

Effect of organic zinc supplementation on growth, nutrient utilization, and plasma zinc status in lambs

Mokhtar MALLAKI, Mohammad Ali NOROUZIAN*, Ali Akbar KHADEM

Department of Animal Science, College of Abouraihan, University of Tehran, Tehran, Iran

Received: 24.05.2014 • Accepted: 23.09.2014 • Published Online: 12.01.2015 • Printed: 09.02.2015

Abstract: Two experiments were conducted to investigate the effect of supplemental zinc (Zn) on performance, nutrient digestibility, and plasma Zn status in Zandi lambs. In experiment 1, 18 male lambs (BW = 21.30 ± 0.55 kg) were fed a basal diet containing 22.8 mg Zn/kg dry matter (DM) with no supplemental Zn (control) or 20 mg of supplemental Zn/kg of DM from Zn sulfate (ZnS) or Zn peptide (ZnP). Average daily gain and dry matter intake were higher for the lambs fed the diet supplemented with ZnP. Feed conversion ratio was significantly ($P < 0.05$) lower in the ZnP group compared to the control and ZnS groups. In experiment 2, an in vitro gas production technique was used to evaluate the effects of zinc source on the gas production parameters. Rate of gas production after 6 h and total production of gas after 24 and 48 h did not differ between groups. In vitro DM digestibility, short-chain fatty acids, and ME content were increased ($P < 0.05$) by ZnP supplementation when compared to the ZnS and control groups. The results of this study showed that feeding ZnP improves performance and digestibility of DM and could result in higher metabolizable energy and short-chain fatty acid yield.

Key words: Zinc, Zn-peptide, gas production, digestibility, Zandi lamb

1. Introduction

Zinc (Zn) is an essential trace element for both animal and microorganisms in the rumen and is required for several metabolic functions as well (1). Although administration of Zn supplement in ruminant diets has been increased in recent years, it is not clear yet which supplement form, an organic or inorganic source, is more effective for improving animal performance. Some researchers (2,3) have reported greater bioavailability for organic Zn sources than that observed for inorganic forms, including Zn oxide and Zn sulfate. It has been demonstrated that organic Zn is transported intact from the intestinal lumen into mucosal cells, increasing tissue supply of Zn and thereby improving animal productivity (4).

Furthermore, studies on the effects of Zn source on nutrients digestibility and ruminal fermentation are scarce (5) and, where available, results are contradictory. However, in this regard, Salama et al. (5) in their study of goats found that digestibility of dry matter (DM), organic matter, and crude protein (CP) increased when the diets were supplemented with 1 g/day organic Zn. In contrast, Mandal et al. (6) and Jia et al. (7) indicated that DM, CP, acid detergent fiber (ADF), and neutral detergent fiber (NDF) digestibility were not affected by dietary Zn in bull

and goat, respectively. In addition, Garg et al. (4) found that supplementation of the basal diet with 20 mg of organic Zn/kg DM improved ADF digestibility in lambs, but DM, CP, and NDF digestibility were not affected.

With the aim of providing more empirical evidence concerning the effect of components providing the element Zn, a prospective study was conducted using growing Zandi lambs as a domestic ruminant model. To accomplish this, diets were supplemented with Zn from organic or inorganic sources and then performance, nutrient digestibility, amount and rate of gas production, and utilization of metabolizable energy (ME) were evaluated. Ruminal fermentation patterns were also studied as the key important parameter for the feeding value of these Zn sources.

2. Materials and methods

In experiment 1, 18 Zandi male lambs (3–4 months of age; 21.30 ± 0.55 kg BW) were randomly assigned into a control group (no supplemental Zn) or 1 of 2 Zn supplementation groups: ZnSO₄ (ZnS) or Zn-peptide (ZnP). Both supplements provided 20 mg Zn/kg DM. Animals were housed in individual pens and fed a total mixed ration (TMR) containing 650 g/kg concentrate mixture (CM),

* Correspondence: manorouzian@ut.ac.ir

200 g/kg alfalfa hay, and 150 g/kg wheat straw (DM basis). The CM contained 700 g/kg ground barley grain, 150 g/kg cotton seed meal, 140 g/kg wheat bran, 6.0 g/kg mineral-vitamin mixture (without Zn), and 4.0 g/kg salt. Lambs were fed the experimental diets in 2 equal portions at 0800 and 1600 hours. The TMR contained basal content of 22.8 mg Zn/kg DM. The feeding trial lasted for 90 days. Feed intake and BW were recorded daily and biweekly, respectively.

