



RESEARCH ARTICLE

Comparison of some hematological and serum biochemical variables in Kangal Akkaraman, Texel and Île De France ewes in lactation period within Sivas province

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Sivas ilinde laktasyon dönemindeki Kangal Akkaraman, Texel ve Île De France koyunlarında bazı hematolojik ve serum biyokimyasal değişkenlerin karşılaştırılması

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Öz

Amaç: Bu çalışmada Sivas ili sınırları içerisinde laktasyon dönemindeki Kangal Akkaraman, Texel ve Île de France koyun ırklarında bazı hematolojik ve biyokimyasal değişkenlerin karşılaştırılması amaçlanmıştır.

Gereç ve Yöntem: Çalışmada klinik olarak sağlıklı, gebe olmayan ve laktasyondaki (40-60 gün) üç ırktan (Kangal Akkaraman = 40, Texel = 40, Île de France = 40) toplam 120 koyun kullanıldı. Hematolojik ve biyokimyasal değişkenlerin analizi otomatik sayma cihazı ve otoanalizör cihazı ile gerçekleştirildi.

Bulgular: Üç koyun ırkında WBC, Hb, MCHC, MPV düzeylerinde anlamlı fark yoktu ($p>0.05$). Kangal Akkaraman koyunda RBC değeri diğerlerinden daha yüksekti ($p<0.05$). PCV değerleri Kangal Akkaraman ve Texel koyunlarında Île de France koyunlarından daha yüksekti ($p<0.05$). MCV ve MCH değerleri Kangal Akkaraman ve Île de France koyunlarında Texel koyunlarından daha düşüktü ($p<0.05$). Kangal Akkaraman koyunlarında PLT ve RDW değerleri daha yüksek olmasına rağmen ($p<0.05$), PDW değeri diğerlerine göre daha düşüktü ($p<0.05$). Texel koyunlarında serum glikoz seviyesi diğerlerine göre daha düşüktü ($p<0.01$). Serum total kolesterol, trigliserid, AST, ALT, albümin/globulin oranı ve Mg düzeylerinde üç koyun ırkı arasında fark yoktu ($p>0.05$). Kangal Akkaraman koyunlarında serum total protein, albümin, globulin ve P düzeyleri diğerlerinden daha yüksekti ($p<0.05$), buna karşın serum Ca düzeyleri diğerlerine göre daha düşüktü ($p<0.05$).

Öneri: Bu sonuçlar Sivas ilinde yetiştirilen bu üç koyun ırkı için referans değer oluşturabilir, hastalıklarının tanı ve prognozu hakkında bilgi verebilir ve gelecekteki araştırmalara temel oluşturmaya yardımcı olabilir.

Anahtar kelimeler: Biyokimya ve Hematoloji, Koyun, Île de France, Kangal Akkaraman, Texel.

Abstract

Aim: In this study, it was aimed to compare some hematological and biochemical variables in Kangal Akkaraman, Texel, and Île de France sheep breeds in the lactation period in Sivas province.

Materials and Methods: A total of 120 ewe from three breeds (Kangal Akkaraman = 40, Texel = 40, Île de France = 40) who were clinically healthy non-pregnant and lactating (40-60 days) were used in the study. Hematological and biochemical variables analysis were performed using automatic cell counting and autoanalyzer devices.

Results: There was no significant difference in WBC, Hb, MCHC, MPV levels between ewe breeds ($p>0.05$). RBC value was higher in Kangal Akkaraman than others ($p<0.05$). PCV values were higher in Kangal Akkaraman and Texel than Île de France ($p<0.05$). MCV and MCH values were lower in Kangal Akkaraman and Île de France compared to Texel ($p<0.05$). Although the PLT and RDW values were higher in Kangal Akkaraman ($p<0.05$), the PDW value was lower than others ($p<0.05$). Serum glucose level was lower in Texel than others ($p<0.01$). Serum total cholesterol, triglyceride, AST, ALT, albumin/globulin ratio, and Mg levels were not different between ewes ($p>0.05$). Serum total protein, albumin, globulin, and P levels were higher in Kangal Akkaraman compared to others ($p<0.05$). However, serum Ca levels were lower in Kangal Akkaraman than others ($p<0.05$).

Conclusion: These results can constitute a reference value in these three sheep breeds reared in Sivas province, and help form a basis for future research.

Keywords: Biochemistry and Hematology, Ewe, Kangal Akkaraman, Île de France, Texel.





