RESEARCH ARTICLE Indian Journal of Animal Research, Volume 55 Issue 7: 767-773 (July 2021)

The Impact of Breed, Testing Time and Metabolic Profile on the Variation of Copper Concentration in Sheep Blood Serum

J. Autukaitė, V. Juozaitienė[1], R. Antanaitis, I. Poškienė,
W. Baumgartner[2], H. Žilinskas, V. Žilaitis

10.18805/IJAR.B-1352

ABSTRACT

Background: Copper supplementation in ruminants deserves special attention because of the narrow margin between deficiency and toxicity. The aim of the study was to analyse the relationship between the Cu concentration and other blood parameter values having taken into consideration their physiological limits based on the variation of copper concentration in the three different sheep breeds.

Methods: Sixty sheep (35±5 kg) kept on the same farm were studied: the Suffolk (n = 20), the Merino (n = 20) and the Lithuanian blackhead (n = 20). The sheep were selected according to the following parameters: 3.5±0.3 years old, not pregnant during the entire investigation period, clinically healthy. All sheep were kept under the same conditions and throughout the year, were fed at the same time the same ration balanced according to their physiological needs. Individual blood samples were collected on the same day, on monthly basis for the period of one year (April 2018 to May 2019). Blood biochemical parameters as well blood haematology and cortisol concentration were analysed.

Result: The highest average value of copper concentration was found in the Merino breed. The study has shown that the lowest number of blood samples with the levels of copper meeting the physiological limit values was found in the native Lithuanian blackhead sheep at the end of the grazing period and in the Merino and the Suffolk breeds - during the coldest winter months. The increase in copper concentration could be related to the stress caused due to the changes in dietary conditions, the variations of ambient temperature and different breed response to these factors.

Key words: Biochemical parameters, Breed, Copper, Haematology, Sheep.

INTRODUCTION

Copper (Cu) is an essential element for life and is required as a co-factor in hundreds of enzymatic reactions involved in red blood cell production, energy generation, hormone formation, collagen synthesis and protection against oxidative damage (Kulkarni *et al.,* 2012; Adams *et al.,* 2018). Ruminants are probably more susceptible to Cu toxicosis than other species. To avoid Cu toxicosis, sheep should be grazed in copper-deficient pastures, which in addition may contain antagonistic minerals, namely sulphur (S), molybdenum (Mo) and iron (Fe). As ruminants have poor homeostatic control over Cu absorption, they have developed certain mechanisms for storing excess Cu in the liver by decreasing Cu excretion in the bile. However, when exposed to Cu concentrations above physiological requirements, ruminants do not modulate Cu excretion in the bile and excessive hepatic Cu accumulation occurs (Lopez-Alonso and Miranda, 2020). Sheep are more susceptible to Cu toxicity than cows or goats - they have a very limited capacity to excrete the Cu *via* the bile duct, as a result of a low capacity to accumulate the Cu bound to metallothioneins in the liver. There are also differences among sheep breeds in Cu tolerance; yet, these have not been traced to variations in the expression of particular copper-binding proteins. The Suffolk and the Merino breeds are particularly at risk.

Veterinary Academy, Lithuanian University of Health Sciences, Tilžės str. 18, Kaunas, Lithuania.
[1]Department of Animal Breeding, Veterinary Academy, Lithuanian University of Health Sciences, Tilžės str. 18, Kaunas, Lithuania.
[2] University Clinic for Ruminants, University of Veterinary Medicine, Vienna, Austria.

Corresponding Author: J. Autukaitė, Veterinary Academy, Lithuanian University of Health Sciences, Tilžės str. 18, Kaunas, Lithuania. Email: jurgita.autukaite@lsmuni.lt.

How to cite this article: Autukaitė, J., Juozaitienė, V., Antanaitis, R., Poškienė, I., Baumgartner, W., Žilinskas, H. and Žilaitis, V. (2021). The Impact of Breed, Testing Time and Metabolic Profile on the Variation of Copper Concentration in Sheep Blood Serum. Indian Journal of Animal Research. 55(7): 767-773. DOI: 10.18805/IJAR.B-1352.

