Biological Trace Element Research
https://doi.org/10.1007/s12011-018-1448-1

Effect of Different Sources of Supplemental Zinc on Performance, Nutrient Digestibility, and Antioxidant Enzyme Activities in Lambs

Reza Alimohamady[1,2] · Hassan Aliarabi[1] · Rupert M. Bruckmaier[2] · Rachael G. Christensen[3]

Received: 3 April 2018 / Accepted: 16 July 2018

Abstract

Zinc (Zn) is an essential element in the growth of all animals and plays structural and catalytic roles in many enzymes and functional proteins. Two completely randomized trials were conducted to evaluate the effects of different sources of zinc on performance, nutrient digestibility, blood mineral profile, and antioxidant enzyme activities in male growing lambs on a barley-based diet. The first trial was conducted for 70 days and consisted of 30 lambs (30.8 ± 2.8 kg mean body weight, 4–5 months of age) which were randomly allocated to five treatments consisting of a basal diet (19.72 mg Zn/kg DM), or the basal diet supplemented with 30 mg Zn/kg DM, added as either zinc-sulfate (ZnSulf; inorganic), zinc-methionine (ZnMet), zinc-proteinate (ZnProt) or zinc-glycinate (ZnGly). For the second trial, to measure the effects of dietary Zn on nutrient digestibility, four lambs from each group of the first experiment were randomly allocated to individual digestibility cages for 12 days (first 7 days as an adaptation period followed by 5 days of sample collection). Among the groups, dietary Zn supplementation above basal level significantly improved average daily gain, average daily feed intake, feed/gain ratio, and superoxide dismutase activity of red blood cells ($P < 0.05$). Glutathione peroxidase activity of lambs supplemented with organic Zn was significantly ($P < 0.05$) higher than inorganic and control groups. At the end of the trial, the concentration of plasma Zn, tri-iodothyronine (T3), thyroxine (T4), and the activity of alkaline phosphatase was increased ($P < 0.05$) in all groups receiving Zn as compared with controls ($P < 0.05$). In addition, thyroxine level in animals supplemented with Zn-methionine and Zn-proteinate was greater than in animals receiving Zn-glycine and Zn-sulfate. The results of the second trial revealed that the supplementation with Zn-methionine and Zn-proteinate increased the digestibility of crude protein (CP) and acid detergent fiber (ADF) compared to groups supplemented with Zn sulfate and control ($P < 0.05$). All organic sources of Zn improved organic matter (OM) digestibility compared to inorganic and control ($P < 0.05$). Results indicated that, regardless of source, supplementation of Zn in growing lambs improved growth performance, blood antioxidants, and thyroid hormone levels. Furthermore, Zn-methionine and Zn-proteinate supplementation appeared to improve the digestibility of CP, OM, and ADF more effectively than Zn-sulfate.

Keywords Zinc · Lamb · Growth · Nutrient digestibility · Antioxidant enzyme · Thyroid hormones

Abbreviations

ADF	Acid detergent fiber
ADFI	Average daily feed intake
ADG	Average daily gain
BW	Body weight
CP	Crude protein
DMI	Dry matter intake
FBW	Final body weight
NDF	Neutral detergent fiber
OM	Organic matter
ZnMet	Zinc-methionine
ZnProt	Zinc-proteinate
ZnGly	Zinc-glycinate
ZnSulf	Zinc-sulfate

✉ Hassan Aliarabi
h_aliarabi@yahoo.com

[1] Department of Animal Science, Faculty of Agriculture, Bu-Ali Sina University, Azadegan Blvd., Hamadan 65178-33131, Iran

[2] Veterinary Physiology, Vetsuisse Faculty University of Bern, 3001 Bern, Switzerland

[3] Animal, Dairy and Veterinary Science, Utah State University, Logan, UT 84322, USA

Introduction

Zinc (Zn) is an essential mineral that plays a vital role in many biological processes, such as enzyme activity, cell membrane stabilization, gene expression, and cell signaling [1, 2]. It is required for structural and functional integrity of more than

Published online: 21 July 2018

2000 transcription factors and 300 enzymes; hence, almost all metabolic pathways are in some way dependent on at least one Zn-requiring protein [3]. Classical signs of severe Zn deficiency are uncommon in ruminants. However, marginal and often undetected Zn deficiency occurs frequently [4–6]. Early work suggested that marginal Zn deficiency can affect growth, reproduction, immune system, and gene expression, especially in fast-growing animals [7, 8]. To ensure that the growing animals fulfill their genetic potential concerning performance and health, Zn is often supplemented to livestock in regions with known Zn deficiency. However, the use of high dietary Zn levels may influence the digestion, absorption, and utilization of other nutrients in the diet and can potentially lead to environmental contamination from excess Zn excretion in the feces [9, 10]. Recently, organic mineral formulations in animal feed supplements have attracted substantial interest of feed manufacturers and animal producers as a way of improving animal performance and health based on higher bioavailability of Zn compared to inorganic salts [4, 11, 12]. However, results on different organic compounds (Zn-chelate, Zn-proteinate, and Zn-complex) are contradictory [13–17]. The inconsistent effects of dietary Zn on growth performance have been attributed to several factors such as differences in the physical-chemical properties of Zn supplements, Zn level in the basal diet, different Zn-dependent enzymes that affect animal performance and presence of other dietary ligands or antagonists [18, 19].

Therefore, the present study investigated the effects of Zn supplementation as Zn-glycinate, Zn-methionine, Zn-proteinate, and Zn-sulfate on performance, thyroid hormones, antioxidant enzymes, and nutrient digestibility of male growing lambs.

