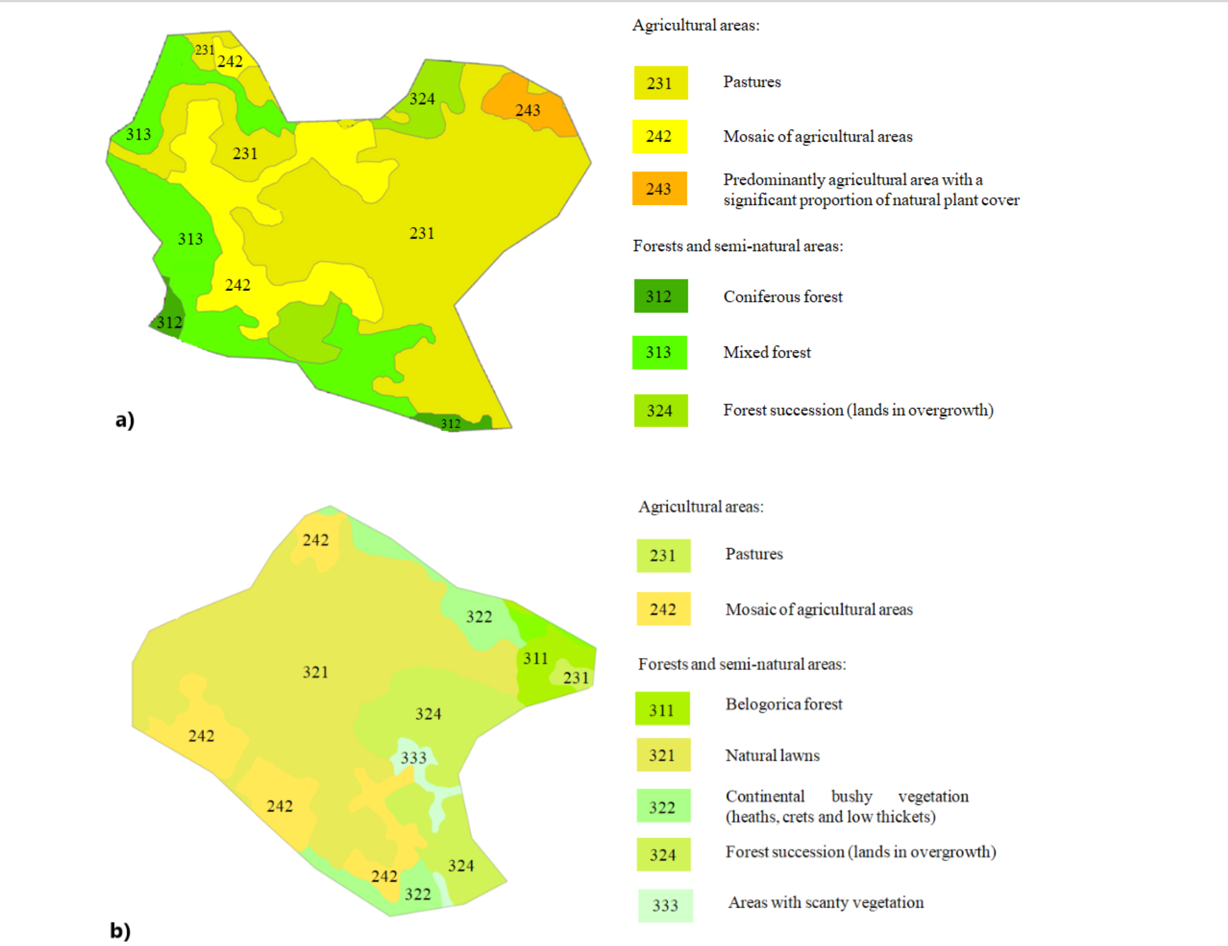

Figure 1 Spatial distribution of land cover and land use patterns in Vlasic (a) and Kupres (b) areas based on Corine Land Cover (CLC) data

