Versatile analysis of some biochemical and hematological parameters of sheep

ABSTRACT

Biochemical, whole blood hematological and macro-mineral values are critical health and disease status parameters, which depend on animals' conditions, age, gender, breed, region, husbandry, geographical differences, seasonal changes, rearing location and diet. In this study, all parameters were measured in blood samples obtained from healthy Akkaraman sheep, composed of lambs and adults of both sexes. Analyses of the parameters were carried out with commercial assay kits. When comparing the values of the biochemical variables, a significant difference ($P<0.05$) between the four groups was observed in the concentrations of the following variables evaluated: glucose, total bilirubin, urea, and creatinin. A significant difference ($P<0.05$) between the four groups was found in the concentrations of the following variables: evaluated red blood cell, mean red blood cell volume, mean red blood cell hemoglobin, platelets and mean platelet volume. When comparing the values of the variables, a significant difference ($P<0.05$) between the four groups was observed in the concentrations of iron. The values of calcium and phosphorus were not found statistically significant. With a summary of the current literature, it could not be found a detailed study on advanced biochemical, mineral and hematological reference values for Akkaraman breeds in Aksaray region. Thus, our goal is to identify and present the values for the total biochemical and hematological parameters of Akkaraman sheep raised in the Aksaray region of Turkey. It is expected that these results may be used as reference values for Akkaraman sheep in this region.

Keywords: Age, akkaraman sheep, biochemical parameters, gender, hematological parameters, mineral assay

INTRODUCTION

The analysis of blood content contributes to detailed check up the organisms. So, blood as a vital tissue, plays a unique role in the metabolic, physiological, nutritional and also pathological status of a living organism. Blood biochemistry is the primary diagnostic tool in determining the health status of and in investigating diseases in human and animal (Braun et al., 2010 and Onasanya et al., 2015). Factors such as nutrition, stress, temperature-climate, seasonal differences, disease, muscle activity, age, sex and race affect the physiological values of parameters biochemically, and these conditions are important in clinical biochemistry (Gunduz, 2000). The determination of specific reference intervals is important for both clinicians and academicians so they can accurately interpret biochemical values in different animal species and breeds (Kaneko et al., 2008; Meyer and Harvey, 2004). The primary use of clinical biochemistry in human and animal are following up on an individual's health status, diagnosing disease and monitoring treatment (Braun et al., 2010).

How to cite this article
Aksoy, NH., Karaşahin, T., Dursun, Ş., Akbulut, NK., Haydardedeoğlu AE., İlgun, R., Büyükleblebici, O. (2021). Versatile analysis of some biochemical and hematological parameters of sheep. *Journal of Advances in VetBio Science and Techniques*, *6*(2), 130-141. https://doi.org/10.31797/vetbio. 944852

Research Article

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Article info
Submission: 29-05-2021
Accepted: 27-08-2021
Online First: 29-08-2021

e-ISSN: 2548-1150
doi prefix: 10.31797/vetbio
- http://dergipark.org.tr/vetbio

Biochemical profiles provide reliable information on the health status of animals and reflect the animal's responsiveness to its internal and external environment (Onasanya et al., 2015). Because there are so many different ovine breeds and production systems differ so much among regions and countries, reference values for sheep definitely show variations. On the basis of data from the "Clinical and Laboratory Standards Institute (CLSI)", it is recommended that reference intervals be produced or validated by each laboratory with its own techniques in a similar as possible population of animals (Braun et al., 2010; CLSI, 2010).

Nowadays the hematological analyzes are easy, quick and dependable keys of clinical monitoring (Braun et al., 2010; Coles, 1986; Onasanya et al., 2015; Polizopoulou, 2010; Rahman et al., 2018). Hematology is a science that deals with whole blood physiology, metabolism, and biochemistry. It investigates the physiological and pathological states of cells and proteins found in blood (Bush 1991; Coles, 1986; Polizopoulou, 2010). Also, interpretation of hematological blood results which crucial for clinicians and academicians mainly depend on the determination of specific reference intervals (Kaneko et al., 2008; Meyer and Harvey, 2004; Simpraga et al., 2013; Vojta et al., 2011). Hematological diagnostic methods and processes have become a necessary and fundamental part of the minimum base of information for determining health status and the diagnostic observation of ovine medical problems. The widespread use of advanced hematological instruments, kits and materials that can process small ruminant blood samples has made blood assays important diagnostic methods in managing sheep health (Polizopoulou, 2010). For healthy ovine as with other mammals, nutritional status, age, sex and breed of the sheep as well as the season and environmental conditions should be considered when determining hematological reference values and ranges, as in biochemical parameters. The value of blood sample diagnosis lies in its ability to identify the effects of diseases on blood cells and platelets. Blood composition is continuously variable. Rapid modifications could occur as a response to multiple physiological events initiated by physiological or pathological stress. In living organisms, especially in mammals, identification of hematological values with specific reference intervals based on breed-specific differences is vital in disease prognosis and treatment processes, in both academic and clinical prospects (Polizopoulou, 2010).