Materials and Methods

All stages of the experiment were observed by the ethical committee of Bu-Ali Sina University and were carried out under veterinary care. This study was designed in two parts to evaluate the effect of different sources of Zn supplementation on performance, thyroid hormones, antioxidant enzymes, and nutrient digestibility of growing Mehraban male lambs. In the first part, 30 male lambs 4–5 months old with an initial body weight of 30.8 ± 2.8 kg, after a 2-week adaptation time to the basal diet and treatment for internal parasites [Albendazole 600 mg, Lorestan Pharmaceutical, Iran), were randomly allocated to five treatments: basal diet containing 19.72 mg Zn/kg DM without supplementary Zn (control), and the basal diet supplemented with 30 mg Zn/kg DM, added as either Zn-sulfate (ZnSulf), Zn-methionine (ZnMet), Zn-proteinate (ZnProt), or Zn-glycinate (ZnGly). Nutrient composition of the basal diet is presented in Table 1. The basal diet was formulated according to National Research Council [20] to meet or exceed lambs requirements except for Zn.

Lambs were kept in individual stalls and had ad libitum access to water and feed. Feed was provided to the animals twice daily at 08:00 hours and 17:00 hours for 70 days. Feed was supplied in an amount so that each lamb would have about 10% orts and feed consumption and refusals were closely monitored and recorded daily. The analytical grade $ZnSO_4 \cdot 7H_2O$ (Merck, Germany) was used as the inorganic source of Zn. The organic sources of Zn were Zn-glycinate (18% Zn; Lalukco Company, Tehran, Iran), Zn-proteinate (15% Zn; Vetaque Company, Tehran, Iran), and Zn-methionine (18% Zn: by a modified method of United States patent with publication number: US7087775 B2). The amount of required Zn was adjusted based on dry matter intake of two consecutive days of individual lambs. The supplemental zinc was provided by addition to a carrier of wheat bran and soybean meal which was presented to the lambs first to ensure complete intake of the Zn supplement. Lambs were weighed at the beginning of the study and then in 2-week intervals until the end of the experiment.

Blood samples were taken by jugular vein puncture and collected in two tubes on days 0, 35, and 70 before the morning feeding; one containing heparin to obtain plasma and red blood cells (RBC) and the other without heparin to obtain serum. Plasma and serum samples were obtained by centrifuging whole blood at 1500×*g* for 15 min. Resulting supernatants were stored at − 80 °C until analysis of biochemical components, enzymes, and hormones.

The Zn, Fe, and Cu contents of feed and plasma samples were estimated utilizing an air-acetylene flame on an atomic absorption spectrophotometer (Varian spectra AA220, Australia) as described by Salama et al. [21] and Rimbach et al. [22], respectively. Serum calcium and phosphorus concentrations were estimated using a commercial kit (Pars Azmon, Iran) and an atomic absorption spectrophotometer (Varian SpectrAA220, Australia).

Total amounts of T3 and T4 in serum were determined by enzyme-linked immunosorbent assay (ELISA) method following manufacturers methods provided by the commercial kit (Pishtaz Teb, Iran) using a plate reader (ELX808, Bio-Tek, USA). Sensitivity and intra-assay coefficients of variation of the T3 assay were 0.3 nmol/L and 6.85%, respectively. Sensitivity and intra-assay coefficients of variation of the T4 assay were 12 nmol/L and 5.22%, respectively.

Whole blood glutathione peroxidase (GPx) activity was measured using detection kits (BiorexFars, Iran) which employ the Paglia and Valentine method [59]. The glutathione peroxidase catalyzes the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of glutathione reductase and NADPH, the oxidized glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP+, leading to the decrease of the absorbance at 340 nm. The superoxide dismutase (SOD) activity of red blood cells (RBC) was measured using an atomic absorption spectrophotometer (Varian SpectrAA220, Australia) after

Table 1 Ingredients and nutrient composition of the basal diet

Nutrients	Feedstuff				Basal diet
	Alfalfa hay (27%)	Barley grain (65%)	Wheat bran (5%)	Soy meal (3%)	
Dry matter (% DM)	90.07	91.96	88.80	91.76	91.13
Organic matter (% DM)	91.70	91.40	93.10	93.41	91.65
Crude protein (% DM)	14.51	11.33	15.92	41.94	13.59
ME (Mcal/kg)	2.10	3.00	2.50	3.00	2.66
NDF (% DM)	51.40	22.70	55.40	30.62	34.62
ADF (% DM)	29.90	11.80	13.13	8.83	18.11
Ca (% DM)	1.71	0.08	0.13	0.31	0.65
P (% DM)	0.25	0.33	0.75	0.60	0.33
Zn (mg/kg DM)	18.06	16.30	52.12	50.09	19.72
Cu (mg/kg DM)	9.50	6.10	12.28	17.22	7.93
Fe (mg/kg DM)	372.60	94.55	140.62	181.60	196.80

ME metabolizable energy (calculated based on NRC, 2007), *NDF* neutral detergent fiber, *ADF* acid detergent fiber, *DM* dry matter, *Ca* calcium, *P* phosphorus, *Zn* zinc, *Cu* copper, *Fe* iron

washing red cells three times with a 0.9% saline solution, according to Biorex kit method (BiorexFars, Iran). This method employs xanthine and xanthine oxidase to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5phenyl-tetrazolium chloride (INT) to form a red formazan dye. SOD activity was then measured by the degree of inhibition of this reaction [60]. For every sample, the antioxidant enzyme activities were calculated and expressed as units per gram of hemoglobin (Hb). Hemoglobin amount was analyzed in the laboratory using an automatic cell counter (Diatron Abacus C, Austria) by routine procedures.

Alkaline phosphatase (ALP), creatine phosphokinase (CPK), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) activities were estimated according to the recommendations of German Society of Clinical Chemistry and International Federation of Clinical Chemistry (IFCC), by a commercial kit (Pars Azmon, Iran) using an atomic absorption spectrophotometer (Varian SpectrAA220, Australia).

Digestibility Trial and Chemical Analyses

After the first trial, four lambs from each group were randomly selected and allocated to individual digestibility cages to study the effects of different sources of Zn on nutrient digestibility. The digestibility trial lasted for 12 days with 7-day adaptation period and subsequent 5-day collection period. Lambs were fed the same diet as in the first trial and daily feed intake, residual of diet and feces were collected, weighed, and recorded. The dietary ingredients and fecal samples were dried at 65 °C for 12 h in a hot air oven and ground to pass through a 1 mm sieve, were analyzed for DM, organic matter (OM), and crude protein (CP) according to standard procedures of AOAC [23]. Organic matter content of samples was estimated by the difference between DM and ash contents. Nitrogen content of samples was measured according to the Kjeldahl method. Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were analyzed according to Van Soest et al. [24] without heat stable alpha amylase and expressed inclusive of residual ash.

