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VARIABILITY OF BLOOD SERUM BIOCHEMICAL PARAMETERS IN KARAKACHAN SHEEP

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Abstract: Karakachan sheep represents an endangered, indigenous sheep breed from Balkan Peninsula. There is relatively little information about the characteristics of this sheep breed in the veterinary scientific literature. The aim of this research was an examination of certain metabolic profile parameters of the Karakachan sheep blood serum, and variability of their concentrations in comparison to age and some other indigenous sheep breeds from Balkans. Examination was conducted on 14 clinically healthy sheep divided in two age groups. Blood samples were collected by puncture of *v. jugularis* and blood serum was separated after spontaneous coagulation. The concentrations of total protein, albumin, calcium, inorganic phosphorus, aspartate amino transferase (AST) and γ -glutamyl transferase (GGT) were determined. In relation to age of Karakachan sheep, statistically significant difference between the calculated mean values of examined parameters was not observed. A statistically significant difference was found between the mean concentrations of the studied parameters in Karakachan sheep and other breeds in total protein (Tsigai, Dubrovnik and Dalmatian sheep), albumin (Dalmatian), calcium and inorganic phosphorus (Tsigai, Dubrovnik) and AST's (Dalmatian, Karakachan sheep from Bulgaria).

Key words: Karakachan sheep, blood serum, biochemical parameters

Introduction

Karakachan sheep is one of the oldest sheep breeds in Europe (*Dervisis et al. 2007*). It was created centuries ago, as a result of selection on the primitive living conditions by Karakachans, a nomadic people who lived in the area of present-day countries of South-Eastern Balkans (Greece, Macedonia, Bulgaria and

Serbia). This sheep breed belongs to a primitive type of mountain sheep with a rough constitution, open fleece (Zackel type) and combined production traits. It is also characterized by pronounced vitality, and resistance. During the intensification of sheep breeding, Karakachan sheep lost its importance in modern production. According to the DADIS (Domestic Animal Diversity Information System), there were 130 females and 20 males in reproduction of this breed and total population was estimated at 150–300 individuals in 2012. Most of this sheep population is concentrated in the area of the Stara Planina nature park in South-Eastern part of Serbia.

Determination and monitoring of metabolic profile parameter values may show whether homeostatic mechanisms can maintain blood composition in physiological limits under different conditions of animal husbandry (*Prodanović et al. 2012*). Proper application of metabolic profile, the evaluation of housing conditions, as well as the composition and quality of the meals, can be a reliable method for assessing the condition of the herd, the diagnosis of health disorders and to indicate the etiology of their occurrence. There is a lot of studies that have examined the influence of gender, age, breed, nutrition and housing on blood biochemistry of sheep (*Jovanović et al. 1983; Hrković et al. 2009; Dias et al. 2010; Ouanes et al. 2011; Kiran et al. 2012*). Knowing the values of blood parameters of indigenous breeds is of particular importance because they allow collecting more information about those breeds which are most often threatened. The racial regression is a phenomenon that is present in all species of domestic animals and the disappearance of the primitive breeds is global problem that has resulted in the loss of genetic variability. In Serbia, there is no information about blood biochemistry of Karakachan sheep, so our work represents the beginning of research that aims to contribute in preservation of this threatened sheep breed.

The aim of this study is to determine the basic blood serum biochemical parameters of Karakachan sheep from Stara Planina mountain area, the influence of age on values of certain parameters, and detection of possible differences between Karakachan and other typical zackel sheep breeds from Balkans.

Material and methods

The studied animals belonged to a Karakachan sheep herd from the Stara Planina Nature Park area, and were in the process of conversion to organic production. In the time of blood sampling, nutrition was based solely on pasture (month of July). The study was conducted on 14 sheep (13 females and 1 male). Based on the age, sheep were divided into two groups of 7 individuals: Group I (1 to 3 years old) and group II (3 to 6 years old). Determination of age was based on dentition (*FAO, 2012*).

Blood was collected by puncture of the jugular vein in vacutainer tubes (10 ml) and spontaneous coagulation and centrifugation (3000 rpm for 10 minutes) led to separation of the blood serum. Samples of blood serum were stored at -18°C until the analysis were performed. The concentration of the following parameters - total protein g / l, albumin g / l, calcium (Ca) mg / l, inorganic phosphorus (P) mmol / L, aspartate aminotransferase (AST) and γ -glutamyl transferase (GGT) IU / l, were determined on a semi-automatic biochemical analyser (Vet evolution, Biosis, Italy).

Basic descriptive data (mean - \bar{x} , standard deviation - SD, standard error of the mean – SEM and variation interval - VI), and t-test for comparison of the values of some biochemical parameters in relation to the age, as well as comparison of the studied parameters in Karakachan sheep with the same values in other sheep breeds was obtained by using Graph Pad Prism 5.0 computer program. The reference values of studied biochemical parameters were taken from *Kaneko et al. 1997*.

