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The effect of age and sex on selected haematological and biochemical parameters in Dalmatian Pramenka lambs

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ABSTRACT

The objective of the present study was to determine the influence of age and sex on selected haematological and blood biochemical parameters in organically raised Dalmatian Pramenka lambs. Haematological and blood biochemical parameters were studied in 40 lambs with an equal representation of both sexes. The research lasted four months. Blood samples were collected once per month, from the beginning of the grazing season when lambs were at the average age of three months, and until the end of the production cycle for lamb meat. The distribution of the results according to age showed that the increase in the age of the lambs significantly reduced ($P<0.05$) the RBC, HGB, HCT and the concentration of GLU, BUN, BIT. The concentration of TP, ALB, GLO and the activity of enzyme AST significantly increased ($P<0.05$) with the increasing age of the lambs. The distribution of results by sex showed that female lambs had significantly higher WBC ($P<0.05$), RBC ($P<0.01$), and HGB ($P<0.05$) concentration, while male lambs had significantly higher values of MCV ($P<0.01$) and concentration of FFA ($P<0.01$). In conclusion, this research demonstrated that age and sex significantly influence most of the haematological and blood biochemical parameters of organically raised Dalmatian Pramenka lambs.

Key words: haematology; serum biochemistry; age; sex; Dalmatian Pramenka lambs

Introduction

Haematological and blood biochemical parameters in the blood are a source of objective and useful information indispensable in assessing the physiological, metabolic, health and nutritional status, and productivity of animals. In combination with the findings of clinical examination they are particularly useful in differential diagnosis or determining disease prognosis, as well as in preventive programmes (BRAUN et al., 2010; POLIZOPOULOU, 2010). However, the

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interpretation may be a challenge in some cases, because it can be significantly influenced by a great number of internal and external factors. To our knowledge, the values of haematological and serum biochemical parameters may be influenced by breed, sex, age, reproductive status, exercise, feeding, circadian variations, the handling procedure of the animals during blood withdrawal, geographical location, degree of excitement and health condition (BÓRNEZ et al., 2009; BRAUN et al., 2010; PICCIONE et al., 2008; ŠIMPRAGA et al., 2013). As variation in blood parameters may occur due to several factors, determination of the effect of age and sex on haematological and serum biochemical parameters is of value when establishing reference values in animals, and for appropriate interpretation of haematological and serum biochemical results.

Sheep production in Croatia has a long tradition. The Dalmatian Pramenka is the most numerous Croatian indigenous sheep breed, with an estimated 73,000 animals (CAA, 2021). These sheep are mostly farmed under traditional, extensive production systems, based on natural pastures with minimum investment in facilities, equipment and nutrition, and they are well adapted to the local environmental conditions and to seasonal variations in food sources. The Dalmatian Pramenka is a sheep breed of combined (milk/wool/meat) properties. However, its main purpose is lamb meat production – lamb's carcasses for spit roasting (VNUČEC et al., 2014).

In spite of the significant number of studies performed on Croatian indigenous sheep breeds in the last few years, published data on the haematology and blood biochemistry of this particular breed are scarce (ANTUNOVIĆ et al., 2010; VOJTA et al., 2011).

Since there are no published data describing the influence of age and sex on the haematological and biochemical parameters of Dalmatian pramenka, the aim of the present study was to investigate the differences in haematological and blood biochemical parameters related to age and sex, in organically raised lambs of the Dalmatian Pramenka breed.

Materials and methods

Animals. The study was performed on a family sheep farm using organic and extensive farming practices located in Radučić (44°02'N latitude, 16°02'E longitude, approximately 278 m asl), 15 km west of Knin, in the hinterland of Dalmatia, the Republic of Croatia. The analysis of haematological and blood biochemical parameters included 40 (forty) organically raised lambs of the indigenous Croatian breed - Dalmatian Pramenka sheep (Croatian: Dalmatinska pramenka), with an equal representation of both sexes (20 male and 20 female lambs), over the period of four months, between March and June. The evaluation period started at the beginning of the grazing season when the lambs were at an average age of three months (lambs born during December and January), and lasted until the end of the lamb meat production cycle. All animals in the herd were registered with the Croatian Agricultural Agency (CAA). The selection of animals was preceded by the collection of complete anamnestic data on the herd, and a clinical examination by means of an assessment of the animals' temperature, pulse, and respiration rates, performed by a licensed veterinarian. All the selected animals, with equal representation of both sexes, were found to be healthy and at the average age of three months. Lambs were kept under the same management conditions – extensively, with their dams, on natural pasture during the day and at night they were housed in a covered shed. Lambs were fed by free grazing on natural pastures, and watered *ad libitum*. No supplementation was given during the study period. Meteorological data for the farm's location were obtained from the Croatian Meteorological and Hydrological Institute (DHMZ), who monitored the air temperature (°C), relative humidity (%) and airflow rate (m/s) for the period during which the samples were taken. The mean daily temperature in March was 8.6 °C, in April 12.4 °C, 17.9 °C in May and 21.1 °C in June. The area has a mean daily relative humidity of 69% in March, 67% in April, 61% May and 70% in June. The mean daily wind speed was in March of 3.7 m/s, April 3.5 m/s, May 2.3 m/s and June of 2.1 m/s. All procedures used in this research

