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Effect of Zinc Supplementation on Haematological Parameters, Biochemical Components of Blood and Rumen Fluid, and Accumulation of Zinc in Different Organs of Goats

Elmas Ulutaş[1,*], Abdullah Eryavuz[2], Aziz Bülbül[2], Abdur Rahman[3,*], İsmail Küçükkurt[4] and Cangir Uyarlar[5]

[1]*Department of Physiology, Faculty of Veterinary Medicine, Yozgat Bozok University, Yozgat, Turkey*
[2]*Department of Physiology, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, Turkey*
[3]*Department of Animal Nutrition, University of Veterinary and Animal Sciences, (Jhang Campus), Jhang, Pakistan*
[4]*Department of Biochemistry, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, Turkey*
[5]*Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, Turkey*

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EU presented the concept of the study while AE supervised it. AB, EU, IK and CU contributed in trials and lab analysis. EU and AR wrote the manuscript.

Key words
Zinc, Oxidative status, Essential minerals, Rumen protozoa, Angora goat.

ABSTRACT

This study was aimed to determine the effects of diet containing high zinc on rumen fermentation, blood parameters, live weight and oxidative status in goats. In this study, twenty four male Angora goats each of 12 months of age, and weighing approximately 35 kg were divided into four groups as: control group (C) fed with basal diet containing 31.76 ppm Zn, experimental group 1 fed with basal diet supplemented with 500 ppm Zn, experimental group 2 fed with basal diet supplemented with 750 ppm Zn and experimental group 3 fed with basal diet supplemented with 1000 ppm zinc sulphate. The investigation was started after 15 days of adaptation period and lasted for 30 days. On days 15 and 30 of the study body weight of the animals was recorded, blood and rumen samples were collected. There were no differences in body weight with different levels of Zn supplementation. Red blood cells (RBC) and haemoglobin levels increased ($p<0.05$) as compared to control group on the 30th day with Zn supplementation. High Zn supplementation increased ($p<0.05$) plasma urea nitrogen and glutathione but decreased ($p<0.05$) leptin and malondialdehyde concentration while other parameters remained unaffected. No difference was observed in ruminal pH between the treatment groups. Ruminal ammonia and number of protozoa were decreased ($p<0.05$) with 700 ppm and 1000 ppm zinc supplementation. Rumen Zn concentration increased ($p<0.05$) in the goats fed with 1000 ppm zinc, whereas there was no difference in rumen Fe and Cu concentration among the treatments, except for 1000 ppm zinc supplementation. High zinc supplementation to diet increased ($p<0.05$) the liver Zn and Fe concentrations and mohair Fe levels but decreased the kidney Cu concentrations. It was concluded that the goats can tolerate the supplementation of high zinc in diet.

INTRODUCTION

Goat farming, a short term profitable business with a significant contribution of animal origin products in human food and has a direct impact on the socio-economic values of human population especially in under developed areas (Dubeuf *et al*., 2004). Goat farming is environmentally friendly and works as preservative of natural habitat and eliminate the weeds and control their growth. It has a prime importance in terms of income in less privileged areas and majority of population is raising goats for their livelihood. Goat rearing is practised mainly as a protein source like mutton, milk and allied products. With these attributes, goat population has increased by 33.8% with major contribution of Asia of 59.4% (Skapetas and Bampidis, 2016) in total worlds goat population of 1.002 billion (FAO, 2018) while share of Asia and Africa in total meat production is 69.8% and 23.8%, respectively (FAO, 2019). The purpose of goat rearing varies geographically either for meat, milk or mohair production.

* Corresponding authors: abdurrehman@uvas.edu.pk; elmas.ulutas@bozok.edu.tr
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Goat is an efficient grazer of herbs and shrubs and need less capital for maintenance and growth. Profitable goat farming is only possible when goat is fed a balanced diet according to his production needs. A specific feeding strategy should be adopted to fulfil the nutrient requirements of goat especially when being reared for specific purpose like for mohair production. Among the other nutrients, minerals have an influencing role in mohair production (Froetscher *et al* 2005). These play various roles in the animal body including metabolic reactions, regulation of osmotic pressure, transport system, enzyme synthesis, hormonal balance (Suttle, 2010; Kundu *et al*., 2014) and has influence on rumen fermentation process in small ruminants (Mallaki *et al*., 2015). Requirement of these minerals vary depending upon the production factors (hair, dairy or mutton) (Suttle, 2010) and these requirements are not well documented in goat feeding especially for micro minerals.

Zinc is a micro mineral and one of the essential elements required for better growth, production, and reproduction of animals by influencing carbohydrate, energy, protein and nucleic acid metabolism that affects both the immune system and general health status (NRC, 1980; Droke *et al*., 1998; Suttle, 2010; Pavlata *et al*., 2011). It is involved in a large number of enzyme systems as a catalytic, co-catalytic or structural component (Reilly, 2004). In the literature, there is a small number of studies on the trace mineral metabolism of the goats among ruminant animal species, compared to cattle and sheep, and these studies are mostly aimed to determine the effects of trace mineral deficiencies on goats or identifying minimum trace mineral requirements. Research data on the effects of zinc supplementation on the performance, nutrient utilization and plasma zinc levels of goats are limited (Jia *et al*., 2008). This study aims to investigate the effects of high levels of zinc supplementation on the haematological and some biochemical parameters as well as on the rumen fermentation in goats ration to improve the health status and performance of goats.

