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Comparison of Some Blood Parameters in female Romanov and Hamdani Sheep Housed under The Same Nutritional Conditions

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ABSTRACT The present study was conducted to compare some blood parameters of adult (2-3 years old) female Romanov and Hamdani sheep breeds subjected to the same climate and feeding conditions. For this purpose, blood samples were taken from 20 clinically healthy adult Romanov and 20 Hamdani sheep. Serum glucose, aspartate aminotransferase, triglyceride, total protein, gamma glutamyl transferase, urea, alanine aminotransferase, high-density lipoprotein, albumin, lactate dehydrogenase, blood urea nitrogen, amylase, alkaline phosphatase, creatine kinase, magnesium, c-reactive protein, creatinine, cholesterol and iron levels were examined. While statistically significant differences were determined in serum glucose, aspartate aminotransferase, triglyceride, gamma glutamyl transferase, urea, lactate dehydrogenase, blood urea nitrogen, alkaline phosphatase, creatine kinase, creatinine and iron levels in Romanov and Hamdani breed sheep (p<0.05), no significant differences were found in other parameters (p>0.05). As a result, it is thought that these data obtained from Romanov and Hamdani sheep breeds can be considered as reference values and will contribute to a better understanding of the metabolic and physiological characteristics of these breeds.

Keywords: Biochemistry, Feeding, Sheep.

ÖZ

Aynı Beslenme Koşullarında Barındırılan Dişi Romanov ve Hamdani Koyunlarında Bazı Kan Parametrelerinin Karşılaştırılması

Mevcut çalışma aynı iklim, aynı besleme koşullarına tabi tutulan yetişkin (2-3 yaş) dişi Romanov ve Hamdani koyun ırklarına ait bazı kan parametrelerinin karşılaştırılması amacıyla yapıldı. Bu amaçla, klinik olarak sağlıklı oldukları belirlenen yetişkin 20 adet Romanov ve 20 adet Hamdani koyundan kan örnekleri alındı. Serumda, glukoz, aspartat aminotransferaz, trigileserid, total protein, gama glutamil transferaz, üre, alanin aminotransferaz, yüksek yoğunluklu lipoprotein, albümin, laktat dehidrogenaz, kan üre azotu, amilaz, alkalen fosfataz, kreatin kinaz, magnezyum, c-reaktif protein, kreatinin, kolesterol ve demir düzeyleri incelendi. Romanov ve Hamdani ırkı koyunlarda serum glukoz, aspartat aminotransferaz, trigiliserid, gama glutamil transferaz, üre, laktat dehidrogenaz, kan üre azotu, alkalen fosfataz, kreatin kinaz, kreatinin ve demir düzeylerinde istatistiksel açıdan önemli fark belirlenirken (p<0.05) diğer parametrelerde anlamlı fark bulunmadı (p>0.05). Sonuç olarak, Romanov ve Hamdani koyun ırklarından elde edilen bu verilerin, referans değerler olarak kabul edilebileceğinin yanı sıra bu ırkların metabolik ve fizyolojik özelliklerinin daha iyi anlaşılmasına katkı sağlayacağı düşünülmektedir.

Anahtar Kelimeler: Besleme, Biyokimya, Koyun.

INTRODUCTION

Sheep breeding has an important place in Türkiye's livestock economy. According to the 2023 data of the Turkish Statistical Institute, there are approximately 42 million sheep in Türkiye (Kirikci 2023). In Türkiye, different native sheep breeds are raised according to the geographical structure and climate. Along with the

breeding of local sheep breeds, breeding studies to obtain hybrid breeds with high productivity characteristics are also continuing. In addition to the breeding studies carried out in Türkiye to produce sheep breeds with high multiple pregnancy rates (Kul and Akcan 2002; Aslan and Emsen 2017), sheep breeds with high calving performance are also imported. By raising sheep breeds with high calving performance, an increase in the sheep population and the

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amount of lamb meat obtained is achieved (Kul and Akcan 2002; Aslan and Emsen 2017).