were in in strict accordance with the European and National Law for the Care and Use of Animals, and under the supervision of the authorised institutional Ethics Committee for Animal Experimentation, Faculty of Veterinary Medicine University of Zagreb, Croatia. Animal care and the conditions of the research followed the recommendation of European Union directive 2010/63/EU (European Commission, 2010). Every effort was made to minimise discomfort and suffering throughout the duration of the study. No sedation or anaesthesia was applied to the animals.

Sampling and measurement. Blood samples for haematological and biochemical analyses were collected from the same animals monthly (March, April, May and June), four times at intervals of a month. From each animal, blood samples were taken by venepuncture of the jugular vein, in the morning between 9.00 a.m. and at 12.00, without prior fasting. For haematological analysis, the blood was sampled into sterile Vacutainer systems containing disodium salt ethylene diamine tetraacetic acid (EDTA) as an anticoagulant. After sample collection, the tubes were inverted several times to ensure adequate anticoagulant mixing, cooled at 4 °C in a portable refrigerator, and analysed within 24 hours from collection. The tests were performed in the Laboratory of Physiology and Radiobiology, Faculty of Veterinary Medicine, University of Zagreb. Haematological indicators were determined using the haematological automatic counter Beckman Coulter ACT Diff Hematology Blood Analyzer (Beckman-Coulter, USA) running veterinary software customized for sheep. The parameters analysed included: total white cell blood count (WBC), total red cell blood count (RBC), haemoglobin (HGB), haematocrit (HCT), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC) and platelet count (PLT). Blood samples for biochemical analysis were sampled into sterile Vacutainer systems with SST II gel, centrifuged immediately and frozen at -20 °C until processing. Concentrations of serum glucose (GLU), free fatty acids (FFA), total cholesterol (CHO), and creatinine (CRE) were measured by commercial kits (Randox, Ireland) using a SABA 18 (AMS, Rome, Italy) biochemical autoanalyser, according to the manufacturer's protocol. Concentrations of total protein (TP), albumin (ALB), total bilirubin (BIT), blood urea nitrogen (BUN), enzymes aspartate aminotransferase (AST) and gamma-glutamyltransferase (GGT) in blood plasma were determined by commercial kits (Diagnostics Ltd., Sisak, Croatia). The globulin (GLO) values were calculated on the basis of the difference between the concentrations of total proteins and albumin.

Statistical analysis. Statistical analysis was performed using the SAS 9.3 software (version 2002–2010 SAS Institute Inc., Cary, NC, USA). The PROC UNIVARIATE statement was used to assess the normality of data distribution of each analysis variable. Levene's median test was performed to test for homogeneity of variance, using the GLM module (PROC GLM) and HOVTEST options. The robust regression procedure (PROC ROBUSTREG) was used to detect outliers and provide resistant (stable) results in the presence of outliers. Trimming and Winsorization (PROC UNIVARIATE) were used for reducing the effects of extreme values in the sample (TRIMMED and WINSORIZED means). The generalized linear mixed model (GLIMMIX) was used for estimation of haematological and blood biochemical parameters. The statistical model included the fixed effects of age and sex. The model included the random effect of animals on repeated measurements over time (RANDOM statement) by using the "residual" and "CS" type structure variance-covariance (compound-symmetry). Results are presented through the 95%-confidence interval (95% CI) with the generally accepted level of statistical significance of $P<0.05$.