MATERIALS AND METHODS

Animals, diets, and experimental design

Ethics Committee approval was obtained from Afyon Kocatepe University Animal Ethics Committee (B.30.2. AKÜ. 0.0.A2.00.000/272) for this study. A total of 24 almost one-year-old healthy male Angora goats with an average live weight of 34.85±3 kg were used. The animals were fed total mix ration two times a day (08:00A.M., 08:00 P.M.) to meet their daily nutrient requirements as described by the National Research Council (2007) and were given free access to water (Tables I, II). Following the 15-day adaptation period (Farenzena *et al*., 2017), blood samples were collected from animals to determine the zinc levels. Goats were divided into four groups each of six experimental animals in a way that the zinc levels were close to each other. The animals were weighed at the beginning of the study, at the 15th and 30th day by using cattle weighing scale (Dikomsan, RCV-600) to determine their body weights. Animals in the control group were fed with basal ration, whereas animals in the experiment group 1, 2 and 3 were fed with basal ration supplemented with 500 ppm, 750 ppm, and 1000 ppm zinc, respectively. Zinc sulphate (zinc sulphate heptahydrate pure, $ZnSO_4.7H_2O$, 1kg, Kartal Kimya, Ankara, Turkey) as a zinc source was added in concentrate ration. Zinc analyses of the ration samples taken from the groups were performed on the Inductively Coupled Plasma Mass Spectrometer (ICP-MS) (Agilent Technologies, ASX-500, Model No. 63286A) using the method specified in the operating instructions of the instrument (Zhou *et al*., 2017).

Table I.- Composition of ration used in the study (% dry matter).

Parameters	Percentage
Ration	
Dry matter (kg/day)	1.2
Ingredients used in ration	
Barley straw	52.74
Alfalfa	32.14
Concentrated feed mix	15.12
Chemical composition of ration	
Metabolic energy (Mcal/kg)	2.16
Crude protein	10.1
Metabolic protein	7.1
Rumen degradable protein	3.9
Bypass protein	2.1

Sample collection

On the 15th and 30th days of the study, the rumen region of the animals was given massage before the morning feeding for mixing of the rumen contents. The local made rumen catheter with an inner diameter of 5-6 mm was inserted into rumen via oesophagus and rumen fluid samples were collected from the ventral ruminal sac with the help of a large volume injector and first 10 ml was discarded to avoid saliva contamination. Blood samples were taken using gel and heparin tube from the internal jugular vein. Plasma and serum samples obtained by the centrifugation (1500 g, 4°C, 15 min) of blood samples (Nüve, NF 1000R) were stored in the freezer at -20°C until the time of analysis. The mohair samples taken from the animals at the end of the experiment were obtained from

the region sheared before the trial. At the end of the trial, the animals were slaughtered in a private slaughterhouse and their liver, pancreas, and kidney tissue samples were collected, frozen at -80°C and then analysed through ICP-MS (Yuan *et al*., 2014).

Table II.- Composition of feed ingredients and nutrients in concentrate mix (%).

	Control	500 ppm	750 ppm	1000 ppm
Feed ingredient				
Barley	50.7	50.7	50.7	50.7
Maize	30	30	30	30
Sunflower seed meal	10	9.47	9.27	9.07
Soybean expeller	7.3	7.61	7.7	7.79
Lime	1.5	1.5	1.5	1.5
Salt	0.5	0.5	0.5	0.5
Zinc sulphate	0	0.22	0.33	0.44
Nutrient composition				
Metaboliseable energy (Mcal/kg)	2.68	2.68	2.68	2.68
Crude protein	15.2	15.2	15.2	15.2
Crude cellulose	5.3	5.3	5.3	5.3
ADF	6.8	6.9	6.9	6.9
NDF	16.7	16.6	16.6	16.7
Ca	0.6	0.6	0.6	0.6
P	0.4	0.4	0.4	0.4
Na	0.2	0.2	0.2	0.2

ADF, Acid detergent fiber; NDF, Neutral detergent fiber; Ca, Calcium; P, Phosphorous; Na, Sodium.

Analysis of rumen samples

Rumen fluid samples were collected and their pH values were measured with digital pH-meter (Hanna Instruments pH meter). Rumen contents were determined by ELISA (Thermo Scientific, Multiskan FC, Finland, Cat No: 51119000/51119050/51119100/51119150) using ammonia assay kit (Sigma, AA0100, USA). Protozoan count of the rumen was based on the values reported by Sulu *et al*. (1988). Zinc level of rumen contents was measured through cautery in ICP-MS (Sineo, MDS-10).

Analysis of haematological parameters

Red blood cells (RBCs), white blood cells (WBCs), haematocrit (Hct) values, and WBC types were determined whereas enzyme-linked immunosorbent assay (ELISA) analyzer was used to determine the haemoglobin (Hb) amount with Drabkin's method spectrophotometrically (Fairbanks and Klee, 1987).

