MAK HILL Publications Journal of Animal and Veterinary Advances

Research Article
doi: 10.59218/makjava.2023.1.7

The Effect of Lactation Period on Some Serum and Milk Biochemical Parameters in Tushin Sheep

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ABSTRACT

In this study, it was aimed to investigate the relationship between serum zinc (Zn), copper (Cu), iron (Fe) and alkaline phosphatase (ALP) and milk Zn, Cu and Fe levels in Tushin sheep during the lactation period. Seventy fout Tushin sheep, aged 2-4, in normal feeding conditions during the lactation period, in the Research and Application Farm of were used in the research. Blood and milk samples were taken from the same sheep included in the study in March, April, May and June 2008. Zn, Fe, Cu and ALP levels were measured in serum of blood samples and Zn, Cu, Fe levels in milk samples were measured by atomic absorption spectrophotometer. The lowest values of serum Zn, Cu, Fe and ALP levels of Tushin sheep by months are respectively; 0.66±0.03 ppm (May), ($p<0.001$); 0.55±0.05 ppm (June), ($p>0.05$); While 1.25±0.13 ppm (May), ($p<0.001$); 80.81±5.81 IU L^{-1} (March), ($p<0.001$); If the highest values 1.21±0.05 ppm (April), ($p<0.001$); 0.80±0.10 ppm (March),($p>0.05$); 2.27±0.16 ppm (March), ($p<0.001$) and 23.28±5.58 IU L^{-1} (May), ($p<0.001$). On the other hand, the lowest values of milk Zn, Cu and Fe levels by months, respectively, the lowest milk Zn levels in May (0.68±0.05 ppm), ($p<0.001$); 0.68±0.05 ppm (March), ($p<0.01$); 0.32±0.03 (June), ($p>0.05$); While 0.65±0.07 ppm (March),($p<0.01$); the highest values were 1.17±0.06 ppm (March), ($p<0.001$); 0.41±0.03 ppm (April), ($p>0.05$); 0.90±0.09 ppm (April), ($p<0.01$). And also, it was determined that there was a positive correlation between serum Zn and ALP and serum Zn and Fe levels and a negative correlation between serum Zn and Cu, milk Zn and Cu and milk Zn-Fe levels. Biochemical parameters are important in determining and controlling the health and disease status of the animal. The most important period affecting milk yield in sheep breeding is the lactation period. Due to the limited number of studies examining serum and milk some biochemical parameters in lactating tuj ewes, it is thought that changes in biochemical parameters of lactating ewes will contribute to the researches. In addition, it was concluded that comprehensive studies should be conducted.

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Key Words
Tushin Sheep, lactation period, biochemical parameters

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Received: 16 February 2023
Accepted: 8 March 2023
Published: 15 March 2023

Citation: Mehtap Odabasi and Ebru Bardas Ozkan, 2023. The Effect of Lactation Period on Some Serum and Milk Biochemical Parameters in Tushin Sheep. J. Anim. Vet. Adv., 22: 1-7, doi: 10.59218/ makjava.2023.1.7

INTRODUCTION

Electrolytes play an important role in many vital physiological systems and processes, especially the normal function of cells in the organism[1]. Electrolytes, due to their role in many processes such as regulating the water balance in the body and the pH value of the blood and the functions of nerve and muscle cells, adversely affect many physiological systems, especially the gastrointestinal system, cardiovascular system, renal system, nervous system and endocrine system, due to their deficiency and excess in the body. It is affected by many diseases and disorders. Iron (Fe) is in the structure of hemoglobin, which carries the oxygen needed by the tissues and it is one of the life-critical electrolytes that can cause morbidity with weakening of the immune system and decreased resistance to infections[2]. Zinc (Zn), which takes part in cellular metabolism, is an essential mineral required for the organism due to its role in the catalytic activity of various enzymes. It is also involved in the immune system, wound healing, protein and DNA synthesis and cell division[3]. Copper (Cu) is an electrolyte that plays an important role in many metabolic processes such as energy and iron metabolism, antioxidant defense, neurotransmitter and neuropeptide synthesis[4]. Alkaline phosphatase (ALP) is a liver enzyme that acts as a catalyst in the cell and is produced intensively in the skeletal system and gastrointestinal tract. ALP has a very important physiological role in terms of bone regeneration and growth. The lactation period, the period that passes after birth in animals until they become pregnant again and the milking period ends, is called lactation. It is one of the important periods for animals due to various physiological disorders such as decreased appetite, decreased growth rate, decreased milk yield, decreased fertility, dental and bone anomalies and decreased resistance to diseases occur due to a lack of mineral substances in animals during lactation[5-7].