Results

Effect of Age and Sex on Haematological Values. The results of analysing age and sex on haematological values are presented in Table 2. Age was an important source of variation for RBC, HGB, HCT and MCV, MCH, MCHC, and PLT count (except the WBC count). The increase in the age of the lambs was followed by a significant

reduction (P<0.05) in RBC, HGB and HCT values. The PLT count and values of MCV, MCH and MCHC varied significantly (P<0.05) with age and showed trend variable values. Significant effects of *sex* were shown for total WBC, RBC, HGB and MCV. The distribution of results by sex showed that female lambs had significantly higher values of WBC (P<0.05), RBC (P<0.01) and HGB (P<0.05) than males, while male lambs had significantly higher values (P<0.01) of MCV than females.

Effect of Age and Sex on Blood Biochemical Values. The results of analysing age and sex on blood biochemical values are presented in Table 4. Significant effects of *age* were shown for TP, ALB, GLO, BUN, CRE, BIT, GLU, FFA, AST and GGT (except the CHO concentration). The increase in the age of lambs was followed by a significant reduction (P < 0.05) in the values of BUN, BIT and GLU, and a significant increase (P < 0.05) in the concentration of TP, ALB, GLO, and the activity of the enzyme AST. Concentrations of CRE and FFA, and the activity of the enzyme GGT significantly varied (P < 0.05) with age and showed trend variable values.

The distribution of results by *sex* showed that male lambs had significantly higher (P<0.01) concentrations of FFA, compared to female lambs.

Discussion

Haematological parameters. In general**,** the values of all the investigated haematological parameters in the Dalmatian Pramenka lambs in the present study were within the reference intervals for the ovine species (KRAMER, 2000; PUGH, 2002; ŠIMPRAGA, 2013), and compare favourably to previously published results by various authors for Croatian native breeds of sheep (ANTUNOVIĆ et al., 2014; SHEK VUGROVEČKI, 2015; ŠIMPRAGA et al., 2013), with the exception of the WBC count (Table 1). The average WBC count was found to be notably higher compared to the reference intervals for sheep available in textbooks (KRAMER, 2000; PUGH, 2002) and study results for some Croatian native breeds of sheep obtained by ANTUNOVIĆ et al. (2014) in pregnant ewes at an average age of three years, SHEK VUGROVEČKI (2015) in female sheep aged between 2-7 years and ŠIMPRAGA et al. (2013) in adult sheep. However, the WBC count presented values similar to those observed by VOJTA et al. (2011), who reported a consistent shift towards higher values in the reference range of WBC in Dalmatian Pramenka sheep. Probably, this higher WBC count in clinically healthy lambs might be a physiological variation considered to be a breed feature of Dalmatian Pramenka sheep. With regard to possible explanations for the higher WBC count, various environmental factors, such as climate or geographic location (ŠIMPRAGA et al., 2013), especially altitude (SOCH et al., 2010) or management practices, organic breeding (SOCH et al., 2010), extensive breeding (OLAYEMI et al., 2009), could also be responsible. On the other hand, haematological parameters according to ŠIMPRAGA et al. (2013) should be maintained within a narrow or at least a well-defined range, since any deviation from the normal range is likely to be caused by an underlying pathology. The interpretation of the results of WBC counts obtained in the present study, and the possible existence of leukocytosis, depends on the selection of the reference interval with which the values of the present study are compared. Further, discrepancies between the present results and published results were smaller, when comparing the present results with those of other Croatian indigenous sheep breeds (Table 1). Also, the interpretation of the results is hampered by the fact that only the results of the total number of leukocytes are available, but not the relative differential leucocyte counts.

Table 1: General haematological parameters in Dalmatian Pramenka lambs and reported reference intervals for sheep

Parameter (unit)	Results (mean ± SD)	Reference intervals						
		Sheep (relevant textbooks)		Croatian native breeds of sheep				
		PUGH (2002)	KRAMER (2000)	ANTUNOVIĆ et al. (2014)	VOJTA et al. (2011)	ŠIMPRAGA et al. (2013)	SHEK VUGROVEČKI (2015)	SHEK VUGROVEČKI (2015)
				Dubrovnik ruda sheep	Dalmatian pramenka sheep	Dalmatian pramenka sheep	Cres sheep	Lika pramenka sheep
WBC (10^9/L)	21.03 ± 9.01	4.0-12.0	4.0-8.0	8.11 ± 1.88	to 36.0	3.00-16.00	4.6-16.6	5.6-17.0
RBC (10^{12}/L)	11.12 ± 1.77	9-15	9-15	-	7-13	7.81-12.77	7.2 -11.4	6.62-9.92
HGB (g/L)	112.61 ± 13.27	90-150	90-150	-	77-127	78.1-134.7	78.3-116.4	74.55-104.7
HCT (L/L)	0.35 ± 0.04	0.27-0.45	0.27-0.45	-	0.237-0.397	0.24-0.41	0.24-0.37	0.22-0.31
MCV (fL)	32.06 ± 4.62	28-40	28-40	-	25-38	28.6-34.8	28.0-36.0	28.8-35.8
MCH (pg)	10.28 ± 1.11	8-12	8-12	-	8.4-11.7	9.54-11.0	9.2-11.8	9.9-11.9
MCHC (g/L)	322.20 ± 20.66	310-340	310-340	-	286-361	284-362	285-362	320-353
PLT (10^9/L)	600.85 ± 194.09	205-705	800-1100	-	70-978	0-731	23-646	75-807

