

# The Effects of Age and Gender on Some Biochemical Serum Parameters in Zom Sheep Raised in the Vicinity of Karacadağ

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## ABSTRACT

A hundred and seventy three clinically healthy Zom sheep aged 1-6 years and grazed on grassland under similar conditions in six villages of the Karacadağ and Ovabağ towns of the central, Bismil and Çınar districts of Diyarbakır province. The effect of gender in the evaluation for serum glucose,  $\gamma$ -glutamyltransferase, and alkaline phosphatase were observed for Zom sheep. The effect of age on the assessment made by taking into consideration the serum cholesterol, direct bilirubin, very low-density lipoprotein, alkaline phosphatase,  $\gamma$ -glutamyltransferase and direct bilirubin and high-density lipoprotein were also observed. In conclusion the age and gender of the Zom sheep needs to be taken into consideration in order to ensure an accurate clinical diagnosis and prognosis.

**Keywords:** Age; Biochemistry; Gender; Zom Sheep

## INTRODUCTION

Sheep breeding is of significance for the Turkish national economy in that it enables the use of low quality grassland and provides input to the food and textile industries (1). The Zom sheep is of particular significance in terms of both the protection of local genetic resources and the provision of genetic material for future animal improvement programmes. The Zom sheep, which are raised in Karacadağ and its vicinity in southeast Anatolia, is a local type of the Akkaraman breed. Its inbreeding in closed herds for centuries by the local people of the region has enabled the adaptation of the Zom sheep to the conditions prevailing in the region, resulting in the evolution of a dual-purpose breed with a higher withers height compared to that of other sheep breeds (2), and with greater resistance to diseases and greater viability. These characteristics have led the farmers of the region preferring the use of Zom sheep as breeder animals (2, 3).

For the interpretation of biochemical serum parameters of an animal species, data is required on the reference value ranges specific to that particular species (1). Reference values have been established for the biochemical parameters of almost all the sheep breeds raised in Turkey, taking into account the differences observed for age and gender. Research has been conducted on the Karayaka sheep (1), Akkaraman sheep (4), Awassi sheep (5), Sakiz x Karayaka sheep (6), Sakiz sheep (7), Merino sheep (8), and Tuj sheep (9). Several other studies are also available for the biochemical parameters of other sheep breeds (10, 11, 12). However, no previous study exists on reference values for the serum biochemical parameters of the Zom sheep. Serum biochemical parameters are used in the assessment of animal diseases, the monitoring of the treatment of animal diseases, the interpretation of nutritional disorders, research aimed at increasing animal productivity, and the collection of information on the physiopathological condition of animals. Therefore, the establishment of refer-

ence values for the biochemical serum parameters of Zom sheep raised in the Karacadağ region bears significance in determining clinical approaches that can be used in these animals for treatment (1, 11, 13, 14).

This study was aimed at establishing reference value ranges for clinically healthy Zom sheep raised in Karacadağ town and its vicinity, by means of the measurement of the levels of certain biochemical serum parameters, and by taking into consideration differences for age and gender.

## MATERIAL AND METHOD

Blood samples were collected from 173 clinically healthy Zom sheep aged 1 to 6 years grazing on grassland under similar conditions in six villages of the Karacadağ and Ovabağ towns of the central, Bismil and Çınar districts of Diyarbakır province, located in the Karacadağ region situated in-between the Diyarbakır, Şanlıurfa and Mardin provinces. The animals were randomly selected from the sheep flocks raised by 9 breeders. Between June and July in 2013, blood samples were collected from the jugular vein of each animal into dry vacuum tubes. For female animals, the sampling period was the end of the lactation period and the beginning of the dry period. After being transferred to the laboratory under cold chain conditions, the blood samples were centrifuged at 3000 rpm for 15 minutes for the extraction of sera. The serum samples were transferred into 1.5 ml Eppendorf tubes and stored at  $-26^{\circ}\text{C}$  in a deep freezer until analysis. Laboratory analyses were performed within two weeks after the collection of the blood samples.