Romanov sheep, which imports to Türkiye have increased in recent years, attract attention with their features such as high calving numbers and the fur-like quality of their skins (Irmak et al. 2022). Morphologically, their legs and tails are shorter than other breeds, their bodies are grayish black in color and their hair structure is short and shiny black in appearance. Anatomically, their heads are wide and long, and the upper part of their noses has an arched appearance. Their bodies are medium-sized and the live weight of adult females is around 45-48 kg (Akcapınar 2000). Romanov sheep, whose homeland is Russia, have adapted to living in cold climates. There are studies on the breeding of Romanov sheep in the Eastern Anatolia Region, which has a colder climate compared to other regions of our country (Kutluca Korkmaz and Emsen 2016). In addition, there are studies on the birth weights, survival rates and production performances of lambs resulting from crossbreeding Tuj, Ivesi and Morkaraman sheep raised in Türkiye with Romanov sheep (Kutluca Korkmaz and Emsen 2016).

Hamdani sheep, which are preferred more than other sheep breeds by the breeders in the Eastern and Southeastern Anatolia regions of our country, are mostly bred in Siirt, Bitlis, Şırnak, Hakkari, Van and their surrounding areas. Hamdani sheep, which have an anatomically large structure, are a fat-tailed breed that is adapted to living in stony terrain and is resistant to even adverse environmental and fattening conditions (Aygün and Demir 2021). Originating from the Hamadan Rizaiye Region of Iranian, this breed is morphologically similar to the Akkaraman sheep. The most distinctive feature of this breed is its ear structures, which are quite long compared to other sheep breeds raised in our country (Şen 2024).

In the evaluation of the general health status of animals by veterinarians, analyses of blood parameters have an important place among the findings that complement and support the clinically observed symptoms (Neira et al. 2024). The examined blood parameters also provide important clues to veterinarians in terms of early diagnosis of diseases, prognosis and control of the effectiveness of the agents to be applied in treatment (Barbosa et al. 2022). Some hematological and biochemical parameters analyzed in blood may vary according to race, age and gender. Therefore, in order to evaluate blood parameters accurately according to animal breeds, reference ranges specific to animal breeds need to be determined (Lautz et al. 2020). In various previous studies, changes in blood values of many farm animals depending on their breed, age, pregnancy, climate, hormonal status and diseases were examined (Li et al. 2023; Sajid et al. 2023). In a study conducted on sheep, it was reported that some blood parameters red blood cells (RBC), hemoglobin (Hb), hematocrit (PCV), thyroglobulin (TG), urea (UREA), glucose (GLUC), creatinine (CRE), albumin (ALB) were higher and some (globulin, iron) were lower in lambs compared to adults (Yiğit et al. 2002). Similarly, Elitok (2012) reported that some hematological (RBC, WBC, Hb, MCV, MCH) and biochemical total protein (TP), cholesterol (CHOL), glucose (GLUC), urea (UREA), creatinine CRE) blood values in Saanen goats changed significantly with age. In a study investigating some biochemical parameters in different sheep breeds, it was reported that the breed factor had an effect on blood parameters (Yılmaz et al. 2022).

Nutrition programs applied in the breeding of farm animals are implemented with two main objectives: milk gain and meat gain. The main feed raw materials to be used in the nutrition program applied to farm animals consist of roughage and concentrated feeds. The basis for increasing animal productivity is the creation of a solid forage base and the preparation of a balanced concentrated diet with complete feeding for the realization of the genetic potential of animals for growth and milk productivity, health and reproduction (Bogolyubova and Zaitsev 2021; Buryakov et al. 2022). Roughage is of great importance in the nutrition of sheep, which spend most of their feeding time in pastures under suitable climatic conditions, as it is suitable for their feeding physiology, is economical and allows a significant portion of the desired yields to be obtained (Arslan and Tufan 2011).