Analysis of some biochemical parameters in whole blood and plasma

Plasma urea nitrogen, glucose and cholesterol levels were measured by ELISA analyzer (Thermo Scientific, Multiskan FC, Finland) using commercial kit (Human, Germany Cat No: 12013, 13002, 12021). In blood samples, serum malondialdehyde (MDA), serum antioxidative activity (AOA) and glutathione (GSH) were determined by using the methods reported by Draper and Hardley (1990), Koracevic *et al*. (2001) and Beutler *et al*. (1963), respectively. Serum and tissue zinc levels (liver, kidney, pancreas, and mohair) were measured through cautery in ICP-MS (Agilent Technologies, ASX-500, Model No. 63286A).

Statistical analysis

Statistical analysis was performed using SPSS version 16.0 software. In terms of the levels of parameters evaluated in the study, ANOVA test was used to determine the significance level of the difference between the mean values of the groups, whereas t-test was applied to reveal the in-group difference with $p<0.05$.

RESULTS

No significant ($p>0.05$) difference was observed between the groups with zinc supplementation of 500 ppm, 750 ppm, and 1000 ppm on the live weight of goats (Table III).

Table III.- Effect of different levels of zinc on live weights of goats (kg) (n=6, ±SEM).

Sampling time	0 ppm	500 ppm	750 ppm	1000 ppm	p
0 day	34.98± 1.18	34.90± 1.30	34.90± 1.46	34.83± 1.27	0.999
15 day	35.10± 1.59	35.21± 1.55	35.23± 1.67	35.85± 1.42	0.989
30 day	35.71± 1.43	35.65± 1.85	35.45± 1.94	36.20± 1.70	0.992

There was a significant difference ($p<0.001$) between the control group and 1000 ppm group in terms of the WBC count measured from the samples obtained on the 30th day of the study. Similarly, a significant difference ($p<0.01$) was observed between the groups in terms of the Hb levels measured from the blood samples obtained on the 30th day of the study in all supplemented groups as compared to control (Table IV).

Table IV.- Effects of different concentrations of zinc on haematological parameters of goats (n=6, ±SEM).

	Sampling time (day)	0 ppm	500 ppm	750 ppm	1000 ppm	p
Erythrocytes/RBC (10^6/ mm^3)	15	15.87±1.01†	15.50±1.01	15.67±0.51	16.25±0.84	0.951
	30	12.72±0.37^a	14.85±0.28^b	15.46±0.47^b	16.18±0.42^c	0.000
Haemoglobin (g /dl)	15	9.28±0.41	8.79±0.35	9.84±0.68	10.45±0.68	0.208
	30	7.93±0.45^c	8.44±0.39^{bc}	9.36±0.21^{ab}	9.70±0.22^a	0.004
Haematocrit(%)	15	24.83±1.35	25.33±1.40	26.66±1.35	27.16±1.13	0.567
	30	24.66±1.14	25.00±0.89	25.83±1.27	25.50±0.67	0.856
Leukocytes (10^3/ mm^3)	15	10.50±0.58	10.80±0.10	10.86±0.10	10.76±0.92	0.989
	30	8.90±0.44	9.70±0.38	9.62±0.90	9.72±0.85	0.809
Neutrophils (%)	15	31.83±2.16†	26.50±2.62	33.16±1.37	32.50±1.99	0.129
	30	26.16±1.24^b	32.83±2.12^{ab}	30.00±2.78^b	36.83±2.34^a	0.018
Lymphocytes (%)	15	65.66±1.90†	70.50±2.47	63.50±1.38	65.33±2.04	0.113
	30	71.83±1.16^a	66.33±2.04^b	68.66±2.88^{ab}	61.33±2.45^b	0.023
Monocytes (%)	15	0.40±0.24	0.83±0.30	1.16±0.16	0.50±0.22	0.140
	30	0.50±0.22	0.33±0.21	0.83±0.30	0.33±0.21	0.434
Eosinophils (%)	15	1.50±0.22	1.13±0.16	1.33±0.33	1.33±0.42	0.619
	30	1.00±0.25	0.73±0.21	0.83±0.21	1.00±0.25	0.076
Basophils (%)	15	0.66±0.33	0.50±0.22	0.83±0.30	0.33±0.21	0.612
	30	0.33±0.21	0.26±0.16	0.26±0.16	0.26±0.16	0.883

^{a,b,c,} The difference between the mean values of different letters in the same order is statistically significant. The difference between the same groups on the 15th and 30th days is significant. †, P<0.05; ††, P<0.01; †††, P<0.001. RBC, Red blood cells.

No significant difference (p>0.05) was found between the groups in terms of plasma cholesterol level, glucose and AOA levels measured from the blood samples obtained on the 15th and 30th day of the study (Table V).

