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RESEARCH

The Effect of Different Zinc Sources on Biochemical Parameters, Intestinal Morphology, Carcass Characteristics and Performance in Finishing Lambs

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Abstract
To investigate the effect of different sources of zinc supplements on blood serum parameters, nutrient digestibility, growth performance, carcass characteristics, and intestinal morphology, 18 male Zandi lambs (with initial body weight of 31 ± 1.2 kg and 120 ± 8 days old) were divided into three groups, six animals each in a completely random design. Experimental treatments include (1) control treatment of basal diet without zinc supplementation, (2) basal diet with 40 mg/kg of zinc supplementation from zinc sulfate source, and (3) basal diet with 40 mg/kg of zinc supplementation with origin it was organic (Zn-peptide). All lambs were kept in individual pens with cemented floor and provision of individual feeding and watering. Mean daily weight gain increased with zinc supplementation ($P < 0.05$), but feed intake and feed conversion ratio were not affected by zinc supplementation in the diet. Zinc supplementation increased the apparent digestibility of the dry matter ($P < 0.05$), but the digestibility of dietary fat, neutral detergent fiber (NDF), and acid detergent fiber (ADF) were not affected by zinc supplementation. In this experiment, the addition of organic and inorganic supplements to the diet of fattening lambs had no significant effect on serum triglyceride, cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL) and glucose concentrations, and carcass traits. The concentration of aspartate aminotransferase (AST) enzyme in the zinc sulfate group was significantly higher than the control and organic zinc groups ($P < 0.05$). Concentrations of blood urea nitrogen were lower in zinc fed lambs, compared to control ($P \leq 0.05$). The villi width in the duodenum was higher in the zinc supplementation treatments ($P < 0.05$). Also, in the ileum section, the height of the villi in the treatment of zinc sulfate supplement was higher, compared to the complement and control ($P < 0.05$). The results of this study showed that Zn supplementation, regardless of its source, improved growth performance in fattening lambs. However, no effect was observed on feed intake and efficiency, carcass traits, and blood parameters.

Keywords Zinc sulfate · Zn-peptide · Intestinal morphology · Performance · Digestibility · Finishing lamb

Introduction

Trace elements such as zinc are one of the ways to improve the health and production of livestock as agents of metabolic improvement. Many researchers have reported that zinc is an essential mineral that plays a vital role in many biological processes, such as enzyme activity, cell membrane stabilization, gene expression, and cellular signaling [26, 32]. The metabolic functions of zinc include carbohydrate metabolism, protein synthesis, energy expenditure, nucleic acid metabolism, production of epithelial tissues, division and regeneration of body cells, improvement of the immune system, and synthesis of some sex hormones [1]. Many studies have shown that the effect of zinc improves the growth function and antioxidant system, immunity, and inflammation of the intestinal microbial community of experimental animals [15, 18, 23, 37]. In lambs, zinc deficiency causes loss of appetite, decreased growth, and skin lesions, prior to an increased risk of infection by immunosuppression [24]. According to the literature, the form and level of Zn supplementation may affect individual animal performance and metabolism. Fadayifar et al. [9] stated that the optimal concentration of zinc in the diet has different results with the mean daily increase in feed efficiency, feed intake, and body

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weight. According to the [21], dietary zinc requirements for growing rams are 24 to 51 mg/kg DM, although according to Suttle [30], these figures have not been conclusively proven. Burton et al. [4]stated that the need for zinc is not constant because growth performance and body size continue to increase in US sheep production systems.

Studies with lambs on fattening diets have shown response to zinc supplementation. As Fadayifar et al. [9] and Mallaki et al. [16] reported lambs supplemented with Zn higher than the recommendations of the NRC, had better performance and higher Zn concentration in different tissues than control lambs, when basal diet contained from 16 to 20 mg Zn/kg DM.

Trace minerals have been typically supplemented as inorganic forms that dissociate in the digestive tract and can interact with other nutrients that decrease bioavailability of these nutrients for animals. The organic forms of trace elements have more effectiveness and increase intestinal absorption and mineral bioactivity. Pal et al. [22] reported that the use of organic zinc supplements increases intestinal absorption and improves the antioxidant status of blood in fed ewes. Ali Arabi et al. [1] and Garg and Vishal-Mudgal [10] reported improved performance, safety, and reproductive performance of ruminants using organic supplements, compared to mineral supplements. Kegley and Spears [14] also stated that organic resources improve carcass quality.

According to various studies, little is known of the effect of different sources of Zn on intestine morphology and performance of male fattening lambs. Therefore, the objective of this study was to evaluate the effect of supplementation with two sources of Zn (Zn peptide and Zn sulfate) in the diet of lambs on productive yield, carcass traits, intestine morphology, and biochemical parameters.