As a result of the feeding of ruminants according to the type of feed raw materials in their daily feed, the cellulotic and amylolytic bacteria in the rumen break down these compounds into simple sugars such as hexose and pentose. As a result of this process, volatile fatty acids (VFA) are formed. Among these fatty acids, acetate, propionate and butyrate stand out and play important roles, especially in milk composition, energy provision and physiological values (Craninx et al. 2007; Li et al. 2012; Dündar and Küçükersan 2021).

clinically healthy animals, determining blood In parameters that may differ between breeds and revealing changes in these parameters is very important for physicians to be able to evaluate blood values correctly. Therefore, as in many parts of the world, there are many studies examining the blood values of sheep breeds raised in our country (Nisbet et al. 2006; Udum et al. 2013; Şimşek et al. 2015). In the present study, the comparison of blood parameters in Romanov and Hamdani sheep was aimed to provide a new perspective to the research on animal health, physiology and productivity. It is thought that analyses of the blood parameters of these two breeds with different genetic structures will not only contribute to a better understanding of the metabolic and physiological characteristics specific to these breeds, but will also shed light on their ability to adapt to various climatic and environmental conditions. In addition, it is anticipated that the data to be obtained will help make scientifically based decisions in critical areas such as breed selection, feeding strategies and health management in sheep farming. In particular, revealing the biochemical and hematological differences between breeds will contribute to the development of healthier and more efficient animal feeding methods and will provide important clues for animal welfare and sustainable animal feeding practices.

MATERIAL AND METHODS

This study was carried out with the approval of the Siirt University Experimental Animals Application and Research Center Animal Experiments Local Ethics Committee dated 27.09.2024 and numbered 2024/07/40. The study was conducted taking into account the ethics committee principles.

Animal Material

In this study, a total of 40 sheep, 2-3 years old female Romanov (n=20) and Hamdani (n=20) breeds, which were found in the Siirt University Veterinary Faculty Research Farm and were determined to be healthy in clinical examination, were used. Before blood samples were taken, general examination findings such as body temperatures, respiratory and pulse rates, examination of mucosal membranes and auscultation of stomach movements of the sheep were evaluated by the veterinarian. As a result of the clinical examination and examination of blood CRP values, it was determined that the sheep were healthy. The feeds given to the sheep had the same content. The nutrient composition of the diet is shown in Table 1.

 Table 1: Content and nutrient composition of the basal diet.

Feed	Amount, %
Lentil straw	40
Alfalfa dry grass	20
Concentrated feed	40
	Nutrient Content
Dry matter	90.00
Crude Protein	12.9
Metabolic protein	9.2
Metabolic Energy, kcal/kg	1.99
Crude fiber	22.4
Raw ash	-
NDF	47.6

Analysis of Blood Parameters

After blood samples were taken from the jugular vena of the animals used in the study into gel biochemistry tubes, their serums were separated by centrifugation at 3000 rpm for 10 minutes. The obtained sera were placed in Eppendorf tubes and stored at -20°C until analyzed. Serum c-reactive protein (CRP), UREA, GLUC, triglyceride (TRIG), total protein (TP), cholesterol (CHOL), ALB, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), amylase (AMYL), high-density lipoprotein (HDL), lactate dehydrogenase (LDH), blood urea nitrogen (BUN), CRE, creatine kinase (CK), magnesium (Mg) and iron (Fe) levels were analyzed using an automatic blood counting device (Beckman Coulter Chemistry Analyzer AU5800, U.S.A).

Statistical Analysis

The data collected in this study were evaluated with various statistical analyzes using the SPSS 22 package program. Shapiro-Wilk normality tests were used to determine the normal distribution of the data. These tests help determine whether the data has a normal distribution and correctly select the appropriate statistical analyses to be used for the research problem. In order to examine the differences in dependent variables according to groups in the study, t-test for unrelated samples was used in cases where normal distribution was provided, otherwise Mann-Whitney "U" test was used. p<0.05 was considered statistically significant.

RESULTS

The t test results for the comparison of some blood parameters (GLUC, AST, TRIG, TP, GGT, UREA, ALT, HDL, ALB, LDH, BUN) in Romanov and Hamdani sheep are presented in Table 2. A statistically significant difference was found between the two races in terms of GLUC, AST, TRIG, GGT, UREA, LDH and BUN levels (p<0.05). TP, ALT, HDL and ALB levels were determined to be similar in the Romanov and Hamdani races (p>0.05).