Most of the elements play important roles in maintaining health, and normal metabolic balance and growth in human and animals (Carlson, 2008; Rucker et al., 2008). Minerals are involved in all biochemical mechanisms, as structural elements and as regulators of almost all metabolic course (Kaneko, et al., 2008). In living organisms, macrominerals fulfill many vital functions in biochemical metabolic processes. Minerals act as catalytic co-factors for normal enzyme functions and essential for constituents. Minerals are important also in the synthesis of many hormones that are required for normal functions of basic biochemical and hematological processes in the body. Arrangement of cell replication and differentiation are other regulatory impacts of minerals. Structurally, minerals can form macro-components of organs and tissues, such as calcium and phosphorus (P) (bones and teeth, tendons). Physiologically, minerals play significant roles in body fluids and tissues, as electrolytes (Na, K, Ca, Mg, etc) concerned with the maintenance of osmotic pressure, acid–base balance, membrane permeability and transmission of nerve impulses (Kaneko, et al., 2008; Suttle, 2010; Yatoo et al., 2013). Phosphorus plays a role in numerous biological processes, including energy metabolism and bone mineralization. P, participates in the structure of DNA and RNA and also takes

mainly part in various biochemical pathways such as glycolysis and beta oxidation of fats. As a component of signal transduction, phosphate is used in cyclic AMP and products of deoxyribonucleoside diphosphates (Raina, 2012). The majority of intracellular P is found either as inorganic phosphate esters, phospholipids in cell membranes, or as phosphorylated intermediate molecules involved in a wide variety of biochemical processes, including energy production (ATP), storage and transfer (Favus, 2006; Suttle, 2010). Serum iron levels have both biochemical and hematological importance. The daily requirements of iron vary according to the age, gender, nutritional and physiological status of the individual. Iron is an essential element in the body, but its effect on the body is like a double-edged knife (Harvey 2008a, 2008b; Suttle 2010). Iron ion is critical for Fe-containing proteins, oxygen transport and storage, respiration, DNA synthesis, Krebs cycle, and various enzymatic reactions. However, the same physical properties that allow iron to function as a cofactor in controlled redox biochemistry also make iron potentially toxic to cells, as it catalyzes the formation of the reactive oxygen species (Harvey 2008a).

The identification and use of specific ranges for breeds and species of animals are required to establish reference sites specific to regions where animals are raised and to their racial values (Altintas and Fidanci, 1993; Braun et al., 2010; Meyer and Harvey, 2004). Since these parameters are vital for animal health and may vary depending on different variables, regional reference values for animals are clinically valuable. The goal is to investigate the reference values for clinically healthy Akkaraman sheep breed raised in Aksaray and the nearby environment, by measuring some parameters and considering age and sex-related differences.

MATERIAL and METHOD

Current study protocol involved to take blood from animals following by ethical rules. Animals were separated into four groups based on gender and age. For biochemical and mineral assays, 15 mL of blood was taken from the vena jugularis, in anticoagulant-free serum tubes. The hemolysis-free serum samples were separated from clotted blood samples by centrifugation and stored at -25 °C till analysis (Coles, 1986). Biochemical variables determined in each sample were total protein (TP), glucose (Glc), albumin (Alb), globulin (Glb), triglycerides (TG), total cholesterol (Tot. Chol.), high-density cholesterol (HDL), low-density cholesterol (LDL), total bilirubin (Tot. Bil.), urea, and creatinine (Crea). Analyses of the parameters were carried out with commercial assay kits (Assel, Italy) and a biochemical analyzer (Humalyzer 3000 semi-analyzer, Germany), according to the method of administration of each parameter described in the commercial kit procedure. Analyses of the serum calcium (Ca), phosphorus (P) and iron (Fe) levels were carried out with commercial assay kit (Assel, Italy) and biochemical analyzer (Humalyzer 3000 semi-analyzer, Germany). For hematological assays, the blood samples were taken from the vena jugularis into EDTA-containing hemogram tubes and commercial test kits (Mindray V-28, China) with the auto-hematology analyzer Mindray, BC-2800-Vet (China) were used.

White blood cell-WBC (10^9/L), hemoglobin-HGB (g/dL), hematocrit-HCT (%), red blood cell-RBC (10^{12}/L), mean red blood cell volume-MCV (fL), mean cell hemoglobin-MCH (pg), mean cell hemoglobin concentration-MCHC (g/dL), red cell distribution width-RDW (%), platelet-PLT (10^9/L), mean platelet volume-MPV (fL), platelet dispersion width-PDW and platelet relative volume-PCT (%) variables were measured.

The descriptive statistics for the properties studied were mean, standard deviation, standard error, minimum values and maximum values. Variations among the groups were analyzed by Student t-test. "One-way ANOVA" was fulfilled to crosscheck the group averages in terms of continuous variables. A Duncan multiple comparison test was used to identify the different groups following the analysis of variance. The data are given as the means ± standard error ($\bar{X}$ ±SE). Statistical significance was accepted as $P<0.05$ level.

RESULTS

In this study, various biochemical values were measured according to gender and age, statistical analyses of these values were performed and the results were examined (Table 1).

Table 1. Biochemical findings for four groups of sheep

Parameter (Unit)	Ewes	Female Lambs	Rams	Male Lambs	*P*
Glucose (mg/dl)	68,91±2,07^{c}	77,04±5,17b^{c}	92,86±5,95^{a}	82,51±2,24^{ab}	0.001
Total Protein (g/dl)	11,27±0,43	10,85±0,39	11,39±0,40	11,76±0,31	>0.05
Albumin (g/dl)	5,46±0,28	5,82±0,31	6,18±0,24	6,16±0,39	>0.05
Globulin (g/dl)	5,32±0,31	4,73±0,31	4,94±0,30	5,21±0,31	>0.05
Triglycerides (µg/dl)	138,71±7,40	129,83±3,43	123,45±5,47	123,91±6,30	>0.05
Total Cholesterol (µg/dl)	76,57±2,97	72,03±5,45	72,31±3,10	67,55±4,91	>0.05
HDL Cholesterol (mg/dl)	45,00±1,62	42,86±3,28	42,28±2,85	43,50±4,03	>0.05
LDL Cholesterol (mg/dl)	29,21±2,75	28,23±2,73	28,89±2,01	23,00±1,71	>0.05
Total Bilirubin (mg/dl)	0,61±0,06^{a}	0,42±0.05^{b}	0,65±0,07^{a}	0,65±0.05^{a}	0.020
Urea (mg/dl)	20,07±1,88^{b}	15,25±1,38^{b}	32,01±2,16^{a}	19,62±1,40^{b}	0.000
Creatinine (mg/dl)	0,89±0,087^{b}	0,71±0,038^{b}	1,24±0,08^{a}	0,91±0,07^{b}	0.000