Statistical Analysis

The data were analyzed according to a completely randomized design (CRD). The model used for analysis was:

$$Y_{ij} = \mu + T_i + Bx_{ij} + e_{ij}$$

where μ is the overall mean, T_i is the effect of treatment i, and e_{ij} is the random error.

The General Linear Model (GLM) procedure (SAS, 9.4) was used for analysis of data. Duncan's multiple range tests were used for comparison of means, considering $P \leq 0.05$ as the significant level. Initial body weight was considered as a covariate for analysis of final body weight and average daily gain.

Results

Blood Minerals

Plasma Zn, Cu, Fe, Ca, and P concentrations are presented in Table 2. On days 35 and 70 of experiment, Zn-supplemented lambs had higher plasma Zn concentrations compared to the control group ($P < 0.01$). However, there were no significant differences of plasma Zn concentrations between Zn-supplemented treatments ($P > 0.05$). Zinc supplementation had no effect on plasma Cu, serum Ca, and P concentrations

Table 2 Effect of dietary zinc supplementation on plasma mineral profile of lambs in different treatments

Item		Treatment					SEM	*P* value
		Control	ZnMet	ZnProt	ZnGly	ZnSulf		Treatment
Zn (mg/L)								
Day	0	0.89	0.88	0.88	0.87	0.89	0.145	0.897
	35	0.93[b]	1.55[a]	1.41[a]	1.50[a]	1.52[a]	0.134	0.018
	70	0.89[b]	1.49[a]	1.48[a]	1.45[a]	1.49[a]	0.138	0.016
Cu (mg/L)								
Day	0	1.02	1.03	1.01	1.02	0.99	0.058	0. 969
	35	1.02	0.95	1.00	1.00	1.03	0.057	0.884
	70	1.02	1.14	1.08	1.06	1.12	0.089	0.880
Fe (mg/L)								
Day	0	1.91	1.94	1.92	1.90	1.92	0.093	0.969
	35	1.83	1.84	1.79	1.83	1.77	0.062	0.892
	70	2.00[a]	1.84[ab]	1.84[ab]	1.86[ab]	1.64[b]	0.069	0.026
Ca (mg/dL)								
Day	0	8.85	8.89	8.86	8.89	8.86	0.167	0.899
	35	8.48	9.42	9.38	9.14	9.47	0.349	0.266
	70	8.82	9.75	9.61	9.36	9.64	0.461	0.634
P (mg/dL)								
Day	0	7.81	7.82	7.78	7.78	7.85	0.194	0.988
	35	8.24	8.04	7.82	8.08	7.98	0.211	0.715
	70	7.89	8.11	7.64	7.81	8.03	0.249	0.710

Means without common superscript letters within rows are significantly different ($P < 0.05$)

Zn zinc, *Cu* copper, *Fe* iron, *Ca* calcium, *P* phosphorus, *ZnMet* zinc-methionine, *ZnProt* zinc-proteinate, *ZnGly* zinc-glycinate, *ZnSulf* zinc-sulfate, *SEM* standard error of mean

($P > 0.05$). On the other hand, Fe concentration in the group supplemented with Zn sulfate decreased significantly compared to control group ($P < 0.05$).

Blood Hormones and Enzymes

At the end of the experiment (day 70), serum T3 and T4 amounts were significantly ($P < 0.05$) higher in Zn-supplemented groups as compared to control (Table 3). However, thyroxine level in groups supplemented with Zn-methionine and Zn-proteinate was significantly higher than in those supplemented with Zn-glycine and Zn-sulfate. Serum ratio of T4/T3 in all of the supplemented groups was significantly lower ($P < 0.05$) than that of the control group. There were also no significant differences in serum T4/T3 ratio in all of the four supplemented groups ($P > 0.05$).

The SOD activity in red blood cells increased ($P < 0.05$) in all supplemented groups compared to control lambs during the period of experiment (Table 3). On the other hand, whole blood GPX activity in lambs supplemented with organic Zn was significantly ($P < 0.05$) higher than in lambs that received inorganic Zn and control groups.

The results for different enzyme activity are presented in Table 3. Alkaline phosphatase activity increased ($P < 0.05$) with Zn supplementation, but no significant differences were observed among Zn-supplemented groups. The activity of ALT and CPK in all groups was found to be statistically comparable among different treatments ($P > 0.05$). However, on day 70, the activity of AST in control group (124.73 U/L) was greater than ZnProt (94.77 U/L), ZnMet (101.52 U/L), ZnGly (104.60 U/L), and ZnSulf (105.99 U/L) groups ($P < 0.05$).

Performance

The performance parameters evaluated including average daily feed intake (ADFI), average daily gain (ADG), final body weight (FBW), and feed conversion efficiency (F/G) are presented in Table 4. Although there were no significant differences between treatments for initial body weight ($P > 0.05$), final weight was significantly higher in lambs supplemented ZnProt and ZnMet compared to control ($P < 0.05$). Zinc supplementation had a significant effect on performance parameters as ADG, ADFI, and F/G of all Zn-fed treatments improved, significantly ($P < 0.05$).