Submitted: 15-02-2021 **Accepted:** 01-05-2021 **Online:** 18-06-2021

Haematological and biochemical analyses in farm animals have been extensively discussed in the study often pointing to a specific differential diagnosis or suggesting a treatment and prognosis (Arfuso *et al.,* 2016). The aim of the study was to analyse the relationship between the Cu concentration and other blood parameter values having taken into consideration their physiological limits based on the variation of copper concentration in the three different sheep breeds.

MATERIALS AND METHODS

Location, animals and study design

The research was performed according to the Law of the Republic of Lithuania No. 8-500 on Protection, Keeping and Use of Animals, dated 06/11/1997 (Valstybės žinios (Official Gazette) No. 108 dated 28/11/1997) and orders of the State Veterinary Service of the Republic of Lithuania on Breeding, Care and Transportation of Laboratory Animals (No. 4-361, dated 31/12/1998) and use of Laboratory Animals for Scientific Tests (No. 4-16, dated 18/01/1999). The study's approval number was PK014606.

The study was conducted on the sheep farm in the Eastern region of Europe, Lithuania, from April 2018 to May 2019. The average ambient temperature during the study year was 10.50 ±1°C, the relative humidity was 77.60 ±2%. Sixty sheep, were selected according to the following criteria: 3.5±0.3 years old, not pregnant during the entire investigation period, clinically healthy, exhibiting the following clinical parameters of an average rectal temperature of 38.5±0.2°C, rumen contractions three to four times per two minutes during the precise clinical examination. The farm conducts the monitoring of infectious diseases. Routine deworming is performed twice a year. The tested sheep were divided into the following three groups: the Suffolk (n = 20), the Merino (n = 20) and the Lithuanian blackhead (n = 20). Average body weight of the sheep was 35±5 kg. All sheep were kept following the loose housing system and were fed at the same time, the same ration, balanced according to their physiological needs. The grazing season was from April to October. The pastures were predominantly grasses and legumes. In addition, in summer, all sheep on the farms at all times had ad libitum access to the complete mineral mixture blocks -"Sheep Rockes" (Tithebarn, UK) (sodium 38%, magnesium 1000 mg/kg, calcium 1%, phosphorus 1%, cobalt 100 mg/kg, iodine 50 mg/kg, manganese 100 mg/kg, selenium 20 mg/kg and zinc 120 mg/kg). In winter, the ewes were kept in barns and fed the grain mixture (300 g/day), grass hay (Timothy grass, red clover, white clover, alfalfa, ryegrass and quack grass). Water was provided ad libitum.

Analytical procedures

The study was conducted from April 2018 to May 2019. Blood samples were collected between 0700 to 0800 hrs after overnight fasting by jugular venipuncture, using vacuum tubes without any anticoagulant. Sixty blood samples were taken once per month on the same day from the identical animals. In the study, 780 blood samples were tested (13 months x 60 animals). Further research was performed in the Lithuanian University of Health Science, Veterinary Academy. The samples were collected in 8°C temperature containers and transported immediately to the laboratory for analysis. Within an hour, the blood samples were centrifuged for 10 min. at 3500 rpm and blood serum was collected and stored in -20°C environment. The blood serum biochemical parameters, such as copper (Cu), zinc (Zn), iron (Fe), urea, glucose (Gl), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), total bilirubin (TBIL), albumin (Alb), creatinine (CREA) and lactate dehydrogenase (LDH) were measured using commercial kits on the Selectra Junior analyser (AC Dieren, The Netherlands, 2006). Cortisol level was measured using the automated analyser TOSOH® AIA-360 (South San Francisco, CA), performing a comparable fluorescent enzyme immunoassay, which is run in small, single-use test cups that contain all necessary reagents. Accuracy and performance data for human and canine cortisol, including analyte recovery and dilutional studies, have been previously evaluated (Higgs *et al.,* 2014). Daily checks, calibration curves and maintenance procedures were carried out according to the System Operator's Manual. For blood haematology sixty blood samples were taken once per month on the same day from the identical animals at the same time like for biochemistry in a vacuum tube system containing 10% EDTA. The values determined were erythrocytes (RBC), haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), leukocytes (WBC) and platelet count (PLT). Haematological parameters were measured using the MS4-5 VET automatic analyser (Melet Schloesing Laboratories, France 2012).