When serum Glc values of healthy groups of different ages and genders were investigated, a higher amount of Glc was detected in males than in females; and the difference was statistically significant ($P<0.05$). Total protein values were the highest in male lambs and the lowest in female lambs, and the amount of protein was higher in ewes than in female lambs; however, neither of these findings were statistically significant ($P>0.05$). It was also observed that the total protein ratios are not age-dependent but sex-dependent, with the protein concentration in males being higher than in females. When the Alb values were examined, it was found that the rams had the highest values and female lambs had the lowest, but these were not statistically significant ($P>0.05$). There were no statistically significant ($P>0.05$) differences between the four groups in the amount of globulin, which was higher in ewes than in female lambs and higher in male lambs than rams. In the present study, regardless of age, measured triglyceride levels were found to be higher in females than in male Akkaraman sheep. The levels of triglycerides, which were also higher in ewes than in female lambs, were slightly different in rams than in male lambs. The triglyceride values of the four groups were not statistically significant ($P>0.05$). Total cholesterol values were observed to be lowest in male lambs and highest in ewes. The cholesterol levels in adults was relatively higher than lambs. Though cholesterol levels were higher in ewes than in rams and lower in male lambs than in female lambs, these results were not statistically significant ($P>0.05$). The ewes had the highest HDL cholesterol compared to the other three groups, but the results were not significant. LDL cholesterol was found to be higher in the adult groups (ewes and rams) than in the lamb groups. The highest LDL cholesterol was in the

ewes, but the difference was not statistically significant. There was no age-related difference in total bilirubin values in males. Looking at gender-dependent differences, the total bilirubin levels were higher in males than in females. In female group, total bilirubin values were higher in ewes than in female lambs and this difference was statistically significant (P<0.05). The higher urea values were found in rams and ewes as compared to lambs (P>0.05). The urea values were found to be statistically significantly and this status applies to all groups. Urea values of rams are higher at statistical significance level compared to all other groups. There is no difference between the other groups. When creatinine was assessed, it was found to be the highest in rams and these results were statistically significant (P<0.05) (Table 1).

With presented study, it could be declared that whole blood hematological parameters in Akkaraman sheep breed (Aksaray region) can be vary on age and gender. The comparative graphics of the total parameters were demonstrated in Figure 1 and the median and mean values and the statistical analysis of the hematological variables of this study are represented in Table 2.

Table 2. Hematological findings of Akkaraman sheep

Parameter (Unit)	Ewes	Female Lambs	Rams	Male Lambs	*P*
WBC (10^9/L)	7,86±0,98	9,08±0,43	9,75±0,55	9,52±1,09	> 0.05
RBC (10^{12}/L)	7,95±0,53^b	9,77±0,34^a	9,96±0,30^a	9,63±0,83^a	0.046
HGB (g/dL)	9,38±0,56	9,33±0,25	9,63±0,36	9,35±0,81	> 0.05
HCT (%)	28,0±1,62	28,95±0,75	29,96±1,27	28,68±2,4	>0.05
MCV (fL)	33,74±0,46^a	29,88±0,67^b	30,83±1,24^b	30,0±0,53^b	0.004
MCH (pg)	11,075±0,20^a	9,55±0,19^b	10,075±0,36^b	9,57±0,17^b	0.000
MCHC (g/dL)	32,05±0,35	32,20±0,41	31,95±0,52	31,52±0,48	>0.05
RDW (%)	22,68±0,42	23,30±0,49	23,70±0,52	24,41±0,51	>0.05
PLT (10^9/L)	261,75±15,31^b	412,75±45,28^a	392,17±46,81^a	401,75±41,18^a	0.031
MPV (fL)	3,96±0,15^a	3,8±0,06^{ab}	3,62±0,05^b	3,58±0,078^b	0.025
PDW (fL)	15,60±0,17	15,27±0,96	15,08±0,21	15,13±0,12	>0.05
PCT (%)	0,10±0,005	0,15±0,016	0,13±0,017	0,21±0,07	>0.05

It was determined that the WBC count was higher in rams and male lambs than in ewes and female lambs. The lowest value was found in ewes; however, the results were not statistically significant. According to the measurements of RBC counts, the highest values belonged to the rams whereas the ewes had the lowest amount of red blood cells. These results were statistically significant (P< 0.05).

The HGB values of Akkaraman sheep were found to be higher in adults than in lambs (both sex). So though the findings were not statistically significant (P> 0.05), there was difference in HGB values depending on age (higher in adults than in lambs), not on sex. HCT values were found to be very close between all groups: no difference was found and these values were found to be statistically insignificant (P> 0.05). When MCV and MCH were examined, it was found that the values were higher in ewes than in rams and lambs of both sexes. Both parameters (MCV P <0.005 and MCH P <0.001) were found to be statistically significant when compared to other 3 groups. The mean MCHC concentrations in female group had higher values than males, but again the data was not found to be statistically significant. The highest RDW value belonged to male lambs whereas the lowest values belonged to ewes. In general, females had lower levels of RDW than males, but the data was statistically insignificant. The highest PLT value was found

in female lambs and the lowest value belonged to ewes. This difference was significant ($P<$ 0.05). MPV was higher in females than males. There was no difference in MPV between the young and adult males. The differences between ewes and rams, and ewes and male lambs were statistically significant ($P<$ 0.05). PDW values were also higher in females than in males, but the difference was not statistically significant ($P>$ 0.05). When relative volumes of PCT were measured and examined statistically, values were highest in lambs and lowest in ewes, but the differences were not statistically significant ($P>$ 0.05). The hematological data obtained in this study will contribute to form of the reference values based on the age and sex of Akkaraman sheep and will also provide valuable clinical information (Figure. 1.).