Results and discussion

The metabolic profile is a diagnostic procedure that determines the concentrations of blood biochemical constituents in order to obtain data on the balance of organic and inorganic substances in the body as well as the function of certain organs. The average concentration values of metabolic profile in blood serum of Karakachan sheep (n = 14) are presented in table 1.

Table 1. Concentrations of studied metabolic profile parameters in blood serum of Karakachan sheep ($\bar{x} \pm \text{SD}$)

Parameter	N	$\bar{x} \pm \text{SD}$	SEM	VI	RV ¹
Total protein (g/l)	14	61,92±7,41	1,98	49,00-76,00	60,00-79,00
Albumin (g/l)	14	31,03±5,50	1,47	26,50-47,30	24,00-30,00
Ca (mmol/l)	14	2,75±0,29	0,08	2,15-3,22	2,70-3,20
P (mmol/l)	14	1,70±0,35	0,09	0,89-2,18	1,62-2,63
AST (UI/l)	14	97,66±28,87	7,72	65,00-172,00	60,00-280,00
GGT (UI/l)	14	60,50±16,08	4,30	25,60-86,90	20,00-52,00

¹Kaneko et al. (1997)

From the table 1 it is observed that the average concentration of total protein and albumin in the blood serum of sheep were within the physiological values. However, from the variation interval is observed that in some animals

(35,71%) the concentrations of total protein values were lower compared to the reference value. In addition to albumin and total protein, for the interpretation of nitrogen metabolism, concentrations of urea and creatinine in the blood are also significant. Given the complexity of protein metabolism, nutrition is considered to be of highest influence on the parameters used in the estimation of nitrogen metabolism in domestic ruminants. The results in this study shows that in some animals was present a mild hypoproteinemia in blood serum which can be regarded as a lack of protein in the diet, or poor quality pasture in the summer period on Stara Planina Mountain. This is supported by the results of *Jovanović et al., (1983)* studies which noted that the largest proteinemia in blood serum of sheep was in early spring, because the pasture is richest in proteins at this period of year.

The average concentrations of calcium (Ca) and inorganic phosphorus (P) in the blood serum of sheep were in physiological interval compared to the reference values. From the variation interval of inorganic phosphorus is observed that in some animals (35.71%) was present hypophosphatemia. However, different reference values for concentration of these minerals - Ca 2-3 mmol / l and P 1-2.5 mmol / l – can be found (*Hindson and Agnes, 2002*). Compared to this literature data, concentrations of these minerals were in range of physiological values. The level of minerals in the blood depends on nutrition, which shows that calcemia can be directly correlated to the category and nutrition of sheep (*Hrković et al. 2009*). Levels of calcemia and phosphatemia are primarily regulated with renal excretion. Hypophosphatemia in serum of Karakachan sheep is most likely the result of the reduced alimentary intake of phosphorus which is in agreement with *Jovanović et al. (1983)*. According to the recommendations of these authors, hypophosphatemia can be corrected by addition of phosphorus in diet of sheep.

Aspartate aminotransferase is an enzyme that is found in the liver and heart muscle, and plays an important role in the metabolism of amino acids. From the results obtained it can be seen that the activity of AST in serum was within the limits of referent values. The average value of γ -glutamyl transferase (GGT) in the blood serum samples of sheep was slightly elevated compared to reference values. Increased activity of this enzyme in clinically healthy sheep can be considered as a consequence of the intensification of metabolic processes and a response of the body to the negative energy balance (*Hrković et al. 2009*). Also, the increased activity of this enzyme in clinically healthy sheep can be consequence of moderate oxidative stress, related to increased degradation of glutathione (*Hodžić et al. 2011*). Furthermore, the increase of GGT can be a sign of chronic liver dysfunction that may be of different etiology. Due to the high concentration instability of these enzymes in the blood of ruminants, their activity is not a reliable diagnostic indicator for determining the nature of the pathological process in the liver (*Reynolds, 1991*).

Table 2. Comparison of studied metabolic profile parameters in relation to age of Karakachan sheep

Parameter	AG ¹	N	$\bar{x} \pm SD$	VI	t
Total protein (g/l)	1	7	58,55±4,88	49,00-63,00	1.91 ^{ns}
	2	7	65,29±8,28	55,00-76,00	
Albumin (g/l)	1	7	29,05±1,99	26,50-31,02	1.47 ^{ns}
	2	7	33,00±7,24	26,80-47,30	
Ca (mmol/l)	1	7	2,62±0,22	2,15-2,81	1.50 ^{ns}
	2	7	2,88±0,31	2,30-3,22	
P (mmol/l)	1	7	1,63±0,40	0,89-2,18	0.72 ^{ns}
	2	7	1,78±0,30	1,30-2,08	
AST (UI/l)	1	7	93,76±36,42	65,00-172,00	0.40 ^{ns}
	2	7	101,57±21,05	80,00-137,00	
GGT (UI/l)	1	7	54,57±5,96	43,40-60,50	1.51 ^{ns}
	2	7	66,43±21,03	25,60-86,90	

¹ age group ^{ns} p>0,05- not significant

Table 3. Comparison of average concentrations of certain metabolic profile parameters in Karakachan sheep with those of the three indigenous breeds in the Balkans.