Introduction

Sheep farming is a productive activity all around in the world, taking advantage of degraded meadows, stubble, fallow, and fields where crop production is limited, turning them into products such as meat, milk, wool, and leather. Rural consumption habits and factors such as Turkey's more suited to sheep farming as the natural resource sector has caused it to become widespread in the country (Kaymakçı and Sönmez 1996). According to recent statistics, there are more than 42 million sheep in Turkey (TUIK 2020). Sheep farming in Turkey has been primarily based on native breeds, from the earliest period in which the country was founded, the crossbreeding of new species to increase milk and wool production, and work on production is done (Kaymakçı et al 2001). For this purpose, Texel and Île de France sheep, which are meat breeds with high productivity, are increasingly popular among sheep breeds imported into the country today and are used in crossbreeding studies (Yılmaz et al 2012). Akkaraman sheep, which is an important breed with economic value in the country, has an important genetic value, has adapted well to the current natural conditions of the country and constitutes approximately 45.8% of the country's sheep population (Koçkaya 2019). Kangal Akkaraman sheep, which is an important species of the Akkaraman breed, is widely produced within the borders of Sivas and neighboring provinces (Koçyiğit et al 2018).

Assessment of hematological and biochemical profiles has become an important tool for diagnostic purposes in human and veterinary medicine. Interpretation of the clinical findings, hematology and biochemistry results and the results of other paraclinical tests may suggest a specific differential diagnosis or prognosis (Braun et al 2010, Polizopoulous 2010, Acharya et al 2015). Measurement of hematological and biochemical values can provide objective information about the status of an animal at the time of sampling, reveal nutritional status, disease conditions, or stress (Pérez et al 2003). Specific reference ranges for each animal species need to be established to compare hematological and biochemical values and to ensure that the results are interpreted appropriately (Meyer and Harvey 2004). Reference ranges, usually defined as values of a particular variable in a healthy population, provide the baseline on which these measurements are interpreted. Well-structured reference intervals are a prerequisite for screening and diagnostic tests aimed at evaluating health and disease in a particular population.

Hematological and biochemical variables that change with lactation in ewes have been reported in previous studies (Mašek et al 2007, Antunović et al 2011). However, we have not found a study comparing some hematological and biochemical variables of Texel and Île de France ewes imported in Turkey and these variables in lactation with the local sheep breed Kangal Akkaraman. In this study, some hematological

and biochemical variables of Kangal Akkaraman, Texel, and Île de France ewes raised within the borders of Sivas province will be presented comparatively.

Material and Methods

The study was conducted with the permission obtained from the Ministry of Agriculture and Forestry (E-80110403-325.04.02-172659) and Sivas Cumhuriyet University Animal Experiments Ethics Committee (Approval No: 65202830-050.04-486). This study was carried out in January 2021 in a commercial enterprise located in Sivas Province (Altitude: 1324, Latitude: 39 ° 51 '35' ', Longitude: 36 ° 20' 22 ' '). A total of 120 non-pregnant and in lactation (40-60 days) ewes in three breeds, aged 2 to 5, were used in the study (Kangal Akkaraman = 40, Texel = 40, Île de France = 40). Ewes were housed under the same feeding and care conditions and fed freely with the addition of hay and grain. All ewes were clinically healthy and free of internal and external parasites.

Blood samples were collected from the jugular vein with 22 G needles at the same time intervals (10.00 a.m.-12.00 p.m.) throughout the study and collected in Vacutainer tubes containing EDTA as anticoagulant for hematology and BD Vacutainer® SST II gel tubes for biochemical analysis. Hematological variables were analyzed with an automatic complete blood count device (Mindray BC 2800) from blood samples taken into tubes containing EDTA within 4 h. White blood cell (WBC), red blood cell (RBC), hemoglobin (Hb), hematocrit (PCV), mean red blood cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelet (PLT), mean platelet volume (MPV) and platelet distribution width (PDW) levels were determined the complete blood count. To extract serum samples, blood samples taken into the gel tubes were allowed to clot for 30 min at room temperature and centrifuged at 5.500 rpm for 10 min. Serum samples were stored at -20°C until biochemical analysis. Serum analysis was done with an autoanalyzer (Mindray BS 200), and total cholesterol, triglyceride, AST, ALT, total protein, albumin, globulin, albumin/globulin, Magnesium (Mg), Calcium (Ca), Phosphorus (P) levels were determined.

Statistical analysis

The data obtained were statistically analyzed using the SPSS 26 program. Results are given as mean \pm S.E.M. The conformity of data to normal distribution was determined by the Kolmogorov-Smirnov test. The analysis was performed using one-way ANOVA and Student-Newman-Keuls (SNK) test was used as post hoc tests. P-value <0.05 was considered statistically significant.