Tushin sheep, which forms the basis of our study, is bred in the northeast of Türkiye, especially in Kars, Ardahan and Iğdır regions. Live weight of sheep is 38-42 kg, lactation period is 124 days; lactation milk yield is 45 kg. It is a combined sheep breed in terms of yield, which is grown in mountainous, high altitude and rough terrain conditions whose pastures are very valuable[8]. Biochemical parameters provide benefits in terms of animal health control and disease follow-up[9]. Although there are a limited number of studies examining the changes in biochemical parameters in Tushin sheep, which is a breed in danger of extinction, no studies examining serum biochemical (Zn, Cu and Fe) parameters during the lactation period have been encountered. In this context, it was aimed to determine the relationship between some serum (Zn, Cu, Fe and alkaline phosphatase (ALP)) and milk (Zn, Cu, Fe) biochemical parameters in milk and serum in the lactation period of Tushin sheep.

MATERIAL AND METHOD

The research material consisted of milk and blood samples taken from Tushin sheep aged 2-4 years in the lactation period, which were kept in the Research and Application farm barns and found to be healthy in the examinations (N: 40°25"0"; E: 43°4"59"), (Table 1). Care was taken to ensure that the animals had the same care, feeding and hygiene conditions. In order to examine the changes in the lactation period, blood and milk samples were taken 4 times in March, April, May and June (Table 1). Cu, Fe, Zn values in blood serum taken during the lactation period and Cu, Fe, Zn values in milk samples were measured in Atomic Absorption Spectrophotometer (AAS). The amount of ALP in the blood serum was measured in an autoanalyzer using Labkit brand kit. In order to examine the changes in the lactation period, blood and milk samples were taken 4 times in the above-mentioned months. Blood samples (10 mL) were taken from the *V. jugularis* of healthy animals in sterile vacuumed heparin-free glass tubes in accordance with hygienic rules and brought to the laboratory with the help of portable coolers.

Method

Preparatıon of serum samples: Serum was separated by centrifugation at 4500 rpm for 12 min in the laboratory. Afterwards, the serums were taken into 1.5 mL polyethylene tubes and stored in deepfreeze at 20°C until analysis. The serums, which were removed from the freezer and thawed at room temperature, were taken into clean glass tubes with 1 mL and homogenized in the mixer by adding 9 mL of 1 N suprapure HNO_3 prepared in deionized water. After a while, the proteins were precipitated by centrifuging the tubes at 4500 rpm for 12 min. The supernatant above (~9.5 mL) was transferred to clean capped polyethylene tubes and used for further analysis[10].

Preparatıon of Milk Samples: The milk samples taken from the animals during the lactation period were taken into sterile glass tubes (10 mL) in the amount indicated in the table below and kept at room temperature for 15 min, then the lids were closed and stored at 20°C in deepfreeze until analysis. Analysis of metals in milk samples was carried out using the AOAC method 999.10 (Detection of Fe, Cu and Zn levels in samples prepared by microwave burning method with AAS)[11]. At the analysis stage, frozen milk samples brought to room temperature were homogenized and taken in Teflon containers in 2 mL amounts. Five milliliter of HNO_3 (65%) and 2 mL of H_2O_2 (30%) were added to the samples and their lids were closed. The samples placed in the microwave oven were processed according to the parameters specified in Table 2. Samples were brought into conditions that could be analyzed in AAS.

At the end of the process, the teflon containers were left to cool under the fume hood for 15 min without opening the lids and at the end of the time, the lid and inner wall of the teflon container were washed down with deionized water. The contents were filtered through filter paper and taken into 15 mL propylene tubes and filled with up to 15 mL of bidistilled water. In this way, the samples were brought into a form that could be analyzed in AAS and they were kept in the refrigerator at +4°C until analysis.Analytical Conditions of AAS: The analytical conditions of AAS are presented in Table 3.

Milk and serum samples prepared for analysis were subjected to the analysis of the specified elements (Zn, Fe, Cu) in the AAS-Flame device. Before the analysis, the instrument was calibrated with at least four different concentration standards prepared for the element to be analyzed. At least 4 calibration solutions of Zn, Fe and Cu elements were prepared by diluting the intermediate stock or standard solution to the element concentration in blood and milk samples. After the calibration of the device, quantification was made for each element using specific wavelengths of light[12] .