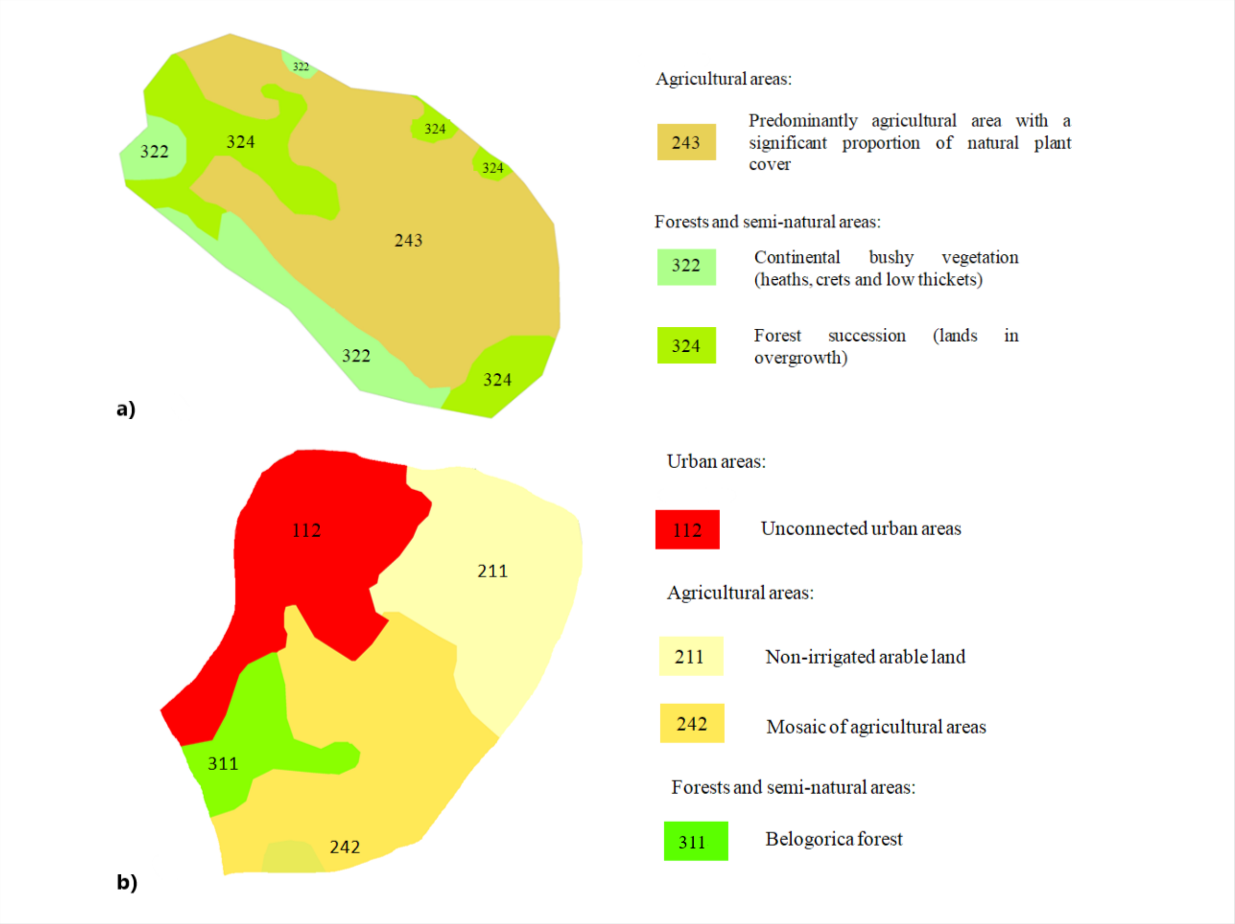

Figure 2 Spatial distribution of land cover and land use patterns in Podvelezje (a) and Nevesinje (b) areas based on Corine Land Cover (CLC) data

Sampling was performed using non-probabilistic approach with two-stage sampling design within each flock. Six healthy female sheep aged between one and six years were randomly selected from each herd. Sheep were raised on pasture during the summer and were provided with hay and silage during the winter, with *ad libitum* water access. Twelve blood samples were collected from each locality during each sampling period, resulting in 24 samples per strain across the two periods (summer and winter). In total, 48 samples were obtained for each of the two examined strains, amounting to 96 sheep blood samples overall. Blood samples were obtained from the jugular vein, utilizing 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® containing NaF and Na_2EDTA). Blood samples intended for hematological analysis were stored at +4°C, while serum samples for biochemical analysis were stored at -20°C until laboratory processing.

Climatological data measurements

Air temperature and relative humidity data were sourced from the Federal Hydrometeorological Institute of Bosnia and Herzegovina for the Vlasic, Kupres and Podvelezje areas, and from the Republic Hydrometeorological Institute of Banja Luka for the Nevesinje area. Data were collected throughout the summer and winter seasons, as well as for the five days preceding blood sampling. To evaluate the influence of temperature on the hematological and biochemical blood parameters, the data on air temperature and relative humidity was used to calculate the Temperature-Humidity Index (THI), according to Finocchiaro et al. (2005):

$$THI = T - \left[\frac{0.55x(1 - RH)}{100}\right]x(T - 14.4)$$

where T stands for air temperature expressed in ℃; RH stands for relative air humidity (%).

The obtained values indicate the following: <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 more = extreme severe heat stress (Marai et al., 2007). According to Ramón et al. (2019) and Carabaño et al. (2021), the threshold value for cold stress was set at 9.8. More detailed information and a more comprehensive methodological approach regarding the climatological data are provided in our previously published study (Ohran et al., 2024).

Blood hematological and biochemical measurements

Hematological parameters assessed included the number of erythrocytes (RBC), hemoglobin concentration (Hb), packed cell volume (PCV), mean value of erythrocyte volume (MCV), mean value of hemoglobin concentration in the erythrocyte (MCHC), mean value of the amount of hemoglobin in the erythrocyte (MCH), red blood cell distribution width (RDW), total number of leukocytes (WBC), total number and percentage of neutrophils (Ne), eosinophils (Eo), basophils (Ba), lymphocytes (Ly) and monocytes (Mo). Additionally, platelet count (PLT) and mean platelet volume (MPV) were measured. Hematological analyses were performed using an ADVIA 120 automated veterinary hematology analyzer (Siemens, USA). Biochemical parameters analyzed included total protein (TP), albumin (ALB), globulin (GLO), total bilirubin (TB), total cholesterol (TC), 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). These analyses were conducted using an Olympus AU400 automated chemistry analyzer (Beckman Coulter, USA). The interpretation of hematological and biochemical blood parameters was based on reference values provided by Aiello et al. (2016).

