



## Comparative investigation of some liver enzyme functions considering age and gender distinctions in healthy Akkaraman sheep

Neşe Hayat Aksoy<sup>a\*</sup>, Tahir Kardeşahin<sup>b</sup>, Şükrü Dursun<sup>c</sup>, N. Kürşat Akbulut<sup>d</sup>, Ali Evren Haydardedeoğlu<sup>e</sup>, Ramazan İlgun<sup>f</sup>, Olga Büyükleblebici<sup>g</sup>

<sup>a</sup> Department of Biochemistry, Faculty of Veterinary Medicine, Aksaray University, Aksaray, Turkey

<sup>b</sup> Department of Physiology, Faculty of Veterinary Medicine, Aksaray University, Aksaray, Turkey

<sup>c</sup> Department of Reproduction and Gynecology, Faculty of Veterinary Medicine, Aksaray University, Aksaray, Turkey

<sup>d</sup> Bahri Dagdas International Agricultural Research Institute, Konya, Turkey

<sup>e</sup> Department of Internal Medicine, Faculty of Veterinary Medicine, Aksaray University, Aksaray, Turkey

<sup>f</sup> Department of Anatomy, Faculty of Veterinary Medicine, Aksaray University, Aksaray, Turkey

<sup>g</sup> Technical Sciences Vocational School, Mersin University, Mersin, Turkey

### ARTICLE INFO

### ABSTRACT

#### Article History

Received 12 / 03 / 2019  
Accepted 27 / 03 / 2019  
Online Published Date 31 / 10 / 2019

#### \* Correspondence to:

Neşe Hayat Aksoy  
Department of Biochemistry,  
Faculty of Veterinary Medicine,  
Aksaray University, Aksaray, Turkey  
e-mail:nhaksoy@gmail.com

#### Keywords:

Age  
Akkaraman sheep  
Gender  
Liver enzymes

Enzymes are proteins that possess catalytic properties, convert substrates into products and are biocatalysts of all biological and metabolic reactions in the body. Biochemical blood variables, mainly enzymes, are critical health and disease status parameters, which vary based on a living organism's conditions (metabolic and physiologic periods), age, gender, breed and diet as well as seasonal changes and regional and geographical differences. It has been well established that the measurement of serum levels of various enzymes is a worthy part of diagnosis. Some enzyme levels, which can serve as biochemical parameters, were measured in serum samples obtained from 220 healthy Akkaraman sheep breed, composed of ewes, rams, and female and male lambs. In the current study, which compared the values of biochemical variables, a significant difference ( $p < 0.05$ ) between the four groups was observed in the concentrations of evaluated alanine aminotransferase, alkaline phosphatase, gamma glutamyl transferase and creatine kinase. Differences in the values of aspartate aminotransferase were not found to be statistically significant.

© 2018 OMU

### 1. Introduction

Enzymes are biocatalysts in specialized protein structures with catalytic properties. A sensitive enzyme analysis can give a sense of overall health or of pathological changes and the nature of any diseases present. Low or high amounts of these enzymes in serum or plasma can indicate the presence of damage

in the cells and the state of the disorders in a living organism (Center, 2007; Hoffmann et al., 2008; Kaneko et al., 2008). In clinical enzymology, enzymes such as transaminases (AST and ALT), creatine kinase (CK), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) are very important for diagnosis (Center, 2007; Srivastava et al., 2007; Hoffmann et al.,