In samples obtained on the 30th day of the study, there was no change in 500 ppm group in terms of plasma urea nitrogen level compared to the control group, however, it was seen to increase (p<0.05) in 750 ppm and 1000 ppm groups (Table V). Similarly, no significant difference (p<0.01) was observed between the groups in terms of plasma GSH levels measured from the samples obtained on the 30th day (Table V). There was a significant difference (p<0.001) between the groups in terms of whole blood MDA levels measured from the samples obtained on the 15th day of the study and a decrease was observed in 500 ppm and 750 ppm groups compared to control group and 1000 ppm group (Table V). There was a significant decrease (p<0.01) in the MDA levels of the experiment groups measured from the samples obtained on the 30th day of the study, compared to the control group (Table V). Zinc level measured from the samples obtained on the 30th day of the study was found to be higher in experimental groups than the control group (p<0.05). The comparison of zinc levels measured from the samples obtained on the 15th and 30th day revealed that the zinc levels of the experimental groups were found to be higher (p<0.01) on the 30th day (Table V).

There was no difference (p>0.05) between the groups in terms of pH values of rumen content obtained at the 15th and 30th day of the study (Table VI). Measurements made on the rumen content samples obtained at the 15th day revealed that there was a decrease (p>0.01) in the ammonia nitrogen in the groups supplemented with zinc, compared to the control group. However, measurements made on the rumen samples taken at the 30th day revealed that there was no difference between the control group and 500 ppm group in terms of the ammonia nitrogen levels but there was a decrease in 750 ppm group and 1000 ppm group which was not significant (p<0.01). A significant difference was found between the groups in terms of the rumen protozoa count measured from the rumen content sample obtained on the 30th day compared to the control group, there was a decrease in 500 ppm, 750 ppm, and 1000 ppm groups and this decrease was found to be similar in 750 ppm and 1000 ppm groups (p<0.001). The rumen zinc level of the rumen content sample obtained on the 15th and 30th days was found to be significantly higher (p<0.05) in the 1000 ppm group compared to the control group (Table VI).

The level of zinc in the liver tissues was found to be significantly higher (p<0.05) in 750 ppm and 1000 ppm groups than the control group (Table VII). There was no change in the groups in terms of copper levels, however,

these levels were found to be significantly higher ($p<0.05$) in the kidney tissues of experimental groups compared to the control group. It was determined that there was a dose-dependent increase ($p<0.05$) in the iron levels in the liver tissue of 750 ppm group compared to the control group (Table VII). A significant increase ($p<0.05$) was observed in the iron level in the mohair tissue of the 500ppm group compared to the control group (Table VII).

Table V.- Effects of different concentrations of zinc on the plasma cholesterol, glucose, urea-N, AOA, whole blood GSH, MDA and serum Zn, Cu and Fe levels in goats (n=6, ±SEM).

	Sampling time	0 ppm	500 ppm	750 ppm	1000 ppm	p
Cholesterol (mg / dl)	15 day	44.21±6.30	42.16±1.79	39.29±3.96	42.38±6.99	0.926
	30 day	40.42±5.18	46.27±5.33	52.48±5.90	40.95±8.34	0.513
Glucose (mg / dl)	15 day	61.52±4.72	46.16±5.09	54.48±2.77	50.66±3.92	0.341
	30 day	48.59±2.44	47.45±4.60	48.85±2.77	47.45±5.55	0.287
Urea-N (mg / dl)	15 day	19.23±1.04	15.31±0.80†	16.09±1.05†	17.57±1.07†	0.059
	30 day	17.88±1.45^{b}	21.93±1.15^{ab}	24.50±1.58^{a}	25.38±1.96^{a}	0.013
AOA (mmol / L)	15 day	6.57±0.69	6.53±052	6.28±0.29	7.11±0.68	0.776
	30 day	7.63±0.79	7.96±043	6.84±0.39	6.93±0.38	0.395
GSH (µmol / L)	15 day	13.41±0.47†	15.27±1.24	16.07±0.64	15.35±0.82	0.182
	30 day	9.68±0.87^{b}	17.75±2.42^{a}	14.29±0.60^{a}	15.62±0.95^{a}	0.005
MDA (nmol / L)	15 day	4.33±0.15^{a}††	3.02±0.10^{c}†††	3.57±0.21^{b}††	4.29±0.12^{a}†††	0.000
	30 day	6.26±0.32^{a}	5.13±0.12^{b}	5.27±0.21^{b}	5.30±0.14^{b}	0.005
Zn (µg / dl)	0 day	98.32±7.2	102.41±11.3	96.00±2.31	108.33±8.23	0.990
	15 day	60.05±7.13	64.31±8.42††	68.15±12.77††	86.50±11.75††	0.659
	30 day	89.22±5.54^{c}	119.91±4.61^{b}	137.20±5.67^{ab}	151.44±2.65^{a}	0.040
Cu (µg / dl)	15 day	42.22±18.64	36.02±6.26	41.64±6.60	48.15±2.28††	0.555
	30 day	42.58±6.61	30.68±10.15	39.03±8.09	21.21±8.69	0.340
Fe (µg / dl)	15 day	2021.81±430.12	2552.62±418.41	2819.91±350.48	3000.62±120.82	0.248
	30 day	3168.52±263.13	3153.13±842.15	2444.93±411.54	2586.54±630.53	0.480

^{a,b,c}, The difference between the mean values of different letters in the same order is statistically significant. The difference between the same groups on the 15th and 30th days is significant. †, P<0.05; ††, P<0.01; †††, P<0.001. AOA, Antioxidant activity; GSH, Glutathione; MDA, Malondialdehyde; Zn, Zinc; Cu, Copper; Fe, Iron.