Table 1. Comparison of some hematological parameters of different sheep breeds

Hematological parameters	Unit	Kangal Akkaraman	Texel	Île de France	P value
WBC	$\times 10^9/L$	9.29 ± 0.66^{NS}	7.64 ± 0.60^{NS}	9.38 ± 0.69^{NS}	0.103
RBC	$\times 10^{12}/L$	9.29 ± 0.37^a	8.33 ± 0.53^{ab}	7.42 ± 0.37^b	0.020
Hb	g/dL	10.54 ± 0.39^{NS}	10.07 ± 0.56^{NS}	8.86 ± 0.45^{NS}	0.051
PCV	%	33.3 ± 1.05^a	32.75 ± 1.71^a	28.20 ± 1.32^b	0.030
MCV	fL	36.25 ± 0.95^a	40.27 ± 1.09^b	38.33 ± 0.65^{ab}	0.014
MCH	pg	11.32 ± 0.21^a	12.25 ± 0.31^b	11.90 ± 0.15^{ab}	0.034
MCHC	g/dL	31.59 ± 0.55^{NS}	30.60 ± 0.20^{NS}	31.26 ± 0.38^{NS}	0.182
RDW	%	16.70 ± 1.35^a	14.04 ± 0.19^b	14.08 ± 0.29^b	0.025
PLT	$\times 10^9/L$	971.71 ± 154.19^a	178.45 ± 22.43^c	587.66 ± 67.23^b	<0.001
MPV	fL	5.58 ± 0.32^{NS}	4.96 ± 0.11^{NS}	4.97 ± 0.11^{NS}	0.054
PDW	%	16.17 ± 0.15^a	16.70 ± 0.17^b	16.58 ± 0.13^{ab}	0.044

NS: Not significant. ^{a, b, c, ab}: Different letters in rows represent the difference between groups. WBC: White Blood Cell, RBC: Red Blood Cell, Hb: Hemoglobin, Hct: Haematocrit, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration, RDW: Red Blood Cell Distribution, PLT: Platelets, MPV: Mean Platelet Volume, PDW: Platelet Distribution Width, L: Liter, g/dL: gram/deciliter, %: percentage, fL: femtoliter, pg: picogram.

Results

Table 1 shows the statistical comparison of hematological variables. There was no statistically significant difference in WBC, Hb, MCHC, MPV values between sheep breeds ($p>0.05$). RBC values of Kangal Akkaraman ewe were statistically higher than Île de France ewes ($p<0.05$). However, RBC values of Texel ewe were not different from those of other sheep breeds ($p>0.05$). Kangal Akkaraman and Texel ewes had higher PCV values than Île de France ewe ($p<0.05$). However, there was no statistically significant difference between Akkaraman and Texel ewes PCV values ($p>0.05$). Kangal Akkaraman ewe had lower MCV and MCH values than Texel ewe ($p<0.05$), but there was no difference between MCV and MCH values of Île de France ewe ($p>0.05$). There was no difference between the MCV and MCH values of Texel and Île de France ewes ($p>0.05$). While there was no statistically significant difference in RDW values in Île de France and Texel ewes ($p>0.05$), it was statistically lower than Kangal ewe ($p<0.05$). Although the lowest PLT number was in Texel ewe, the highest value was observed in Kangal Akkaraman ewe ($p<0.001$). Furthermore, PDW values were the highest in

Texel ewe, the lowest was determined in Kangal Akkaraman ewe ($p<0.05$).

Table 2 shows the statistical comparison of biochemical variables. There was no statistically significant difference between the three sheep breeds in serum total cholesterol, triglyceride, AST, ALT, Mg, and albumin/globulin ratio values ($p>0.05$). While there was no statistically significant difference between the serum glucose levels in Kangal Akkaraman and Île de France ewes ($p>0.05$), the serum glucose values of these two ewes were higher than that of the Texel ewe ($p<0.01$). Serum total protein, albumin, and globulin levels were higher in Kangal Akkaraman ewe compared to Texel and Île de France ewe ($p<0.01$, $p<0.001$, $p<0.05$). However, there was no statistically significant difference between Texel and Île de France ewes ($p<0.05$). Serum Ca levels were lower in Kangal Akkaraman ewe compared to Texel and Île de France ewes ($p<0.05$). However, there was no statistically significant difference between Texel and Île de France ewes ($p>0.05$). Serum P levels were higher in Kangal Akkaraman ewe compared to Texel and Île de France ewes ($p<0.01$). However, there was no statistically significant difference between Texel and Île de France ewes ($p>0.05$).