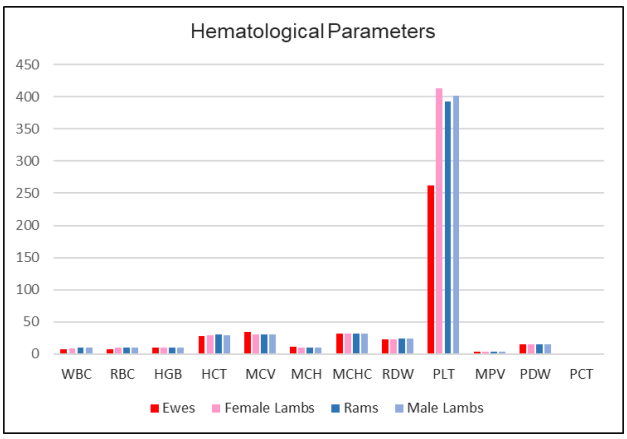

Figure 1. Levels of hematological parameters in four groups of sheep

The values of the iron levels for all groups found statistically significant differences ($P<0.05$) (Table 3).

Table 3. Macromineral levels of Akkaraman sheep

Parameter (*SI* Unit)	Ewes	Female Lambs	Rams	Male Lambs	*P*
P (mmol/L)	2,19±0,13	2,40±0,11	2,00±0,14	2,40±0,12	>0.05
Ca (mmol/L)	3,8±0,22	3,53±0,23	3,20±0,17	3,37±0,719	>0.05
Ca / P Ratio	1,74	1,47	1,60	1,40	
Fe (*μ* mol/L)	22,46±1,11 ^{ab}	19,52±1,35^{b}	24,25±1,40^{a}	24,90±1,50^{a}	0,028

Differences between groups in the values of Ca and P were not statistically significant ($P>0.05$). There was no significant correlation between ewes and rams and the values were statistically insignificant. The Ca levels did not show any significant differences between all four groups. High Ca values detected in ewes were not statistically significant compared to the other groups.

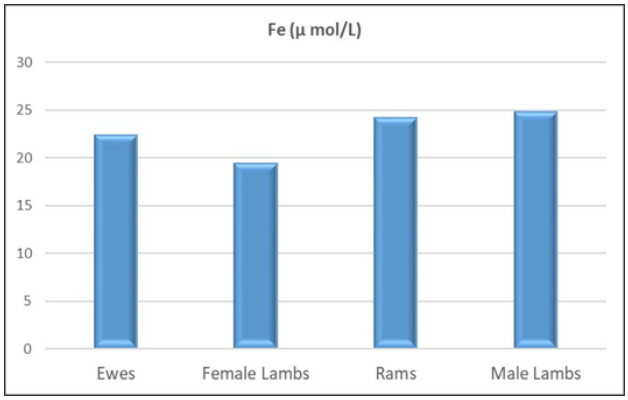

Figure 2. Levels of Fe in four groups of Akkaraman sheep

The highest Fe level was in male lambs. It's found that, ewes had higher Fe values compared to female lambs, which had the lowest iron levels measured ($P<0.05$) (Figure 2 and 3).

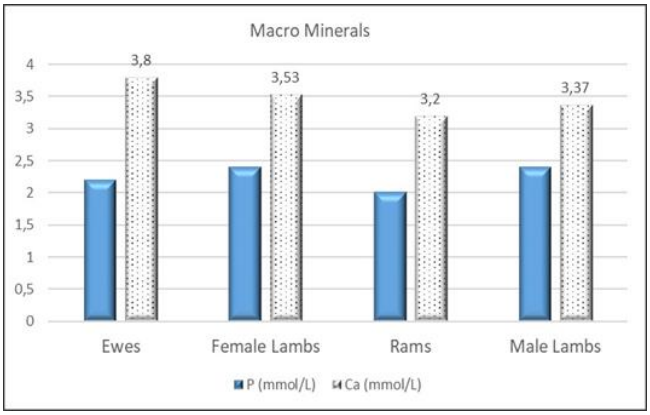

Figure. 3. Serum Ca and P Levels in Akkaraman sheep

DISCUSSION

Blood analyzes are the indispensable distinctive pieces in identifying the health status and following up the treatment in investigating diseases in living beings. At this point, determining the changes in the hematological and biochemical values and mineral levels of the Akkaraman sheep breeds will be very important in terms of the diagnosis and interpretation of disease in these animals as well as the choice of treatment and the follow-up it. Thus, our goal is to identify and present the values for the some important and vital hematological, biochemical parameters and mineral levels of Akkaraman sheep raised in the Aksaray region of Turkey. It is also a major aim of the study to compare the data obtained in the current study with previously identified reference intervals for other sheep breeds.