Table 2: T test results for comparison of some blood parameters in Romanov and Hamdani sheep.

T Test	Group	N	Average	S. deviation	sd	t	р
	R	20	54.2550	7.25037	38	3.178	0.003*
GLUC (mg/dl)	Н	20	47.1000	6.98510			
۸ ۳ (۱۱۱ /۱)	R	20	78.9350	8.87108	38	-7.643	0.000*
AST (10/L)	Н	20	124.1700	24.93710			
TRIC (mg/dl)	R	20	25.6500	7.92913	38	4.794	0.000*
i kig (ilig/ul)	Н	20	15.7000	4.82428			
$TD(\alpha(d))$	R	20	72.1490	5.83697	38	1.909	0.064
IP (g/ul)	Н	20	68.8240	5.15715			
	R	20	44.1000	6.08622	38	-5.365	0.000*
	Н	20	58.2000	10.05563			
UDEA (mg/dl)	R	20	43.2315	6.10855	38	7.105	0.000*
OREA (IIIg/ul)	Н	20	29.5605	6.06065			
۸۱ Ͳ (III /I)	R	20	19.2200	2.87267	38	0.333	0.741
	Н	20	18.8400	4.21881			
HDI (mg/dl)	R	20	28.5000	5.59605	38	1.812	0.078
IIDE (IIIg/ul)	Н	20	25.3000	5.57343			
AIR(mg/dl)	R	20	27.2650	2.59437	38	0.472	0.640
ALD (IIIg/ul)	Н	20	26,8345	3.14921			
IDH (mg/dl)	R	20	399.4500	54.01703	38	-2.548	0.015*
LDH (ling/ul)	Н	20	441.0000	48.98012			
PUN(ma/dl)	R	20	20.2000	2.85804	38	7.248	0.000*
	Н	20	13.7500	2.76967			

* p <0.05 R: Romanov; H: Hamdani.

The results of the Mann Whitney "U" test for the comparison of some blood parameters (AMYL, ALP, CK, Mg, CRP, CRE, CHOL, Fe) in Romanov and Hamdani sheep are presented in Table 3.

While statistical significance was found between the ALP, CK, CRE and Fe levels of both sheep breeds (p<0.05), AMYL, Mg, CRP and CHOL levels were determined to be similar (p>0.05).

Table 3: Mann Whitney "U" test results for comparison of a	some blood parameters in Romanov and Hamdani sheep.
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Mann Whitney 'U'	Group	Ν	Average Rank	Total Rank	U	Z	р
AMYL (IU/L)	R	20	21.55	431.00	179.000	-0.571	0.568
	Н	20	19.45	389.00			
ALP (IU/L)	R	20	24.33	486.50	123.500	-2.070	0.038*
	Н	20	16.68	333.50			
CK (IU/L)	R	20	16.48	329.50	119.500	-2.178	0.029*
	Н	20	24.53	490.50			
Mg (mg/dl)	R	20	21.83	436.50	173.500	-0.717	0.473
	Н	20	19.18	383.50			
CRP (mg/dl)	R	20	18.00	360.00	150.000	-1.426	0.154
	Н	20	23.00	460.00			
CRE (mg/dl)	R	20	24.88	497.50	112.500	-2.372	0.018*
	Н	20	16.13	322.50			
CHOL (mg/dl)	R	20	20.78	415.50	194.500	-0.149	0.881
	Н	20	20.23	404.50			
Fe (mg/dl)	R	20	14.75	295.00	85.000	-3.113	0.002*
	Н	20	26.25	525.00			

* p<0.05 R: Romanov; H: Hamdani.

DISCUSSION AND CONCLUSION

Changes in the values of hematological and biochemical blood parameters in the body can be caused by physiological factors such as gender, age and race, as well as environmental factors such as stress, nutrition, diseases and climatic conditions (Kaya and Dönmez 2019; Pehlivan et al. 2020; Khan et al. 2024). The findings obtained from the analysis of hematological and biochemical blood parameters provide veterinarians with important information about the general health status of animals. In order for physicians to interpret these findings correctly, reference values for breeds-specific blood parameters need to be determined (Meyer and Harvey 2004).