WBC - total white cell blood count; RBC - total red cell blood count; HGB - haemoglobin; HCT - haematocrit; MCV - mean cell volume; MCH - mean cell haemoglobin; MCHC - mean cell haemoglobin concentration; PLT - platelet count

The effect of age on haematological parameters. In this study, *age* was an important source of variation for RBC, HGB concentration, HCT and MCV, MCH, MCHC values and PLT count (Table 2). The HGB blood content and RBC count decreased significantly with the age of the lambs, HCT value decreased on the last month of the study, and PLT count and the value of MCV, MCH and MCHC showed trend variable values. By contrast, a higher HGB content in older animals in comparison to younger was reported by ADDASS et al. (2010) in 4 year old or older sheep compared to younger sheep (aged 1.5 – 4 years) and BÓRNEZ et al. (2009) in 70 day- compared to 30 day-old lambs. The reason for the higher HCT values in younger lambs could be attributable to functional changes in haematopoiesis, or due to haemoconcentration caused by dehydration (KRAMER, 2000). Our results, the age related differences in MCHC, are consistent with the report by EGBE-NWIYI et al. (2000). From observation of the differences in PLT count, a significant decrease in PLTs was recorded exclusively in lambs at the average age of five months, and it cannot be explicitly correlated with the effect of age. Variations in PLT count have been reported by various authors. Increased PLTs in the blood with increasing age were observed by ANTUNOVIĆ et al. (2012) in lambs, and by MOHRI et al. (2007) in calves. Higher values of PLT in foals than in adult individuals were reported by MIKNIENĖ et al. (2014). A reduction in PLTs with ageing was described in mares (SATUE et al., 2009). Since MCHC is derived from the HGB concentration and HCT values, it is expected that any changes in HGB and erythrocyte values will result in changes in the MCHC value. Of all the haematological parameters, the WBC count was without significant age dependent changes (Table 2).

The effect of sex on haematological parameters. Among all the haematological parameters studied, *sex* was an important source of variation for total WBC, RBC, HGB and MCV values (Table 2). Significantly higher values of MCV were found in males in comparison to female lambs, while female lambs showed significantly higher WBC, RBC and HGB values in comparison to male lambs. This result

corresponds to results achieved by BORJESSON et al. (2000) and NJIDDA et al. (2014). On the other hand, higher HGB and RBC values in females were not found in most other studies, such as the findings of BADAWI and AL-HADITHY (2014) and EGBE-NWIYI et al. (2000). The differences in the RBC values may be explained by the effect of stress (splenic contraction), hormonal influences, hydration status, dietary differences, or adaptations to a harsh environment (BORJESSON et al., 2000).

Table 2: Mean value (95%-confidence interval) of haematological parameters in lambs of the Dalmatian Pramenka breed according to age and sex