As mentioned earlier, the unique biochemical blood profile can be influenced by age, gender, nutrition, breed, species, metabolic periods, and environmental and seasonal conditions, which are reflected in its biochemical structure (Cruz et al., 2017). Braun and co-workers recommended that, because there are many different breeds and breeding systems in sheep, each laboratory specify their own reference values and ranges. As a result, there must be different reference ranges (Braun et al., 2010). They and other scientists suggested that seasonal impact is often difficult to discern from a variety of misleading factors, such as food supply and the reproductive condition of female animals (Braun et al., 2010; Yokus et al., 2004, and 2006; Yokus and Cakir, 2006). A study that appraised the physiological alterations caused and influenced by age and gender on biochemical variables in male and female Dorper sheep at different ages. They concluded that age has a significant influence on the values of most biochemical parameters of Dorper sheep aged 15 to 121 days, but that there is no effect based on sex (Cruz et al., 2017). In clinical biochemistry, monitoring of fasting blood Glc levels in every biochemical assay is crucial for tracking health-disease states in all metabolic pathways, especially vital tissues such as the liver. Similar to our study, which we found the variability of glucose levels depending on age and gender in all four groups statistically significant, Durak et al. (2015) demonstrated that the serum Glc levels of the Zom sheep, were significantly lower in the female group than those of male group (P<0.05). Similarly, the researchers declared that for Glc, they found significant differences between groups of different gender and age (Durak et al., 2015). In the current study, while the Glc results were found to be within normal limits for ewes and rams (Kaneko, 2008), they were higher than in the studies performed in different geographical regions, such as Iraq (Fartosi et al., 2010) and Pakistan (Kiran et al., 2012). It was declared that in a study on Akkaraman sheep raised in the Ankara region of Turkey (Altinsaat, 2001), the amount of Glc in healthy ewes and rams was lower, than our adult male and female sheep's glucose results. The possible differences in Glc levels could be attributed to age, gender, physiological and endocrinological changes, nutrition, the individual metabolic activity of animals and geographical differences (Braun et al., 2010; Burtis et al., 2012; Carlos et al., 2015; Kaneko, 2008). Besides these, in three different studies, performed in tropical regions, the biochemical variables glucose and total cholesterol were investigated. Compared with the current study, Glc and Hb values were lower and cholesterol levels were higher. Considering that these studies were conducted in tropical regions, it is understandable that their results are similar to each other and differ from the results of our study. It is certain that gender, geographical distribution, ecological and geological differences, nutritional properties and health conditions can influence hemoglobin levels (Bhat et al., 2014; Kiran et al., 2012; Pradhan, 2016). These values which were obtained in

studies conducted with different sheep breeds; female Karakachan sheep (Stevanović et al., 2015), Dalmatian pramenka sheep (without sex and age differences, total mean) (Vojta et al., 2011), Tsigai sheep (total mean values) (Antunović et al., 2009) and female Lika pramenka sheep (Vugrovečki et al., 2017) had lower total protein, Alb and globulin values than our results. The total protein and its components measured in ewes in the current study were higher than that in the studies conducted in Adıyaman province, Turkey (Kurt et al., 2008) and in Ankara, Turkey (Altinsaat, 2001). In another study the age of the sheep affected the blood chol., trig., urea, crea., prot., and glob. values as well as the Alb concentrations; however, the difference between the different age groups was not significant (Carlos et al., 2015). When the values of determined by Carlos et al., (2015) are compared with our results; in ewes and rams, Glc, tot. prot., alb and glob levels were lower, whereas tot. chol., trig. and urea values were higher. These all differences may due to physiological variations between the breeds as well as geographical distinctions between the different regions. Furthermore, these unsimilarities in the results probably stem from also the different management conditions, climates and nutrition levels of the animals (Vugrovečki et al., 2017). The level of cholesterol, a health indicator for many issues, especially cardiovascular diseases, is the sine qua non of the body (Bruss, 2008; Nelson, and Cox, 2006). Literature information is insufficient with region-specific prior studies of Akkaraman sheep with triglyceride, total cholesterol, HDL, and LDL cholesterol parameters. In studies conducted in different geographical regions, the overall mean values of cholesterol were higher than in our study whereas the overall triglyceride mean values were lower (Fartosi et al., 2010; Kiran et al., 2012). In the study conducted in seven different districts of Adıyaman province, Turkey, the overall mean values of lipid parameters were close to the current study regardless of gender difference (Kurt et al., 2008). In the present study, total bilirubin, urea and creatinine levels, which are closely related to renal and hepatic health, the circulatory system and fluid-electrolyte balance, were also investigated in Kurt's research (2008), while the overall mean values of the urea levels were approximately the same, and creatinine and total bilirubin levels were lower, Altinsaat et al (2001), declared lower levels of the overall mean values of urea, creatinine and bilirubin. Because creatinine is formed by the degradation of phosphocreatine for energy release in skeletal muscle, the serum creatinine is an important marker proportional to the muscle mass (Kreutzer and Turk, 2008; Meyer and Harvey 2004). In the current study, creatinine was the highest in rams and generally higher in males than in females, and these differences were statistically significant ($P<0.05$). In the current study, bilirubin and urea values were highest in rams. So, Akkaraman adults had higher values of urea, crea and bil than in lambs.

Examinations that complement physiological findings and biochemical analyses are extremely important for veterinarians. Descriptive and determinant biochemical investigations carried out in animals raised in different countries, or in different regions of the same country, as well as clinical studies, also provide useful information (Altintas and Fidancı, 1993; Kaneko et al., 2008). These mentioned parameters are used in diagnosing diseases, revealing nutritional disorders, and following up on treatments. They also help to pioneer and assist in future research on these animals. In this completed study, we observe that some values can change with age, some can differ by gender and some parameters can be influenced by both. After all, when all parameters are taken into consideration, differences in region, breed, sex, age, season and nutritional sources affect biochemical values and can cause changes. The

identification and monitoring of these parameters reflecting the metabolic profile show that animals' homeostatic mechanisms maintain blood composition at physiological limits under different conditions (breeds, species, regions, feed-nutrition regimes and age and sex characteristics).