Table 3 Effect of dietary zinc supplementation on some enzyme activities and hormone concentrations of lambs in different treatments

Item		Treatment					SEM	*P* value
		Control	ZnMet	ZnProt	ZnGly	ZnSulf		Treatment
ALP (U/L)								
Day	0	237.56	238.88	229.90	228.45	228.52	8.51	0.832
	35	22.59^{b}	266.30^{a}	261.84^{a}	253.43^{a}	261.67^{a}	7.38	0.002
	70	233.69^{c}	272.26^{a}	259.86^{ab}	243.28^{bc}	261.40^{ab}	7.55	0.011
SOD (U/mg Hb)								
Day	0	80.93	80.77	81.17	78.52	78.95	7.57	0.985
	70	87.97^{b}	117.43^{a}	128.61^{a}	112.65^{a}	118.97^{a}	8.29	0.024
GPX (U/mg Hb)								
Day	0	48.96	51.60	47.85	53.32	54.09	6.19	0.913
	70	55.41^{b}	88.70^{a}	90.34^{a}	82.31^{a}	72.48^{ab}	6.45	0.004
AST (U/L)								
Day	0	85.79	83.13	83.66	80.39	80.79	5.09	0.942
	35	88.65	94.54	95.73	94.32	95.07	2.98	0.472
	70	124.73^{a}	101.52^{b}	94.77^{b}	104.60^{b}	105.99^{b}	3.64	0.001
ALT (U/L)								
Day	0	22.08	22.25	20.08	21.00	19.74	2.31	0.912
	35	22.41	21.42	18.44	20.84	21.00	2.56	0.857
	70	26.14	24.73	23.08	24.89	24.23	2.47	0.935
CPK (U/L)								
Day	0	78.74	86.02	78.57	81.55	81.38	3.93	0.674
	35	87.67	89.76	91.42	89.87	92.30	3.55	0.907
	70	99.69	101.23	101.78	103.66	96.16	3.05	0.508
T4 (nmol/L)								
Day	0	16.70	16.68	16.71	16.62	16.79	0.146	0.935
	70	16.40^{c}	17.09^{a}	17.06^{a}	16.71^{b}	16.74^{b}	0.106	0.006
T3 (nmol/L)								
Day	0	1.56	1.56	1.57	1.56	1.58	0.027	0.977
	70	1.47^{b}	1.66^{a}	1.65^{a}	1.60^{a}	1.58^{a}	0.029	0.007
T4/T3								
Day	0	10.70	10.68	10.67	10.64	10.67	0.203	0.926
	70	11.19^{a}	10.34^{b}	10.36^{b}	10.44^{b}	10.61^{b}	0.190	0.022

Means without common superscript letters within rows are significantly different ($P < 0.05$)

ALP alkaline phosphatase, *SOD* superoxide dismutase, *GPx* glutathione peroxidase, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *CPK* creatine phosphokinase, *T4* thyroxine, *T3* tri-iodothyronine, *ZnMet* zinc-methionine, *ZnProt* zinc-proteinate, *ZnGly* zinc-glycinate, *ZnSulf* zinc-sulfate, *SEM* standard error of mean

Nutrient Digestibility

Nutrient digestibility results are shown in Table 5. The digestibility of DM and NDF was not affected by treatment ($P > 0.05$). However, our findings revealed that supplementation of the diet with ZnMet and ZnProt increased digestibility of CP and ADF compared to groups supplemented with Zn sulfate and control ($P < 0.05$). Furthermore, organic sources improved OM digestibility compared to inorganic Zn and control ($P < 0.05$).

Discussion

Blood Minerals

Zinc concentration in blood plasma or serum is the most widely used indicator of Zn status. Plasma zinc concentrations normally respond to zinc supplementation, especially in lambs consuming diets with a low or marginal Zn level [8, 25]. The average concentrations of Zn (days 35 and 70) in blood plasma were 0.91 and 1.52 mg/L for control and Zn-methionine

Table 4 Effect of dietary zinc supplementation on growth performance of lambs in different treatments

Item	Treatment					SEM	*P* value
	Control	ZnMet	ZnProt	ZnGly	ZnSulf		
Initial body weight (kg)	30.23	31.22	30.83	30.80	31.05	0.74	0.893
Final body weight (kg)	42.90^{b}	46.27^{a}	45.97^{a}	45.18^{ab}	45.70^{ab}	0.97	0.083
Average daily gain (g/day)	181^{b}	216^{a}	215^{a}	205^{a}	209^{a}	5.35	0.001
Average dry matter intake (g/day)	1440^{b}	1520^{a}	1532^{a}	1499^{a}	1517^{a}	17.36	0.008
Feed conversion efficiency (feed/gain)	8.01^{a}	7.08^{b}	7.10^{b}	7.31^{b}	7.26^{b}	0.173	0.004

Means without common superscript letters within rows are significantly different ($P < 0.05$)

ZnMet zinc-methionine, *ZnProt* zinc-proteinate, *ZnGly* zinc-glycinate, *ZnSulf* zinc-sulfate, *SEM* standard error of mean

groups, respectively, whereas the normal level of Zn in sheep plasma ranges between 0.8 and 1.2 mg/L [8]. Current zinc data indicate that Zn level in the blood of control lambs is close to minimum value but was not indicative of Zn deficiency. Furthermore, higher plasma Zn level in the zinc supplemented groups as compared to control animals suggested that adding 30 mg Zn/kg DM from organic or inorganic sources to diet of growing lambs containing 19.72 mg Zn/kg DM might increase plasma Zn concentration. Similar to our findings, previous evidence demonstrated that Zn supplementation could increase the plasma Zn concentration in lambs [17, 26] and male goats [15]. In contrast, no significant difference was observed in the plasma Zn concentration in adult sheep supplemented daily with 75 or 150 mg of Zn either as chelated Zn or inorganic Zn [27] and calves supplemented with 80 or 120 mg Zn/kg DM as Zn-sulfate on a basal diet containing 29.7 mg Zn/kg DM during 60- and 90-day feeding trials [28].

Hill and Matrone [29] stated that Zn and some trace minerals have similar physico-chemical properties, and Zn may disrupt the homeostasis of other essential elements. In the present study, there was no effect of either Zn-sulfate, Zn-methionine, Zn-proteinate, or Zn-glycinate supplementation on plasma Cu concentration. However, serum Cu levels have been reported [30, 31] to be decreased due to supplementation of ZnO (250 or 1000 mg Zn/kg) in the basal diet of male buffalo calves, and with supplementation of different organic or inorganic Zn sources (360 mg per day) in yearling cattle. A likely explanation for the inconsistent results might be due to higher levels of Zn supplementation used by these researchers. In contrast, present results are supported by literature [14, 32] in which any effect on serum Cu concentration in lambs supplemented with 20 or 40 mg Zn/kg DM through different sources was not reported. Similarly, no significant effect on serum Cu concentration was observed for finishing goats [33] and calves [13] supplemented with Zn either as organic or inorganic forms.