Table VI.- Effects of different concentrations of zinc on rumen fluids, number of protozoa, pH, ammonia nitrogen, Zn, Cu and Fe in goats (n=6, ±SEM).

	Sampling time	0 ppm	500 ppm	750 ppm	1000 ppm	P
pH	15 day	6.92±0.10	6.96±0.03	6.80±0.06	6.91±0.06	0.479
	30 day	6.87±0.04	7.00±0.05	6.76±0.07	6.93±0.03	0.056
Ammonia nitrogen (mg / dl)	15 day	15.16±0.51^{a}	12.85±0.64^{b}	12.31±0.35^{b}	11.19±0.67^{b}	0.001
	30 day	15.37±0.98^{ab}	13.21±1.40^{b}	12.29±0.53^{c}	9.18±1.02^{d}	0.003
Number of protozoa (10^{3}/ ml)	15 day	394.16±16^{a}	345.00±42^{a}	260.14±21^{b}†††	247.08±24^{b}††	0.003
	30 day	375.27±25^{a}	266.53±12^{b}	205.83±21^{c}	186.11±15^{c}	0.000
Zn (µg / ml)	15 day	0.77±0.13^{b}	1.04±0.20^{ab}	1.22±0.26^{ab}	1.57±0.16^{a}	0.048
	30 day	0.96±0.08^{b}	1.12±0.19^{ab}	1.35±0.17^{ab}	1.51±0.11^{a}	0.037
Cu (µg / ml)	15 day	0.30±0.08	0.32±0.09†††	0.45±0.10††	0.40±0.04†††	0.636
	30 day	0.51±0.12^{b}	0.64±015^{b}	0.87±0.08^{ab}	1.38±0.03^{a}	0.034
Fe (µg / ml)	15 day	1.81±0.11^{a}	1.33±0.11^{ab}	1.50±0.14^{ab}	0.83±0.28^{b}††	0.038
	30 day	1.45±0.17	1.32±0.61	1.34±0.14	1.64±0.39	0.902

^{a,b,c,} The difference between the mean values of different letters in the same order is statistically significant. The difference between the same groups on the 15th and 30th days is significant. †, P<0.05; ††, P<0.01; †††, P<0.001. Zn, Zinc; Cu, Copper; Fe, Iron.

Table VII.- Effects of different concentrations of zinc on accumulation of zinc (ppm) in different rgans of goats (n=6, ±SEM).

	Tissue	0 ppm	500 ppm	750 ppm	1000 ppm	P
Zn (ppm)	Liver	77.95±7.75^{b}	93.13±7.76^{ab}	109.52±8.23^{a}	114.03±12.06^{a}	0.043
	Kidney	33.17±2.76	26.86±2.63	25.56±1.40	28.65±2.58	0.161
	Pancreas	45.35±1.63	45.18±2.47	49.19±3.04	48.96±1.95	0.461
	Mohair	120.90±10.80	125.70±3.06	137.72±15.39	156.30±36.90	0.608
Cu (ppm)	Liver	0.66±0.10	0.44±0.17	0.50±0.12	0.10±0.05	0.305
	Kidney	8.48±0.73^{a}	6.30±0.69^{b}	6.63±0.34^{b}	6.85±0.31^{b}	0.050
	Pancreas	1.79±0.14	1.50±0.05	1.80±0.17	1.92±0.16	0.220
	Mohair	7.84±0.46	10.01±1.08	9.04±0.48	8.00±0.34	0.103
Fe (ppm)	Liver	255.67±29.28^{b}	300.50±43.71^{ab}	390.19±46.40^{a}	231.79±28.54^{b}	0.038
	Kidney	92.78±6.87	99.80±16.43	85.23±8.08	93.41±6.66	0.804
	Pancreas	29.68±3.03	33.38±2.36	36.57±1.88	30.52±3.50	0.310
	Mohair	1073.00±236.70^{b}	1702.90±338.50^{a}	1180.10±158.90^{ab}	738.40±13.42^{b}	0.050

^{a,b}, The difference between the mean values of different letters in the same order is statistically significant.

DISCUSSION

The Zn supplementation is known to have positive effect on performance parameters in goats (Malcolm-Callis *et al*., 2000). In the present study, no difference was found between the groups in terms of live weights recorded on the same days. This result did not match with the studies by Malcolm-Callis *et al*. (2000) who observed that 100 ppm zinc supplementation reduces feed consumption and live weight and by Aksoy *et al*. (2002) observed that 500 mg zinc supplementation once a week per animal increases the live weight in lambs. However, it is compatible with the studies where the feed of the goats was supplemented with 250 ppm zinc (Eryavuz *et al*., 2002), feed of the sheep was supplemented with 1000 ppm zinc (Bonhomme *et al*., 1980) and 2100 ppm zinc (Henry *et al*., 1997), and feed of the cattle was supplemented with 1000 ppm zinc (Miller *et al*., 1989; Froestchel *et al*., 1990). In these studies, it was demonstrated that zinc supplementation had no effect on feed consumption and live weight. As a matter of fact, it has been documented that zinc supplementation to the feed of ruminant animals fed with feed containing optimum zinc does not have an effect on live weight (White *et al*., 1994). This also indicates that the 1000 ppm zinc supplementation has no negative effect on live weight and feed consumption in goats, as in cattle (Miller *et al*., 1989; Froestchel *et al*., 1990) and sheep (Henry *et al*., 1997). Long-term studies on goats may reveal the effect of high zinc supplementation on the feeding performance.