Data analysis and statistics

Blood parameters were grouped by physiological limits into three classes, where the middle class refers to the norm (Class 2, Cu values 46-140) and the two extreme ones denote, respectively, the values under or above the physiological limits (Class 1, Cu values $<$46; Class 3, Cu values $\geq$141) (Table 1). In the analyses, the breed, the month of study and blood parameters were considered as categorical variables. The level of significance was $P<0.05$ for all tests.

Data were analysed using the software IBM SPSS Statistics (version 25.0, IBM, Munich, Germany). The descriptive statistics of data were presented as mean ± standard error of the mean of a sample. The normality of all data recorded in the study was assessed by Shapiro-Wilk normality test. A multiple comparison (Bonferroni test) was applied to compare the Cu value among the breeds. The GLM repeated measures technique (general linear model) was used to assess the changes in serum Cu concentration (µg/dL). The difference between the proportions was assessed using the two-proportion z-test procedure. The Chi Square (χ^2) statistics was used to determine the relationship between the following categorical variables: Cu concentration among the breeds and Cu concentration in respect to other blood parameter values having taken into consideration their physiological limits. To analyse the factors contributing to the Cu level in the blood of sheep, multivariable logistic regression models were used, applying a backward stepwise logistic model to eliminate all non-significant explanatory variables. Categorical variables

Table 1: Sheep blood parameters grouped by the concentration according to the physiological limit values (Kramer, 2000; Radostits *et al*., 2000).

Blood indices	Class 1 (Cu < 46 μg/dl)	Class 2 (Cu 46-140 μg/dl)	Class 3 (Cu ≥141 μg/dl)
Urea mmol/l	<3	3-10	>10
Crea μmol/l	<70	70-210	>105
TBIL μmol/l	<1.71	1.71-8.55	>8.55
AST U/l	>60	60-280	<280
GGT U/l	<20	20-52	>52
LDH U/l	<238	238-440	>440
ALB g/l	<24	24-30	<30
Gl mmol/l	<1.7	1.7-3.6	<3.6
Fe μg/dl	<166	166-222	>222
Zn μg/dl	>80	80-120	>120
WBC × 10^9/L	<4	4-12	>12
RBC × 10^{12}/L	<9	9-15	>15
MCV f/l	<28	28-40	>40
MCH pg	<8	8-12	>12
MCHC g/dL	<310	310-340	>340
HGB g/l	<90	90-150	>150
HCT %	<27	27-45	>45
PLT × 10^9/L	<100	100-800	>800
Cortisol μg/dl	<1.40	1.40-3.10	>3.10

(the breed, the month of study and certain blood parameters) were continuously removed from the models according to the significance of the Wald criterion. Finally, two independent variables were used in the model.

RESULTS AND DISCUSSION

The impact of breed on Cu level in blood serum

In sheep, breed is known to have a strong impact on the susceptibility to Cu disorders. Certain breeds have been classified as tolerant or resistant in terms of Cu toxicity. As a result, it is possible to improve the resistance of sheep to Cu deficiency and excess by appropriate cross breeding and selection programs (Stevanovic *et al.,* 2015). In recent years, copper toxicity of sheep has been increasing with the intensification of sheep industry, increased housing of ewes and use of breeds susceptible to Cu poisoning, such as the Texel and the Merino (Anchordoquy *et al.,* 2017). The results of data analysis showed a large variation (Cv = 64.03%) in the concentration of Cu in blood samples (n = 780). The value of Cu according to physiological limits (46-140 μg/dl) was found to be normal in 427 samples and in 353 samples (24.4%) it was higher (≥141 μg/dl). Most samples (80.1%) with normal Cu concentration were found in the Lithuanian blackhead sheep. Some samples (with normal Cu concentration) were found in the Suffolk sheep (by 4.2% less) and for the Merino breed it was by - 10.6% less compared to the Lithuanian blackhead sheep (Fig 1). The highest average value of Cu concentration (above the norm) was found in the Merino breed. Two breeds (the Merino and the Suffolk) of the three used in the present research may be relatively susceptive to Cu content. These genetic differences in Cu metabolism may be related to the efficiency of dietary absorption, biliary excretion of endogenous Cu or even the amount of feed intake (Stevanovic *et al.,* 2015).