Table 1: Monthly distribution of milk and blood samples collected from Tushin sheep in the lactation period

Months	Serum	Milk	Total
March 2008	23	23	46
April 2008	19	16	29
May 2008	15	16	31
June 2008	15	13	22
The General Total	74	68	118

Table 2: Microwave oven parameters

Stage	Temperature (°C)	Power (watts)	Duration (min)
1	160	80%	15
2	210	80%	15
3	100	80%	15

Table 3: Analytical conditions of AAS

Elements	Wavelength (nm)	Slit opening (nm)	Lamp current (mA)	Acetylene (L/dk)	Weather (L/dk)
Fe	248.3	0.2 H	30	2.0	17
Cu	324.8	0.5 H	30	2.3	18
Zn	213.9	1 H	25	2.0	17

*Fe:İron; Cu: Copper; Zn: Zinc

Measurement of blood serum Alkalıne Phosphatase (ALP) actıvıty: The ALP activities of the serum samples were measured 1 week after the samples were taken Serum ALP activities were measured in a Sinnova brand autoanalyzer using a commercial ALP kit from LABKIT brand (Lod No. 30130). After centrifuging and separating serum, 20 microliters were taken with an automatic pipette, mixed with 1.20 microliters of reagent and incubated at 37°C and the activities were calculated by reading at 405 nm[13].

Statistical analysis: The results obtained were evaluated by analysis of variance using OneWay ANOVA (SPSS, 12.0) between groups. In addition, the differences between the data averages of the groups were compared with the Duncan test. Comparison was subjected to statistical analysis against threshold of 0 and results are given as mean±standard error (X±SE).

RESULTS AND DISCUSSION

Correlation Coefficients between Serum Zn-Serum ALP, Serum Zn-Serum Cu, Serum Zn-Serum Fe, Serum Cu, Serum Fe, Milk Zn-Milk Cu, Milk Zn-Milk Fe, Milk Zn-Serum Zn, Serum Cu-Milk Cu and Serum Fe-Milk Fe ($p<0.01$), (Table 4).

In the months of analysis, the lowest serum Zn mean was 0.66±0.03 ppm in March and the highest value was 1.21±0.05 ppm in May. When serum Zn levels were examined, significant differences were found between months ($p<0.001$), (Fig.1).

The lowest values of serum ALP averages, which were found to be very close to each other in March and April, were determined in March with 80.81±5.81 IU L^{-1} and the highest values were determined (Fig. 2).

In the sheep examined in this study, the lowest serum Cu values were 0.55±0.05 ppm in June and the highest values were 0.80±0.10 ppm in March. The differences in serum Cu values in March-April-May and June were not statistically significant ($p>0.05$), (Fig. 3).

The lowest serum Fe levels were determined as 1.25±0.13 ppm in May and the highest levels were determined as 2.27±0.16 ppm in April. Again, significant differences were found in terms of serum Fe values between all months of analysis ($p<0.01$), (Fig. 4).

Table 4: Correlation Coefficients between Serum Zn-Serum ALP, Serum Zn-Serum Cu, Serum Zn-Serum Fe, Serum Cu, Serum Fe, Milk Zn-Milk Cu, Milk Zn-Milk Fe, Milk Zn-Serum Zn, Serum Cu-Milk Cu and Serum Fe-Milk Fe

	Compared parameters								
	Serum				Milk			Serum	
Months	Zn-SerumALP	Zn-Serum Cu	Zn-Serum Fe	Cu-Serum Fe	Zn-Milk Cu	Zn-Milk Fe	Zn-Serum Zn	Cu-Milk Cu	Fe-Milk Fe
March	0.880**	-0.257	0.105	0.178	0.421**	-0.031	-0.329	-0.379	-0.017
April	0.755**	-0.157	0.332	0.195	-0.096	-0.129	-0.018	-0.292	-0.411
May	0.466	0.157	0.377	0.093	-0.176	-0.632**	-0.126	-0.041	-0.277
June	0.852**	-0.078	0.279	0.276	0.133	-0.2	-0.603	-0.255	-0.167