Material and Methods

Animals, Treatments, and Feeding

This study was conducted in Pars Talachin Farm located in Robat Karim town (25 km southwest of Tehran, Iran). All animal procedures were conducted under protocols approved by the Institutional Animal Care and Use Committee of the University of Tehran (protocol no. 19867617). Eighteen Zandi male lambs (with initial BW of 31 ± 1.2 kg and 120 ± 8 days old) were used as livestock. These animals were divided into 3 groups of 6 lambs in a completely randomized design based on body weight. Experimental treatments include (1) control (basal diet without zinc supplementation), (2) basal diet + 40 mg/kg of zinc supplementation from zinc sulfate source, and (3) basal diet + 40 mg/kg of zinc supplementation from organic source (Table 1). The analytical grade $ZnSO_4$ (Merck, Germany) was used as the inorganic source of Zn. The organic source of Zn was in the form of Zn-peptide with 15% Zn (Vetaque Company, Tehran, Iran). Both supplements provided 40 mg Zn/kg DM.

Table 1 Ingredients and chemical composition of the basal total mixed ration fed to experimental lambs

Ingredient	% DM	Composition	% DM
Alfalfa hay	30	Crude protein (% DM)	14.96
Wheat straw	5	ME (Mcal/Kg DM)	2.82
Barley	43	NDF (% DM)	34.4
Wheat bran	16	Calcium (% DM)	0.80
Soybean meal	5	Phosphorus (% DM)	0.50
Vitamin-Mineral mix[a]	0.3	Zinc (mg/kg DM)	24.2
Salt	0.2	Copper (mg/kg DM)	7.6
Di-calcium phosphate	0.5	Iron (mg/kg DM)	135.6

[a]Each kg contained vitamin A, 400,000 IU; vitamin D3, 100,000 IU; vitamin E, 200 mg; Ca, 180 g; P, 70 g; Mg, 30 g; Na, 50 g; Mn, 5000 mg; I, 100 mg; Fe, 3000 mg; Cu, 300 mg; Co, 100 mg; and Se, 20 mg plus 400 mg antioxidant

The diets were formulated according to the [21] guidelines, and animals were fed a total mixed ration ad libitum. All lambs were kept in individual pens with cemented floor and provision of individual feeding and watering. Feed was supplied twice daily at 0800 and 1600 h. The amount of supplements was adjusted daily based on DMI of individual lambs. Diets were manually mixed and weighed into each lamb feed trough, and refusals were manually removed each day and weighed. Lambs were gradually introduced to the ration in order to minimize the risk of gastrointestinal disorders.

Measurements

Lambs were individually weighed on a digital scale (100 kg capacity with 100 g precision), at the beginning of the study and then weighed bi-weekly. During the experiment, feeders were checked daily, and dry matter intake (DMI) was individually recorded. The fattening period was 85 days, during which ADG was recorded, and feed conversion (FC) was calculated. Blood samples were collected from all the lambs at start and days 42 and 85 of experiment from the jugular vein. Whole blood was centrifuged at 3000 × g for 15 min, then serum was removed and frozen at − 20 °C for glucose, nitrogen urea, triglycerides, cholesterol, albumin, cortisol, testosterone, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) to be analyzed using commercial kits (Pars Azmoun, Tehran, Iran) and autoanalyzer (BT 3500 model, Spain).

During the final 3 days of the feeding experiment, diets, refusals, and daily stools were sampled and combined in lambs during the 3-day collection period to determine apparent in vivo digestibility using AIA as an indigestible internal

marker. Feed and fecal samples were ground to pass through a 1-mm plate and then stored in sealed plastic bags at room temperature. The N contents of feed and fecal samples were measured by the Kjeldahl method and crude protein (CP) was calculated as N × 6.25 [2]. Neutral detergent fiber (NDF) in feed and feces were determined by a fiber analyzer using the methods of Van Soest et al. [33]. Ash was determined by complete combustion in a muffle furnace at 450 °C for 8 h [2]. Ash samples were then boiled in 100 mL of 2N HCl for 5 min and filtered through Whatman No. 541 filter paper in a vacuum system. Samples and filter paper were again ashed for 8 h. Dry matter and nutrient digestibilities were calculated using the following equations [34]: Dry matter digestibility = 100 − [100 (AIA in feed/AIA in feces)]; Digestibility of nutrient = 100 − [(AIA in feed / AIA in feces) × (nutrient in feces /nutrient in feed)] × 100.