2008). Alanine aminotransferase (ALT) is a cytosolic enzyme found in many vertebrate species, especially homo sapiens, with the highest concentration in the liver and smaller concentrations in the heart, skeletal muscle and kidneys. This enzyme has variable ranges that depend on tissue and animal breeds, species, subspecies, regional changes, age and gender differences. Pathological increases in serum ALT levels are detected in the inflammation or destruction of any of the tissues where the enzyme is highly concentrated (Hoffmann et al., 2008; Kaneko et al., 2008). Aspartate aminotransferase (AST) activity is found in the liver in amounts similar to that in skeletal and cardiac muscle. The serum AST level can be about 1.5 times higher in newborns than in adults. In myocardial infarctions (MI), viral hepatitis, toxic liver necrosis, shock and hypoxia-associated circulatory failure, the serum AST level can increase by 10 ~ 100 times more than normal (Calbreath, 1992; Kaplan et al., 1996; Bishop et al., 2000; Srivastava et al., 2007; Hoffmann et al., 2008; Burtis et al., 2012). Gamma glutamyl transferase (GGT), which transfers amino acids from cell membranes, is used as a diagnostic test because it is affected by tissue specificity. The tissue distribution of GGT has been found especially in the liver, spleen, lung, pancreas and intestines as well as in the mammary glands of dogs, cattle, goats and sheep (Goldberg, 1980; Calbreath, 1992; Kaplan et al., 1996; Bishop et al., 2000; Srivastava et al., 2007; Hoffmann et al., 2008; Burtis et al., 2012). The highest GGT activity in the liver is found in cattle, horses, sheep and goats (Hoffmann et al., 2008). Alkaline phosphatase (ALP) has a catalytic effect in bone mineralization. ALP is found in all tissues (especially bones, placenta, intestines, the spleen and the kidneys). However, it is present in higher amounts in the liver and bones. The most abundant are bone ALP iso-enzymes in children and adults and liver ALP iso-enzymes (Calbreath, 1992; Kaplan et al., 1996; Hoffmann et al., 2008; Burtis et al., 2012). ALP activity is elevated physiologically in young animals (regardless of sex) where bone development is active, and is raised pathologically as a result of liver and heart disease (Hoffmann et al., 2008). During the contraction of the muscle, creatine kinase (CK) catalyzes the transfer of phosphate by providing the formation of creatine phosphate-ATP. While the height of serum CK activity in healthy individuals is affected by age, sex, race, body mass, obesity and physical activity, other genetic differences have less effect. Children have higher CK values than adults, and males have higher CK levels than females (Calbreath et al., 1992; Anderson et al., 1993; Kaplan, 1996; Bishop et al., 2000; Burtis et al., 2012).

To accurately evaluate metabolic profiles, it is essential to compare with reference range values appropriate for the region and the breed. Accordingly,

it is crucial to determine and standardize the specific values for each breed and region (Braun et al., 2010). The main purpose of this study was to determine the reference intervals for some important enzyme variables for male and female Akkaraman sheep of different ages.

## 2. Materials and methods

### Ethical scope

This study was directed in accordance with the principles of the "Local Ethics Committee" in the framework of the ethics confirmed by the "Bahri Dagdas International Agricultural Research Institute" Directorate of Local Ethics Committee of Animal Experiments (14.01.2015 / 35 and 0088).

### Sample collection

The blood samples were collected within the framework of ethical rules for animals in private enterprises located in Aksaray, Turkey. The animal samples consisted of 220 healthy Akkaraman sheep, which came from Aksaray and its nearby environment. The animals were separated into four groups based on gender and age, each group including 55 sheep. 220 totally healthy sheep, composed of females (n=55 lambs and 55 ewes) and males (n=55 lambs and 55 rams), were used as animal samples. 15 mL of blood was taken from the vena jugularis and blood samples were centrifuged and (Coles, 1986) used for analysis.

### Enzyme assays

Enzymatic parameters identified in each sample were ALT, AST, GGT, ALP and CK. Analyses of these enzymes were carried out with a commercial assay kit (Assel, Italy) and a Humalyzer-3000 (Germany) biochemical analyzer according to the method of the commercial kit procedure.

### Statistical analysis

Descriptive statistics for the properties studied were mean, standard deviation, standard error, and minimum and maximum values. Data were analyzed using the statistical software SPSS 15.0 for Windows™ (SPSS Inc., Chicago, IL, USA). Differences among the groups were analyzed by student t-test. "One-way ANOVA" was performed to compare 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  $\pm$  standard error ( $X \pm SH$ ). Statistical significance was accepted as  $p < 0.05$  level.

## 3. Results

The results and statistical mean values of enzymatic measurements of the sheep used in this study are represented in Table 1 and Fig. 1, respectively.