Table 5 Effect of dietary zinc supplementation on nutrient digestibility (%) of lambs in different treatments

Item	Treatment					SEM	*P* value
	Control	ZnMet	ZnProt	ZnGly	ZnSulf		
DM (%)	65.42	67.00	68.54	67.22	65.99	1.15	0.257
CP (%)	68.92^{b}	73.03^{a}	73.38^{a}	72.26^{ab}	68.31^{b}	1.32	0.042
OM (%)	75.22^{b}	78.05^{a}	79.26^{a}	78.08^{a}	74.84^{b}	0.82	0.012
NDF (%)	43.13	48.17	47.34	45.94	44.95	1.77	0.378
ADF (%)	28.32^{b}	33.97^{a}	34.22^{a}	31.43^{ab}	27.02^{b}	1.45	0.009

Means without common superscript letters within rows are significantly different ($P < 0.05$)

DM dry matter, *CP* crude protein, *OM* organic matter, *NDF* neutral detergent fiber, *ADF* acid detergent fiber, *ZnMet* zinc-methionine, *ZnProt* zinc-proteinate, *ZnGly* zinc-glycinate, *ZnSulf* zinc-sulfate, *SEM* standard error of mean

Inorganic Zn supplementation reduced plasma Fe content which is supported by Garg et al. [14], who observed a reduced effect of Zn supplementation on serum Fe status in male lambs. In addition, these researchers stated that Zn-methionine supplementation was also associated with reduction in Fe level. The difference in Zn response might be due to higher level of Zn in their basal diet compared to the present study (34 vs 19.72, respectively). In contrast, Aliarabi et al. [26] and Mandal et al. [13] did not see similar response on blood Fe content in relation to dietary Zn levels in lambs and calves, respectively.

The concentrations of serum Ca and P were found to be similar in all the groups as reported by Garg et al. [14] in lambs supplemented with 20 mg/kg of Zn in their diet. The values were found to be within the normal reference range for ruminants [61]. Our results are also supported by Daghash and Mousa [34] who did not observe any effect of Zn supplementation on serum Ca and P in buffaloes with supplementation of 50 and 100 mg Zn/kg.

Blood Hormones and Enzymes

Zinc effects on thyroid hormones are complex including both synthesis and mode of action [35]. Zinc plays an important role in the detoxification of oxygen-derived free radicals and may thus protect the thyroid gland from toxic species [36].

Considering the improvement in antioxidant status of Zn-fed lambs in the present study, it may be possible that at least a part of the thyroid hormones decrease in control lambs might be due to the impairment of antioxidant functions and thyroid hormones synthesis. Lower T4 and T3 concentrations in control group compared to supplemented lambs support the previously reported result [37] which showed decreased serum T3 and T4 concentrations in Zn-deficient animals. It is unclear why lambs fed Zn-proteinate and Zn-methionine tended to have higher concentration of T4 than lambs fed a similar quantity of Zn from Zn-sulfate and Zn-glycinate. However, results of the present study support that the concept that Zn-proteinate and Zn-methionine may be metabolized differently from inorganic Zn and, thus, may alter some metabolic processes differently [38]. Conversely, it has been indicated that supplemental Zn (35 mg Zn/kg) either from inorganic or organic sources did not affect plasma T3 and T4 concentrations in crossbred calves [39]. On the other hand, Kececi and Keskin [40] observed reduced concentration of serum T3 and T4 due to supplementation of 290 mg Zn/kg diet as zinc sulfate in healthy male Merino lambs and Angora goats. Zinc is also required for the activity of the enzyme 1,5′-deiodinase, which converts biologically T4 to T3 [41, 42]. In the present study, Zn supplementation also had significant effect on ratio of T4/T3 with lower value in all supplemented lambs. Lower T4 to T3 ratios in supplemented groups in our study show that 5′-deiodinase activity was improved by organic and inorganic zinc supplementation.

The oxidative enzymes, such as Zn-Cu-superoxide dismutase and glutathione peroxidase, cooperate to eliminate free radicals and products of their decomposition. Superoxide dismutase produces hydrogen peroxide during its scavenging action. The peroxides formed by the SOD action are detoxified by glutathione peroxidase which reduces hydrogen peroxide to water [43]. For the present study, regardless of source, Zn supplementation increased RBC-SOD activity that might be possibly related to the improvement of the synthesis, stability, or slowdown of degradation [44]. The higher SOD activity observed in Zn supplemented groups is in agreement with Fadayifar et al. [32] who observed that 20 mg Zn/kg DM supplementation was required for obtaining higher SOD activity in lambs. Similarly, Nagalakshmi et al. [45] reported superior SOD activity in lambs fed 15 and 30 mg/kg Zn supplemented diets as organic or inorganic sources compared to control diet-fed lambs. In contrast, Manadal et al. [39] observed a similar SOD activity among control group receiving 32.5 mg/kg Zn compared to the crossbred calves supplemented with 35 mg/kg Zn from Zn-sulfate or Zn-propionate. On the other hand, among the treatment groups, GPx activity in lambs supplemented with organic Zn were significantly higher than GPx activity in control group. It was reported that Zn deficiency may lead to an increase in free radical production and decrease in activity of SOD and GPx of Zn-deficient rats [46, 47]. This indicated that the 19.72 mg/kg DM level of Zn in the basal diet is not sufficient for adequate GPX and SOD activities in lambs, and addition of 30 mg Zn/kg DM as organic sources can improve antioxidant balance.