Statistical analysis

To assess the differences in hematological and biochemical blood parameters between the examined areas, a one-way analysis of variance (ANOVA) was employed. For *post hoc* comparison between the groups, the Tukey's test was used (SPSS Statistics version 20, IBM Corp, USA). To compare hematological and biochemical parameters between seasons within the same area (summer vs. winter), a paired t-test was applied, as these are dependent samples (i.e., the same individuals observed in different seasons) (MedCalc Software Ltd. version 20.115, Belgium). The threshold for statistical significance was set a $P < 0.05$.

RESULTS

Climatological data

The climatological data for the examined areas are shown in Table 1. THIavg in investigated areas indicated no significant heat stress, except for Podvelezje, where mild heat stress was observed during summer. However, THIavg-5daysBC and THImax-5daysBC indicated strong to extremely strong heat stress observed during summer in the Podvelezje and Nevesinje areas, breeding areas of the Hercegovacka strain. Conversely, during winter, nearly all THI readings indicated cold stress in all areas, with extremely severe conditions in Vlasic and Kupres, breeding areas of the Dubska strain.

Table 1 Climatological data of the examined areas

	Hercegovacka strain				Dubska strain			
	PODVELEZJE		NEVESINJE		VLASIC		KUPRES	
	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
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

Summer: June–September; Winter: November–February

AvT –average temperatures throughout the summer and winter season; AvRH –average relative humidity during the entire summer and winter season; THIavg –THI calculated according to the average values of temperature and relative humidity during the entire summer and winter season; AvT-5daysBC –average temperature values five days before collection; MaT-5daysBC −maximum temperature values five days before collection; MiT-5daysBC − minimum temperature values five days before collection; AvRH-5daysBC –average values of relative humidity five days before collection; RHmax-5daysBC −maximum values of relative humidity five days before collection; THIavg-5daysBC − THI calculated from the average values of temperature and relative humidity five days before collection; THImax-5daysBC − THI calculated with maximum values of temperature and relative humidity five days before collection

Hematological parameters

Most parameters of the erythrocyte lineage, including RBC, Hb, PCV, MCH and RDW, in all investigated areas were within the reference values, even under conditions of thermal stress. However, during summer, a significant increase in Hb content, MCH, and MCHC were noted only in the Nevesinje area compared to winter (Table 2). A significant increase in the levels of Ne during winter in the Vlasic and Kupres areas compared to summer is shown in Table 3. In contrast, significantly higher WBC count were found in the Podvelezje area compared to the Nevesinje area during summer (Table 2). Basophil counts were significantly higher during summer across all areas, except Vlasic, compared to winter (Tables 2 and 3), while eosinophils levels were significantly higher in Nevesinje compared to Podvelezje during summer. PLT values exhibited a statistically significant elevation during winter compared to summer within the Vlasic area (Table 3), as did MPV, including the Kupres area.

Biochemical parameters

During summer, the serum biochemical analysis of examined sheep revealed a significant increase in TP, ALB and GLO in all investigated areas (Tables 4 and 5). TP values were significantly higher in the Nevesinje area compared to the Podvelezje area during summer (Table 4). AST values exceeded reference levels in both seasons, with statistically higher levels during summer in Kupres compared to Vlasic, and in Nevesinje compared to Podvelezje (Tables 4 and 5). In the central part of the country, a significant increase in CK and GGT was recorded, during summer and winter, respectively (Table 5). Significantly higher values were found in the Vlasic area during winter compared to summer (Table 5), where the Dubska pramenka strain was exposed to severe cold stress. Significantly higher Ca values were found during summer compared to winter in the Nevesinje and Kupres areas (Tables 4 and 5). Changes in electrolyte concentrations were obsereved under both cold and heat conditions. During winter, a significant increase in the value of Mg was recorded in the Vlasic area, while Ca values were significantly higher in Nevesinje and Kupres during summer compared to winter.

Table 2 Seasonal changes in hematological parameters within and between the Nevesinje and Podvelezje areas in the Pramenka sheep breed