**: p<0.01, materiality for March, April, May and June

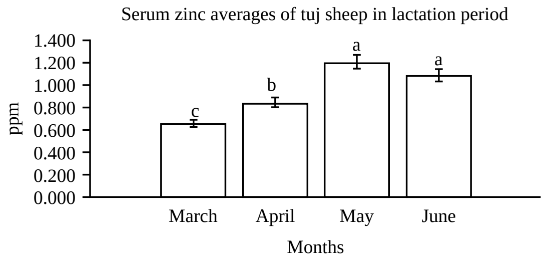

Fig. 1: Serum Zn levels (X±SE) in lactating Tushin sheep
a, b, c: The difference between values with different letters is significant (p<0.001)

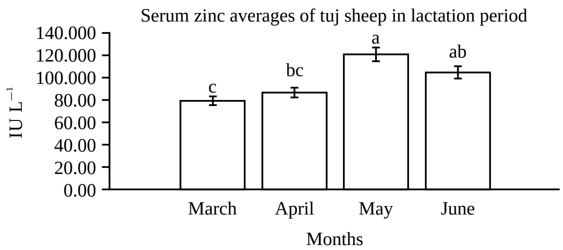

Fig. 2: Serum ALP activities (IU L^{-1}) (X±SE) in lactating Tushin sheep
a, b, c: The difference between values with different letters is significant (p<0.001)

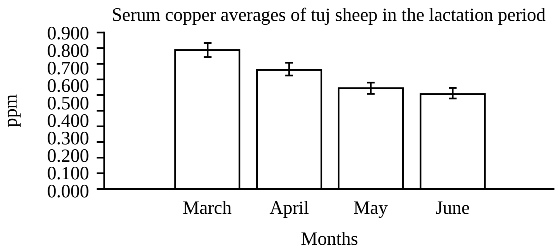

Fig. 3: Serum Cu (ppm) levels (X±SE) in lactating Tushin sheep
There is no difference between the serum Cu values by months (p>0.05)

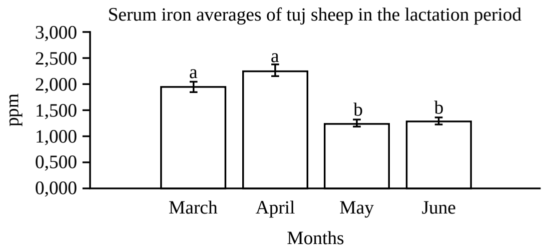

Fig. 4: Serum Fe (ppm) levels (X±SE) in lactating Tushin sheep
a, b: Differences between values with different letters are significant (p<0.01)

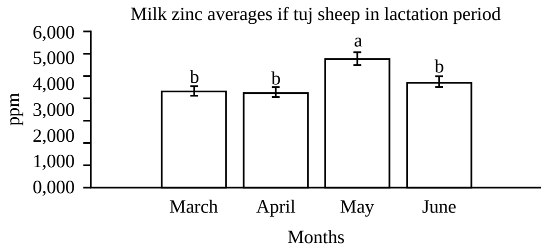

Fig. 5: Milk Zn (ppm) levels (X±SE) in lactation Tushin sheep
a, b, c: The difference between values with different letters is significant (p<0.001)

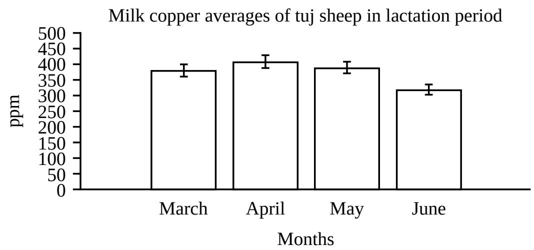

Fig. 6: Milk Cu (ppm) levels (X±SE) of lactation Tushin sheep
There is no difference between the values according to the months (p>0.05)

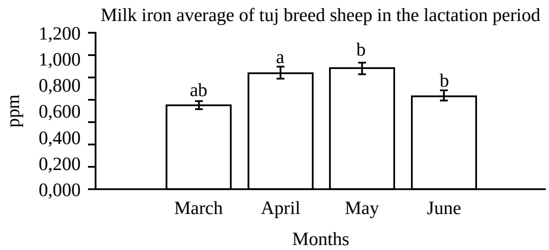

Fig. 7: Milk Fe (ppm) levels (X±SE) in lactation Tushin sheep
a, b, c: The difference between values with different letters is significant (p<0.001)

The lowest milk Zn levels were determined in April with 3.67±0.19 ppm and the highest values were determined in May with 4.99±0.26 ppm. Significant differences were found between the months of analysis of milk Zn levels (p<0.001), (Fig. 5).