At the end of feeding period, lambs were slaughtered following a 12-h feed removal, according to the standard slaughter protocol in experimental abattoir of the farm of college of agriculture.

Directly after slaughtering, carcass weight, tail, visceral fat, liver, kidney, heart, lungs, testis, and empty gastrointestinal tract were measured with a digital scale to the nearest 5 g.

After slaughtering the lambs, the gastrointestinal tract was removed from the esophagus to the end of the colon. For histological examinations of the intestines, from the middle part of the three regions of the duodenum, jejunum, and ileum of the small intestine, pieces with a length of 1 cm sampled to evaluate the length, depth, and width of the villi. Each sample was placed separately in closed-sealed containers containing 10% neutral formalin. The samples were transferred to Kerman Veterinary Pathology Center for histomorphometry and histological study. In the laboratory, tissue samples were molded transversely in paraffin using Luke Hart molds and with the help of Sakura rotary microtome (model SRM 200 CW made in China), sections with a thickness of 5 µm were prepared and using hematoxylin–eosin dye were mixed, and tissue changes were examined under a light microscope. The measured indices were measured at different magnifications using a Nikon microscope (model YS100 made in Japan) with calibrated and calibrated lenses (three samples in each group and five tissue sections from each sample and at least four microscopic fields were counted and measured in each tissue section).

Statistical Analysis

The statistical analysis was performed using the GLM procedure [29]. Experimental parameters which were determined on different days (performance and blood serum parameters) were analyzed as repeated measures with the MIXED procedure of SAS in a completely randomized design.

The following model was used:

$$Y = \mu + ti + Tj + (t \times T)ij + eijk$$

where

Y	mean of observation
μ	overall mean
ti	treatment
Tj	day of observation
$(t \times T)ij$	interaction between treatment and days of observation
$eijk$	random residual

For both repeated and non-repeated measures, Duncan's multiple range test was used to detect statistical significance between treatments using a significance level of 0.05.

Results

Performance

The results of dry matter intake (DMI), daily weight gain, final live weight, and feed conversion ratio (FCR) are presented in Table 2. Zinc supplementation caused a significant increase in final body weight of lambs. Mean daily weight gain was also affected by zinc supplementation, but feed intake and feed conversion ratio were not affected by zinc supplementation in the diet ($P < 0.05$).

Diet Digestibility

No significant effects were observed for the digestibility of ADF, NDF, and ether extract (Table 3). However, the use of zinc supplementation increased the apparent digestibility of the dry matter ($P < 0.05$).

Table 2 The effect of different groups on feed intake and performance of experimental lambs

Measurement	Control	Zinc sulfate	Zinc organic	SEM	*P* value
Initial body weight (kg)	31.3	31.8	31.6	1.2	0.94
Final body weight (kg)	44.8[b]	46.6[a]	46.1[a]	1.1	0.03
Average gain (g/day)	180.1[b]	196.7[a]	192.8[a]	9.2	0.02
Average dry matter intake (g/day)	1600.0	1680.2	1652.3	60.9	0.39
Feed conversion ratio (FCR)	8.89	8.55	8.57	0.96	0.09

Means with different superscript letters in rows are significantly different ($P < 0.05$)

Table 3 Effect of different sources of zinc on apparent nutrient digestibility in lambs (based on DM)

Measurement	Control	Zinc sulfate	Zinc organic	SEM	*P* value
Dry matter (%)	70.73[b]	72.30[a]	72.10[a]	1.40	0.04
CP (%)	68.31	69.70	68.40	1.28	0.41
NDF (%)	39.37	45.21	40.52	2.64	0.36
ADF (%)	37.18	38.71	37.72	1.60	0.54
EE (%)	36.81	40.60	43.31	2.10	0.53

Means with different superscript letters in rows are significantly different ($P < 0.05$)

Blood Serum Parameters

The results for blood serum metabolites are presented in Table 4. The supplement had no significant effect on serum ALT activity, glucose, triglyceride, cholesterol, LDL, HDL, albumin, total protein, and cortisol concentrations. The concentration of AST enzyme in the zinc sulfate group was significantly higher than the control and organic zinc groups ($P < 0.05$). Concentrations of serum urea nitrogen were affected by zinc supplementation experimental groups and were lower in zinc fed lambs ($P \leq 0.05$).

Carcass Traits

Experimental data on the carcass characteristics of experimental animals are presented in Table 5. The results of these parameters did not show a significant difference between the experimental groups. However, the carcass data among the experimental treatments are consistent with the results of growth and yield data in this experiment (Table 2).