Hercegovacka strain								
	Nevesinje (n=24)		P	Podvelezje (n=23)		P	P_{bl}	
	S (n=12)	W (n=12)		S (n=12)	W (n=12)		S	W
RBC (10^{12}/l)	9.14 ± 0.25	8.73 ± 0.34	0.391	8.92 ± 0.49	8.79 ± 0.41	0.844	0.985	0.999
Hb (g/l)	107.48 ± 2.70	94.33 ± 3.30	**0.016**	100.00 ± 10.35	94.82 ± 4.40	0.635	0.944	1.000
PCV (%)	28.40 ± 0.85	27.41 ± 0.87	0.471	29.25 ± 1.47	28.75 ± 1.29	0.767	0.970	0.811
MCV (fl)	31.10 ± 0.57	31.58 ± 0.62	0.551	33.02 ± 0.93	32.80 ± 0.74	0.892	0.311	0.543
MCH (pg)	11.80 ± 0.24	10.79 ± 0.11	**0.003**	11.24 ± 1.04	10.74 ± 0.13	0.751	0.971	0.991
MCHC (g/l)	379.66 ± 7.88	344.33 ± 5.67	**0.002**	341.73 ± 32.25	330.64 ± 5.88	0.778	0.762	0.342
RDW (%)	15.47 ± 0.36	15.96 ± 0.32	0.373	15.86 ± 0.21	15.41 ± 0.31	0.123	0.680	0.722
WBC (10^9/l)	9.03 ± 0.60	8.96 ± 0.34	0.940	13.36 ± 1.38	9.48 ± 0.90	**0.027**	**0.005**	0.941
Ne (10^9/l)	1.81 ± 0.39	1.94 ± 0.23	0.788	1.52 ± 0.19	1.59 ± 0.21	0.801	0.908	0.823
Eo (10^9/l)	1.66 ± 0.24	1.22 ± 0.18	0.247	0.99 ± 0.18	0.91 ± 0.17	0.782	0.084	0.552
Ba (10^9/l)	0.36 ± 0.04	0.18 ± 0.02	**0.002**	0.42 ± 0.07	0.24 ± 0.03	**0.048**	0.841	0.151
Mo (10^9/l)	0.69 ± 0.23	0.44 ± 0.08	0.491	1.22 ± 0.27	0.74 ± 0.14	0.174	0.200	0.143
Ly (10^9/l)	4.52 ± 0.40	5.03 ± 0.31	0.366	9.20 ± 1.07	5.78 ± 0.55	**0.011**	**0.000**	0.625
Ne (%)	19.26 ± 3.07	21.63 ± 2.33	0.568	11.74 ± 1.24	17.82 ± 2.36	**0.036**	0.109	0.725
Eo (%)	18.12 ± 2.35	13.76 ± 2.12	0.279	8.23 ± 1.70	9.08 ± 1.12	0.735	**0.003**	0.241
Ba (%)	3.98 ± 0.45	1.94 ± 0.17	**0.009**	3.09 ± 0.48	2.56 ± 0.29	0.398	0.610	0.154
Mo (%)	8.18 ± 3.13	4.93 ± 0.90	0.491	8.06 ± 1.23	7.69 ± 1.01	0.859	1.000	0.139
Ly (%)	49.92 ± 3.78	51.44 ± 4.72	0.830	68.33 ± 2.03	60.86 ± 1.77	**0.028**	**0.000**	0.238
PLT (10^9/l)	594.92 ± 45.83	605.25 ± 39.75	0.893	727.36 ± 150.2	652.18 ± 56.11	0.766	0.522	0.906
MPV (fl)	14.16 ± 0.78	14.70 ± 0.91	0.719	13.56 ± 0.87	17.70 ± 0.88	**0.006**	0.999	0.053

Statistical significance: $P < 0.05$, S – summer, W – winter, P – P value for comparisons within the area between the summer and winter periods, P_{bl} - P value for comparisons between areas within the season - summer and winter periods

Table 3 Seasonal changes in hematological parameters within and between the Vlasic and Kupres areas in the Pramenka sheep breed

Dubska strain								
	Vlasic (n=24)		**P**	**Kupres (n=23)**		**P**	**P_{bl}**	
	S (n=12)	W (n=12)		S (n=12)	W (n=12)		S	W
RBC (10^{12}/l)	10.71 ± 0.56	9.99 ± 0.24	0.182	10.68 ± 0.50	9.81 ± 0.32	0.145	0.992	0.958
Hb (g/l)	99.60 ± 14.82	103.50 ± 3.53	0.883	118.22 ± 5.41	107.11 ± 3.69	0.111	0.474	0.940
PCV (%)	33.29 ± 2.60	28.83 ± 1.11	0.168	31.93 ± 1.58	30.77 ± 1.15	0.544	0.997	0.852
MCV (fl)	29.90 ± 1.02	28.77 ± 0.68	0.378	29.95 ± 0.81	31.33 ± 0.54	0.251	0.928	0.167
MCH (pg)	9.70 ± 1.44	10.27 ± 0.19	0.770	11.06 ± 0.15	10.86 ± 0.11	0.382	0.784	**0.028**
MCHC (g/l)	326.80 ± 49.22	359.40 ± 4.35	0.519	371.22 ± 7.26	349.11 ± 6.93	0.065	0.653	0.954
RDW (%)	16.18 ± 0.37	16.46 ± 0.36	0.622	15.92 ± 0.22	16.53 ± 0.58	0.172	0.942	0.948
WBC (10^9/l)	7.23 ± 0.78	8.93 ± 0.59	0.088	8.83 ± 0.75	8.97 ± 0.83	0.912	0.790	1.000
Ne (10^9/l)	1.65 ± 0.17	2.63 ± 0.40	**0.005**	1.48 ± 0.31	2.61 ± 0.36	0.054	0.857	0.998
Eo (10^9/l)	0.92 ± 0.12	0.81 ± 0.20	0.635	0.88 ± 0.19	0.47 ± 0.07	0.152	1.000	0.840
Ba (10^9/l)	0.17 ± 0.04	0.13 ± 0.02	0.533	0.30 ± 0.08	0.13 ± 0.01	0.075	0.379	0.994
Mo (10^9/l)	0.42 ± 0.08	0.45 ± 0.07	0.831	0.59 ± 0.12	0.44 ± 0.08	0.362	0.977	1.000
Ly (10^9/l)	4.22 ± 0.47	4.76 ± 0.26	0.385	5.57 ± 0.53	5.24 ± 0.66	0.747	0.512	0.943
Ne (%)	22.75 ± 1.81	28.80 ± 2.96	0.094	16.42 ± 3.00	29.07 ± 3.58	**0.023**	0.114	0.999
Eo (%)	12.19 ± 1.11	8.98 ± 1.99	0.173	10.24 ± 2.11	5.56 ± 1.01	0.359	0.955	0.922
Ba (%)	2.15 ± 0.41	1.47 ± 0.23	0.247	3.37 ± 0.73	1.46 ± 0.14	**0.028**	0.260	0.991
Mo (%)	5.64 ± 0.97	5.00 ± 0.77	0.595	6.18 ± 0.98	4.94 ± 0.97	0.418	1.000	0.1000
Ly (%)	55.88 ± 2.45	54.19 ± 2.90	0.591	63.38 ± 3.28	58.00 ± 4.19	0.405	0.198	0.960
PLT (10^9/l)	347.80 ± 40.40	620.60 ± 63.8	**0.005**	410.66 ± 58.71	480.00 ± 47.53	0.180	1.000	0.243
MPV (fl)	16.03 ± 1.28	8.92 ± 0.79	**0.003**	14.63 ± 1.50	8.93 ± 0.76	**0.004**	0.936	0.999