Table 2. Comparison of some biochemical parameters of different sheep breeds

Biochemical parameters	Unit	Kangal Akkaraman	Texel	Île de France	P value
Glucose	mg/dL	61.39 ± 1.44 ^a	53.07 ± 0.90 ^b	66.45 ± 4.07 ^a	0.003
Total Cholesterol	mg/dL	61.14 ± 3.76 ^{NS}	62.50 ± 3.46 ^{NS}	53.52 ± 2.60 ^{NS}	0.125
Triglyceride	mg/dL	13.34 ± 1.24 ^{NS}	14.82 ± 2.42 ^{NS}	12.93 ± 1.58 ^{NS}	0.745
ALT	u/L	21.54 ± 1.24 ^{NS}	19.35 ± 1.61 ^{NS}	19.43 ± 1.58 ^{NS}	0.519
AST	u/L	88.61 ± 2.75 ^{NS}	84.78 ± 3.87 ^{NS}	86.35 ± 3.88 ^{NS}	0.754
Total Protein	g/dL	8.71 ± 0.15 ^a	7.89 ± 0.18 ^b	7.80 ± 0.14 ^b	0.001
Albumin	g/dL	2.98 ± 0.06 ^a	2.79 ± 0.04 ^b	2.65 ± 0.06 ^b	<0.001
Globulin	g/dL	5.72 ± 0.17 ^a	5.11 ± 0.18 ^b	5.15 ± 0.11 ^b	0.013
Albumin/Globulin	g/dL	0.53 ± 0.02 ^{NS}	0.56 ± 0.02 ^{NS}	0.52 ± 0.01 ^{NS}	0.297
Mg	mg/dL	2.89 ± 0.14 ^{NS}	3.28 ± 0.07 ^{NS}	3.17 ± 0.13 ^{NS}	0.069
Ca	mg/dL	8.62 ± 0.18 ^a	9.13 ± 0.18 ^b	9.23 ± 0.08 ^b	0.018
P	mg/dL	6.01 ± 0.19 ^a	5.18 ± 0.13 ^b	5.14 ± 0.24 ^b	0.004

NS: Not significant. ^{a, b, ab}: Different letters represent the difference between groups. AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase, Mg: Magnesium, Ca: Calcium, P: Phosphorus, mg/dL: milligram/deciliter, g/dL: gram/deciliter, u/L: unite/liter.

Discussion

Routine clinical evaluation of blood variables has become an important tool for diagnosing health problems. The interpretation of hematological and biochemical parameters with clinical findings for each parameter can help veterinarians reach a definitive diagnosis in different sheep breeds (Braun et al 2010, Polizopoulos 2010). This study was compared some hematological and biochemical variables in Kangal Akkaraman and Texel and Île de France ewes. It has been reported that the hematological and biochemical parameters of animals may vary according to factors such as age and sex (Íriadam 2004, Şimşek et al 2015). Compared to other sheep breeds serum hematological and biochemical reference values for domestic sheep may differ greatly. This situation can be associated with breed characteristics, physiological status, geographical conditions, temperature, humidity, climatic conditions, feed, and grass quality (Rahman et al 2018).

WBC value is affected by bacterial, viral, and parasitological diseases, malnutrition, and stress (Kısadere et al 2018). On

the other hand, a slight decrease in WBC, RBC and Hb levels was reported in the blood of lactating ewe (Antunović et al 2011). Similar results have been reported in Baladi goats (El-Tarabany et al 2018). A decrease in the number of basophils, monocytes, and WBC in sheep blood during lactation indicates that they migrate from blood to milk (Antunović et al 2017). In this study, WBC values for three sheep breeds were within normal reference limits and there was no difference between them. In a previous study, it was reported that a significant decrease in RBC, PCV, and Hb values was observed in lactating ewe and does (Salem 2017). The decrease in RBC, Hb, and PCV during lactation can be attributed to the hemodilution effect resulting from the increase in plasma volume and/or increased water mobilization to the mammary gland via the vascular system (El-Sherif and Assad 2001). In our study, RBC, PCV, RDW, and PLT values were lower in Île de France ewe compared with Kangal Akkaraman ewe. In addition, RDW and PLT values were lower in Texel ewe than in Kangal ewe. In addition to hemodilution, increased RDW may indicate that anisocytosis is high in Kangal Akkaraman ewe (Constantino 2013). MCV, MCH, and MCHC are cha-