Zinc has been claimed to be associated with erythrocyte and haemoglobin production, and male goats living at high altitudes with increased erythrocytosis and blood haemoglobin levels have been emphasized to have higher serum zinc levels (Gonzales *et al*., 2011). Similarly in the studies reported by Dönmez and Keskin (1999) that the 250 ppm and 500 ppm by Sobhanirad and Naserian (2012) zinc supplementation in goats and cattle increase the RBC and Hb levels, a significant increase was found in the RBC and Hb levels measured from the samples obtained on the 30th day of the study in the zinc supplemented groups compared to the control group. Our results were also in accordance with the studies reported by Sobhanirad and Naserian (2012) that 500 ppm zinc supplementation to the ration of dairy cows and 250 ppm zinc supplementation to the ration of goats by Dönmez and Keskin (1999) increased the RBC and Hb levels. However, in a study, reported by Miller *et al*. (1989) that 1000 ppm zinc supplementation to the feed of cattle has no effects on the relevant parameters which were not compatible with our results. In a study by Ott *et al*. (1966), it was reported that the Hb and Hct levels of the lambs consuming 4000 ppm and 6000 ppm zinc, was increased, which was attributed to the haemoconcentration. In the present study, Hb levels were seen to increase but no change was observed in Hct value, indicating that the 1000 ppm zinc supplementation to the feed of goats had no negative effects such as haemoconcentration. In a study by Dönmez and Keskin (1999), they fed Angora goats with the control ration containing 35 ppm and a treatment ration supplemented with 250 ppm zinc for six months and declared that there was no change in the RBC, Hb, and Hct levels until the third month. However, they further observed that there was a decrease in the relevant values of the control group during the last sampling period of the study and they attributed this to the restriction of the

movement of animals due to the pasture conditions. Ülger and Coşkun (2003) reported in their study that the amount of zinc in the RBCs is approximately ten times higher than the zinc amount in the plasma because RBCs are rich in enzymes such as zinc-containing carbonic anhydrase. Considering this fact, adding zinc to the feed of goats with a smaller RBC diameter and a higher number of RBC (Reece and Swenson, 2008), compared to the sheep, can be said to have a positive effect on the number of RBC and the amount of Hb and to have the ability to prevent the decrease in the number of RBC due to movement restriction.

In the present study, 500-1000 ppm zinc supplementation resulted in no difference between the groups in terms of plasma total cholesterol levels measured from the samples obtained on the 15th and 30th days. It is stated that zinc has a structural and functional characteristic for lipid enzymes and lipid metabolism is impaired in the case of zinc deficiency (Li *et al*., 2013). Furthermore, high zinc intake reduces the lecithin-cholesterol acyltransferase enzyme activity as well as cholesterol, cholesterol esters, and plasma lipids. Indeed, the addition of 1000 ppm zinc to the ration has been shown to reduce plasma cholesterol esters by 10% and 500-1000 ppm zinc supplementation has been shown to reduce the cholesterol concentration by about 10% (Jenkins and Kramer, 1992). In a study by Malcolm-Callis *et al*. (2000), they added 20 ppm, 100 ppm, and 200 ppm zinc sulphate to the rations of feeder cattle and found that this zinc sulphate supplementation did not change the serum cholesterol levels. Similar to the findings of the present study, it was reported by Sobhanirad and Naserian (2012), that dairy cows with 500 ppm zinc did not change the total cholesterol level. On the other hand, in a study by Jenkins and Kramer (1992), in which 500 and 1000 ppm zinc was added to the milk of the calves, plasma cholesterol levels of these calves were reported to decrease. In this context, the study results revealed that 500-1000 ppm zinc has no effect on the cholesterol level.

No significant difference was observed between the groups in terms of the glucose level measured from the blood samples obtained on the 15th and 30th days. Avcı *et al*. (2013) added 250 ppm zinc to the feed of different breeds of sheep for a month and found that plasma glucose level did not change compared to control groups. Similarly, in other studies of Angora goat and calves by Puchala *et al*. (1999) and Mandal and Dass (2010), zinc added to the ration was reported to have no effect on the plasma glucose level.