Identifying the biochemical and hematological values of the Akkaraman sheep breeds is crucial for the monitoring general health status, diagnosis of diseases and also choosing of the treatment and to follow this cure. In a study conducted in Bangladesh, obtained that hematological values found significantly different between adult and lamb and between male and female indigenous sheep, respectively health, similarly with our results (Rahman et al., 2018). In 2014, it's determined that the hemogram values obtained, which have great regional and geographical differences, nonetheless have approximate values (with the exception of WBC). The mean WBC values obtained in the current study were high when compared to those in some other (Njidda et al., 2014) and close to normal values in others (Kaneko, 2008), considering both gender and age differences (Celebi and Uzun, 2000). Compared to some other studies, in our research the hemoglobin values were higher in both genders and at different age ranges (Kiran et al., 2012). In present study, RBC values in rams were higher than those obtained by Njidda et al., whereas the values in the females were close (2014). In a hematological study performed in healthy Akkaraman Kangal lambs in the Sivas region of Turkey, the RBC was within the limits of the hemogram values (for lambs) obtained in the present study, the WBC was lower, and the HGB, HCT, MCV and PLT were higher than this study's (Kockaya and Ozsensoy, 2016). The MCV, MCH, MCHC values found in this study were close to those of some other studies carried out in Turkey. The current HCT values were lower in different age and gender groups as compared to the results obtained from Tuj and Morkaraman sheep (Celebi and Uzun, 2000). In our study, the HCT values of four groups were close to each other and similar to the literature (Kaneko, 2008). Firstly, Vojta and co-workers (2011) and later Simpraga and colleagues (Simpraga et al., 2013) and lastly in 2017 (Vugrovečki et al., 2017) presented a model study for reference intervals of organically raised dalmatian pramenka sheep by the robust method. In 2013, it's specified that, these sheep have special hematological and biochemical reference ranges which depend on the conditions, breeding, and food supply, environmental and seasonal influences. According to their findings, it seemed some similarity of values with our results. Although not statistically significant some of our findings (WBC, RBC, HGB, HCT), also differ between upper and lower limit of this study (Simpraga et al., 2013). In the present study, differences in RBC and PLT were found to be significant only in sheep compared to other groups ($P<0.05$). Significant differences in MPV, MCV and MCH were found to be especially in adults statistically significant ($P < 0.05$). Often, blood values are effecting by many factors, genetic and non-genetic. As already mentioned, all these diversities in hematologic parameters may be due to maturity of sheep, metabolism and hormonal differences of sexes. And also depend on the feed, stress, hormonal influence, and environmental status (Vugrovečki et al., 2017). As Etim et al., pointed out, we also believe that it's so vital to build basic indicators for blood variables on the basis of many factors and also perform further researches to define all influences of these factors on these (2014).

Many of the minerals are cofactors of vital catalytic proteins in all metabolic pathways. The most of trace minerals should also be determined in ruminant animals to assign if deficiencies, imbalances and toxicities are present (Balamurugan, et al., 2017; López-Alonso, 2012; McDowell and Arthington,

2005). Since marginal mineral deficiencies can affect growth, development, reproduction, and production and are not frequently diagnosed, animals are considered to be equally as important as mineral deficiencies in which they show clinical signs that can be detected and treated (Suttle, 2010). In our research, the Ca and P values obtained are slightly higher, and Fe values were found lower, than the general reference values. In the study where iron, calcium and phosphorus levels were examined in Sivas-Akkaraman Kangal lambs without discriminating sex, it was observed that in the healthy control group had lower levels compared to our study (Kockaya and Ozsensoy, 2016). Calcium and phosphorus values of our groups were higher and iron levels were observed lower, when compared to the study conducted in Akkaraman sheep raised in the districts of Adıyaman (Kurt et al., 2008). In different studies comparing some biochemical parameters of sheep, reported that calcium and phosphorous values were lower than our study and there were no statistical differences (Angelov et al., 2013; Gürsu and Aygün, 2014; Stevanović et al., 2015; Stojković et al., 2014). As mineral deficiencies may influence all metabolic mechanisms include growth, development and reproduction, they are evenly important as mineral deficiencies in the most living beings display clinical indicators that can be defined and cured (Suttle, 2010). According to these comparisons, it is clear how effective the diet, the type and breed of the animal, regional differences, seasonal differences and lactation period and pregnancy (health status), and also the gender differences are on blood mineral levels (Rucker et al., 2008; Suttle, 2010).

CONCLUSION

It is especially important to know the values of the blood parameters of indigenous breeds, which form a critical component of the economy of our country. Biochemical values obtained in breed studies and regional studies are of great importance among the studies complementing, supporting and strengthening clinical findings. Detection and follow-up of health status provides important information on diagnosis, early diagnosis, etiology, pathogenesis of disease, disease detection and control of the selection and usefulness of applied treatment methods to veterinarians working in the field. It is significant to determine the reference ranges of domestic and region specific breeds' blood parameters, which form a dominant and precious component of the economy of our country. According to the findings presented in the literature, there are variations in the hemato-biochemical reference values for Akkaraman sheep breed, (in Aksaray region) compared to other countries and regions and other sheep species. In sight of all these evaluations, detect and determine the blood values differences would further emphasize the need to establish appropriate health baseline values (physiological, hemato-biochemical, metabolic, pathological, etc.) for livestock in Turkey in establishing the physiological status of farm animals. It is therefore important to perform serum hemato-bio-chemistry and mineral profiles in livestock in order to detect and prevent imbalances that can lead to reduced production and reproductive disturbances and as a result, economic losses.

At this point, the identification, calculation and use of biochemical reference intervals for species variations in breed and region will be the most useful. In this regard, we believe that this study will aid and help to propagate future research. In further, it is planned to make researches with more detailed variables in order to create reference intervals in the hematological and biochemical blood values of Akkaraman sheep.

ACKNOWLEDGMENT

Authors' Contributions: Dr. Aksoy, actively worked in every stage of the study, identified the principles of the study, conducted the laboratory studies, made the calculations and general evaluations and realized and wrote the manuscript. Dr. Karasahin and Dr. Dursun participated in fieldwork and worked at all stages of blood taking from animals, and statistical calculations. The other authors have been helpful in revising some parts of the article.

Ethical approval: Bahri Dağdaş International Agricultural Research Institute, Directorate of Local Ethics Committee of Animal experiments (14.01.2015/35, No 0088)

Financial support This project was supported and financed by Aksaray University Scientific Research Projects Coordination Unit (P No: 2015-084).

Conflict of interest: The authors have nothing to disclose. There is no conflict of interest exist.