Parameter (unit)	Mean value (95% CI)					
	Age				Sex	
	3 (n=40)	4 (n=40)	5 (n=40)	6 (n=40)	F (n=20)	M (n=20)
WBC (10^9/L)	18.39 (15.59-21.69)^a	19.94 (16.80-23.67)^a	19.10 (16.50-22.10)^a	17.47 (15.14-20.15)^a	21.63 (18.52-25.25)*	16.40 (13.94-19.29)*
RBC (10^{12}/L)	11.66 (11.01-12.26)^a	11.35 (10.67-12.00)^a	11.18 (10.61-11.72)^{ab}	10.49 (9.90-11.04)^b	11.75 (11.34-12.16)**	10.94 (10.44-11.42)**
HGB (g/L)	121.47 (117.80-125.03)^a	113.90 (109.83-117.83)^b	112.93 (109.41-116.35)^b	105.51 (101.96-108.94)^c	116.17 (113.22-119.05)*	110.96 (107.48-114.34)*
HCT (L/L)	0.37 (0.35-0.38)^a	0.35 (0.34-0.37)^a	0.36 (0.35-0.37)^a	0.34 (0.33-0.35)^b	0.36 (0.35-0.37)	0.35 (0.34-0.36)
MCV (fL)	31.54 (30.82-32.33)^a	31.04 (30.32-31.81)^{ac}	30.12 (29.51-30.77)^b	30.30 (29.68-30.98)^c	30.03 (29.34-30.77)**	31.50 (30.69-32.39)**
MCH (Pg)	10.45 (10.25-10.66)^a	10.06 (9.88-10.24)^b	9.68 (9.54-9.83)^a	9.76 (9.62-9.90)^c	9.90 (9.76-10.04)	10.05 (9.89-10.22)
MCHC (g/L)	330.47 (323.62-337.50)^a	332.48 (315.76-329.49)^b	313.09 (307.60-318.78)^c	311.92 (306.56-317.46)^c	320.20 (313.19-327.55)	318.37 (311.53-325.52)
PLT (10^9/L)	684.59 (618.90-750.29)^a	612.98 (539.97-685.99)^a	528.17 (470.32-586.03)^b	676.76 (620.26-733.26)^a	636.42 (580.56-692.28)	614.83 (553.58-676.08)

3, 4, 5, 6 - age in months; F - female gender, M - male gender; n - number of lambs; a, b, c - means within a column with different superscripts differ significantly by months ($P < 0.05$); *, ** - means within a column differ significantly ($P < 0.05$, $P < 0.01$) by sex; WBC - total white cell blood count; RBC - total red cell blood count; HGB - haemoglobin; HCT - haematocrit; MCV - mean cell volume; MCH - mean cell haemoglobin; MCHC - mean cell haemoglobin concentration; PLT - platelet count

Biochemical blood parameters. In general, the interpretation of the reported results of biochemical blood parameters obtained in the present study depended crucially on the selection of reference intervals with which they were compared (Table 3). Namely, the average serum values of ALB, BUN, BIT, GLU and GGT activity were higher, but the values of TP, CRE, GLO and CHO were approximately similar when compared with the reference intervals previously reported for sheep (Table 3). However, the major biochemical parameters in the present study were consistent with or approximately similar to those reported for Croatian native breeds of sheep (SHEK VUGROVEČKI, 2015; ŠIMPRAGA et al., 2013; VOJTA et al., 2011). The average FFA concentration and AST activity were higher than or consistent with the reference intervals reported for sheep (Table 3). Unfortunately, due to the scarcity of data related to FFA values in Croatian indigenous sheep breeds, and the lack of reference intervals, it was not feasible to compare the results of studies with confidence. Further, the often strong and readily detectable impact of external factors on blood biochemical parameters (ŠIMPRAGA et al., 2013) should not be dismissed.

The effect of age on blood biochemical parameters. Generally, *age* had an effect on all serum biochemical parameters evaluated in the present study, with the exception of the CHO value (Table 4). The serum concentration of the TP is used as an indicator of the animal's nutritive status and the synthetic capacity of the liver. The increasing TP concentrations with advanced age were consistent with the results obtained by ANTUNOVIĆ et al. (2004 and 2005), BICKHARDT et al. (1999) and

BORJESSON et al. (2000). The increase in TP (with GLU, CHO and FFA) concentrations during the period of intense growth can be explained by the anabolic effect of growth hormones and increased energy needs (EL-BARODY et al., 2002). The ALB values, that are considered a reliable indicator of the long-term protein status, or hydration status in conjunction with HCT, tended to increase after the average age of 5 months. Increased concentrations of plasma GLO may be related to the high protein content, while the lower serum concentrations of proteins in younger animals might be related to lower GLO concentrations and incomplete development of the immune system (KANEKO et al., 2008). Lower plasma ALB in younger animals may be explained by the greater demand for this protein during the intense growth phase.