It is known that the plasma urea nitrogen level in ruminants is highly influenced by the amount and type of protein in the ration and by the deamination of amino acids in the liver (Ayaşan, 2009). In the present study, the plasma urea nitrogen level did not change on the 15th day of the study and a significant increase was observed in the 1000 ppm group, in particular, on the 30th day. This finding is similar with the study by Eryavuz *et al*. (2002) in which 250 ppm zinc was supplemented to the ration of Angora goats and with another study conducted on two different sheep breeds (Avcı *et al*., 2013). This effect can be explained by the fact that a high level of zinc reduces protein digestion by decreasing activation of rumen microorganisms and passage of more proteins into the lower digestive organs. Puchala *et al*. (1999) demonstrated that the addition of zinc did not change the plasma urea level. Similarly, in a study by Mandal and Dass (2010) in which 35 ppm zinc was added to the feed of calves, it has been shown that the mean serum urea levels did not change on the 30th day if the periods were not considered (but there was a significant increase in the total period average).

Reduction of MDA levels in experimental groups compared to the control group in our study is matching up with the antiperoxidative activity of zinc on lipids and the case where the zinc stabilizes the membrane structures by antagonizing redox active metals such as iron and copper (Shaheen and El-Fattah 1995). In contrary to our finding, in a study by Nagalakshmi *et al*. (2009) on lambs, 15 ppm zinc supplemented to the basal ration was observed to be sufficient to protect the oxidant-antioxidant balance and the addition of higher zinc (45 ppm) was declared to have no effect in terms of reducing the oxidative stress. In the literature, it was reported that zinc supplementation decreased MDA and elevated GSH, however, Avcı *et al*. (2013) found that the change in the MDA and GSH levels were not significant in their study in which 250 ppm zinc was added to the feed of different breed sheep. The findings of the present study are in line with the finding that the addition of zinc to the feed of sheep does not lead to a change in total AOA reported by Avcı *et al*. (2013), whereas it is in contrary to the finding that AOA level increases in rats which are given feed with high zinc levels (Jing *et al*., 2007). The fact that there is no significant difference between the groups in terms of AOA level, which is an important indicator of oxidative stress, indicates that there is no feeding and environment causing oxidative stress in animals.

Serum zinc level was particularly determined in the formation of groups and was within normal limits which were 80-120 µg/dL (Altıntaş and Fidancı, 1993). In the samples obtained on the 15th day, serum zinc levels were under the normal limits, except for the 1000 ppm group. On the 30th day sampling, serum zinc levels were found to be within the normal limits in control and 500 ppm groups, however, 750 ppm and 1000 ppm groups were found to

have significantly higher serum zinc levels (Table V). In a study by Sobhanirad and Naserian (2012), 18 Holstein Friesians were divided into three groups; control, 500 ppm zinc sulphate and 500 ppm zinc methionine and serum zinc levels measured at the end of the 15th day were found as 133, 243 and 284 µg/dL. Serum zinc levels were reported to be higher compared to the zinc sulphate similar to the results of the studies by Ott *et al*. (1966) and Stake *et al*. (1975).

In the present study, it was found that serum copper and iron levels were not affected by the supplementation. In a study by Aksoy *et al*. (2002) in which 500 mg zinc oxide was administered orally to the lambs once a week for a total of 12 weeks, it was reported that serum copper levels significantly decreased and there was no change in iron levels. Our findings are similar with their findings in terms of iron levels, but contrary to in terms of copper levels. It has been further reported that the addition of 250 ppm zinc to the ration of the goats by Eryavuz *et al*. (2001), administration of 500 mg zinc per goat daily by Pechova *et al*. (2009) and addition of 1000 ppm zinc to the feed of cattle by Miller *et al*. (1989) have no effect on the copper levels and our findings are compatible with this.

The pH levels of the rumen samples obtained on the 15th and 30th day were found to be within the range of 6.76-7.00, which was similar with the studies by Eryavuz *et al*. (2002) and Cecava *et al*. (1993) in which they observed that zinc supplementation had no effect on the rumen pH levels. Run *et al*. (2013) emphasized in their *in vitro* study that rumen pH levels did not change with the zinc supplementation. Contrary to this, Önder and Keçeci (2003) in their research on Merino breed sheep have suggested that the zinc decreases the rumen pH levels. In a study by Arelovich *et al*. (2000) on dairy cows, a similar reduction was observed and the reason for this reduction in pH was reported to be due to the high level of zinc inhibiting urea hydrolysis and reducing ammonia production.

The addition of high levels of zinc to the feed of ruminant animals has been reported to cause changes in rumen fermentation (Bonhomme *et al*., 1980; Arelovich *et al*., 2000; Bateman *et al*., 2004). In several studies, the addition of zinc to the feed of ruminants was reported to affect the number and function of rumen microorganisms and the level of ammonia was accordingly affected by this situation (Arelovich *et al*., 2000; Bateman *et al*., 2004). In the samples obtained on the 15th and 30th day, the level of ammonia nitrogen was found to decrease in the zinc-added groups compared to the control group in the present study. It may be due to the ability of zinc to reduce the breakdown of amino acids and increases the post-ruminal transmission of the amino acid in the ration (Froetschel *et al*., 1990). This was compatible with the study by Eryavuz *et al*. (2002) in which the addition of 250 ppm zinc added to the feed of the Angora goats decreased the rumen ammonia nitrogen level. It has been reported that high levels of zinc in sheep affect the urea and nitrogen balance, while 860 ppm zinc has significantly reduced the rate of NH_3 formation (Arelovich *et al*., 1998). In a study by Mousa (2014) the reason for the effect of the addition of zinc on the ammonia level in rumen content of sheep and goats was reported to be due to the decrease in the release of ammonia from urine. On the other hand, in another *in vitro* study, 40 ppm zinc addition to the feed containing 37.60 ppm did not change the ammonia levels (Run *et al*., 2013) which can be attributed to the low dosage use.