Although milk Cu averages in March and May are quite close to each other; the lowest milk Cu level was determined as 0.32±0.03 ppm in June and the highest average was determined in April with 0.41±0.03 ppm. Considering the milk Cu averages, it was observed that the difference between the groups was not significant in March-April-May and June (p>0.05), (Fig. 6).

Although milk Fe averages were found to be quite close to each other in April and May, the lowest milk Fe level was determined as 0.65±0.07 ppm in March and the highest average was 0.90±0.09 ppm in April. Significant difference was found between milk Fe values between months (p<0.01), (Fig. 7).

DISCUSSION

In this study, mean serum Zn levels of Tushin sheep were determined as 0.66±0.03, 0.85±0.07, 1.21±0.05 and 1.09±0.08 ppm in March, April, May and June, respectively. It was observed that our data were higher than the values reported for Morkaraman, Tushin and Merinos sheep and were compatible with the data of Akkaraman, Melez and Valaska sheep. According to these evaluations, serious differences were found between serum Zn values[14] of sheep showing Zn deficiency and values considered critical for deficiency and values considered critical according to different researchers[15]. Although it is not possible to explain exactly what factors might have caused these differences, it has been concluded that they may have arisen from differences in the methods used by the researchers or from other factors causing changes in serum Zn levels[14, 16, 18]. Some researchers[13,16] indicate that serum Zn levels increase with the progression of pregnancy in sheep, while others[17] indicate that serum Zn levels are higher in cows in the postpartum period compared to the prenatal period. In addition, the differences between the serum and milk Zn averages of March, April, May and June were found to be significant ($p<0.001$) in our study and in some studies on this subject[18]. It is compatible with the differences determined for the months.

Pregnancy and lactation affect Zn requirement. Lactation animals need more Zn due to high milk yield. Again, some researchers stated that the addition of Zn to the ration increased the amount of Zn in the milk of cattle and pigs. In also our study, milk Zn levels were measured within normal limits as 3.73±0.15, 3.67±0.19, 4.99±0.26 and 4.08±0.21 ppm in March, April, May and June, respectively. However, it was emphasized that milk Zn content is an important indicator of body Zn values in animals. Khan *et al*.[19] reported the Zn level as 1.29±0.05 ppm in the winter season and 0.56±0.05 ppm in the summer season in their analysis in sheep milk. In studies investigating mineral concentrations in milk in dairy cows, the amount of Zn in milk was reported as 3.64±0.16 ppm, 4.6±0.4 ppm[19] and 4.2 ppm[20]. Aganga *et al*.[21] stated that the Zn level in the milk of Tswana sheep and goats was higher than the Cu and Fe levels[20]. In addition, Bedo *et al*. found a statistically insignificant decrease in Zn concentrations in the milk of cattle during the lactation period[21]. The serum ALP averages of the sheep used in the study for the months of March, April and June were determined to be lower than the 168-217, 119-165 IU L^{-1} change limits of the values reported in the literature[22]. Likewise, it is thought that the factors affecting the level of Zn, which plays an important role in ALP synthesis, also affect ALP activities[23]. However, it is compatible with the values (98-128) stated by Spears[24]. Unshelm and Flock[25] determined that serum Zn and ALP activities of sheep in some months showed significant changes compared to other months. In our study, we have also found significant differences between the serum ALP activities of March, April, May and June ($p<0.001$). According to the results of this study, while serum Cu levels of sheep were found to be normal in March and April, the data for May and June are close to critical levels. While the serum Cu level is lower than normal in some animals, the average serum Cu levels of sheep in May and June are within normal values. In our study, the feeding of animals is based on concentrated feed in March and April and pasture feed in May and June. It is possible that this diet is effective in the higher serum Cu levels in March and April compared to May and June. In this study, while the milk Cu averages were consistent with the winter reports of Khan *et al*.[19] It was found to be higher than the reports in other literature[20]. It is possible that the reason for this is due to regional, seasonal and racial differences in studies or the methodologies used by the researchers[20]. In a study conducted by Johnson reported that the Cu concentration in the milk of many farm animals was lower than the serum Cu concentration. Similarly, in our study, a statistically insignificant negative correlation was found between serum and milk Cu levels. It is possible to attribute this to the passage of Cu in the serum into the milk during the lactation period. It is possible to attribute this to the passage of Cu in the serum into the milk during the lactation period. The serum Fe levels we obtained were within the limits reported in the literature. Likewise, it has been reported that Fe deficiency in grazing sheep is caused by parasitic invasions rather than nutritional deficiencies.