During the sixth fortnight of the feeding period, diets and refusals were sampled daily, and fecal samples were collected from individual lambs for determination of *in vivo* digestibility. Diet and fecal samples were dried in a forced-air oven at 60 °C for 48 h to determine DM content (8). The samples were ground (2 mm) and analyzed for CP (8) with a Kjeltach nitrogen analyzer (Tecator, Sweden). Sequential detergent fiber analysis was used to determine NDF and ADF concentrations as described previously (9).

Digestibility of DM was determined using acid insoluble ash (AIA) as an internal marker according to the procedures described by Van Keulen and Young (10). Briefly, fecal samples were composited across the 4-day sampling period for individual animals. After determination of DM, CP, NDF, and ether extract (EE), feed and fecal samples were ashed at 450 °C for 8 h and the ash was then boiled in 100 mL of 2 N HCl for 5 min. Samples were then 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 (10):

Dry matter digestibility = $100 - [100 (\text{AIA in feed}/\text{AIA in feces})]$,

Digestibility of nutrient = $100 - [(\text{AIA in feed}/\text{AIA in feces}) \times (\text{nutrient in feces}/\text{nutrient in feed})] \times 100$.

Blood samples were collected from all lambs at day 0 and subsequently at an interval of 30 days from the jugular vein for determination of mineral and enzyme activities.

In experiment 2, *in vitro* gas production (11) was used to estimate *in vitro* DM digestibility (IVDMD), ME, and short-chain fatty acid (SCFA) production. Ruminal fluid obtained from 4 animals 3 h after morning feeding was mixed and strained through 4 layers of cheesecloth into a prewarmed thermos and transported to the laboratory. The lambs were fed a TMR (60:40 forage:concentrate; DM basis) and 0.6% mineral and vitamin premix (without Zn). The TMR contained 20.1 mg Zn/kg DM from forage and grain. The lambs were fed twice daily at 0700 and 1900 hours and had free access to water. Incubation medium was prepared as described by Menke et al. (11). Samples, each of 200 ± 0.2 mg DM, were incubated in 100-mL glass bottles in which 30 mL of the incubation medium was added. To each bottle, one of the following treatments was applied: 1) control (C; no supplemental zinc), 2) 20 ppm

of Zn as ZnS, or 3) 20 ppm of Zn as ZnP. Samples were incubated in triplicate and cumulative gas production was monitored at 2, 4, 6, 8, 10, 12, 15, 19, 24, 30, 36, 48, 72, and 96 h after incubation. Three bottles with incubation medium only were used as blanks to correct the gas production values for gas release from the rumen contents. The gas production data were fitted to the following model of France et al. (12):

$$A = b \times [1 - e^{-k(t-L)}],$$

where A is the volume of gas production at time t, b is the asymptotic gas production (based on mL/200 mg DM), k is the rate of gas production per hour from the slowly fermentable feed fraction b, and the time lag (L) is the discrete lag time prior to gas production.

The rate of gas production (RGP) at 4 and 6 h was calculated from recorded volumes of gas produced before and after these times (13). For example, RGP at 4 h was calculated as:

$$\text{RGP}_{4h} \text{ [(mL/g DM)/h]} = \frac{(\text{volume of gas produced at 6 h} - \text{volume of gas produced at 2 h})}{4 \times \text{sample weight (mg)}}$$

IVDMD was determined after 96 h of incubation by recovery of the undigested fraction using sintered crucibles. Recovered residues were washed under running water and subsequently dried at 100 °C for 24 h (13).

ME in MJ/kg DM was estimated according to the equation of Menke and Steingass (14):

$$\text{ME} = 2.20 + 0.136 \text{ IVGP}_{24} \text{ (mL/200 mg DM)} + 0.057 \text{ CP (DM\%)},$$

where IVGP_{24} is 24-h gas volume and CP is the crude protein content of the feed sample.

SCFAs were estimated by the equation of Makkar (15):

$$\text{SCFA (mmol/g DM)} = 0.0222 \text{ (mL gas at 24 h)} - 0.00425.$$

Data were analyzed in a completely randomized design using the MIXED procedure of SAS (16). For variables measured over time (average daily gain, dry matter intake, and feed conversion ratio), time was added to the model as a repeated factor. Duncan's multiple range test was used to detect statistical significance between treatments using a significance level of 0.05.