Analyses for serum glucose (GLU), alkaline phosphatase (ALP), aspartate aminotransferase (AST), cholesterol (CHOL), creatine kinase (CK), lactate dehydrogenase (LDH), albumin (ALB), direct bilirubin (DBIL), creatinine (CREA), alanine aminotransferase (ALT), urea, total bilirubin (TBIL), high-density lipoprotein (HDL), total protein (TP), amylase (AMY),  $\gamma$ -glutamyltransferase ( $\gamma$ -GT), triglyceride (TG), blood urea nitrogen (BUN), globulin (GLB), indirect bilirubin (IBIL), low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) were performed by an enzymatic colorimetric method using a modular P-800 biochemistry autoanalyzer (Roche Diagnostics GmbH, Mannheim, Germany) using original commercial autoanalyzer test kits.

With an aim to establish reference values for certain bio-

chemical parameters in Zom sheep, two main groups were established, one for gender and the other for age (A). The animals included in these two main groups were assessed under further subgroups. The main group for gender was divided into further subgroups for female animals (F), male animals (M), female young animals (FY), male young animals (MY), female adult animals (FA), and male adult animals (MA) (Table 1). The main group for age was divided into further subgroups for young animals (Y), adult animals (A), young female animals (YF), young male animals (YM), adult female animals (AF), and adult male animals (AM) (Table 2).

In the groups established for age, the selection criteria for young animals were their age being below 2 years and the females not having given birth previously, while the selection criteria for adult animals were their age being above 2 years and the females having given birth before but not being pregnant during the study period.

## Statistical analysis

Statistical comparisons were made between the main groups F and M, the subgroups FY and MY, and the subgroups FA and MA in the groups established for gender, and between the main groups Y and A, the subgroups YF and YM, and the subgroups AF and AM in the groups established for age. The statistical analyses for the biochemical serum parameters investigated were performed using the independent samples t-test.

All values were expressed as mean $\pm$ standard deviation ( $x\pm$ SD) with a  $P < 0.05$  considered as being significant. The SPSS 18.0 software package was used for the statistical analysis of data.

## RESULTS

The serum biochemistry findings obtained for gender are presented in Table 1, while the serum biochemistry findings obtained for age are given in Table 2. It was determined that the Zom sheep included in the study did not differ for serum AST, CK, LDH, ALB, CREA, ALT, urea, TBIL, TP, AMY, TG, BUN, GLB, IBIL or LDL levels between the gender and age groups ( $p < 0.05$ ). The assessment of the effects of gender demonstrated that the serum GLU levels of the animals included in group F were significantly lower than those of group M ( $P < 0.05$ ). No significant difference existed between the subgroups FY and MY. The comparison