Parameter	$\bar{x} \pm SEM$			
	Karakachan sheep (N=14)	Dubrovnik sheep (N=10) ¹	Tsigai (N=15) ²	Dalmatian sheep (N=114) ³
Total protein (g/l)	61,92±1,98	79,90±2,79**	74,97±1,22**	77,00±0,6**
Albumin (g/l)	31,03±1,47	32,40±0,19	30,31±1,31	38,00±0,30**
Ca (mmol/l)	2,75±0,08	3,01±0,04*	2,55±0,03*	-
P (mmol/l)	1,70±0,09	1,31±0,12*	1,68±0,06	-
AST (UI/l)	97,66±7,72	97,00±6,72	102,20±4,90	127,00±2,20**
GGT (UI/l)	60,50±4,30	52,60±11,08	46,80±2,97*	59,00±1,30

¹Antunović et al. (2009), ²Antunović et al. (2011), ³Vojta et al. (2011)

* p<0,05 – significant, ** p<0,001 – very significant

From the results shown in table 2, it can be observed that there is no statistically significant difference in concentration of examined parameters average values between the two age categories. These results are in agreement with the results obtained by *Kiran et al., (2012)* and suggesting that age has no substantial effect on the concentration of specific blood parameters.

Statistically significant difference between the values of certain parameters in Karakachan sheep blood serum and other indigenous breeds of the Balkans were observed (table 3.).

The average total protein concentration in the blood serum of the Karakachan sheep was significantly lower ($p < 0.001$) compared to other breeds. The average albumin concentration of Karakachan sheep was significantly lower ($p < 0.001$) compared to Dalmatian sheep. The difference in the protein can be explained by the fact that the authors sampled blood of Dalmatian breed in late spring when the pasture is still rich in proteins (*Vojta et al. 2011*).

The average concentration of calcium in the blood serum of the Karakachan sheep was statistically lower ($p < 0.05$) compared to Dubrovnik sheep, and higher than the calcium blood serum concentration of Tsigai ($p < 0.05$). Phosphatemia in Karakachan sheep was statistically higher ($p < 0.05$) compared to Dubrovnik sheep. These differences in the concentrations of minerals in blood between examined sheep breeds can be attributed to the different representation of the mineral matter in the soil where animals are grazed.

The mean concentration of AST in Karakachan sheep blood serum was significantly lower ($p < 0.001$) compared to the activity of this enzyme in the Dalmatian breed. The obtained values of AST in Karakachan sheep from Bulgaria were 121.29 ± 32.35 (*Angelov et al. 2013*). By comparing these values with the results of AST in our sample, we obtained statistically significant differences ($p < 0.05$), indicating that the value of AST may vary even within the same breed of sheep.

Conclusion

The concentrations of examined metabolic profile parameters in Karakachan sheep were within the physiological values.

Age status has no significant effect on the concentrations of blood serum biochemical parameters in Karakachan sheep. Nutrition, soil and pasture quality where the sheep graze has a significant effect on the concentration of proteins and minerals in the blood serum.

There was variation of the AST and GGT concentrations of Karakachan sheep in comparison with other breeds, as well as within the same breed.

Varijabilnost biohemijskih parametara krvnog seruma karakačanske ovce

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Rezime

Karakačanska ovca je ugrožena autohtona rasa ovaca sa Balkanskog poluostrva, o čijim rasnim karakteristikama postoji relativno malo podataka. Cilj ovog rada je bio da se u uzorcima krvnog seruma ispituju određeni biokemijski parametri karakačanske ovce i ustanove odstupanja njihovih vrednosti u odnosu na starost karakačanske ovce, kao i u odnosu na vrednosti istih parametara kod drugih autohtonih rasa ovaca Balkana. Ispitivanjem je obuhvaćeno 14 klinički zdravih ovaca podeljenih u dve starosne grupe. Uzorci krvi uzimani su punkcijom *v. jugularis* iz kojih je, nakon spontane koagulacije i centrifugovanja, izdvojen krvni serum. Određivane su koncentracije ukupnih proteina, albumina, kalcijuma, neorganskog fosfora, aspartat amino transferaze (AST) i γ -glutamyl transferaze (GGT). U odnosu na starost karakačanske ovce, nije ustanovljena statistički značajna razlika između izračunatih srednjih vrednosti ispitivanih parametara. Statistički značajna razlika je ustanovljena između srednjih vrednosti koncentracija ispitivanih parametara karakačanske ovce i drugih rasa za: ukupne proteine (cigaja, dubrovačka i dalmatinska ovca), albumin (dalmatinska), kalcijum i neorganski P (cigaja i dubrovačka) i aktivnosti AST-a (dalmatinska, karakačanska ovca iz Bugarske).

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