Kaneko and colleagues reported that glucose values in sheep were between 50 and 80 mg/dl (Kaneko 2008). In the presented study, it was found that glucose values in Romanov breed sheep were within the reported range, while in Hamdani breed they were below the reported range. In addition, it was determined that the difference in glucose levels between both races was significant (p<0.05). It is thought that this situation may be related to differences in metabolism.

Enzymes are biocatalysts that have a protein structure and catalytic properties. In the clinical evaluation of enzymes, enzymes such as transaminases (AST, ALT, GGT) are very important for the diagnosis of diseases (Center 2007; Hoffman et al. 2008). The fact that blood parameters are within previously established reference ranges is one of the indicators that the examined animal is healthy. ALT and AST synthesized in hepatocytes and GGT and ALP synthesized in bile duct epithelial cells are known as liver enzymes. Additionally, LDH is one of the enzymes used in

the diagnosis of hepatocellular damage. However, it is recommended to consider multiple biomarkers instead of a single biomarker in the evaluation of pathophysiological processes of the liver (Kaya et al. 2024). In the presented study, AST, GGT and LDH levels were found to be higher in the Hamdani breed compared to the Romanov breed, while ALP levels were found to be higher in the Romanov breed. These differences may be associated with metabolic activities in the liver. In addition, while AST, GGT, LDH levels were close to the values reported by Kaneko et al. (2008) (60-280, 20-52, 238-440 U/L), respectively, ALP levels were lower than those reported in the relevant literature (68-387 U/L). This situation can be considered specific to the studied breeds.

In the presented study, CK levels of Hamdani breed sheep were found to be higher compared to Romanov breed. CK levels of both breeds are higher than the value reported by Kaneko et al. (2008). CK levels are thought to be characteristic values of Romanov and Hamdani sheep breeds. In a study in which some biochemical parameters were determined in blood samples of Kıvırcık sheep, triglyceride levels of clinically healthy Kıvırcık sheep aged between 1 and 6 years in 5 different farms were determined as 33.22 ± 1.96 , 41.61 ± 11.0 , 14.63 ± 5.43 , 29.98 ± 6.93 , 28.56 ± 2.50 mg/dl in 5 groups, respectively (Udum et al. 2013). In the presented study, TRIG levels of Romanov sheep were found to be higher than those of Hamadani sheep (p<0.05), which is consistent with the findings of Udum et al. (2013).

Urea nitrogen (BUN) and creatinine levels are the most commonly used tests to evaluate kidney function. Conditions such as excessive protein intake, amino acid infusion, and accelerated catabolism may cause BUN levels to increase. In clinically healthy, 2-3 year old Karayaka sheep, BUN levels were reported as 10-33 mg/dl and creatinine levels were reported as 0.12-2.1 mg/dl (Nisbet et al. 2006). In the presented study, BUN levels of Romanov sheep were found to be statistically higher than Hamdani sheep (p<0.05). Although BUN values of both breeds were within the range reported by Nisbet et al. (2006), it is thought that the difference between the breeds may be related to protein metabolism.

In a study conducted on blood parameters of Dorset, Lincoln, Hampshire, Black Head German and Border breed meat sheep at the Bandırma Sheep Breeding and Research Institute, triglyceride levels were reported as 22.3 ± 3.0 -22.7 ± 2.1% mg; total cholesterol levels were reported as 66.3 ± 4.3 - 94.6 ± 3.5% mg and HDL-cholesterol levels were reported as $42.6 \pm 5.9 - 60.7 \pm 2.9\%$ mg. In the same sheep, according to lipoprotein electrophoresis, LDL levels were reported to be between $22.7 \pm 1.6 - 29.6 \pm 2.3\%$ and HDL levels were reported to be between $57.2 \pm 4.3 - 64.4 \pm$ 2.9% (Gündüz and Mert 1997). In another study conducted on Morkaraman sheep raised on pasture in the Adıyaman region and known to be clinically healthy, the mean triglyceride value was measured as 36.88 ± 19.41 mg/dL, total cholesterol value as 74.48 ± 22.33 mg/dl, and LDL level as $35.6 \pm 15.15 \text{ mg/dl}$ (Kurt et al. 2008). When the findings in Table 2 and Table 3 are examined, TRIG findings are similar to previous studies conducted with sheep, while CHOL, LDH and HDL findings differ from previous studies (Gündüz and Mert 1997; Kurt et al. 2008). This situation can be explained by the differences in the sheep breeds studied, the feeding programs applied, and the geographies where the studies were conducted. In the present study conducted on Romanov and Hamdani sheep fed under equal conditions, the statistical difference in serum TRIG and LDH levels is thought to be due to the characteristic features of the investigated sheep breeds (p<0.05).