Table 3: General biochemical parameters in Dalmatian Pramenka lambs and reference intervals for sheep reported in relevant textbooks

Parameter (unit)	Results (mean ± SD)	Reference intervals								
		Sheep					Croatian native breeds of sheep			
		AIKEN (2007)	PUGH (2002)	RADOSTITS et al. (2000)	ANNISON (1960)	KANEKO et al. (2008)	VOJTA et al. (2011) Dalmatian pramenka sheep	ŠIMPRAGA et al. (2013) Dalmatian pramenka sheep	SHEK VUGROVEČKI (2015)	
									Cres sheep	Lika pramenka sheep
TP (g/L)	72.19± 6.65	-	-	-	-	60-79	65-89	66.8-87.4	73-104	66.7-91.0
ALB (g/L)	37.13± 4.32	-	-	-	-	24-30	31-44	28.5-44.7	24-40	35.4-47.5
GLO (g/L)	35.00± 6.82	-	-	-	-	35-57	34-45	38.3-42.7	49-64	31.3-43.50
BUN (g/L)	8.23± 3.18	2.9-7.1	1.32-3.32	-	-	2.86-7.14	0.00-9.1	3.5-7.8	1.84 - 7.01	6.7-10.9
CRE (µmol/L)	101.76± 10.73	44-150	106.20-168.15	-	-	106-168	91-125	98-144	77.7-125.3	74.5-103.2
BIT (µmol/L)	11.27± 7.42	0-2	1.71-8.55	-	-	1.71-8.55	0.5-14	-	2.4-9.5	5.0-11.0
CHO (mmol/L)	2.13± 0.90	1.0-2.6	1.352-1.976	-	-	1.35-1.97	0.8-2.5	-	0.97-2.22	0.74-2.47
GLU (mmol/L)	5.07± 0.58	2.0-3.0	-	-	-	2.73-4.43	2.7-6.9	2.9-4.3	1.7-5.2	1.4-3.7
FFA (mmol/L)	0.36± 0.14	-	-	0.1-0.35	0.1-0.9	-	-	-	-	-
AST (U/L)	117.99 ± 31.64	32-97	60-280	-	-	60-280	79-174	66.2-129.3	52.3-120.1	110.7-241.5
GGT (U/L)	69.67± 13.30	0-32	20-52	-	-	20-52	31-87	31.7-71.7	39.21-98.0	14.3-80.0

TP - total protein; ALB - albumin; GLO - globulin; BUN - blood urea nitrogen; CRE - creatinine; BIT - total bilirubin; CHO - total cholesterol; GLU - glucose; FFA - free fatty acids; AST - aspartate aminotransferase; GGT- gamma-glutamyltransferase

Urea is used as an indicator of alimentary protein supply and/or protein utilization. The variations in BUN value may be explained by the different protein intake, endogenous protein catabolism, the state of hydration, hepatic urea synthesis, and renal urea excretion (KOHN et al., 2005). The significant decline in the blood BUN values in lambs at the average age of five and six months, can be attributed to poor nutritional protein status in animals undergoing malnutrition or undernutrition, or intense growth and increased protein anabolism. BORJESSON et al. (2000) and MADUREIRA et al. (2013) reported higher BUN values in sheep up to 12 months of age compared to

sheep older than 12 months. The CRE is considered useful in evaluating the protein status, kidney function and muscle damage; in conjunction with BUN and TP (DIAS et al., 2010). The quantity of CRE formed depends on the total body content of creatine, which in turn depends on dietary intake, rate of synthesis of creatine, and muscle mass, or it is related to hydration status (BORJESSON et al., 2000). The value of CRE levels throughout the study indicated a trend variable value. The decline in CRE levels in lambs at the average age of five months may be related to low protein intake. NJIDDA et al. (2014) and BICKHARDT et al. (1999) reported an increase in lamb CRE with increasing age. In the present study, the BIT value was observed to be higher than the normal range during the third month of the lamb's life, which was followed by a significant decrease in the fourth month, and relatively stable values until the end of the experimental period. Other researchers (BICKHARDT et al., 1999) also reported a decline in the BIT level with increasing age, or lower serum concentration in young animals compared to adults (GURGOZE and ICEN, 2010). The variation in BIT value may be due to decreased food intake, and increased values may indicate hepatobiliary diseases (DIAS et al., 2010). Of all the biochemical parameters considered in the present study, age related differences were not found only in the CHO levels (Table 4). This is in contrast with the reports by ANTUNOVIĆ et al. (2004), who observed lower serum concentrations in younger sheep and an increase in the CHO level with increasing age (ANTUNOVIĆ et al., 2005). The levels of GLU in association with FFA concentrations are considered valuable indicators of the energy status, where high levels of GLU in relation to FFA generally indicate a negative energy balance and, conversely, low levels indicate a positive balance (DIAS et al., 2010). The decline in the content of GLU with the increasing age of the lambs corroborates the findings of ANTUNOVIĆ et al. (2004 and 2005), but contradicts BICKHARDT et al. (1999) and NJIDDA et al. (2014). Interferences in GLU concentrations in the present study may be related to the greater nutritional demand during the lamb's intense growth phase and insufficient quality of feed supply, indicating a negative energy balance (BHAT et al., 2014). In response to this negative energy balance, the serum FFA concentration increases above the reference limit (SNOJ et al. 2014), reflecting body reserves mobilisation. The trend variable value in FFA concentrations throughout the study corroborates the results of PICCIONE et al. (2010) in goat serum. In sheep, AST is poorly specific to the liver and maybe used as a marker of liver damage only when other possible causes of variation have been eliminated (BRAUN et al., 2010). The steadily increasing trend of serum AST activity with the increasing age of the lamb corroborates the findings of AL-FARTOSI et al. (2010), but contradicts NJIDDA et al. (2014). The increasing serum AST activity may be attributed to increasing muscle mass during the intense growth phase; or, alternatively, to liver and skeletal muscle damage (KANEKO et al., 2008), but also with increasing physical activity (SICILIANO et al., 1995). The GGT activity in sheep is mainly used as a sensitive indicator for liver disease, indicating cholestasis and bile duct damage. The trend variable of GGT serum activity in the present study corroborated with the results of BICKHARDT et al. (1999) who observed a decrease in serum GGT activity with increasing age, and MADUREIRA et al. (2013) who reported higher GGT activity in the blood of the sheep in comparison with lambs.