KAYNAKLAR

Altinsaat, C. (2001). Relationship between vitamin B12 and folic acid levels and some hematological and biochemical values in Akkaraman sheep. *Ankara Universitesi Veteriner Fakultesi Dergisi*, *48*, 141-145.

Altintas, A., & Fidancı U.R. (1993). Normal biochemical values of pets and human blood. *Ankara Universitesi Veteriner Fakultesi Dergisi*, *40*, 173-186.

Angelov, G., Dimitrova, I., Mehmedov, T., Stamberov, P., Stancheva, N., Georgieva, S., & Nakev, Zh. (2013). Comparative study of some biochemical indicators in Karakachan and Copper-Red Shumen sheep breeds. *Agricultural Science and Technology*, *5*(4), 391-393.

Bhat, S.A., Mir, M.R., Reshi, A.A., Ahmad, S.A., Husain, I., Bashir, S., & Khan, H.M. (2014). Impact of age and gender on some blood biochemical parameters of apparently healthy small ruminants of sheep and Goats in Kashmir valley. *International Journal of Agricultural Sciences and Veterinary Medicine*, *2*, 2-8.

Braun, J.P., Trumela, C., & Bézille, P. (2010). Clinical biochemistry in sheep: A selected review. *Small Ruminant Research*, *92*, 10–18.

Bruss, M.L. (2008). Clinical Biochemistry of Domestic Animals, *Chapter 4: Lipids and Ketones*. 6th Edition Academic Press, Elsevier. pp.81-116 (ISBN: 978-0-12-370491-7).

Burtis C.A., Ashwood, E.R., & Bruns, D.E. (eds) (2012). Tietz Textbook of Clinical Chemistry and Molecular Diagnosis (5th edition). Elsevier. pp. 909. (ISBN: 978-1-4160-6164-9).

Bush, B.M. (1991). *Interpretation of Laboratory Results for small animal*. Clinician Blackwell scientific Publication, London.

Carlos, M.M.L., Leite, J.H.G.M., Chaves, D., Vale, A.M., Façanha, D.A.E., Melo, M.M., & Soto-Blanco, B. (2015). Blood parameters in the Morada Nova sheep: influence of age, sex and body condition score. *The Journal of Animal & Plant Sciences*, *25*(4), 950-955.

Carlson, G.P., & Bruss, M. (2008). Clinical Biochemistry of Domestic Animals, Academic Press, Elsevier, 6th Edition. *Chapter 17: Fluid, Electrolyte, and Acid-Base Balance* pp.529-530. (ISBN: 978-0-12-370491-7).

Celebi, F., & Uzun, M. (2000). Some haematological values of Tuj and Morkaraman sheep. *Veteriner Bilimleri Dergisi*, *16*(1), 103-108.

CLSI, Clinical and Laboratory Standards Institute (2010). *Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory*, Approved Guideline, 3rd ed. CLSI, Wayne, PA, USA.

Coles, E.H. (1986). *Veterinary Clinical Pathology* 4th Edition. W.B. Saunders Co. Philadelphia.

Cruz, R.E.S., Rocha, F.M., Sena, C.V.B., Noleto, P.G., Guimarães, E.C., José, A.G.J.A., & Mundim, A.V. (2017). Effects of age and sex on blood biochemistry of Dorper lambs. *Semina-ciencias agrarias journal*, *38*, 3085.

Durak, M.H., Erkan, R.E.C., Celik, R., Yokus, B., Kurt, D., & Gurgoze, 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*(2), 33-39.

Etim, N.A., Williams, M.E., Akpabio, U., & Offiong, E.E.A. (2014). Haematological Parameters and Factors Affecting Their Values. *Agricultural Science*, *2*(1), 37-47.

Favus, M.J., Bushinsky, D.A., & Lemann, J.Jr., (2006). American Society for Bone and Mineral Research, *Chapter 13: Regulation of Calcium, Magnesium, and Phosphate Metabolism*. pp.76-117.

Gunduz, H. (2000). Seasonal variations of some biochemical parameters in Holstein cows. *Yüzüncü Yıl Üniversitesi Veteriner Fakultesi Dergisi, 11*(2), 50-53.

Gürsu, G., & Aygün, T. (2014). Serum Calcium, Potassium, Phosphorus and Cobalt Levels of Awassi Ewes Maintained at Village Conditions during Lactation Period. *Asia-Pacific Chemical, Biological & Environmental Engineering Society Procedia*, *8*, 6-10. https://doi.org/10.1016/j.apcbee.2014.01.072.

Harvey, J.W., (2008a). *Chapter 7: The Erythrocyte: Physiology, Metabolism, and Biochemical Disorders.* Clinical Biochemistry of Domestic Animals. 6th Edition Academic Press, Elsevier, pp.173-240 (ISBN: 978-0-12-370491-7).

Harvey, J.W., (2008b). *Chapter 9: Iron Metabolism and Its Disorders.* Clinical Biochemistry of Domestic

Animals, 6th Edition Academic Press, Elsevier, pp.259-286 (ISBN: 978-0-12-370491-7).

Kaneko, J.J. (2008). *Chapter 3: Carbohydrate Metabolism and Its Diseases.* In Clinical Biochemistry of Domestic Animals, 6th Edition Academic Press, Elsevier, pp.45-80 (ISBN: 978).

Kaneko, J.J., Harvey, J.W., & Bruss, M.L. (2008). Clinical Biochemistry of Domestic Animals 6th Edition. Academic Press N.Y.

Kiran, S., Bhutta, A.M., Khan, B.A., Durrani, S., Ali, M., Ali, M., & Iqbal, F. (2012). Effect of age and gender on some blood biochemical parameters of apparently healthy small ruminants from Southern Punjab in Pakistan. *Asian Pacific Journal of Tropical Biomedicine, 24,* 304-306. doi:10.1016/S2221-1691(12)60028-8.