**Table 1.** Gender-related biochemical parameters in Zom sheep (x±SD).

Parameter	Gender		Gender Young		Gender Adult		Mean (n=173)
	F (n=95)	M (n=78)	FY (n=36)	MY (n=33)	FA (n=59)	MA (n=45)	
GLU(mg/dl)	39±15*	41±13*	41±16	41±13	39±15	41±13	40±14
ALP (U/l)	150±73	215±94	190±80	243±85	132±61**	199±97**	177±88
AST (U/l)	99±18	95±16	108±20	95±16	95±16	95±17	97±17
CHOL(mg/dl)	69±13	67±15	71±15	65±16	68±12	69±14	68±14
CK (U/l)	145±56	142±48	155±60	156±49	141±59	134±46	144±55
LDH (U/l)	945±213	872±184	1056±201	874±181	895±201	871±189	914±204
ALB (g/dl)	3.2±0.3	3.4±0.3	3.3±0.2	3.4±0.3	3.1±0.3	3.3±0.3	3.3±0.3
DBIL (mg/dl)	0.03±0.0	0.02±0.0	0.03±0.0	0.02±0.0	0.04±0.0	0.02±0.0	0.03±0.0
CREA (mg/dl)	0.8±0.2	0.8±0.2	0.8±0.2	0.8±0.2	0.8±0.2	0.8±0.2	0.8±0.2
ALT (U/l)	18±6	19±5	18±7	18±4	17±5	19±5	18±5
UREA (mg/dl)	30±6	32±6	31±7	33±5	30±5	32±6	31±6
TBIL (mg/dl)	0.1±0.1	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.1	0.1±0.0	0.1±0.1
HDL (mg/dl)	47±12	43±11	48±12	45±15	47±12	41±8	45±12
TP (g/dl)	7.7±1	7.8±1	7.8±1	7.5±1	7.6±1	7.9±1	7.7±1
AMY (U/l)	14±7	12±7	12±6	13±7	14±8	11±7	13±7
γ-GT (U/l)	49±15	49±12	48±12	52±12	50±17*	47±12*	49±14
TG (mg/dl)	23±14	22±12	27±12	21±9	21±13	22±14	22±13
BUN (mg/dl)	14±3	16±3	15±3	15±2	14±2	16±3	15±3
GLB (g/dl)	4.2±1	4.3±1	4.4±0	4.1±1	4.1±1	4.4±0	4.2±1
IBIL (mg/dl)	0.08±0.0	0.09±0.0	0.08±0.0	0.09±0.0	0.08±0.0	0.08±0.0	0.08±0.0
LDL (mg/dl)	18±6	20±7	18±7	18±7	18±6	22±7	19±7
VLDL(mg/dl)	5±2	5±2	5±2	5±1	4±2	5±2	5±2

The same superscripts (\* and \*\*) in the same row denote significant difference at ( $p < 0.05$ ,  $P < 0.01$ ) respectively.

F=Femine. M=Male. FY=Femine Young. MY=Male Young. FA=Femine adult. MA=Male adult.

of the subgroups FA and MA demonstrated that in the FA group, the serum ALP level was lower ( $P < 0.01$ ) and the  $\gamma$ -GT level was significantly higher ( $P < 0.05$ ). Gender was confirmed not to have caused any statistical difference for the other parameters investigated (Table 1).

The assessment of the effects of age revealed that the serum CHOL and DBIL levels of group Y were lower than that of group A, while the VLDL levels were higher ( $P < 0.05$ ). The comparison of the subgroups YF and AF demonstrated that in subgroup AF, serum ALP levels were lower and DBIL levels were higher in comparison to subgroup YF. The serum  $\gamma$ -GT levels of subgroup YF were found to be lower than that of subgroup AF ( $P < 0.05$ ). On the other hand, the comparison of the subgroups YM and AM showed that in subgroup YM, serum DBIL ( $P < 0.01$ ) and VLDL ( $P < 0.05$ ) levels were lower and serum HDL levels were significantly higher ( $P < 0.01$ ), compared to subgroup AM.

It was ascertained that age did not cause any statistically

significant difference for the other parameters investigated (Table 2).

## DISCUSSION

The Zom sheep included in the present study were selected among healthy sheep raised by 9 farmers in 6 villages located in Karacadağ and its vicinity. The biochemical serum parameters of sheep are known to vary with multiple factors, including disease, breed, age, gender, season, muscle activity, nutrition, gestation, heat, and stress (6, 13, 15). The mean values determined for the biochemical parameters investigated in the present study (Tables 1 and 2) were found to fall within the reference value ranges previously reported for sheep by other researchers (4, 5, 7, 10, 11). Biochemical serum parameters have common use in the clinical assessment of domestic animals.