**Table 1.** Enzyme level findings of four groups of Akkaraman sheep.

Parameter (Unit)	Ewes	Female Lambs	Rams	Male Lambs	P
AST (IU/L)	69.05±3.65	61.23±4.32	64.38±4.45	64.08±3.64	>0.05
ALT (IU/L)	10.26±0.70b	13.48±1.21a	12.25±0.55ab	11.94±0.40ab	0.038
ALP (IU/L)	153.51±7.68b	255.20±30.34a	201.93±15.45ab	202.40±19.56ab	0.006
GGT (IU/L)	61.07±8.16bc	44.41±4.26c	75.09±6.70ab	82.79±5.92a	0.000
CK (IU/L)	76.27±4.63b	72.57±5.81b	109.67±11.69a	89.14±5.57b	0.002

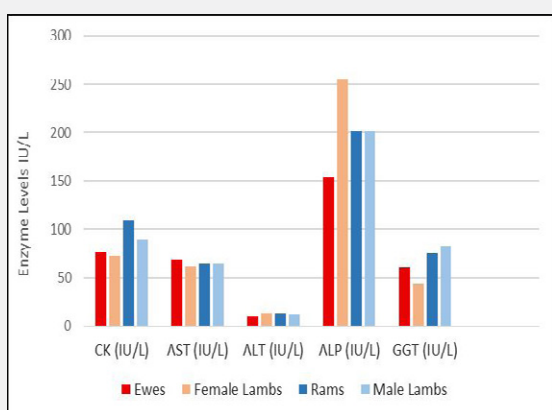


Fig. 1. Levels of liver enzyme parameters in four groups of sheep.

AST levels were found to be relatively higher in ewes than in the other three groups. The results were not statistically significant. When the ALT values were examined, it was found that the values obtained from the ewes were lower than those of female lambs and male lambs and adults. The ALT values were highest in female lambs. The results were statistically significant ( $p < 0.05$ ). In the current study, ALP values were statistically significantly higher in female lambs than in both the ewes and the male groups ( $p < 0.05$ ). Overall, the female lambs had the highest values and the ewes had the lowest values; these results were statistically significant ( $p < 0.05$ ). While no significant age-related differences were observed in males, ALP values in females increased as age decreased. GGT levels were found to be considerably lower in female lambs than in male lambs. In adults, the amount of GGT was higher in rams than in ewes. GGT values were higher in male lambs than in rams whereas in female lambs, they were lower than ewes; these findings were statistically significant ( $p < 0.05$ ). The highest CK value measured was found in the rams and the lowest in the female lambs. It was found that the difference was not significant between ewes and female lambs whereas the large difference between rams and male lambs was statistically significant ( $p < 0.05$ ).

#### 4. Discussion

The values of some serum enzymes that were measured, studied and presented in this study are the basis for further research into indigenous sheep breeds. In this completed study, the activities of AST, ALT, ALP, GGT and CK were investigated to establish an extensive biochemical perspective for the investigation and thus health status was viewed in detail. The main purpose of liver tests in sheep is to clarify the liver functions that effect the total health of the organism. The serum values of these enzymes are often routinely used for assessing liver function (Braun et al., 2010). The principal utilization of clinical biochemistry based on enzymes in sheep are health management and the follow-up of health status, the diagnosis of diseases and the monitoring of treatment, for which selected examples are investigated in this study. Biochemical and enzymological profiles provide reliable information on the health status of animals and also reflect the responsiveness of an animal to its internal and external environments. Braun and co-workers recommended that, because there are many different breeds and breeding systems in sheep, each laboratory specify its own reference values and ranges. Thus, they claim that there must be different reference ranges (Braun et al., 2010). Some of the scientists also emphasized that the effect of seasonal change is often very hard to discern from many misleading determinants, such as feeding conditions and the reproductive condition of female animals (Yokus et al., 2004.; Braun et al., 2010). Measuring the activities of liver enzymes requires sensitive analysis involving the biochemical parameters of the relevant organism. In the current study, AST levels were higher in females than in males, there was a variation due to age difference in females and there was no difference between male age groups; these results were not statistically significant. In a study conducted in 2015, the AST and ALP values of Akkaraman sheep (gender and age not specified) in the healthy control group are consistent with the current study's total means results (Gunes et al., 2015). Some studies in the literature show enzyme amounts higher than the AST value and slightly lower than the GGT value of our