Intestinal Histology

As shown in Table 6, villus width at the duodenum was higher ($P < 0.05$) with Zn sulfate supplementation, compared with the organic and control. Supplementation with Zn sulfate also increased ($P < 0.05$) the villus height at the ileal section. Supplemental organic or $ZnSO_4$ had no effect on other intestinal morphological traits.

Discussion

In agreement with our study, many complementary studies have reported no significant effects of zinc supplement source on dry matter intake and feed canversion ratio. Salama Ahmed et al. [27]added 1 g of methionine zinc to the diet of dairy goats for 21 weeks and found that dry matter intake was not affected. In Fadayifar et al.'s [9] experiment, the use of 41 parts per million zinc elements from zinc sulfate sources and zinc proteinate in the diet of growing lambs had no significant effect on daily dry matter intake. Bun et al. [3] also did not report a significant effect of organic zinc supplementation on ADG or DMI. Zaboli et al. [38] also reported that consumption of 21 and 41 parts per million zinc supplements in the form of zinc oxide and zinc nano oxide had no effect on feed conversion ratio between different treatments. However, it has been reported that supplementation of diets with Zn-methionine improved feed intake in goats [27] and lambs [16], which is in contrast to our findings.

The average final body weight and daily gain of the lambs during the experimental period was increased with Zn

Table 4 Effect of different treatments on blood serum metabolite of experimental lamb

Parameters	Control	Zinc sulfate	Zinc organic	SEM	*P* value
Glucose (mg/dl)	65.8	66.2	64.4	1.24	0.04
Blood urea nitrogen (mg/dl)	13.4[a]	11.8[b]	7.4[c]	0.26	0.0001
Triglycerides (mg/dl)	18.55	18.33	17.11	0.80	0.40
Cholesterol (mg/dl)	42.08	57.66	53.33	1.03	0.21
HDL (mg/dl)	20.55	23.66	24.55	1.48	0.16
LDL (mg/dl)	12.94	11.77	10.77	0.41	0.12
Albumin (g/dl)	3.4	3.85	3.73	0.07	0.10
Total protein (g/dl)	6.61	6.81	6.57	0.10	0.25
AST (U/l)	86.33[c]	104.88[a]	97.11[b]	4.97	0.05
ALT (U/l)	17.94	20.36	16.66	1.69	0.11
Cortisol (µg/dl)	0.54	1.45	2.00	0.08	0.16

Means with different superscript letters in rows are significantly different ($P < 0.05$)

Table 5 Carcass characteristics of lambs with different experimental treatments

Parameter	Control	Zinc sulfate	Zinc organic	SEM	*P* value
Warm carcass weight (kg)	17.37	18.07	17.74	0.84	0.45
Dressing percentage (%)	46.6	46.1	46.5	0.15	0.85
Visceral fat (% of carcass)	0.35	0.55	0.52	0.04	0.24
Fat tail (% of carcass)	8.13	6.02	8.40	0.40	0.32
Testis (%of carcass)	0.83	0.91	0.79	0.04	0.53
Liver (%of carcass)	1.48	1.46	1.47	0.03	0.91
Kidneys (%of carcass)	0.25	0.26	0.27	0.01	0.11
Heart (%of carcass)	0.30	0.42	0.38	0.01	0.30
Lungs (%of carcass)	0.81	1.26	0.93	0.02	0.54
Digestive system (%of carcass)	10.04	11.46	9.65	0.39	0.72

supplements as compared to control. Similar to our results, adding 40 mg of organic zinc source to barley diets containing 22.47 mg of zinc increased the final body weight, and daily weight gain, which has been reported due to deficiency in the basic diet of this element (Fadayifar et al. 2012) [9]. Also, zinc supplementation in lambs [1] and goat [12] had reported an improvement in daily weight gain. On the other hand, supplementation of the diet with different sources of zinc had no effect on growth performance of calves [35], lambs [9], and goats [27] relative to the control. Daghash and Mousa [7] reported that addition of zinc to the basal diet of growing animal increase the activity of zinc metalloenzymes such as RNA and DNA polymerases and thymidine kinase. These enzymes are responsible for the growth and development of skeleton and synthesis of body protein.

Inconsistent results of Zn source on nutrient utilization have been reported in previous studies in sheep [10, 16], goats [12], and calves [17]. Garg and Vishal-Mudgal [10] found that supplementation of basal diet with 20 mg/kg Zn as Zn-methionine improved ADF digestibility in growing lambs, but dry matter, organic matter, crude protein, ether extract, hemicellulose, and NDF digestibility were not affected, whereas Mallaki et al. [16]reported 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. Various factors might contribute to the disparities among the above reports, such as concentration of zinc supplement, its organic or inorganic, type of diet (ratio of forage to concentrate), chemical properties, type of organic zinc supplement, animal species, and factors affecting the solubility and stability of this element in the gastrointestinal tract [13].