Blood glucose concentrations, which show a broad range

**Table 2.** Age-related biochemical parameters in Zom sheep (x±SD)

Parameter	Age		Age Female		Age Male		Mean (n=173)
	Y (n=69)	A (n=104)	YF (n=36)	AF (n=59)	YM (n=33)	AM (n=45)	
GLU(mg/dl)	41±15	40±14	41±16	39±15	41±13	41±13	40±14
ALP (U/l)	214±86	159±84	190±80*	132±61*	243±85	199±97	177±88
AST (U/l)	102±19	95±16	108±20	95±16	95±16	95±17	97±17
CHOL(mg/dl)	68±16*	69±13*	71±15	68±12	65±16	69±14	68±14
CK (U/l)	156±54	138±54	155±60	141±59	156±49	134±46	144±55
LDH (U/l)	972±210	885±195	1056±200	895±201	874±181	871±189	914±204
ALB (g/dl)	3.3±0.3	3.2±0.3	3.3±0.2	3.1±0.3	3.4±0.3	3.3±0.3	3.3±0.3
DBIL (mg/dl)	0.03±0.0*	0.03±0.0*	0.03±0.0*	0.04±0.0*	0.02±0.0**	0.02±0.0**	0.03±0.0
CREA(mg/dl)	0.8±0.2	0.8±0.2	0.8±0.2	0.8±0.2	0.8±0.2	0.8±0.2	0.8±0.2
ALT (U/l)	18±6	18±5	18±6.7	17±5	18±4	19±5	18±5
UREA(mg/dl)	32±6	31±6	30±7	30±5	33±5	32±6	31±6
TBIL (mg/dl)	0.1±0.0	0.1±0.1	0.1±0.0	0.1±0.1	0.1±0.0	0.1±0.0	0.1±0.1
HDL (mg/dl)	47±14	45±11	48±12	47±12	45±15**	41±8**	45±12
TP (g/dl)	7.7±1	7.7±1	7.8±1	7.6±1	7.5±1	7.9±1	7.7±1
AMY (U/l)	13±6	13±8	12±6	14±8	13±7	11±7	13±7
γ-GT (U/l)	50±12	49±15	48±12*	50±17*	52±12	47±12	49±14
TG (mg/dl)	25±11	21±13	27±12	21±13	21±9	22±14	22±13
BUN (mg/dl)	15±3	15±3	15±3	14±2	15±2	16±3	15±3
GLB (g/dl)	4.2±1	4.3±1	4.4±0	4.1±1	4.1±1	4.4±0	4.2±1
IBIL (mg/dl)	0.09±0.0	0.08±0.0	0.08±0.0	0.08±0.0	0.09±0.0	0.08±0.0	0.08±0.0
LDL (mg/dl)	18±6	20±7	18±7	18±6	18±7	22±7	19±7
VLDL(mg/dl)	5±1*	5±2*	5±2	4±2	5±1*	5±2*	5±2

The same superscripts (\* and \*\*) in the same row denote significant difference at ( $p < 0.05$ ,  $P < 0.01$ ) respectively.

Y=Young, A=Adult, YF=Young Female, AF=Female adult, YM=Young Male, AM=Adult Male.

in domestic animals, are regulated by the hypoglycaemic and hyperglycaemic hormones (16). Blood glucose levels are also known to be related to genetic predisposition (16). Glucose concentrations may alter with the secretion of catecholamines, and may also increase secondarily, as a result of the stress of muscle and liver enzymes induced by myopathy and hypoxia (17). In several research studies serum glucose levels have been reported to be lower at the end of the lactation period and during the dry period in sheep (9, 12) and during the summer months in cattle (14). Some researchers have suggested that gender does not to affect glucose levels in sheep (1, 6, 18) and red deer (19).

Eshratkhah (20) indicated that, while gender significantly affected serum glucose concentrations, age had no such effect in sheep (20, 21). Some other researchers (6, 7, 8) suggested that serum glucose concentrations were affected by age and were significantly lower in adult animals, as a result of reduced glucose production in the liver that could

be related to increased levels of toxic substances in the liver with ageing or the parasitic infection of the liver (8). In the present study, it was determined that the mean serum glucose levels of all Zom sheep, irrespective of their gender and age, fell within the previously reported reference value ranges (5, 7, 9, 11). Glucose concentrations were observed to have been significantly affected by the gender factor ( $P < 0.05$ ) (Table 1), which was in agreement with the reports of Eshratkhah (20) and Kiran (21). On the other hand, glucose concentrations were ascertained not to have been affected by age, which was not in agreement with the results of previous studies (6, 7, 8). This was attributed to the blood samples having been collected in late lactation or in the early dry period, which also resulted in the serum glucose levels of the animals included in group F being lower. However, the serum glucose levels of the Zom sheep found below the reference values, were not statistical significance, suggesting that a genetic adaptation may have developed for glucose in

Zom sheep. Further studies are required to elucidate this aspect.