Impact of season on the changes in the blood serum Cu levels

The highest serum Cu concentration was observed in January (147.1 ± 12.20 μg/dl) and the lowest - in May (97.6 ± 18.85 μg/dl) in 2019 in the Lithuanian blackhead sheep breed (P<0.001) (Fig 2). Having assessed the changes in Cu concentration in sheep breeds according to the physiological limits of this blood parameter, it was found that most blood samples showed the values that met the physiological limits were collected in the first month of the study, *i.e.* in April 2018 (90-100%) and in the last two months of the study, *i.e.* in April and May 2019 (80.1-100%) (Fig 2). The lowest number of blood samples with Cu values meeting the physiological limits was collected from the Lithuanian blackhead sheep at the end of the grazing period, *i.e.* in October 2018 (26.3% of the samples) and in the Merino breed (10%) and the Suffolk breed (31.3%) in January 2018. The increase in Cu concentration can be related with stress, mineral deficiencies, ambient temperature and possibly higher humidity in these months (Ramirez *et al.,* 2001).

Relationship between Cu concentration and blood parameters

Table 2 represents the percentage distribution of blood samples according to the concentration of Cu and blood parameters. For the sheep with normal Cu concentration, the percentage of blood samples that corresponded to the recommended norm was found to be from 60.9% (by Fe level) to 82.3% (by CREA level). The Chi Square (χ^2) test of independence demonstrated the relationship between the Cu concentration and the concentrations of blood Fe, CREA and the activity of GGT ($P \leq 0.001$), MCH and MCHC (P = 0.005), HGB (P = 0.006), GI (P = 0.028), PLT (P = 0.029) and ALB (P = 0.041). The analyses showed that the increase in Cu above the normal range was associated with a significant decrease in PLT below the normal range. This proves the fact that Cu has a direct effect on iron metabolism and thus, it indirectly affects the biosynthesis of haemoglobin (Cybulski *et al.,* 2009). When the concentrations of Cu exceed the limit values, it enters the secretory pathway to bind to metallothionein in the Golgi body and is stored in the lysosomes, which thus protect the cell from free Cu. When the concentration of Cu in the blood increases, the liver can take a large amount of Cu from the blood and if the Cu level exceeds the tolerance limit, the activities of various enzymes can be inhibited, which can cause liver cell denaturation necrosis and Cu excretion dysfunction (Suttle *et al.,* 2002). When the Cu level in the liver is excessive, a large amount of it enters into the bloodstream and then - into the red blood cells and the intracellular Cu concentration continues to rise so that the concentration of glutathione in red blood cells is reduced, erythrocyte fragility is increased and haemolysis occurs (Shen *et al.,* 2010).

Blood glucose values are also known to be related to genetic predisposition (Durak *et al.,* 2015). Glucose concentrations may alter with the secretion of catecholamines and may also increase or decrease secondarily. Lowest concentration of glucose and albumin