3. Results

3.1. Performance

The results of growth rate and performance are shown in Table 1. Average daily gain and dry matter intake increased ($P < 0.05$) with Zn supplementation and were higher for the lambs fed with ZnP as compared with those fed with ZnS and the control diet. Feed required per unit of weight gain was lower ($P < 0.05$) in the ZnP group compared to the control and ZnS groups.

Table 1. Effect of different sources of zinc on performance of growing Zandi lambs.

Measurement	Treatment			SEM	P-value
	Control	ZnS	ZnP		
Average daily gain (g/day)	159.3 ^b	153.6 ^b	201.4 ^a	7.2	0.004
Average dry matter intake (g/day)	1105.2 ^c	1194.3 ^b	1301.2 ^a	53.2	0.001
Feed conversion ratio	7.93 ^a	7.44 ^a	6.66 ^b	0.65	0.002

Treatments: control; ZnS: zinc sulfate; ZnP: zinc peptide.

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

3.2. Blood Zn status and enzyme activity

The data pertaining to blood Zn concentration of lambs on various Zn-containing diets are detailed in Figure 1. The Zn plasma concentration for ZnP (155.2 $\mu\text{g/dL}$) and ZnS (149.7 $\mu\text{g/dL}$) groups were greater than in the control (134.6 $\mu\text{g/dL}$) group ($P < 0.05$), as illustrated in Figure 1. Moreover, the concentration of Zn in plasma was increased when the experimental diet was supplemented with ZnP as compared to ZnS ($P < 0.05$).

The results for the serum enzymes activities are presented in Figure 1. There were no differences in serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) activities among treatments ($P < 0.05$).

3.3. In vivo digestibility and gas production parameters

The digestibility of DM, organic matter, and EE was not affected by treatment (Table 2). However, our findings revealed that supplementation of the diet with ZnP increased digestibility of CP and NDF ($P < 0.05$).

The effects of different sources of Zn on in vitro gas production, IVDMD, ME, and the production of SCFAs

are shown in Table 3 and Figure 2. The rates and total production of gas were not different among the groups. Asymptotic gas production (b) tended to be higher ($P = 0.08$) for the ZnP group compared with the ZnS and control groups. There were no differences among treatments for the fractional rate of gas production (k) and lag time (L).

The IVDMD and ME content were increased ($P < 0.05$) with the diet supplemented with ZnP as compared to the ZnS and control groups. The concentration of SCFAs was increased ($P < 0.05$) following supplementation of the diet with either ZnP or ZnS as compared to the control diet.

4. Discussion

4.1. Performance

The improved dry matter intake observed in this study following Zn supplementation is in agreement with the previous findings of Garg et al. (4). However, it has been reported that supplementation of diets with Zn-methionine had no effect on feed intake in goats (5), growing lambs (17,18), and beef steers (6), which is in contrast to our findings and other reports (4).

The average daily gain of the lambs during the 90-day experimental period was increased with ZnP as compared to both ZnS and the control. Similar to our results, Dey and Garg (19) observed a higher growth rate in weaned albino rats supplemented with Zn-methionine as compared to those fed ZnCl. On the other hand, supplementation of the diet with organic sources of zinc had no effect on growth performance of calves (2,20), lambs (18), goats (5), and guinea pigs (21) relative to the control or inorganic Zn supplements.

Our data revealed that addition of 20 mg/kg DM of ZnP to the basal diet containing 22.8 mg Zn/kg DM improved the feed conversion ratio. Similarly, Garg et al. (4) and Fadayifar et al. (18) observed improved feed efficiency in finishing lambs fed organic Zn as compared to lambs fed ZnS or a control diet. In contrast, observations in calves (22) and steers (6) showed that diet Zn supplementation had no effect on feed efficiency. In small animal feeding trials, Shinde et al. (21) did not find any difference in feed

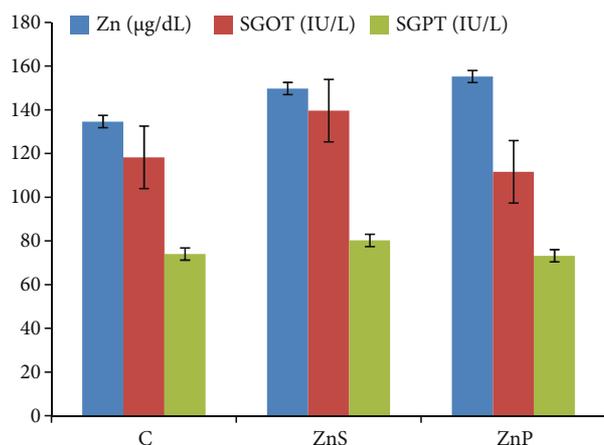


Figure 1. Effects of Zn sources on plasma zinc concentration and enzyme activities of lambs (treatments: C: control, ZnS: zinc sulfate, ZnP: zinc peptide). ^{a,b,c}: Columns with different superscripts differ ($P < 0.01$).