The presence of multiple isoenzymes of alkaline phosphatase enables the use of this enzyme as an indicator in the diagnosis of diseases (22). Each tissue has different alkaline phosphatase isoenzymes. To date, hepatic, renal, intestinal, placental and bone alkaline phosphatase isoenzymes have been identified, yet, as the majority of these isoenzymes are characterized by a short plasma half-life, the alkaline phosphatase activity in the blood circulation is indicated to originate, to a large extent, from the liver and bone tissues (15, 23). Serum alkaline phosphatase activity, which is reported to greatly vary between animal species, is indicated to be affected by several factors, including age, gender, diet, hunger, and environmental changes (15). Some researchers have suggested the alkaline phosphatase enzyme concentrations of sheep are not affected by gender (1, 6) or age (18). Perez *et al.* (24) reported alkaline phosphatase enzyme levels to be higher in males, compared to females, and highest in the young compared to adults. High levels of this enzyme have been attributed to higher muscular activity and better body condition (24) feeding on zinc-deficient feed (25) and high osteoblastic activity in young animals (12, 26). On the other hand, in a study carried out in goats, Daramola *et al.* (27) determined that alkaline phosphatase levels were higher in adult animals. In the present study, it was ascertained that serum alkaline phosphatase levels were significantly affected by both gender ( $P < 0.01$ ) and age ( $P < 0.05$ ). In this respect, the present study was found to be in agreement with previous studies relating to gender (12,24) and age (6, 11, 26).

The  $\gamma$ -GT enzyme is found in the kidneys, pancreas, liver, spleen and small intestine. Although this enzyme exists in contact with the membrane in the cellular cytosol of several tissues, an increase in its serum levels generally suggests the presence of diseases related to the bile ducts or liver (15, 19). Ramos *et al.* (28) reported that in Arogenesha sheep, serum  $\gamma$ -GT levels decreased with age. In several other studies, it has been suggested that neither gender (6, 19, 24) nor age (7, 19) have an effect on serum  $\gamma$ -GT levels. In their study on the effects of age and gender in Kangal dogs, Çınar *et al.* (29) demonstrated that  $\gamma$ -GT levels were higher in adult females and young males, compared to young females. Furthermore, Piccione *et al.* (30) reported that age significantly affected  $\gamma$ -GT levels in Girgentana goats, and suggested that serum  $\gamma$ -GT levels being higher in adults, when compared to

young animals, could be related to physical stress. The serum  $\gamma$ -GT levels measured in the present study demonstrated a statistically significant effect of both gender and age on this parameter ( $P < 0.05$ ) (Tables 1 and 2). Therefore, the results obtained for serum  $\gamma$ -GT levels in the present study did not comply with either previous results suggesting that this enzyme is not affected by the gender (6, 19, 24) and age (7, 19, 24) or results reported by Ramos *et al.* (28) suggesting lower levels to be observed in adults. On the other hand, the results of the present study were found to be in agreement with previous reports demonstrating higher serum  $\gamma$ -GT levels in adults, compared to the young (24, 29, 30).