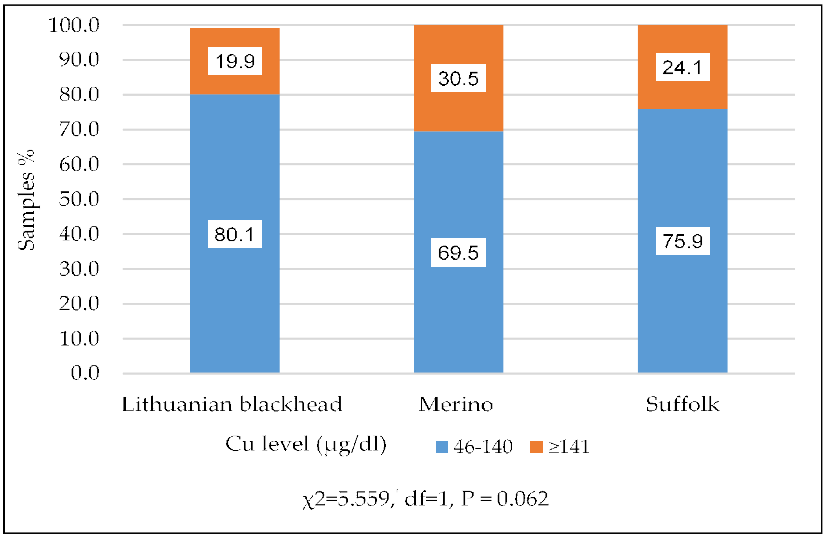

Fig 1: Copper concentration distribution in blood serum by sheep breeds.

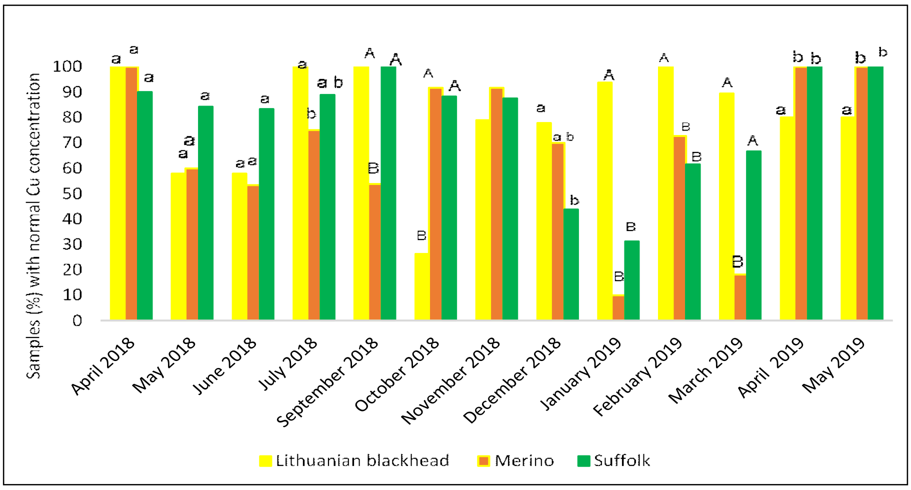

Fig 2: Cu levels in the blood serum of sheep.

Differences in proportions marked with different letters are statistically significant: ^{a,b}- P<0.05, ^{A,B} - P<0.01.

Table 2: Relationship between the Cu values and other blood parameter physiological limit values.