The effect of sex on blood biochemical parameters. *Sex* dependent differences in blood biochemical parameters values were only found in FFA concentrations, with higher values in males in comparison to female lambs (Table 4). This observation of sex dependent differences in FFA concentrations contradicts CRUZ et al. (2017), who observed no sex-related differences. Namely, when comparing most published reports to the results of sex-associated changes in blood biochemical parameters in the present study, our results differed from the findings of sex-related differences in the serum values of TP, GLO, CRE (MADUREIRA et al., 2013) and CHO (AL-FARTOSI et al., 2010).

Table 4: Mean value (95%-confidence interval) of biochemical parameters in lambs of the Dalmatian Pramenka breed according to age and sex

Parameter (unit)	Mean value (95% CI)					
	Age				Sex	
	3 (n=40)	4 (n=40)	5 (n=40)	6 (n=40)	F (n=20)	M (n=20)
TP (g/L)	69.14 (66.87-71.42)^{a}	66.86 (64.47-69.25)^{a}	76.14 (74.19-78.09)^{b}	76.03 (74.17-77.89)^{b}	71.58 (70.09-73.08)	72.50 (70.73-74.28)
ALB (g/L)	35.71 (34.62-36.76)^{a}	36.09 (34.96-37.19)^{a}	40.20 (39.38-41.00)^{b}	39.27 (38.46-40.06)^{b}	37.86 (37.34-38.38)	37.87 (37.19-38.54)
GLO (g/L)	33.36 (31.41-35.42)^{a}	30.37 (28.51-32.36)^{b}	35.88 (34.07-37.78)^{a}	36.63 (34.86-38.48)^{a}	33.47 (32.23-34.75)	34.47 (32.94-36.08)
BUN (g/L)	10.43 (9.59-11.33)^{a}	11.22 (10.25-12.27)^{a}	7.17 (6.67-7.70)^{b}	3.93 (3.67-4.21)^{c}	7.48 (7.07-7.91)	7.55 (7.06-8.08)
CRE (µmol/L)	100.05 (96.66-103.34)^{a}	104.68 (101.26-107.99)^{b}	96.59 (93.58-99.52)^{a}	104.80 (102.16-107.39)^{b}	101.00 (98.94-103.02)	102.18 (99.69-104.60)
BIT (µmol/L)	16.08 (12.83-21.56)^{a}	7.63 (6.83-8.60)^{b}	7.88 (7.20-8.70)^{b}	7.30 (6.75-7.99)^{b}	9.17 (8.40-10.07)	8.39 (7.63-9.30)
CHO (mmol/L)	2.11 (1.91-2.37)^{a}	1.87(1.69-2.09)^{a}	1.95 (1.80-2.14)^{a}	2.00 (1.85-2.18)^{a}	2.00 (1.87-2.16)	1.96 (1.81-2.13)
GLU (mmol/L)	5.59 (5.39-5.78)^{a}	5.01 (4.78-5.23)^{bc}	5.15 (4.97-4.84)^{b}	4.90 (4.70-5.09)^{c}	5.17 (5.01-5.33)	5.17 (4.98-5.35)
FFA (mmol/L)	0.41 (0.35-0.48)^{a}	0.33 (0.28-0.39)^{ab}	0.38 (0.33-0.43)^{a}	0.27 (0.24-0.31)^{b}	0.31 (0.28-0.33)**	0.38 (0.34-0.43)**
AST (U/L)	98.91 (91.32-107.81)^{a}	108.81 (99.11-120.69)^{ab}	116.14 (107.20-126.71)^{b}	115.10 (106.68-124.97)^{b}	113.34 (106.63-120.96)	106.99(99.80-115.30)
GGT (U/L)	74.01 (68.80-79.62)^{a}	64.55 (59.77-69.71)^{b}	69.23 (64.91-73.84)^{ab}	66.31 (62.39-70.47)^{b}	67.16 (63.34-71.19)	69.74 (65.36-74.40)