study (Stevanović et al., 2015). Compared with a study by Kurt et al. (Fartosi et al., 2010), our measured AST and ALT values were lower, our measured ALP and GGT values were higher and, the CK values of both studies were similar. In another study, values of AST, ALT and ALP in both males and females are in general much lower than the values we obtained (Durak et al., 2015). A study conducted by Durak et al. shows that Zom sheep did differ significantly ( $p < 0.05$ ) between gender and age groups in terms of serum AST, CK, ALT and GGT levels. They found that ALP and GGT were affected by age, while ALP levels were significantly affected by sex. They also detected high GGT levels in adults as compared to young Zom sheep (Durak et al., 2015). If the results for Zom sheep are compared with our results without considering gender and age, the AST, ALT and CK levels were lower than in this study; ALP values were nearly the same and GGT values were higher than in this study. In another study, much lower GGT levels were found in Lika pramenka sheep (ewes) as compared to our GGT results (Vugroveci et al., 2017). A study presented in 2015 used healthy sheep of the Morada Nova breed and some biochemical data were monitored based on gender, age and body condition score. Carlos and colleagues claimed that age affected the serum ALT and AST levels in the Morada Nova sheep. The activity of ALT was found to be higher in males (Carlos et al., 2015). These situations could be connected to the differences in hepatic activity in different age periods. In the current study, apparent height in female lambs was significant for ALT and ALP ( $p < 0.05$ ). In males, the effect on the ALT level is insignificant, whereas the ALP level is minimal but significant. Another study (Cruz et al., 2017) investigated the effect of age and gender on the biochemical parameters of Dorper sheep, measuring AST, ALP and GGT values among 15- and 121-day-old lambs. They showed that the highest AST value was in 3 different groups ( $p < 0.05$ ) in ages ranging from 45 to 121 days, and they did not find any significant differences in ALT between the four groups. In a study linking these differences to developing muscle activity and metabolic activity, they stated that gender did not affect biochemical variability. In a study managed by Yokus and colleagues of female adult sheep, ALT,

AST, ALP, GGT and CK were studied as enzymatic parameters (Yokus et al., 2006). In this study, which was conducted in southeast Turkey in April, AST, ALT and CK were lower than our study's (ewes) results but ALP and GGT values were found to be higher. Despite the fact that both studies took place during the same season and had similar feeding conditions for animals, there were differences in enzyme values between different breeds (Sakiz-Awassi crossbreed sheep / Akkaraman sheep breed) based in different geographical regions (southeast Turkey / Mid-Anatolia) in the same country. In three different studies in similar tropical regions, the biochemical variables ALT, AST and LDH were investigated. Compared to the current study, ALT and AST levels were higher. Given that the working regions are tropical areas, it is not surprising that these three studies were similar and differ from our study. Factors including animal gender, geographical distribution, ecological and geological differences, nutritional properties and health conditions can influence hemoglobin levels (Kiran et al., 2012; Bhat et al., 2014; Pradhan, 2016). After comparing the current study with these other studies, the following conclusion can be reached: changeable ALT and AST concentrations indicate a great variation in ALT and AST levels among different ovine in different geographical regions. As can be clearly seen here, differences in enzyme activity depend on the animals, species and breeds as well as age, gender and environment.

As presented in the study, liver enzymes, which are vital to life, possess notable age and gender differences in ovine. The main purpose of this study was to determine the reference intervals of selected clinic enzymologic variables for males and females in different ages of Akkaraman sheep. This paper focused on the major application of enzymologic biochemistry in ovine in tracking health status, and on establishing regional reference values and ranges for Akkaraman sheep.

#### Acknowledgement

This work was supported by Aksaray University Scientific Research Projects Coordination Unit (Project No: 2015-084).

#### REFERENCES

- Anderson, S.C., Cockayne, S., 1993. Clinical chemistry concepts and applications, W.B. Saunders Company, Philadelphia.
- 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 India. *Int. J. Agric. Sc Vet. Med.* 2, 22-27.
- Bishop, M.L., Duben-Engelkirk, J.L., Fody, E.P., 2000. Clinical chemistry principles, procedures, correlations, lippincott Williams and Wilkins, Philadelphia