Blood indices	Blood indices class	Percentage of blood samples by Cu (µg/dl)		χ^2 test
		46-140	≥ 141	
Urea mmol/l	<3	80.00	20.00	χ^2=4.646, df=2, P=0.087
	3-10	73.50	26.50	
	>10	82.60	17.40	
Gl mmol/l	<1.7	65.40	34.60	χ^2=7.118, df=2, P=0.028
	1.7-3.6	78.50	21.50	
	>3.6	71.30	28.70	
AST U/l	<60	100.00	0.00	χ^2=4.216, df=2, P=0.12
	60-280	75.60	24.40	
	>280	55.60	44.40	
Fe µg/dl	<166	78.70	21.30	χ^2=13.621, df=2, P=0.001
	166-222	60.90	39.10	
	>222	64.70	35.30	
CREA µmol/l	<70	60.00	40.00	χ^2=13.819, df=2, P=0.001
	70-105	82.30	17.70	
	>105	69.40	30.60	
TBIL µmol/l	<1.71	80.00	20.00	χ^2=1.265,df=2, P=0.531
	1.71-8.55	74.70	25.30	
	>8.55	75.00	25.00	
ALB g/l	<24	75.40	24.60	χ^2=6.374, df=2, P=0.041
	24-30	79.00	21.00	
	>30	68.80	31.20	
GGT U/l	<20	25.00	75.00	χ^2=18.224, df=2, P<0.001
	20-52	66.70	33.30	
	>52	80.40	19.60	
WBC × 10y /L	<4	66.70	33.30	χ^2=1.908, df=2, P=0.385
	4-12	76.20	23.80	
	>12	77.80	22.20	
RBC × 10^{12}/L	<9	83.30	16.70	χ^2=4.888, df=2, P=0.087
	9-15	74.30	25.70	
	>15	40.00	60.00	
HGB g/l	<90	78.30	21.70	χ^2=10.148, df=2, P=.006
	90-150	77.20	22.80	
	>150	50.00	50.00	
HCT %	<27	82.80	17.20	χ^2=1.069, df=2, P=0.586
	27-45	74.20	25.80	
	>45	71.40	28.60	
MCV fL	<28	76.00	24.00	χ^2=0.678, df=2, P=0.713
	28-40	75.00	25.00	
	>40	50.00	50.00	
MCH pg	<8	100.00	0.00	χ^2=10.686, df=2, P=0.005
	8-12	76.20	23.80	
	>12	67.30	32.70	
Cortisol µg/dl	<1.40	79.20	20.80	χ^2=2.207, df=2, P=0.332
	1.40-3.10	75.80	24.20	
	>3.10	72.50	27.50	
Zn µg/dl	80-120	63.90	36.10	χ^2=2.897, df=1, P=0.089
	>120	76.50	23.50	

Table 2: Continue..........

Table 2: Continue..

LDH U/l	238-440	40.00	60.00	χ^2=3.426, df=1,
	>440	75.80	24.20	P=0.064
PLT × 10y /L	<100	62.70	37.30	χ^2=4.780, df=1,
	100-800	77.20	22.80	P=0.029
MCHC g/dL	<310	93.50	6.50	χ^2=10,686, df=2,
	310-340	63.60	36.40	P=0.005
	>340	72.50	27.50	

of the examined animals may be a logical consequence of any nutritional deficiencies (Durak *et al.,* 2015). An alternative hypothesis is that the concentration of blood creatinine might have been related with the trace elements and liver enzymes. This effect would be induced by the reduction in the glomerular filtration rate (Yokus *et al.,* 2006). Compared to the reference values the average activity of GGT in the samples of sheep blood serum was slightly elevated, in 19.6% of samples, its normal range was observed in 33.3% and the decreased value was found in 75.0%. The increased activity of this enzyme in clinically healthy sheep can be considered as a consequence of the intensification of metabolic processes and may be related to Cu content (Stevanovic *et al.,* 2015).

The concentration of cortisol can be different for each individual because some animals secrete more stress hormones than others. Of all domestic animals, sheep are the most sensitive to the effects of stress (Behera *et al.,* 2018). Based on the results of this study, it can be stated that the increase in the amount of Cu in the blood serum does not have a significant effect on the increase in the amount of cortisol, there is no relationship between serum Cu and plasma cortisol concentrations.

CONCLUSION

Based on the results of this study, it can be concluded that copper metabolism in the Merino breed is poorer compared to that observed in other two of the studied breeds. The lowest number of blood samples with copper levels that met the physiological limits was collected from the Lithuanian blackhead sheep at the end of the grazing period and from the Merino and the Suffolk breeds – in the coldest month of the year. The increase in Cu concentration could be associated with stress due to altered dietary conditions, changes in ambient temperature, mineral deficiencies, especially antagonists. The concentration of Cu in the blood serum of sheep was associated with blood Fe, CREA and the activity of GGT, MCH and MCHC, HGB, Gl, PLT and ALB. This proves the fact that Cu has a direct effect on Fe metabolism. When the concentration of Cu in the blood increases, the liver can take a large amount of Cu from the blood and the activities of various enzymes can be inhibited.