Statistical significance: $P < 0.05$, S – summer, W – winter, P – P value for comparisons within the area between the summer and winter periods, P_{bl} - P value for comparisons between areas within the season - summer and winter periods

Table 4 Seasonal changes in blood biochemical parameters within and between the Nevesinje and Podvelezje areas in the Pramenka sheep breed

Hercegovacka strain								
	Nevesinje (n=24)		P	Podvelezje (n=23)		P	P_{bl}	
	S (n=12)	W (n=12)		S (n=12)	W (n=12)		S	W
TP (g/l)	86.21 ± 1.89	70.63 ± 1.68	**0.001**	76.83 ± 2.32	67.61 ± 1.73	**0.005**	**0.010**	0.536
ALB (g/l)	34.02 ± 0.55	31.65 ± 0.78	**0.033**	33.43 ± 0.84	29.24 ± 0.84	**0.006**	0.966	0.223
GLO (g/l)	52.16 ± 2.19	38.98 ± 1.7^{A}	**0.001**	46.33 ± 3.63	38.34 ± 1.76	**0.037**	0.307	0.992
TB (μmol/l)	2.41 ± 0.46	1.71 ± 0.33	0.199	2.36 ± 0.50	1.57 ± 0.24	0.223	0.996	0.988
TC (mmol/l)	2.41 ± 0.14	2.10 ± 0.15	0.058	2.30 ± 0.16	2.06 ± 0.09	0.218	0.945	0.998
GLU (mmol/l)	2.99 ± 0.13	2.83 ± 0.15	0.336	2.76 ± 0.09	3.20 ± 0.16	0.113	0.362	0.212
UREA (mmol/l)	6.75 ± 0.19	3.28 ± 0.29	**0.001**	3.18 ± 0.15	5.05 ± 0.29	**0.003**	**0.000**	0.100
CRE (μmol/l)	36.60 ± 2.27	54.27 ± 4.27	**0.001**	37.38 ± 1.66	46.88 ± 2.06	**0.005**	0.989	0.406
AST (U/l)	164.89 ± 11.08	142.59 ± 11.99	0.149	128.22 ± 3.82	140.80 ± 7.77	0.123	**0.009**	0.999
GGT (U/l)	30.14 ± 7.70	41.08 ± 5.35	0.348	32.21 ± 4.63	36.85 ± 4.40	0.482	0.993	0.918
CK (U/l)	209.43 ± 23.06	198.22 ± 25.39	0.705	280.15 ± 41.29	546.80 ± 129.5	0.095	0.646	**0.004**
NEFA (mmol/l)	0.71 ± 0.19	0.44 ± 0.06	0.209	1.33 ± 0.27	0.57 ± 0.16	**0.046**	0.467	0.813
BHB (mmol/l)	0.33 ± 0.03	0.44 ± 0.08	0.144	0.82 ± 0.08	0.43 ± 0.06	0.001	0.112	0.997
Ca (mmol/l)	2.60 ± 0.03	2.46 ± 0.03	**0.040**	2.44 ± 0.05	2.42 ± 0.04	0.769	0.103	0.931
P (mmol/l)	1.68 ± 0.08	1.43 ± 0.12	0.102	1.52 ± 0.07	1.91 ± 0.19	0.080	0.706	0.182
Na (mmol/l)	141.44 ± 1.98	144.46 ± 1.01	0.196	145.19 ± 1.79	147.21 ± 1.31	0.317	0.531	0.284
Cl (mmol/l)	104.55 ± 1.03	108.00 ± 0.93	**0.040**	106.01 ± 1.03	107.49 ± 1.21	0.329	0.802	0.988
Mg (mmol/l)	0.98 ± 0.08	1.08 ± 0.03	0.285	1.06 ± 0.09	1.09 ± 0.04	0.728	0.905	0.998