Kockaya, M., & Ozsensoy, Y. (2016). Determination of some blood parameters and macro elements in coccidiosis affected Akkaraman Kangal lambs. *Journal of Asian Scientific Research, 6*(9), 138-142.

Kreutzer, K.V., & Turk, J.R. (2008). *Chapter: 27: Clinical Biochemistry in Toxicology*. In Clinical Biochemistry of Domestic Animals, Academic Press, Elsevier, 6th Edition. pp.821-837.

Kurt, D., Yokus, B., Cakır, D.U., & Denli, O. (2008). Investigation Levels of Certain Serum Biochemistry Components and Minerals of Pasturing Akkaraman Sheeps in Adıyaman Province. *Dicle Universitesi Veteriner Fakültesi Dergisi, 1*(2), 34-37.

López-Alonso, M. (2012). Trace minerals and livestock: Not too much not too little. *ISRN Veterinary Science, 4,* 704825. Doi: 10.5402/2012/704825.

McDowell, L.R., & Arthington J.D. (2005). *Minerals for grazing ruminants in tropical regions*, No. Ed.4, pp.86.

Meyer, D.J., & Harvey, J.W. (2004). *Veterinary Laboratory Medicine: Interpretation and Diagnosis*, Third ed. Saunders, St. Louis.

Nelson, D.L., & Cox, M.M. (2006). *Chapter 10: Lipids.* In Lehninger Principles of Biochemistry. Fourth Edition.

Njidda, A.A., Shuai'bu, A.A., & Isidahomen, C.E. (2014). Haematological and Serum Biochemical Indices of Sheep in Semi-Arid Environment of Northern Nigeria. *Global Journal of Science. Agriculture and Veterinary, 14*(2), 48-56.

Onasanya, G.O., Oke, F.O., Sanni, T.M., & Muhammad, A.I. (2015). Parameters Influencing Haematological, Serum and Bio-Chemical References in Livestock Animals under Different Management Systems. *Open Journal of Veterinary Medicine, 5,* 181-189. http://dx.doi.org/10.4236/ojvm.2015.58025.

Polizopoulou, Z.S. (2010). Haematological tests in sheep health management. *Small Ruminant Research, 92,* 88–91.

Pradhan, B.C. (2016). Effect of age and sex on some blood biochemical parameters of apparently healthy small ruminants of central Odisha, India. *World Journal of Pharmaceutical Research, 5*(4), 1321-1330.

Rahman, K., Islam, S., Ferdous, J., Uddin, H., Hossain, M.B., Hassan, M.M., & Islam, A. (2018). Determination of hematological and serum biochemical reference values for indigenous sheep (*Ovies aries*) in Dhaka and Chittagong Districts of Bangladesh. *Veterinary World, 11*(8), 1089-1093.

Raina, R., Garg, G., Sethi, S.K., Schreiber, M.J., & Simon, J.F., et al. (2012). Phosphorus Metabolism. *Journal of Nephrology & Therapeutics, S3*:008. doi:10.4172/2161-0959.S3-008.

Rucker, R.B., Fascetti, A.J., & Keen, C.L. (2008). *Chapter 22: Trace Minerals.* Clinical Biochemistry of Domestic Animals, Academic Press, Elsevier, 6th Edition. pp.663-693 (ISBN: 978-0-12-370491-7).

Simpraga, M., Smuc, T., Matanovic, K., Radin, L., Vugrovecki, A.S., Ljubici, I., & Vojta, A. (2013). Reference intervals for organically raised sheep: Effects of breed, location and season on hematological and biochemical parameters. *Small Ruminant Research, 112*, 1–6.

Stevanović, O., Stojiljković, M., Nedići, D., Radoja, D., Nikolić, V., Prodanović, R., Ivanov, S., & Vujanac, I. (2015). Variability of blood serum biochemical parameters in karakachan sheep. *Biotechnology in Animal Husbandry, 31*(1), 55-62.

Stojković, J., Ilić, Z., Petrović, M.P., Petrović, V.C., Muslić, D.R., Kurčubić, V., & Đoković, R. (2014). The content of calcium, phosphorus and magnesium in the blood serum of sheep depending on the season and physiological state. *Biotechnology in Animal Husbandry, 30*(4), 601-610.

Suttle, N.F. (2010). *Mineral Nutrition of Livestock, 4th the fertility of rams II*. Macro and microscopic changes in the Edition. Cabi Publishing, USA. pp. 54, 122-354.
http://dx.doi.org/10.1079/9781845934729.0000.

Vugrovečki, A.S., Vojta, A., & Šimpraga, M. (2017). Establishing reference intervals for haematological and biochemical blood variables in Lika pramenka sheep. *Veterinarski arhiv, 87*(4), 487-499.

Vojta, A., Shek-Vugrovečki, A., Radin, L., Efendić, M., Pejaković, J., & Šimpraga, M. (2011). Hematological and biochemical reference intervals in Dalmatian Pramenka sheep estimated from reduced sample size by bootstrap resampling. *Veterinarski arhiv, 81*(1), 25-33.

Yokus, B., Cakir, D.U., & Kurt, D. (2004). Effects of seasonal and physiological variations on the serum major and trace element levels in sheep. *Biological Trace Element Research, 101*(3), 241-55.

Yokus, B., Cakir, D.U., Kanay, Z., Gulten, T., & 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.

Yokus, B., & Cakir, D.U. (2006). Seasonal and Physiological Variations in Serum Chemistry and Mineral Concentrations in Cattle. *Biological Trace Element Research*, *109*(3), 255-266.

Yatoo, M.I., Saxena, A., Kumar, P., Gugjoo, M.B., Dimri, U., Sharma, M.C., & Jhambh, R. (2013). Evaluation of serum mineral status and hormone profile in goats and some of their inter-relations. *Veterinary World. 6*(6), 318-320, doi: 10.5455/ vetworld.2013.318-320.