characteristics of RBC that show average cell size, average cell Hb content, and average cell Hb concentration, respectively (Jones and Allison 2007). In our study, the higher MCV and MCH values in Texel ewe compared to Kangal Akkaraman ewe show that erythrocyte volume and erythrocyte Hb are higher in Texel ewe than in Kangal Akkaraman ewe. PDW is a more specific indicator of platelet activation than MPV, as it is not elevated during single platelet distension caused by platelet swelling, and therefore the combined use of MPV and PDW can predict coagulation activation more efficiently (Vagdatli et al 2010). In our study, PDW values were below the reference values in three sheep breeds. However, the PDW value in Texel ewe was higher than Kangal Akkaraman ewe. PDW values of Île de France ewe were not different from other sheep breeds. There was no difference in MPV value between the three sheep breeds. It can be predicted that Texel ewe in lactation is more prone to blood clotting tendency than others. These results may stem from many factors such as an adaptation to Sivas province conditions (geographical location, season, day length, altitude), milk production, breed characteristics, genetics, drug management, and seasonal change (Reece et al 2015).

Biochemical parameters can help veterinarians assess the health status and metabolic activities of animals. Many physiological factors can affect the physiological levels of blood variables (Yiğit et al 2002, Bozdoğan et al 2003, Şimşek et al 2015). Blood triglycerides are an important component in milk fat synthesis (Nazifi et al 2002). Negative energy balance during lactation decreases triglycerides and cholesterol (Antunović et al 2011). In addition, although there was no difference in serum cholesterol levels in different periods of lactation in Baladi goats in a previous study (El-Tarabany et al 2018), another study showed that cholesterol levels increased during late lactation in Istrian × East Friesian crossbreed ewes (Mašek et al 2007). In this study, there was no difference between animals' serum total cholesterol and triglyceride levels. The increase in AST and ALT activity in the blood of lactating sheep indicates an increase in hepatic metabolism (Antunović et al 2011). In this study, there was no difference between the three sheep breeds in serum AST and ALT levels. Glucose is essential in the production of proteins and lipid metabolism. Blood glucose level decreases due to the continuous loss of energy with milk in lactating ewe. Increased glucose usage for milk lactose synthesis and inadequate nutritional intake impair glucose homeostasis (Pambu-Gollah et al 2000). In this study, serum glucose level was higher in Kangal Akkaraman and Île de France ewes than Texel ewe. Lactation is considered a stress on protein metabolism (Amer et al 1999). Significant reduction in protein profile may be due to anemia (Radostits 2007) and milk production requirement (Abdelrahman and Aljumaah 2012). In our study, serum total protein, albumin and globulin, were higher in Kangal Akkaraman ewe compared to Texel and Île de France ewes, but these parameters were not different

between Texel and Île de France ewes. Interestingly, the albumin/globulin ratio was not different between the groups.

Although the serum Ca level was the lowest, the P level was the highest in Kangal Akkaraman ewe, there was no difference in Ca and P levels between the other two sheep breeds. The decrease in Ca concentration in the blood results from the restriction of nutrient intake due to the social hierarchy in pen (Larsen et al 1986). In addition, it is related to the increased secretion of Ca through milk and rearrangement in the bone following delivery and early lactation in the ewe (Liesegang et al 2007). Abdelrahman et al (2002) also found lower Ca concentrations in the blood of lactating sheep than reference values. It was found that the P-inorganic concentrations in the serum of ewes increased following delivery (Özyurtlu et al 2007). Similar findings were found about Ca and P-inorganic levels in blood samples taken from Istrian × East Friesian crossbreed ewes in lactation (Mašek et al 2007). Because of the physiological mechanism, Ca ion is mobilized from bone and blood to milk in a similar way as P ion during lactation. However, P ion concentration in the blood is higher, as more Ca passes into milk than P. It was found that the P-inorganic concentrations in the serum of ewes increased following delivery. These changes in biochemical parameters that are within the reference values (Altıntaş and Fidancı 1993, Turgut 2000, Kurt et al 2008) may result from adaptation to Sivas province conditions (geographic location, season, day length, altitude) and breed characteristics. In addition, genetics, season changes, feed quality, and differences in feed conversion ratio can affect biochemical data.

Conclusion

These results are the first results comparing the hematological and biochemical variables between Kangal Akkaraman ewe and Texel and Île de France ewes in the lactation within Sivas province. Although some hematological and biochemical parameters are within the reference limits; statistical differences were observed between sheep breeds. These values can serve as a reference value in Kangal Akkaraman, Texel, and Île de France ewes in Sivas province and can help form the basis for the diagnosis, prognosis, and future research of different diseases.

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Conflict of Interest

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Ethical Approval

The study was conducted with the permission obtained from the Ministry of Agriculture and Forestry (E-80110403-325.04.02-172659) and Sivas Cumhuriyet University Animal Experiments Ethics Committee (Approval No: 65202830-050.04-486).

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