3, 4, 5, 6 - age in months; F - female gender, M - male gender; n - number of lambs; a, b, c - means within a column with different superscripts differ significantly by months ($P < 0.05$); *, ** - means within a column differ significantly ($P < 0.05$, $P < 0.01$) by sex; TP - total protein; ALB - albumin; GLO - globulin; BUN - blood urea nitrogen; CRE - creatinine; BIT - total bilirubin; CHO - total cholesterol; GLU - glucose; FFA - free fatty acids; AST - aspartate aminotransferase; GGT- gamma-glutamyltransferase

Conclusion

In conclusion, age and sex showed a significant influence on some haematological and blood biochemical parameters of organically raised lambs of the indigenous sheep breed Dalmatian Pramenka, and this should be considered when interpreting results and assessing the physiological, metabolic, health and nutritional status and productivity of lambs. Further, it is necessary to take the age and sex of sheep into consideration, before the physiological limits for the concentrations of certain blood parameters have been specified with the aim of establishing reference intervals for sheep. The limitation of this study is that only one farm was tested, which is certainly not representative of all lambs of the Dalmatinska Pramenka breed from organic farms. Further effort should be addressed to more comprehensive studies with a larger number of lambs raised on different organic farms, and considering additional blood parameters (alkaline phosphatase, creatine kinase, minerals and trace elements).

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Conflict of interest statement

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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LJUBIČIĆ, I., S. VINCE, A. SHEK VUGROVEČKI, S. MILINKOVIĆ TUR, M. ŠIMPRAGA: Utjecaj dobi i spola na odabrane hematološke i biokemijske pokazatelje u janjadi pasmine dalmatinska pramenka. Vet. arhiv 92, 691-702, 2022.

SAŽETAK

Cilj rada bio je istražiti utjecaj dobi i spola na odabrane pokazatelje u krvi janjadi pasmine dalmatinska pramenka uzgajane u ekološkim uvjetima. Četrdeset janjadi u prosječnoj dobi od tri mjeseca, s ravnomjernom zastupljenošću oba spola, obuhvaćeno je istraživanjem koje je uspostavljeno s početkom sezone izlaska na pašu (ožujak), a trajalo je do kraja proizvodnog ciklusa uzgoja janjadi za klanje (lipanj), ukupno četiri mjeseca. Uzorci krvi za hematološku i biokemijsku pretragu uzimani su svakog mjeseca (u četiri navrata) u razmaku od 30 dana. Raspodjela rezultata hematoloških i biokemijskih pokazatelja u krvnom serumu prema dobi janjadi, pokazala je da je povećanje dobi janjadi pratilo statistički znakovito smanjenje ($P<0,05$) koncentracije RBC, HGB, HCT, GLU, BUN i BIT te povećanje ($P<0,05$) koncentracije TP, ALB, GLO i aktivnosti enzima AST. Raspodjela rezultata prema spolu janjadi, pokazala je da je ženska janjad imala statistički znakovito veći WBC ($P<0,05$), RBC ($P<0,01$), HGB ($P<0,05$) od muške janjadi, dok je muška janjad imala veći MCV ($P<0,01$) i koncentraciju FFA ($P<0,01$) u usporedbi s ženskom janjadi. Zaključeno je da dob i spol znakovito utječu na promjene vrijednosti većine hematoloških i biokemijskih pokazatelja u serumu janjadi dalmatinske pramenke iz ekološkog uzgoja.

Ključne riječi: hematološki pokazatelji; biokemijski pokazatelji; dob; spol; janjad pasmine dalmatinska pramenka