Fe content of milk samples belonging to the sheep included in the study was determined as 0.65±0.06, 0.90±0.09, 0.93±0.09, 0.72±0.03 ppm in March, April, May and June, respectively. The highest milk Fe levıtel was measured in May. In this study, the milk Fe levels of the sheep sampled were lower than the data of some researchers[1] and higher than those of some[21, 22]. It is thought that the difference in the data in the studies may be due to the differences in race and region. However, Weissreported that Fe concentration in milk of cattle, sheep and buffaloes decreased significantly. Some researchers have determined that Fe concentration in milk of sheep decreases significantly with the progress of lactation. In addition, it was stated that Fe concentration in milk is generally low due to its binding to lactoferrin[23]. Fe deficiency anemia develops very rapidly in suckling puppies and this anemia is not frequently encountered in lambs and calves. It is highly probable that the differences in the amount of Fe in serum and milk between months are related to the amount of Fe in the diet, its form and the factors affecting its absorption[24].

Zinc interacts with some substances in the organism. It has been reported that there is an antagonistic interaction between Zn and Cu, Fe, Ca and Cd. A negligible negative correlation was found in the correlation analysis between serum Cu level and Zn values of the animals included in the study. It is possible to say that the reason for this is that Zn binds to Cu receptors in intestinal mucosa cells and reduces the absorption of Cu[25]. It is possible to explain the positive correlation between serum CuZn levels in May with the decrease in the availability of Cu in immature plants due to the fact that the pasture is in the early vegetation period in these months. Although there is a positive correlation between serum Cu and Fe levels in the months of sampling in sheep, this is statistically significant. Ramirez-Perez *et al*.[25] on the other hand, reported that high Fe amount in the soil in grazing sheep decreased the plasma Cu level by decreasing Cu absorption. Again, Grace *et al*. stated that the high amount of Fe in the diet reduces the plasma Cu level. Similarly, Theil and Calvert[26] reported that the plasma Fe levels and transferrin saturation ratios increased in sheep given excessive Cu and that giving too much Cu increased plasma Fe levels but had no effect on Fe stores[27-28]. In our study, it is possible to associate the detection of a positive relationship between Fe and Cu in serum with the hemolysis of some samples.The amount of minerals in the milk of different animal species is affected by factors such as the chemical forms of the elements, age, interactions with other minerals, the amount taken in the ration, the breed of the animal and adaptation[29]. A positive correlation was found between milk Cu and milk Zn levels in March and June. It is possible to say that this positive relationship is due to the fact that the animals are rich in concentrated feed in March and the plants in the pasture in June are rich in these substances. The fact that there is a negative relationship between Cu and Zn levels in milk in April and May suggests the possibility that Cu absorption is affected and decreased by many factors including Zn as well as the chemical form of Cu in the ration[6] . Smith *et al*. reported a decrease in Cu, Zn and Fe concentrations in milk with the progression of lactation. Chu and Cox stated that the Cu concentration in the milk of rats that fed by a diet containing high amounts of Zn decreased. It has been stated that the Cu concentration in the milk of cattle is lower than the amount of Zn in the milk, but several times higher than the serum Cu concentration. Bedö *et al*. stated that Zn and Cu concentrations tended to decrease during lactation, but this decrease was insignificant. High amount of Fe in the ration reduces Cu absorption. The presence of Fe in reduced form increases the need for Cu. During grazing, when soil-contaminated grasses or grasses in silage feed containing high Fe are consumed, Cu absorption shows a significant decrease[31].

CONCLUSION

As a result, it was concluded that serum Zn, Fe, Cu and ALP and milk Zn, Fe and Cu values of Tushin sheep may change in some months according to lactation and there is a positive correlation between serum Zn and ALP values and serum Zn and Fe. In herd management, it is critical to monitor biochemical parameters in order to determine and monitor the yield level and disease status during the lactation period. In addition, our current results will be realized in this field. Although it is thought that it will contribute to the researches, it has been concluded that it would be beneficial to carry out comprehensive studies.

ACKNOWLEDGMENTS

This study was a part of my thesis and Ethics approval was received from the Local Ethics Committee (Date: 06.09.2009; No:16/147). Also This study was presented as a poster at the 35th National Congress of the Turkish Society of Physiological Sciences on September 30-October 3, 2009.

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