Alkaline phosphatase is a Zn-dependent enzyme involved in calcium absorption, growth, and development of growing animals and has been used as an indicator of Zn status [48]. The lower ALP activity of the control lambs is consistent with the study of Cho et al. [49] who reported decreased ALP activity in Zn-deficient animals. The present results are also in agreement with Liu et al. [50] who observed a significant increase in ALP activity due to dietary Zn in goats fed a basal diet (containing 45.9 mg Zn/kg) supplemented with 20, 40, or 80 mg Zn/kg. Similarly, Kumar et al. [51] observed a significant increase in ALP activity attributed to dietary Zn in bulls fed a basal diet (containing 32.5 mg Zn/kg) supplemented with 35 or 70 mg Zn/kg. However, no significant effect in growing lambs was noted among different Zn sources, as reported by Aliarabi et al. [26].

The activities of ALT, AST, and CPK in blood plasma or serum are routinely measured to assess liver and muscle lesions [52]. Manadal et al. [39] observed the comparable AST and ALT activity among crossbred calves supplemented with different sources but similar level (35 mg/kg Zn) and control group receiving 32.5 mg/kg Zn. This indicates that the Zn concentration in basal diet of the present study (19.72 mg/kg) was not sufficient to inhibit tissue lesion. On the other hand, Daghash and Mousa [34] observed higher AST activity in buffalo calves supplemented with 50 or 100 mg Zn/kg, which is not in agreement with our results.

Performance

In the present experiment, lambs fed the control diet with basal level of 19.72 mg Zn/kg DM gained less weight than the Zn-supplemented lambs, suggesting that the basal level of Zn was insufficient for optimum growth. Based on our results, this might be due to impairment of feed intake, alkaline phosphatase activity, thyroid hormone concentration, and antioxidant status. According to NRC, Zn recommendations for lambs with 30 to 50 kg BW and an ADG of 250 g/day is about 40 mg/day. The lambs in control group received, on average, about 28 mg/day Zn which is less than NRC recommendations, which could affect the performance. However, when 30 mg Zn/kg DM was added, average daily intake of Zn increased to about 75 mg/day, which is higher than NRC recommendations. These results suggest that there may be a higher Zn requirement for lambs in the present study. In addition, an increased Zn requirement above that recommended by NRC has been suggested for goats and cattle [6, 13, 53]. Similar to our results, it is reported that lambs consuming a diet supplemented with 20 or 40 mg Zn/kg DM from Zn-proteinate and Zn-sulfate had greater ADG and final body

weight compared with lambs consuming a basal diet containing 22.5 mg Zn/kg DM [26]. In contrast, supplementation of the diet with different sources of Zn had no effect on daily weight gain of lambs [54], calves [55, 56], and goats [33] compared to the control or inorganic Zn supplements.

Considering the effect of Zn on feed intake, our results are consistent with those reported by Mallaki et al. [17] who found that DMI was greater in lambs receiving an additional 20 mg/kg Zn as sulfate or peptide (basal diet containing 22.8) than in those not supplemented with Zn. In contrast, some authors reported that Zn supplementation did not affect DMI in calves [56] and goats [21, 57].

Based on feed efficiency in the present experiment, supplementation of 30 mg/kg Zn to the basal diet containing 19.72 mg Zn/kg DM improved the feed conversion ratio. Similarly, Mallaki et al. [17] and Jia et al. [6] reported the improvement of feed efficiency in finishing lambs fed organic and inorganic Zn as compared to control diet. In contrast, Spears et al. [58] in calves and Mandal et al. [13] in bulls did not report the difference in feed conversion ratio using 35 and 25 mg/kg Zn, respectively.

Nutrient Digestibility

Conflicting results of Zn source on nutrient digestibility have been reported in previous studies in sheep [14, 17, 21, 54], goats [6], and bulls [39]. Many factors might contribute to the discrepancies among the above reports, such as chemical characteristics of organic Zn sources used, dietary Zn levels, animal species, and factors affecting Zn solubility and stability in gastrointestinal tract. The present results indicate that the three organic Zn sources were more effective than the inorganic Zn-sulfate with Zn-proteinate and Zn-methionine more effective than Zn-glycinate in improving of CP and OM digestibility. Jia et al. [6] found that supplementation of a diet containing 22.3 mg Zn/kg DM with 20 mg/kg Zn as Zn-methionine improved ADF digestibility in cashmere goats, but DM, CP, and NDF digestibility were not affected, whereas Mallaki et al. [17] stated that supplementation of a diet containing 22.8 mg Zn/kg DM with 20 mg/kg Zn as Zn-proteinate improved NDF and CP digestibility. Comparable digestibility of DM, OM, CP, EE, NDF, and hemicellulose was also observed in study of Garg et al. [14], while ADF digestibility was improved significantly in lambs receiving 20 mg Zn/kg DM as Zn-methionine compared to Zn-sulfate and control lambs.

Conclusions

In conclusion, our data suggest that regardless of source, supplementation of Zn in growing lambs improved performance as compared to non-supplemented control lambs consuming a basal diet containing 19.72 mg/kg Zn. The improvement of performance in Zn-supplemented lambs might be due to increased feed intake, improved Zn-dependent enzyme activity, increased thyroid hormone production and improved antioxidant status. Furthermore, Zn-methionine and Zn-proteinate supplementation improved CP, OM, and ADF digestibility compared to Zn-sulfate.