Statistical significance: $P < 0.05$, S – summer, W – winter; P – P value for comparisons within the area between the summer and winter periods; P_{bl} - P value for comparisons between areas within the season - summer and winter periods

Table 5 Seasonal changes in blood biochemical parameters within and between the Vlasic and Kupres area in the Pramenka sheep breed

Dubska strain								
	Vlasic (n=24)		**P**	**Kupres (n=23)**		**P**	**P_{bl}**	
	S (n=12)	W (n=12)		S (n=12)	W (n=12)		S	W
TP (g/l)	78.01 ± 1.88	65.27 ± 1.26	**0.008**	81.08 ± 1.95	64.90 ± 1.57	**0.002**	0.703	0.906
ALB (g/l)	34.65 ± 0.93	26.90 ± 1.16	**0.001**	36.43 ± 1.20	28.00 ± 0.88	**0.006**	0.545	0.921
GLO (g/l)	43.36 ± 1.57	38.37 ± 1.54	**0.028**	44.68 ± 1.14	36.90 ± 1.57	**0.003**	0.981	0.992
TB (µmol/l)	2.21 ± 0.66	2.40 ± 0.33	0.534	3.83 ± 0.62	2.48 ± 0.32	**0.031**	0.311	0.991
TC (mmol/l)	2.04 ± 0.19	2.19 ± 0.16	0.527	2.34 ± 0.08	2.19 ± 0.16	0.454	0.997	1.000
GLU (mmol/l)	3.12 ± 0.10	3.18 ± 0.12	0.672	3.00 ± 0.08	3.00 ± 0.08	0.999	0.846	0.538
UREA (mmol/l)	4.99 ± 0.62	1.45 ± 0.28	**0.003**	3.40 ± 0.19	1.74 ± 0.13	**0.001**	**0.006**	0.666
CRE (µmol/l)	46.18 ± 1.42	58.58 ± 3.64	**0.014**	33.30 ± 1.58	57.16 ± 3.43	**0.001**	**0.000**	1.000
AST (U/l)	124 ± 62 ± 4.07	129.76 ± 8.61	0.643	167.76 ± 9.05	129.90 ± 5.21	**0.004**	**0.005**	1.000
GGT (U/l)	32.42 ± 6.00	64.91 ± 6.35	**0.003**	16.08 ± 2.69	41.79 ± 2.99	**0.001**	0.194	**0.002**
CK (U/l)	286.16 ± 33.14	205.15 ± 20.08	0.054	350.22 ± 62.79	161.61 ± 23.65	**0.021**	0.742	0.988
NEFA (mmol/l)	0.84 ± 0.22	0.26 ± 0.04	**0.033**	1.66 ± 0.44	0.36 ± 0.09	**0.015**	0.255	0.842
BHB (mmol/l)	0.39 ± 0.03	0.31 ± 0.03	0.052	0.56 ± 0.04	0.38 ± 0.03	**0.015**	0.139	0.955
Ca (mmol/l)	2.44 ± 0.05	2.36 ± 0.07	0.423	2.61 ± 0.06	2.37 ± 0.05	**0.013**	0.096	0.888
P (mmol/l)	1.93 ± 0.14	1.81 ± 0.14	0.496	1.35 ± 0.11	1.50 ± 0.06	0.258	**0.002**	0.128
Na (mmol/l)	142.79±1.93	147.57± 1.05	0.055	149.26 ± 2.19	147.57 ± 1.03	0.576	0.133	1.000
Cl (mmol/l)	105.48±1.56	109.51 ± 0.78	0.094	112.69 ± 1.08	110.47 ± 1.53	0.302	**0.001**	0.962
Mg (mmol/l)	0.93 ± 0.06	1.06 ± 0.03	**0.008**	0.93 ± 0.11	1.16 ± 0.03	0.058	1.000	0.524

Statistical significance: $P < 0.05$, S – summer, W – winter; P – P value for comparisons within the area between the summer and winter periods; P_{bl} - P value for comparisons between areas within the season - summer and winter periods

DISCUSSION AND CONCLUSION

Heat stress exerts significant effects on hematological parameters in sheep, reflecting physiological adaptations and potential challenges to health and productivity (Marai et al., 2007). Elevated Hb, MCH, and MCHC may be due to a compensatory mechanism to enhance oxygen delivery to tissues during heat stress. Similarly, Njidda et al. (2014) and Sejian et al. (2013) also observed higher Hb concentrations in heat-stressed sheep. Sejian et al. (2013) also observed a significant increase in the Hb and PCV% values due to short-term heat stress in Malpura sheep. Alam et al. (2011) reported a significant increase in the RBC, PCV%, Hb%, total leukocyte count (TLC) and differential leukocyte count (DLC) in the other ruminant species, i.e., goats. On the other hand, several reports indicated a reduction in the number of RBCs and, thus, PCV and Hb values during heat stress values (Temizel et al., 2009; Sivakumar et al., 2010; Kumar et al., 2011). These variations in the results might be due to the differences in the design of the experiments, long-term (study under natural environment) versus short-term exposure of the animals (either in a climatic chamber for 6 h duration carried out by Sejian et al. (2013), or short 6–8 h exposure by Alam et al. (2011) to heat stress). The higher levels of Hb during summer could be due to hemoconcentration resulting from the loss of water through panting and increased respiratory rate to dissipate heat from the body in hot and humid climates (Reddy et al., 2018). Habibu et al. (2018) stated that increased values of MCHC could be indicators of poor dynamics of body fluids in conditions of heat stress. Karthik et al. (2021) in their study showed that there were no differences in MCV, MCH and MCHC during heat stress compared to thermoneutral conditions in sheep in extensive housing. Examining the erythrocyte parameters, our results suggest that heat stress causes more alterations on a red bloodline than cold stress since no statistically significant changes were observed in the breeding areas where cold stress was observed.