One of the serum indicators of hepatic failure and extra-hepatic obstruction is direct bilirubin (DBIL) (31). Increased bilirubin concentrations have shown that species variations to be important for the use of indirect/direct bilirubin levels in diagnosis (15). Bilirubin is generated as a result of the breakdown of haemoglobin and microsomal cytochromes. While prehepatic icterus is characterized by increased indirect bilirubin (IBIL) levels, hepatic and posthepatic icterus are characterized by increased DBIL levels (11, 15). Several studies have shown that gender and age do not affect DBIL levels in sheep (1, 6, 12) and goats (24). To the best of the authors' knowledge, there is no literature suggesting ovine serum DBIL levels to be affected by age. On the other hand, Gurgoze and Icen (32) have reported that DBIL levels were affected by age in purebred Arabian mares. In the present study, it was determined that, in Zom sheep, DBIL levels were not affected by gender ( $P < 0.05$ ). This finding was in agreement with previous reports suggesting serum DBIL levels not to be affected by the gender factor (1, 6, 12). The assessment of all age groups demonstrated that serum DBIL levels were significantly higher in the adult animals ( $P < 0.05$ ,  $P < 0.01$ ) (Table 2). Further research is needed to elucidate age-dependent alterations that occur in DBIL levels in Zom sheep.

Lipoproteins are closely associated with cholesterol levels. Increased cholesterol levels are the result of increased HDL and/or VLDL levels. Increase in cholesterol levels mainly arises from increase in HDL levels, and to a less extent, from increase in VLDL levels (33). Cholesterol concentrations, which increase during the dry period in sheep, are regulated by a number of complex factors (10). While some literature reports suggest that neither gender (1,6,18,20) nor age (6, 18, 21) affect cholesterol levels, some other reports suggest that

serum cholesterol levels decrease with age (20, 24, 28, 29). Perez *et al.* (24) reported that, in the Spanish ibex (mountain goat), cholesterol levels were higher in the young, compared to adults, and in females, compared to males. On the other hand, some researchers (20, 35) reported that serum cholesterol levels increase with age. In the present study, while serum cholesterol levels were ascertained not to have been affected by gender, they were shown to have been significantly affected by age. In this respect, the findings of the present study in Zom sheep were in agreement with previous reports suggesting serum cholesterol levels not to be affected by gender (1, 6, 18, 20, 34) and to increase with age (20, 35). On the other hand, the findings obtained in the present study were found to disagree with research suggesting cholesterol levels to decrease with age (24, 29, 34). This difference was attributed to the Zom sheep in the present study to have been grazed on pastures in mountainous areas, and therefore, their muscular activity to have occurred at a higher level, as well as to cholesterol levels increasing in the summer season (36) and to several other factors, including breed, genetics (37) and geography.

Studies on the blood lipid profile of domestic animals have shown the existence of variations between species and even within species (20, 38). While plasma lipoprotein levels may vary with body weight gain (39), they may also alter with age (34). In a study conducted by Eshratkhah *et al.* (36) in Moghani sheep, it was reported that, while HDL and VLDL levels were not affected by gender, HDL levels decreased and VLDL levels did not alter with age (40). Eshratkhah *et al.* (34) reported that, in sheep, serum HDL levels decreased in late lactation and during the dry period. The same researchers (38) reported that, in male goats, VLDL levels decreased with age. Noguchi (41) demonstrated that, in humans, LDL and VLDL levels increased and HDL levels decreased with age. In the present study, it was ascertained that HDL and VLDL levels were not affected by gender, while the serum HDL levels of the Zom sheep included in Subgroup MA had significantly decreased in comparison to the levels measured in Subgroup MY ( $p < 0.01$ ). In this respect, the present study complied with the research mentioned above (34, 40, 41). In the current study in Zom sheep, it was observed that, serum VLDL levels were significantly affected by age, and it was determined that, of the subgroups MY and MA the latter presented with higher levels, and of the groups Y and A, the former presented with higher levels ( $P < 0.05$ ) (Table 2). In

this respect, the findings obtained in the present study for serum VLDL levels conformed with the report of Noguchi (41), but differed from the results of some other previous research carried out in sheep and goats (34, 38, 40).

In conclusion, in view of biochemical parameters being studied to ensure the accurate clinical diagnosis and prognosis of animal diseases and the fact that no literature is available on reference biochemical parameters for Zom sheep, the present study is considered to contribute to veterinary medical literature by providing reference values for future biochemical research that would enable the comparison of normal values with those measured in cases of disease. The present study carried out in Zom sheep demonstrated the need for further research for the interpretation of serum glucose, direct bilirubin and lipid levels.

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