References

1. Swain PS, Rao SBN, Rajendran D, Dominic G, Selvaraju S (2016) Nano zinc, an alternative to conventional zinc as animal feed supplement: a review. Anim Nutr 2:134–141
2. Uniyal S, Garg AK, Jadhav SE, Chaturvedi VK, Mohanta RK (2017) Comparative efficacy of zinc supplementation from different sources on nutrient digestibility, hemato-biochemistry and antioxidant activity in guinea pigs. Livest Sci 204:59–64
3. Ranasinghe P, Wathurapatha WS, Ishara MH, Jayawardana R, Galappatthy P, Katulanda P, Constantine GR (2015) Effects of zinc supplementation on serum lipids: a systematic review and meta-analysis. Nutr Metab 12:1–16
4. Spears JW (1989) Zinc methionine for ruminants: relative bioavailability of zinc in lambs and effects on growth and performance of growing heifers. J Anim Sci 67:835–843
5. Sandoval M, Henry PR, Littell RC, Cousins RJ, Ammerman CB (1997) Estimation of the relative bioavailability of zinc from inorganic zinc sources for sheep. Anim Feed Sci Technol 66:223–235
6. Jia W, Jia Z, Zhang W, Wang R, Zhang S, Zhu X (2008) Effects of dietary zinc on performance, nutrient digestibility and plasma zinc status in cashmere goats. Small Rumin Res 80:68–72
7. Spears JW, Weiss WP (2008) Role of antioxidants and trace elements in health and immunity of transition dairy cows. Vet J 176:70–76
8. Suttle NF (2010) Mineral nutrition of livestock, 4th edn. CABI Publishing, New York
9. Bao YM, Choct M, Iji PA, Bruerton K (2007) Effect of organically complexed copper, iron, manganese, and zinc on broiler performance, mineral excretion, and accumulation in tissues. J Appl Poult Res 16:448–455
10. Mohanta RK, Garg AK (2014) Organic trace minerals: immunity, health, production and reproduction in farm animals. Indian J Anim Nutr 31:203–212
11. Ammerman CB, Baker DP, Lewis AJ (1995) Bioavailability of nutrients for animals: amino acids, minerals, vitamins. In: Academic press. San Diego, California
12. Spears JW (2003) Trace mineral bioavailability in ruminants. J Nutr 133:1506–1509
13. Mandal GP, Dass RS, Isore DP, Garg AK, Ram GC (2007) Effect of zinc supplementation from two sources on growth, nutrient utilization and immune response in male crossbred cattle (Bos indicus×Bos taurus) bulls. Anim Feed Sci Technol 138:1–12
14. Garg AK, Vishal M, Dass RS (2008) Effect of organic zinc supplementation on growth, nutrient utilization and mineral profile in lambs. Anim Feed Sci Technol 144:82–96
15. Jia W, Xiaoping ZH, Wei ZH, Jianbo CH, Cuihua G, Zhihai J (2009) Effects of source of supplemental zinc on performance, nutrient digestibility and plasma mineral profile in cashmere goats. Asian-Australas J Anim Sci 22:1648–1653
16. Wang RL, Liang JG, Lu L, Zhang LY, Li SF, Luo XG (2013) Effect of zinc source on performance, zinc status, immune response and rumen fermentation of lactating cows. Biol Trace Elem Res 152:16–24