In our study, we observed elevations in Ba and Eo levels in the Hercegovacka and Dubska Pramenka sheep strains during the summer period. It has been reported that Eo and Ba are responsive immune cells to heat stress, but they show differential responses (Park et al., 2021). Consistently, several studies have reported that animals have increased Ba counts following exposure to heat stress (Mitchell et al., 1992; Altan et al., 2000). As a part of type 2 immunity, the Ba counts in blood and mesenteric lymph nodes also may be expanded during parasite infection (Roland et al., 2014, Reitz et al., 2018). In addition, eosinophilia is a reliable diagnostic clue for helminthic and bacterial infections (Ramirez et al., 2018). Based on cellular and biological aspects of Ba and Eo, there is a higher chance that several parasitic infections could occur in a stressful environment. Therefore, we could not rule out the possibility that heat-stressed sheep in Nevesinje and Podvelezje areas suffered parasitic infections during high-temperature conditions in this study. However, our results should be interpreted with caution, given that the significant differences in Eo values found may be the result of allergic reactions in the examined sheep herds. Namely, there are differences in geological composition of the land in the Nevesinje area (Figure 2) compared to the Podvelezje area, i.e., a substantially larger share of alpine forests and a mosaic of agricultural areas. The presence of these geological determinants could also mean the presence of an enormous number of allergens with which the tested animals could come into contact in the summer period, and this could potentially cause elevated values of Eo. Therefore, it seems that the increased values of Eo and Ba during the summer season are not specific indicators of heat stress, and may be the result of different adaptation abilities among breeds, but also the presence of a greater number of parasites and allergens in the summer months.

Heat stress affects both erythrocyte and leukocyte parameters, while cold stress mainly impacts leukocytes (Banerjee et al., 2014). Leukocyte counts may vary during thermal stress, either increasing or decreasing, depending on factors such as species, exposure duration, and adaptation level (Habibu et al., 2018). The results of our study indicate the elevated levels of Ne during the winter season in the areas of Vlasic and Kupres, which confirms the findings of Banerjee et al. (2014), since extremely strong cold stress was established in these areas. While our findings hint at a connection between leukocyte fractions and thermal stress, it is crucial to acknowledge that these changes may not solely stem from the thermal stressors. Nonetheless, the elevated THI values during winter (Table 1) necessitate further exploration and discussion regarding their potential impact on leukocyte dynamics.

Thrombocytes, recognized for their role in clot formation and aggregation, serve as essential mediators of both innate and adaptive immunity (Ferdous et al., 2015; Habibu et al., 2018). In our study, PLT values exhibited a significant elevation during winter compared to summer in the Vlasic area. Correspondingly, Casella et al. (2013) demonstrated that lactating Bruna cows displayed diminished PLT and plateletcrit (PCT) values during periods characterized by elevated THI. Moreover, reduced PLT counts have been documented in cattle subjected to heat stress conditions (Mazzullo et al., 2014), although Wojtas et al. (2014) did not observe significant alterations in sheep. Consistently, several studies have reported decreased PLT counts in cattle with rising environmental temperatures and/or during hot seasons (Casella et al., 2013; Giri et al., 2017). Thrombocytopenia observed during severe weather conditions has been correlated with hemodilution, thrombopoietic suppression, increased platelet aggregation, and augmented consumption and destruction of PLT (Roland et al., 2014; Habibu et al., 2018). Consistent with the findings of Velayudhan et al. (2022), thrombocytic indices, such as high MPV and platelet distribution width (PDW), alongside low PLT and PCT during periods of high THI and/or summer, suggest a megakaryocytic response characterized by accelerated production and release of larger PLT into circulation (Kocatürk et al., 2010; Habibu et al., 2018). These mechanisms may be interpreted as adaptive responses to heat stress exhibited by the investigated crossbred dairy cows.

During thermal stress, animals undergo complex biochemical and metabolic responses to maintain internal stability and cope with the physiological challenges imposed by high or low environmental temperatures (Sejian et al., 2018). In our research, higher values of TP, ALB and GLO were noticed during the summer period compared to the winter period in all investigated areas, emphasizing that TP was higher in the Nevesinje area compared to Podvelezje. This may be the result of increased relative humidity in the area of Nevesinje compared to Podvelezje, since high ambient relative humidity makes thermolysis much more difficult and indirectly leads to dehydration through excessive sweating, loss of electrolytes and difficulty breathing. This occurs due to the negative effects of high humidity on the dissipation of body heat because of the decline in the effectiveness of radiation, conduction and convection and the effectiveness of evaporative cooling (Aggarwal et al., 2013).