Table 2. Effect of different sources of zinc on nutrient utilization of growing Zandi lambs.

Measurements	Treatment			SEM	P-value
	Control	ZnS	ZnP		
Dry matter (%)	66.5	64.9	69.9	1.72	0.19
Organic matter (%)	69.3	67.5	72.5	1.52	0.15
CP (%)	62.3 ^b	54.6 ^c	67.3 ^a	1.55	0.003
NDF (%)	41.1 ^b	35.5 ^c	45.2 ^a	2.61	0.01
EE (%)	95.9	95.4	96.5	0.51	0.13

Treatments: control; ZnS: zinc sulfate; ZnP: zinc peptide.
Means with different superscript letters in rows are significantly different ($P < 0.05$).

Table 3. Effect of different sources of zinc on in vitro ruminal fermentation parameters, gas production parameters, metabolizable energy, and short-chain fatty acid production in growing Zandi lambs.

Item	Treatment			SEM	P-value
	Control	ZnS	ZnP		
RGP4h	8.54	9.12	9.68	2.9	0.36
RGP6h	6.85	7.25	8.14	3.9	0.43
GP24h	55.6	61.3	65.8	4.6	0.35
GP48h	64.7	70.9	75.9	4.6	0.29
b (mL/200 mg DM)	78.5 ^b	83.5 ^a	85.3 ^a	1.75	0.03
k (%/h)	0.076	0.077	0.078	0.003	0.92
L (h)	1.13	1.14	1.06	0.056	0.66
IVDMD (%)	69.8 ^b	67.5 ^b	79.0 ^a	1.6	0.0002
ME (MJ/kg DM)	10.89 ^b	10.14 ^b	12.15 ^a	0.13	0.0002
SCFA (mmol/g DM)	1.1 ^b	1.3 ^a	1.4 ^a	0.02	0.0002

Treatments: control; ZnS: zinc sulfate; ZnP: zinc peptide.
Means in the same row with different letters are significantly different ($P < 0.05$).
DM: Dry matter, RGP: rate of gas production (in milliliters per 200 mg DM per hour at 4 and 6 h), GP: gas production (in milliliters per 200 mg at 24 and 48 h), b: asymptotic gas production (in milliliters per 200 mg DM), k: fractional rate of gas production (per hour), L: lag phase (per hour); IVDMD, in vitro dry matter degradability (%), ME: metabolizable energy (in MJ per kilogram DM), SCFA: short chain fatty acid (millimole per gram DM).

efficiency in guinea pigs supplemented with Zn through either organic or inorganic sources. However, Dey and Garg (19) observed an improvement in feed efficiency of weaned albino rats given organic Zn as compared to ZnS-supplemented and nonsupplemented groups.

Low performance in previous reports (17,18,20) using different sources of zinc could be due to the low levels of supplemented zinc in those studies.

4.2. Blood Zn status and enzyme activity

Results of this study further showed that the level of Zn in the plasma was increased in Zn-supplemented groups as compared to the control and in the ZnP group as compared to the ZnS group. Contrary to this result, Ryan et al. (3) did not find any difference in the plasma Zn concentration in adult sheep that were supplemented daily with 75 and 150 mg of Zn either as Bioplex Zn (chelated

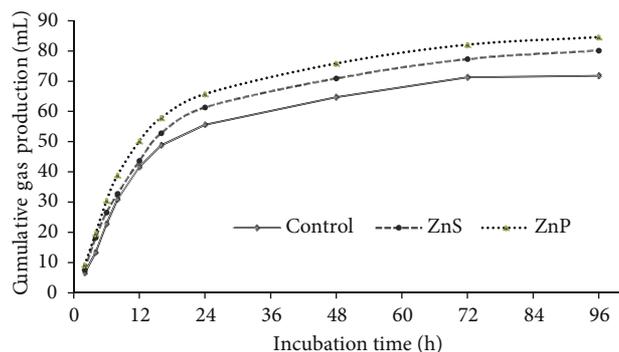


Figure 2. Gas production profiles of the diets of lambs supplemented with different sources of Zn.