17. Mallaki M, Norouzian MA, Khadem AA (2015) Effect of organic zinc supplementation on growth, nutrient utilization, and plasma zinc status in lambs. Turk J Vet Anim Sci 39:75–80
18. Salim HM, Lee HR, Jo C, Lee SK, Lee BD (2012) Effect of sex and dietary organic zinc on growth performance, carcass traits, tissue mineral content and blood parameters of broiler chicken. Biol Trace Elem Res 147:120–129
19. Zakaria HA, Jalal M, Al-Titi HH, Souad A (2017) Effect of sources and levels of dietary zinc on the performance, carcass traits and blood parameters of broilers. Rev Bras Cienc Avic 19:519–526
20. NRC (2007) National Research Council nutrient requirements of small ruminants: sheep, goats, Cervids, and New World camelids. National Academy Press, Washington, DC
21. Salama AAK, Caja G, Albanell E, Such X, Casals R, Plaixats J (2003) Effects of dietary supplements of zinc methionine on milk production, udder health and zinc metabolism in dairy goats. J Dairy Res 70:9–17
22. Rimbach G, Walter A, Most E, Pallauf J (1998) Effect of microbial phytase on zinc bioavailability and cadmium and lead accumulation in growing rats. Food Chem Toxicol 36:7–12
23. AOAC (2000) Association of Official Analytical Chemist. In: Horwitz W (ed) Official methods of analysis of AOAC international, 17th edn. AOAC International, Maryland-Gaithersburg, USA
24. Van Soest PJ, Robertson JB, Lewis BA (1991) Methods of dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. J Dairy Sci 74:3583–3587
25. Wieringa FT, Dijkhuizen MA, Fiorentino M, Laillou A, Berger J (2015) Determination of zinc status in humans: which indicator should we use? Nature 7:3252–3263
26. Aliarabi H, Fadayifar A, Tabatabaei MM, Zamani P, Bahari A, Farahavar A, Dezfoulian AH (2015) Effect of zinc source on hematological, metabolic parameters and mineral balance in lambs. Biol Trace Elem Res 168:82–90
27. Ryan JP, Kearns P, Quinn T (2002) Bioavailability of dietary copper and zinc in adult Texel sheep: a comparative study of the effects of sulfate and bioplex supplementation. Ir Vet J 55:221–225
28. Ramulu SP, Nagalakshmi D, Kumar MK (2015) Effect of zinc supplementation on haematology and serum biochemical constituents in Murrah buffalo calves. Indian J Anim Res 49:482–486
29. Hill CH, Matrone G (1970) Chemical parameters in the study of in vivo and in vitro interaction of transition elements. Fed Proc 29: 1474–1481
30. Attia AN, Awadalla SA, Esmail EY, Hady MM (1987) Role of some microelements in nutrition of water buffalo and its relation to production 2 Effect of zinc supplementation Assiut. Vet Med J 18:91–100
31. Rojas LX, McDowell LR, Cousins RJ, Martin FG, Wilkinson NS, Johnson AB, Velasquez JB (1995) Relative bioavailability of two organic and two inorganic zinc sources fed to sheep. J Anim Sci 73: 1202–1207
32. Fadayifar A, Aliarabi H, Tabatabaei MM, Zamani P, Bahari AA, Malecki M, Dezfoulian AH (2012) Improvement in lamb performance on barley based diet supplemented with zinc. Livest Sci 144: 285–289
33. Zaboli K, Aliarabi H, Bahari AA, Abbasalipourkabir R (2013) Role of dietary nano-zinc oxide on growth performance and blood levels of mineral: a study on in Iranian angora (markhoz) goat kids. Int Advis Board 2:19–26
34. Daghash HA, Mousa SM (1999) Zinc sulfate supplementation to ruminant rations and its effects on digestibility in lamb; growth, rectal temperature and some blood constituents in buffalo calves under heat stress. Assiut Vet Med J 40:128–146
35. Ertek S, Cicero A, Caglar O, Erdogan G (2010) Relationship between serum zinc levels, thyroid hormones and thyroid volume following successful iodine supplementation. Hormones 9:263–268
36. Rabeh NM, El-Ghandour HA (2016) Effect of iron, zinc, vitamin E and vitamin C supplementation on thyroid hormones in rats with hypothyroidism. Int J Nutr Food Sci 5:201–210
37. Gupta RP, Verma PC, Garg SL (1997) Effect of experimental zinc deficiency on thyroid gland in guinea pigs. Ann Nutr Metab 41: 376–381
38. Spears JW (1996) Organic trace minerals in ruminant nutrition. Anim Feed Sci Technol 58:151–163
39. Mandal GP, Dass RS, Varshney VP, Mondal AB (2008) Effect of zinc supplementation from inorganic and organic sources on growth and blood biochemical profile in crossbred calves. J Anim Feed Sci 17:147–156
40. Kececi T, Keskin E (2002) Zinc supplementation decrease total thyroid hormone concentration in small ruminants. Acta Vet Hung 50:93–100
41. Wada L, King JC (1986) Effect of low zinc intakes on basal metabolic rate, thyroid hormones and protein utilization in adult men. J Nutr 116:1045–1053
42. Nishiyama S, Futagoishi-Suginohara Y, Matsukura M, Nakamura T, Higashi A, Shinohara M, Matsuda I (1994) Zinc supplementation alters thyroid hormone metabolism in disabled patients with zinc deficiency. J Am Coll Nutr 13:62–67
43. Guan T, Song J, Wang Y, Guo L, Yuan L, Zhao Y, Gao Y, Lin L, Wang Y, Wei J (2017) Expression and characterization of recombinant bifunctional enzymes with glutathione peroxidase and superoxide dismutase activities. Free Radic Biol Med 110:188–195
44. Jarosz M, Olbert M, Wyszogrodzka G, Młyniec K, Librowski T (2017) Antioxidant and anti-inflammatory effects of zinc. Zinc-dependent NF-κB signaling. Inflam Mopharmacol 25:11–24
45. Nagalakshmi D, Dhanalakshmi K, Himabindu D (2009b) Effect of dose and source of supplemental zinc on immune response and oxidative enzymes in lambs. Vet Res Commun 33:631–644
46. Shaheen AA, El-Fattah AA (1995) Effect of dietary zinc on lipid peroxidation, glutathione, protein levels and superoxide dismutase activity in rat tissues. Int J Biochem Cell Biol 27:89–95
47. Sidhu P, Garg ML, Dhawan DK (2005) Protective effects of zinc on oxidative stress enzymes in liver of protein-deficient rats. Drug Chem Toxicol 28:211–230
48. Wang KK, Cui HW, Sun JY, Qian LC, Weng X (2012) Effects of zinc on growth performance and biochemical parameters of piglets. Turk J Vet Anim Sci 36:519–526
49. Cho YE, Lomeda RAR, Ryu SH, Sohn HY, Shin HI, Beattie JH, Kwun IS (2007) Zinc deficiency negatively affects alkaline phosphatase and the concentration of Ca, Mg and P in rats. Nutr Res Pract 1:113–119
50. Liu HY, Sun MH, Yang GQ, Jia CL, Zhang M, Zhu YJ, Zhang Y (2015) Influence of different dietary zinc levels on cashmere growth, plasma testosterone level and zinc status in male Liaoning cashmere goats. J Anim Physiol Anim Nutr 99:880–886
51. Kumar N, Verma RP, Singh LP, Varshney VP, Dass RS (2006) Effect of different levels and sources of zinc supplementation on quantitative and qualitative semen attributes and serum testosterone level in crossbred cattle (Bos indicus × Bos taurus) bulls. Reprod Nut Dev 46:663–675
52. Alimohamady R, Aliarabi H, Bahari A, Dezfoulian AH (2013) Influence of different amounts and sources of selenium supplementation on performance, some blood parameters, and nutrient digestibility in lambs. Biol Trace Elem Res 154:45–54
53. Spears JW, Kegley EB (2002) Effect of zinc source (zinc oxide vs zinc proteinate) and level on performance, carcass characteristics, and immune response of growing and finishing steers. J Anim Sci 80:2747–2752
54. Nagalaksmi D, Himabindu D (2013) Effect of zinc supplementation from organic and inorganic sources on performance, nutrient utilization and carcass characteristics in lambs. Indian J Anim Sci 83: 411–418

55. Kessler J, Morel I, Dufey PA, Gutzwiller A, Stern A, Geyer H (2003) Effects of organic zinc sources on performance, zinc status and carcass, meat and claw quality in fattening bulls. Livest Prod Sci 81:161–171
56. Wright C, Spears J (2004) Effect of zinc source and dietary level on zinc metabolism in Holstein calves. J Dairy Sci 87:1085–1091
57. Puchala R, Sahlu T, Davis JJ (1999) Effects of zincmethionine on performance of angora goats. Small Rumin Res 33:1–8
58. Spears JW, Harvey RW, Brown JR (1991) Effects of zinc methionine and zinc oxide on performance, blood characteristics, and antibody titer response to viral vaccination in stressed feeder calves. J Am Vet Med Assoc 199:1731–1733
59. Paglia D, Valentine W (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. Lab Clin Med 70:158–169
60. Arthur JR, Boyne R (1985) Superoxide dismutase and glutathione peroxidase activities in neutrophils from selenium deficient and copper deficient cattle. Life Sci 36:1569–1575
61. Underwood EJ, Suttle NF (1999) The mineral nutrition of livestock, 3rd edn. CABI Publishing Co., New York