In a study conducted by Şimşek et al. on adult Hasak and Hasmer breed sheep, serum GLUC values were found to be 57.36 ± 2.80 - 58.37 ± 2.34 mg/dl, TP values were found to be 10.24 ± 0.26 - 9.77 ± 0.37 g/dl, UREA values were found to be 30.44 ± 3.61 - 37.09 ± 3.19 g/dl, ALB levels were found to be 4.05 \pm 0.15 - 3.68 \pm 0.17 g/dl and CRE values were found to be $1.05 \pm 0.04 - 1.00 \pm 0.05 \text{ mg/dl}$, respectively (Şimşek et al. 2015). When these findings are considered with the findings in the current study, while serum GLUC and UREA are in similar reference ranges, differences are observed between serum TP, ALB and CRE values. It is thought that this situation is due to the different sheep breeds investigated and the geographies where the experiments were conducted. In the present study, a statistically significant difference was found between the GLUC, UREA and CRE values obtained from the analysis of serum samples obtained from Romanov and Hamdani sheep (p<0.05). It is thought that the differences in these investigated parameters are due to breed-related changes and are characteristic values of Romanov and Hamdani sheep breeds.

Although minerals are found in low concentrations in living organisms, they play important roles in the continuity of many physiological events such as enzymatic activity, regulation of osmotic pressure in the cell, hormonal activity, collagen formation, oxygen transport, fertility, body weight gain and disease prevention. Nutritional diseases occur due to the deficiency of mineral substances and cause great economic losses for the growers (Kayri and Irmak 2021). Levels of macro and micro elements in serum give quite different results in animal studies. Serum mineral levels vary depending on age, breed, gender, the content of the pastures where animals graze, and especially nutrition (Autukaitė et al. 2020; Fan et al. 2020). In the current study conducted on Romanov and Hamdani sheep, no difference was found in serum magnesium values, while a statistically significant difference was found in serum iron levels (p<0.05). The difference in serum iron levels among these breeds living in the same climate and fed the same diet suggests that this may be due to differences in iron binding capacities in the sheep examined.

As a result, while statistically significant differences were determined in serum GLUC, AST, TRIG, GGT, UREA, LDH, BUN, ALP, CK, CRE and Fe levels in Romanov and Hamdani breed sheep (p<0.05), no significant differences were found in TP, ALT, HDL, ALB, AMYL, Mg, CRP and CHOL levels (p>0.05). It is thought that these data obtained will provide a new perspective on research on animal health, physiology and productivity. It is thought that these analyses performed on the blood parameters of these two breeds with different genetic structures will not only contribute to a better understanding of the metabolic and physiological characteristics specific to these breeds, but will also help illuminate their ability to adapt to various climatic and environmental conditions. It is expected that the data obtained will enable scientifically based decisions to be made in critical areas such as breed selection, feeding strategies and health management in sheep breeding. In particular, it is thought that revealing the biochemical and hematological differences between breeds will contribute to the development of healthier and more efficient animal nutrition methods, as well as provide important clues for animal welfare and sustainable nutrition programs.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

AUTHOR CONTRIBUTIONS

Idea / Concept: VK Supervision / Consultancy: VK, MI Data Collection and / or Processing: MI, Kİ, ÖYÇ Analysis and / or Interpretation: VK, Kİ Writing the Article: VK, MI, Kİ, ÖYÇ Critical Review: MI, Kİ, ÖYÇ

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