Blood parameters were not affected by the source of zinc except for BUN concentration and AST activity (Table 3). This is in agreement with the finding in lambs [16, 28] and dairy cows [25], where it was reported that serum parameters were not affected by different zinc sources. Lower blood urea nitrogen level in lambs fed zinc supplements may be explained by the increase in the more digestion and efficiency of ruminal protein of feed, as Chavan et al. [5]showed that feed nitrogen retention

Table 6 Effects of experimental treatments on small intestinal morphology (μM)

Parameter	Control	Zinc sulfate	Zinc organic	SEM	*P* value
Duodenum					
Villus height	8.30	579.77	591.11	570.55	0.24
Villus width	6.27	299.44^{ab}	326.36^{a}	291.11^{b}	0.0008
Crypt depth	6.06	296.24	296.66	282.22	0.17
Height/crypt depth	1.2	1.95	1.99	2.02	0.15
Jejunum					
Villus height	8.82	690.23	690.34	687.44	0.42
Villus width	5.90	317.88	318.77	301.11	0.07
Crypt depth	5.80	305.55	307.77	298.88	0.53
Height/crypt depth	1.14	2.25	2.24	2.30	0.13
Ileum					
Villus height	9.61	650.12^{ab}	663.33^{a}	645.22^{b}	0.01
Villus width	4.50	324.44	327.77	326.66	0.86
Crypt depth	3.99	304.44	306.77	305.55	0.92
Height/crypt depth	1.5	2.13	2.16	2.11	0.73

Means with different superscript letters in rows are significantly different ($P < 0.05$)

in kids was higher in the Zn group, as compared to the control. It can be inferred that a threshold level of Zn is required in diet for optimum ruminal N metabolism, and zinc supplementation could improve N utilization which is supported by the highest live body gain recorded in Zn treated lambs (Table 2).

Zinc sulfate supplementation increases serum AST concentration, compared to organic source and control. In consistency with our result, it was reported that the concentration of AST enzyme in buffalo calves that fed diet supplemeneted with zinc was higher than control [7]. However, contrary to these findings, Mandal et al. [17]no significant differences were observed in the concentrations of AST and ALT in fattening calves fed with organic and inorganic zinc sources (35 mg/kg level with basal diet containing 32.50 mg/kg zinc). The increase of blood AST concentration may be related to higher growth rate due to protein synthesis process [7] in lambs fed zinc rather than the control group. Davidson [8] reported that the function of AST enzyme is the transfer of amino group from amino acid to synthesise another one and plays an important role in gluconeogenesis. Furthermore, an increase of AST concentration is a response to the increase need for gluconeogenesis [7].

There is not enough information regarding the effect of zinc supplementation on carcass traits in fattening animals. Similar to our results, adding 32.5 mg from Zn methionine and (or) Zn oxide to barley diets containing 32.5 mg of zinc did not affect hot and cold carcass weight and commercial yield carcass [24]. However, Nourian Server et al. [20]reported that the use of zinc supplementation increased hind and fore leg length in lambs fed Zn-Met supplement.

The results of the current study show that Zn supplementation, especially zinc sulfate, was able to modify intestinal morphology and, thus, could be recommended for improving gastrointestinal development in finishing lambs. Increasing the width and height of the villi, as observed in this study, increases the level of absorption of nutrients in the intestine [36]. Jafarpour et al. [11] reported significant increases in the villus height in the duodenum and jejunum as a result of Zn-Met supplementation. Also, Li et al. [15]reported that Zn influenced cell replication and the growth of enterocytes of the villus epithelium in animals. In another study, an increase in villi height in the small intestine with consumption of high levels of zinc has also been reported due to increased protein synthesis and cell proliferation [19]. However, in the study of Swinkels et al. [31] and Cheng et al. [6], they did not observe the effects of zinc-methionine and zinc sulfate supplementation on intestinal morphology, which is not consistent with the results of the present study.

Conclusion

The results of this study revealed that supplementing diets with Zn improved growth performance of fattening lambs, as well as the surface of intestinal villi, width in the duodenum and height of the ileom villi. But in this experiment, no effect was observed on feed intake and efficiency, carcass traits, and blood parameters.

Author contributions SHME conducted the experiment, analyzed the data and wrote the first draft; MAN and AN conceptualized the experiment, supervised the project and edited the final version of the manuscript and All authors reviewed the manuscript.

Declarations

Competing interests The authors declare no competing interests.

Conflict of Interest The authors declare no competing interests.

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