Froetschel *et al*. (1990) reported that the addition of 1142 ppm zinc reduced the number of protozoa, and Bonhomme (1990) observed that the addition of 500 ppm or 1000 ppm zinc may cause protonation by killing the protozoa in the rumen. Similar to these studies, it was found that the number of protozoa decreased due to the addition of a high amount of zinc to the feed and the highest decrease was observed in the group supplemented 1000 ppm zinc. In an *in vitro* study, it was reported that the addition of zinc chloride to the rumen content of goats and sheep reduced the number of protozoa (Mousa, 2014). It has been demonstrated that rumen protozoa receive an excessive amount of zinc to their structure, even if they are toxic to themselves, and are therefore fragmented if there is too much zinc content in the environment because Rumen protozoa cannot control consumption in contrast to bacteria (Bonhomme, 1990). Considering the fact that faunated animals have lower rumen ammonia levels than defaunated animals (Eryavuz *et al*., 2002), the low number of protozoa in goats consuming a high amount of zinc may lead to a decrease in the rumen ammonia level. The findings obtained in this study show that zinc can be used to transfer the benefits obtained from defaunation to field conditions (Eryavuz, 2000).

The zinc levels of rumen fluid in the zinc-supplemented groups were found to be higher than those in the control group and these high levels were observed to be significant in the group supplemented with 1000 ppm zinc ($p<0.05$). This result was similar with the study by Eryavuz *et al*. (2002) who reported that zinc rumen fluid added to the feed of goat increased the zinc levels. In an *in vitro* study by Run *et al*. (2013) it has been documented that as the zinc level added to the rumen content increases, the zinc level measured in the rumen content also increases. In the present study, the rumen fluid zinc levels of the animals in the control group were within the rumen fluid zinc levels (0.20-1.00 µg/mL), which have been reported to provide optimum microbial growth in feed containing 50 ppm zinc in the dry matter (Reid *et al*., 1987). The rumen fluid zinc

levels of the groups supplemented with zinc were below the level (2.35 µg/mL) reported by Kennedy *et al*. (1993) in cattle fed with 920 ppm zinc but above the level (1.12 µg/mL) reported in Angora goats fed with 250 ppm zinc (Eryavuz *et al*., 2002). Similar to rumen fluid zinc levels, the addition of zinc to the feed increased the copper levels in the rumen content and the increase in the rumen content of the goats given 1000 ppm zinc supplementation was found to be significant ($p<0.05$). The reason for this result can be attributed to the decrease in the number of protozoa in the rumen. In fact, Ivan *et al*. (1992) reported that the level and absorption of copper in the rumen fluid increased due to the decrease in the number of protozoa in the rumen.

Ruminants show high tolerance towards the high amount of zinc intake because zinc is a mineral with low toxicity (Suttle, 2010). In the present study, the effects of high dietary zinc on the zinc, copper and iron levels in mohair, liver, kidney, and pancreas samples were also investigated and a significant increase was observed in zinc levels with the addition of 750 ppm and 1000 ppm zinc in liver samples. In mohair samples, zinc levels were observed to increase depending on the amount of addition, but this increase was not statistically significant. In the kidney samples, a significant decrease was found in the copper levels of groups given high zinc supplementation, but a higher amount of zinc supplementation was found not to contribute to this decrease. Liver iron levels were found to increase significantly with the addition of 750 ppm zinc, however, there was no such increase in the 1000 ppm zinc addition in the liver. In a study by Eryavuz *et al*. (2002) after the addition of 250 ppm zinc to the feed of the goats, an increase in the mohair zinc levels was observed four months later. In line with these findings, there was a nonsignificant increase in the mohair zinc levels obtained in the present study. Compatible with the study by Sandoval *et al*. (1997) who described that 700 ppm, 1400 ppm, and 2100 ppm zinc supplementation increased the level of liver zinc depending on the amount of addition in sheep, the addition of zinc to the feed was found to increase the liver zinc levels in the present study. However, an increase in the pancreas and kidney tissue was also reported in the same study. The difference between this and the present study can be attributed to differences in animal species and the amount of zinc additions used in trials. The results obtained from this study showed that high zinc supplementation reduces the storage of copper in the liver and kidney in goats fed with high zinc supplement for 30 days but increases iron storage in mohair and liver.

CONCLUSION

Zinc has influenced the WBC count, Hb concentration, blood urea nitrogen, MDA levels, and zinc availability in supplemented groups. Similarly, zinc supplementation decreased rumen ammonia nitrogen, rumen protozoa, Cu concentration in kidney tissue and increases Fe in mohair up to 500 ppm. It is concluded that goats can tolerate Zn level even up to 750 ppm in the diet.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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