- Braun, J.P., Trumela, C., Bézille, P., 2010. Clinical biochemistry in sheep: A selected review *Small Ruminant Research*. 92,10–18.
- Burtis, C.A., Ashwood, E.R., and Bruns, D.E., 2012. *Tietz textbook of clinical chemistry and molecular diagnosis* (5<sup>th</sup> edition). Elsevier, St. Louis, USA. 2238 pp, 909 illustrations. ISBN: 978-1-4160-6164-9.
- Calbreath, D.F., 1992. *Clinical chemistry a fundamental textbook*, W.B. Saunders Company, Philadelphia.
- 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. *J. Anim. Plant Sci.* 25, 950-955.
- Center, S.A., 2007. Interpretation of liver enzymes. *Vet. Clin. North Am. Small Anim. Pract.* 37, 297-333.
- Coles, E.H., 1986. *Veterinary Clinical Pathology* 4<sup>th</sup> Edition. W.B. Saunders Co. Philadelphia.
- Cruz, R.E.S., Rocha, F.M., Sena, C.V.B., Noletto, P.G., Guimarães, E.C., José Antônio Galo, J.A., Mundim, A.V., 2017. Effects of age and sex on blood biochemistry of Dorper lambs. *Semina: Ciências agrárias*. 38,3 085.
- 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. *J. Vet. Med.* 70, 33-39.
- Fartosi, Kh.G., Al-Talib, Y.J., Ali, Sh., 2010. Comparative study of some serum biochemical parameters of cattle and sheep of the marshes in the south of Iraq. *Al- Qadisiya J. Vet. Med. Sci.* 9, 78-83.
- Goldberg, D.M., 1980. Structural, functional and clinical aspects of  $\gamma$ -glutamyltransferase. *CRC Crit. Rev. Clin. Lab. Sci.* 12, 1-58.
- Gunes, V., Meral, O., Fidanci, U.R., Kismali, G., Sel, T. 2015. Serum lipid profile and enzyme levels in Akkaraman sheep with high nitrate in drinking water. *Ankara Univ. Vet. Fak. Derg.* 62, 247-253.
- Hoffmann, W.E., Solter, P.F., 2008. Chapter.12: Diagnostic enzymology of domestic animals. In *clinical biochemistry of domestic animals*, 6<sup>th</sup> Edition Academic Press, Elsevier. pp:351-378.
- Kaneko, J.J., Harvey, J.W., Bruss, M.L., 2008. *Clinical biochemistry of domestic animals* 6<sup>th</sup> Edition. Academic Press N.Y.
- Kaplan, L.A., Pesce, A.J. 1996. *Clinical chemistry, theory, analysis, correlation.*, Mosby, St. Louis, Missouri. pp:65-82.
- 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 Pac. J Trop. Biomed.* 2, 304-306.
- Kurt, D., Yokus, B., Cakir, D.U., Denli O., 2008. Investigation levels of certain serum biochemistry components and minerals of pasturing Akkaraman sheep in Adıyaman province. *Dicle Üniv. Vet. Fak. Derg.* 1, 2, 34-37.
- Pradhan, B.C., 2016. Effect of age and sex on some blood biochemical parameters of apparently healthy small ruminants of central Odisha, India. *J. Pharma. Res.* 5, 83-90.
- Srivastava, T., Chosdol, K., 2007. *Clinical Biochemistry: Clinical enzymology and its applications*, New Delhi – 110 029.
- Stevanović, O., Stojiljković, M., Nedić, D., Radoja, D., Nikolić, V., Prodanović, R., Ivanov, S., Vujanac, I., 2015. Variability of blood serum biochemical parameters in karakachan sheep. *Biotech. Ani. Husbandry.* 31, 55-62.
- Vugrovečki, A.S., Vojta, A., Šimpraga, M., 2017. Establishing reference intervals for haematological and biochemical blood variables in Lika pramenka sheep. *Veterinarski Arhiv.* 87, 487-499.
- Yokus, B., Cakir, D.U., Kurt, D., 2004. Effects of seasonal and physiological variations on the serum major and trace element levels in sheep. *Biol. Trace Elem. Res.* 101, 241-55.
- Yokus, B., Cakir, DU., Kanay, Z., Gulten, T., Uysal, E., 2006. Effects of seasonal and physiological variations on the serum chemistry, vitamins and thyroid hormone concentrations in sheep. *J. Vet. Med.* 53, 271-276.