Zn) or inorganic Zn (ZnSO_4). Salama et al. (5) also did not find any effect on serum Zn concentrations in dairy goats that were supplemented with 1 g of Zn-methionine per day with a diet containing 44.7 mg Zn/kg DM. Mandal et al. (6) also did not find any difference in the serum Zn levels in cattle calves supplemented with 35 mg Zn/kg DM as ZnSO_4 or Zn-propionate in a basal diet containing 32.5 mg Zn/kg DM.

Our result suggests that adding 20 mg Zn/kg DM from organic or inorganic sources to finishing lambs' diet containing 22.8 mg Zn/kg DM will increase plasma Zn concentration. Similar to our findings, previous evidence demonstrated that supplementation of Zn could increase the plasma Zn concentration in male goats (5) and finishing lambs (18).

No changes in SGOT and SGPT activities were observed while Zn was supplemented to the basal diets of lambs. Contrary to these findings, Daghash and Mousa (23) observed higher SGOT and SGPT activity in buffalo calves supplemented with 50 or 100 ppm Zn. However, similar to our findings, Mandal et al. (6) did not find any effect of Zn supplementation on SGOT and SGPT activity in lambs fed a basal diet supplemented with 20 mg/kg Zn either through inorganic (ZnSO_4) or organic [Zn-methionine AA complex (Zn-meth)] sources.

4.3. In vivo digestibility and gas production parameters

The unchanged digestibility of DM, organic matter, and EE with Zn supplementation in the present study is not consistent with previous studies in steer (6) or lambs (4) in which organic and inorganic Zn supplements were used. In this regard, Salama et al. (5) observed that supplementation of dairy goats with 1 g/day ZnMet increased digestibility of DM and OM. Consistent with our results, Salama et al. (5) observed that the apparent digestibility of CP was higher in dairy goats receiving a

diet supplemented with 1 g/day organic Zn. However, in the present experiment, supplementation of 20 mg organic Zn/kg DM improved the digestibility of NDF, suggesting a positive role for organic Zn supplementation in fiber digestion. In agreement with this, Garg et al. (4) found that supplementation of a diet containing 34 mg Zn/kg DM with 20 mg of organic Zn/kg DM improved ADF and cellulose digestibility.

In this study, supplementation of either organic or inorganic zinc had no effect on most gas production parameters except for asymptotic gas production (b), which was higher with ZnP as compared to ZnS and the control. Similarly, Vazquez-Armijo et al. (13) observed increased asymptotic gas production by adding 5.6 mg/kg Zn to a basal diet containing 22.5 mg/kg Zn when compared to the control group (with no addition of Zn supplementation). Sobhanirad et al. (24) reported that supplementation of rumen fluid with Zn supplements had no effect on gas production parameters during wheat straw fermentation. However, these authors reported that supplementation of rumen fluid with Zn-methionine increased gas production during alfalfa hay fermentation.

Salama et al. (5) and Jia et al. (7) found no differences in the in vivo digestibility of DM in goats supplemented with Zn, which does not agree with our findings that Zn supplementation increased IVDMD as compared to the control. The increased IVDMD with Zn supplementation in the current study is consistent with the report of Vazquez-Armijo et al. (13), who reported that adding 5.6 mg Zn/kg DM to a basal diet containing 22.5 mg/kg Zn increased IVDMD. In contrast, Arelovich et al. (25) observed a negative effect of high levels of Zn supplementation on IVDMD.

The ME content and production of SCFAs were increased with ZnP compared with ZnS and the control, which is in agreement with Arelovich et al. (25). Increased ruminal production of SCFAs with ZnP is related to increased energetic efficiency of ruminal fermentation and could explain some of the benefits associated with chelated Zn supplements (25).

In conclusion, supplementation of growing lambs with 20 mg Zn/kg DM as ZnP in a basal diet containing 22.8 mg Zn/kg DM significantly improved performance, digestibility of CP and NDF, IVDMD, and ME and SCFA production.

Acknowledgment

The authors would like to acknowledge the financial support of the University of Tehran for this research under grant number 27628/06/01.