REFERENCES

Adams, S., Hongjiao, M., Che, D., Hailong, J., Rui, H., Bao, Z., Danquah, K. and Guixin, Q. (2018). Effect of dietary copper levels on the growth performance and nutrientutilization in fattening pigs. Indian Journal of Animal Research. 54(5): 573-577.

Anchordoquy, J.M., Anchordoquy, J.P., Nikoloff, N., Pascua, A.M. and Furnus, C.C. (2017). High copper concentration produce genotoxicity and cytotoxicity in bovine cumulus cells. Environmental Science and Pollution Research. 24: 20041-20049.

Arfuso, F., Fazio, F., Rizzo, M., Marafioti, S., Zanghì, E. and Piccione, G. (2016). Factors affecting the hematological parameters in different goat breeds from Italy. Annals of Animal Science. 16: 743-757.

Behera, R., Chakravarty, A.K., Sahu, A., Kashyap, N., Rai, S. and Mandal, A. (2018). Identification of best temperature humidity index model for assessing impactof heat stress on milk constituent traits in Murrah buffaloes under subtropical climatic conditions of Northern India. Indian Journal of Animal Research. 52: 13-19.

Cybulski, W., Jarosz, L., Chalabis-Mazurek, A., Jakubczak, A., Kostro, K. and Kursa, K. (2009). Contents of zinc, copper, chromium and manganese in silver foxes according to their age and mineral supplementation. Polish Journal of Veterinary Science.12: 339-345.

Durak, M.H., Erkan, R.E.C., Celik, R., Yokus, B., Kurt, D. and Gürgöze, S. (2015). The effects of age and gender on some biochemical serum parameters in zom sheep raised in the vicinity of Karacadağ. Israel Journal of Veterinary Medicine. 70: 33-39.

Higgs, P., Costa, M., Freke, A. and Papasouliotis, K. (2014). Measurement of thyroxine and cortisolin canine and feline blood samples using two immunoassay analysers. Journal of Small Animal Practice. 55: 153-159.

Kramer, J.W. (2000). Normal hematology of cattle, sheep and goats. In: A Textbook of Schalm's Veterinary Hemattology. 5th Edn, Philadelphia, Lippincott Williams and Wilkins. pp. 1075-1084.

Kulkarni, R.C., Shrivastava, H.P. and Mandaland, A.B. (2012). Effect of copper supplementation on serum trace mineral status and cholesterol in broiler chickens. Indian Journal of Animal Research. 3: 313-314.

López-Alonso, M. and Miranda, M. (2020). Copper supplementation, a challenge in cattle. Animals. 10: 1890.

Radostits, O.M., Gay, C.C., Blood, D.C. and Hinchcliff, K.W. (2000). In: A Textbook of the biochemical values for cattle, sheep and pigs. 9th Edn, London. pp. 1819-1822.

Ramirez, R.G., Haenlein, G.F. and Nunez-Gonzalez, M.A. (2001). Seasonal variation of macro and trace mineral contents in 14 browse species that grow in northeastern Mexico. Journal Small Ruminant Research. 39: 153-159.

Shen, X.Y., Li, X. and Zhang, R.D. (2010). Studies on unsteady gait disease of the Tibetan gazelles. Journal of Wildlife Disease. 46: 560.

Stevanović. O., Stojiljković, M., Nedić. D., Radoja, D., Nikolić, V., Prodanović, R., Ivanov, S. and Vujanac, I. (2015). Variability of blood serum biochemical parameters in karakachan sheep. Journal Biotechnology and Animal Husbandry. 31: 55-62.

Suttle, N.F., Lewis, R.M. and Small, J.N.W. (2002). Effects of breed and family on rate of copper accretion in the liver of purebred Charollais, Suffolk and Texel lambs. Journal of Animal Science. 75: 295-302.

Yokus, B., Cakir, D.U., Kanay, Z., Gulten, T. and Uysal, E. (2006). Effects of seasonal and physiological variations on the serum chemistry, vitamins and thyroid hormone concentrations in sheep. Journal of Veterinary Medicine. 53: 271-276.