AST values exceeded reference levels during both seasons, with significantly higher values at the Kupres and Nevesinje area during the summer season compared to winter. Hrkovic-Porobija et al. (2017) observed significant variations in AST concentration between tissues and blood serum. They noted that when tissues abundant in AST undergo lesions, the enzyme was discharged into circulation, leading to increased AST activity in blood serum. Based on ours and others results, it seems that elevated serum AST values may indicate low heat tolerance in combination with the nutritional status, housing conditions and physiological state of the animal. Among other liver enzymes, elevated levels of CK during the summer period compared to winter in the area of Kupres were found. Chulayo et al. (2014) stated that increased CK activity in sheep might have been caused by physical stress during the summer period. However, our results should be interpreted with caution, given that the significant differences in CK values found may be the result of some individual factors such as age, growth, reproduction, animal breeding system and diet.

Thermal stress can also have effects on electrolyte concentrations. Nazifi et al. (2003) found higher values of Mg in conditions of cold stress compared to heat stress, which is also our finding, since significantly higher values were found in the Vlasic area during winter compared to summer, where the Dubska Pramenka strain was exposed to severe cold stress. According to Hamzaoui et al. (2013), usual reaction to heat stress is a decrease in Na, Ca and P levels and an increase in Cl concentration. However, elevated values of Ca during summer compared to winter in the Nevesinje and Kupres areas were found. The level of minerals in the blood depends on the diet, which indicates that the calcemia may be directly correlated with the category and diet of sheep (Hrkovic et al., 2009).

The findings of this study demonstrated notable variations in the blood parameters of Pramenka sheep exposed to thermal stress, underscoring the significant impact of breeding area characteristics, including geological composition, spatial distribution, and land cover. Key results indicated that while some variations in hematological and biochemical parameters were observed, none of the measured values exceeded established physiological reference ranges. This suggests a remarkable adaptive capacity of the studied Pramenka strains to cope with thermal stress, likely rooted in their long-term evolutionary adaptation to local environmental conditions. These results contribute a valuable insight into the resilience of indigenous breeds and reinforce the importance of preserving such animals within their native habitats, where they have naturally developed tolerance to climatic extremes. Practical applications of this research include breeding and management strategies toward the conservation and sustainable utilization of local genetic resources, particularly in the context of climate change. The study supports the promotion of location-specific livestock breeding practices that leverage the inherent adaptability of native breeds. However, our study was confined to a specific region and a limited number of animals, which may restrict the generalizability of the findings. Future research should expand to include larger populations across diverse geographic areas and incorporate genetic and molecular analyses to further elucidate the mechanisms underlying thermal resilience in indigenous sheep breeds.

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CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTIONS

Conception: HO, AS, VB, AH; Design: HO, AS, VB, LŠ; Supervision: LŠ, AH; Fundings: HO, VB, AH; Materials: HO, KB, VB, IA, MO, LŠ, AH; Data Collection and/or Processing: HO, AS, KB, VB, IA, MO; Analysis and/or Interpretation of the Data: HO, AS, KB, IA, MO, LŠ, AH; Literature Review: AS, IA; Writing: HO, AS, AH; Critical Review: HO, VB, IA, LŠ, AH.

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EVALUACIJA UČINKA SEZONE I PODRUČJA UZGOJA NA KRVNE PARAMETRE PRAMENKE IZLOŽENE UVJETIMA TERMALNOG STRESA

SAŽETAK

Cilj istraživanja je procjena sezonskih varijacija krvnih parametara kod dva soja ovce pramenke, dubskog i hercegovačkog, na četiri područja Bosne i Hercegovine pod uvjetima termalnog stresa. Istraživanje je uključilo 48 ovaca s Vlašića i Kupresa (područja uzgoja dubskog soja) i Nevesinja i Podveležja (područja uzgoja hercegovačkog soja). Istraživanje je provedeno u ljetnoj i zimskoj sezoni, a obuhvatilo je ukupno osam stada (po dva na svakom području). Hematološki parametri su uključili broj eritrocita, leukocita i trombocita, srednji volumen trombocita, ukupni broj granulocitnih i agranulocitnih leukocita, hemoglobin, širinu distribucije eritrocita i parametre eritrocita. Biohemijske analize su obuhvatile bilirubin, ukupni holesterol, glukozu, ureu, kreatinin, kreatin kinazu, neesterificirane masne kiseline, beta-hidroksibutirat, proteine, jetrene enzime i elektrolite. Rezultati su pokazali signifikantno više vrijednosti eritrocitnih parametara u ljetnoj sezoni za područje Nevesinja ($P<0.05$) u usporedbi s Podveležjem. Važno je naglasiti signifikantno povišenu vrijednost bazofila u ljetnoj sezoni za sva područja, osim Vlašića, u usporedbi sa zimskom sezonom. Nadalje, vrijednosti ukupnih proteina, albumina i globulina su bile signifikantno povišene u svim područjima u ljetnoj u usporedbi sa zimskom sezonom. Rezultati istraživanja naglašavaju znatan utjecaj koji područje uzgoja u smislu geološkog sastava, prostorne distribucije i pokrivača imaju na specifične krvne parametre ispitivane kod ovaca u periodima karakteriziranim nepovoljnim temperaturnim uvjetima.

Ključne riječi: Krvni parametri, ovca, pramenka, sezona, termalni stres