References

1. Eryavuz A, Burk AD. Effects of supplemental zinc concentration on cellulose digestion and cellulolytic and total bacterial numbers in vitro. *Anim Feed Sci Technol* 2009; 151: 175–183.
2. Kessler J, Morel I, Dufey FA, Gutzwiller A, Stern A, Geyes H. Effect of organic zinc sources on performance, zinc status, and carcass, meat, and claw quality in fattening bulls. *Livest Prod Sci* 2003; 81: 161–171.
3. Ryan JP, Kearns P, Quinn T. Bioavailability of dietary copper and zinc in adult Texel sheep: a comparative study of the effects of sulfate and bioplex supplementation. *Irish Vet J* 2002; 55: 221–224.
4. Garg AK, Mudgal V, Dass RS. Effect of organic zinc supplementation on growth, nutrient utilization and mineral profile in lambs. *Anim Feed Sci Technol* 2008; 144: 82–96.
5. Salama AAK, Caja G, Albanell E, Such X, Caslas R, Plaixats. Effects of dietary supplements of zinc-methionine on milk production, udder health and zinc metabolism in dairy goats. *J Dairy Res* 2003; 70: 9–17.
6. Mandal GP, Dass RS, Isore DP, Garg AK, Ram GC. 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* 2007; 138: 1–12.
7. Jia W, Jia Z, Zhang W, Wang R, Zhang S, Zhu X. Effects of dietary zinc on performance, nutrient digestibility and plasma zinc status in Cashmere goats. *Small Rum Res* 2008; 80: 68–72.
8. Association of Official Analytical Chemists. *Official Method of Analysis*, Vol. 1. 15th ed. Arlington, VA, USA: AOAC.
9. Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber and neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. *J Dairy Sci* 1991; 74: 3583–3597.
10. Van Keulen J, Young BA. Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. *J Anim Sci* 1977; 44: 282–287.
11. Menke KH, Raab L, Salewski A, Stingass H, Fritz D, Schneide W. The estimation of the digestibility and metabolizable energy content of ruminant feedstuffs from the gas production when they are incubated with rumen liquor in vitro. *J Agr Sci* 1979; 93: 217–222.
12. France J, Dijkstra J, Dhanoa MS, López S, Bannink A. Estimating the extent of degradation of ruminants feeds from a description of their gas production profiles observed in vitro: derivation of models and other mathematical considerations. *Brit J Nutr* 2000; 83: 143–150.
13. Vázquez-Armijo JF, Martínez-Tinajero JJ, López D, Salem AZM, Rojo R. In vitro gas production and dry matter degradability of diets consumed by goats with or without copper and zinc supplementation. *Biol Trace Elem Res* 2011; 144: 580–587.
14. Menke KH, Steingass H. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. *Anim Res Dev* 1988; 28: 7–25.
15. Makkar HPS. In vitro gas methods for evaluation of feeds containing phytochemicals. *Anim Feed Sci Technol* 2005; 123: 291–302.
16. SAS Institute. *User's Guide. Version 9.1: Statistics*. Cary, NC, USA: SAS Institute; 2004.
17. Droke EA, Gengelbach GP, Spears JW. Influence of level and source (inorganic vs organic) of zinc supplementation on immune function in growing lambs. *Asian Austral J Anim Sci* 1998; 11: 139–144.
18. Fadayifar A, Aliarabi H, Tabatabaei MM, Bahari A, Malecki M, Dezfoulian AH. Improvement in lamb performance on barley based diet supplemented with zinc. *Livestock Sci* 2012; 144: 285–289.
19. Dey A, Garg AK. Effect of supplementation of zinc methionine complex on growth rate and feed efficiency in weaned rats. *Indian Vet Med J* 2004; 28: 117–120.
20. Wright CL, Spears JW. Effect of zinc source and dietary level on zinc metabolism in Holstein calves. *J Dairy Sci* 2004; 87: 1085–1091.
21. Shinde P, Dass RS, Garg AK, Chaturvedi VK, Kumar R. Effect of zinc supplementation from different sources on growth, nutrient digestibility, blood metabolic profile and immune response of male guinea pigs. *Biol Trace Elem Res* 2006; 112: 247–262.
22. Spears JW, Harvey RW, Brown TT. 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* 1991; 199: 1731–1733.
23. Daghash HA, Mousa SM. 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* 1999; 40: 128–146.
24. Sobhanirad S, Valizadeh R, Aghel H. Effect of organic and inorganic zinc supplements on degradability of wheat straw and alfalfa hay by using gas production techniques. In: *Proceedings of the 13th Animal Science Congress of the Asian-Australian Association of Animal Production Societies*; 2008. pp. 24–26.
25. Arelovich HM, Owens FN, Horn GW, Vizcarra JA. Effects of supplemental zinc and manganese on ruminal fermentation, forage intake, and digestion by cattle fed prairie hay and urea. *J Anim Sci* 2000; 78: 2972–2979.