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Changes in blood and tissues zinc concentration in response to organic and inorganic sources in growing Baluchi lambs

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Abstract

The objective of this study was to find zinc (Zn) concentration in blood and different tissues in Baluchi lambs fed different levels of organic and inorganic sources of this element. Thirty-six male growing lambs with an average weight 26.5±0.3 kg were divided into six groups and fed basal diet (no Zn supplementation, BD), 50 or 100 ppm zinc sulphate monohydrate (Zn-S), zinc proteinate (Zn-P) and their mixture (Zn-P+S, 50 mg/kg each). Results showed that serum Zn concentration was significantly ($P<0.05$) high in lambs fed 100 mg/kg Zn-P or mixture of Zn-P-S at the rate of 50 mg/kg each. Zn in heart and testis was significantly ($P<0.05$) high in lambs fed Zn-S (100 mg/kg) and Zn-P-S. In kidney, liver, muscle and spleen, Zn concentration was significantly ($P<0.05$) high in lambs fed with Zn-P, Zn-S and Zn-P-S. The results showed that Zn fed as organic source at the level of 100 mg/kg or at the rate of 50 mg/kg in combination with $ZnSO_4$ showed higher bioavailability in serum and tissues.

Keywords: Lambs; Zinc proteinate; Zinc sulfate monohydrate; Zinc concentration

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Introduction

Zinc (Zn) is a central component of hundreds of metalloenzymes such as dehydrogenases, alkaline phosphatase and superoxide dismutase (Keen and Graham, 1989; Coleman, 1998). Zn has also many biologically significant interactions with hormones. It plays a role in the production, storage and secretion of individual hormones, and has involvement in the immune system and electrolyte balance (Phiri et al., 2007). It also has a role in keratinization, calcification, and wound healing and somatic and sexual development (Kendall et al., 2000). Deficiency of trace elements affects almost all physiological processes like growth, reproduction, immunity, milk production and other systems of animals (Kundu et al., 2014).

Supplemental Zn is usually added to animal diets in form of inorganic Zn (ZnO and $ZnSO_4$). Recently, organically bound Zn supplements are used in animal diets. It was reported that Zn absorption from some organic sources appears to be higher than inorganic sources when supplemented at high concentrations (Spear, 2003).

The recommended Zn level is 20-33 mg/kg in sheep diet which is necessary for optimum productivity (Nutrient Requirements of Small Ruminants, 2007). In practice, feed manufacturers use higher concentration than those specified for small ruminant to achieve maximum performance (NRC, 2007). Lambs supplemented with Zn at the rate of 360 mg/kg of diet from Zn lysine had much higher Zn concentrations in kidney, liver and pancreas than lambs received Zn SO_4, ZnO or zinc methionine (Rojas et al., 1995). Liver and plasma Zn concentrations were also higher in calves that were supplemented with 300 mg/kg of diet from a combination of zinc lysine and zinc methionine than in calves supplemented with ZnO (Kincaid et al., 1997). Gowda et al. (2014) also reported that the

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supplementation of organic source of Zn in the diet of lambs was effective in improving their bioavailability of this element in the body. However, considerable reports on feeding organic trace minerals instead of inorganic forms to animals for the benefit of higher bioavailability have been published (Henry et al, 1992; Rojas et al., 1996). Higher tissue concentrations of Zn-proteinate was also seen in calves (Wright and Spears, 2001) and lambs (Cao et al., 2000) that were fed high concentrations of zinc proteinate and $ZnSO_4$. The objective of this experiment was to investigate the response of different concentration of organic and inorganic Zn sources and their mixture in the blood and different tissues of lambs.

Materials and Methods

Experimental animals and diets

Thirty-six, 120-day-old, Baluchi male lambs with an average body weight of 26.5±0.3 kg were randomly allocated to six groups; each lamb was individually kept in a separate pen with concrete floor. The lambs were provided drinking water *ad libitum* by using plastic bucket. The dietary treatments were: 1) the basal diet (Table 1) containing 33 mg Zn/kg DM, without supplementary Zn (BD), 2) BD plus 50 mg Zn/kg DM as Zn-phosphate (Alltech Inc., Nicholasville, KY), 3) BD plus 100 mg Zn/kg DM as Zn-phosphate, 4) BD plus 50 mg Zn/kg DM as Zn-sulphate (Sigma-Aldrich, Seelze, Germany), 5) BD plus 100 mg Zn/kg DM as Zn-sulphate, 6) BD plus 50 mg Zn/kg Zn-phosphate + 50 mg Zn/kg Zn-sulphate. The basal diet was provided according to NRC (2007) to meet or exceed lamb requirements except Zn (Table 1). The experiment lasted for 60 days.

Determination of Zn in blood

Blood samples were collected from jugular vein using vacationer tubes containing sodium heparin at the end of 60 days of the experimental period. Blood in Zn-free no-additive tubes was allowed to clot at ambient temperature (15 to 21°C), centrifuged (3500 × g for 10 min), and serum was stored at -20°C for further analysis. Serum Zn concentration was determined by deproteinization of 0.5 ml serum samples with 1 ml of 100 ml/l trichloracetic acid, centrifuged at 2,500 × g for 10 min. The supernatant was diluted 1:5 with deionized water (Case, 2002) then analyzed on atomic absorption spectrophotometer (Chemtech Analytical CTA 2000, Analytical Co., Kempston, UK).

Determination of Zn in tissue

Upon termination of experiments, lambs were killed by exsanguinations and tissues were quantitatively removed. Liver, heart, kidney, lung, muscle, spleen and testis were excised and frozen for further mineral analyses. Zn determination was performed by a modified method of Parker et al. (1963) using a Perkin-Elmer Model 603 atomic absorption spectrophotometer (Perkin Elmer Corp., Norwalk, CT).

Table 1: Ingredients of basal diet (%, dry matter basis)

Feedstuffs	Composition (%)
Alfalfa hay	55
Barely, ground	20
Corn, ground	15.75
Soybean meal	1.8
Cottonseed meal	1.8
Wheat bran	4.5
Urea	0.2
Calcium carbonate	0.45
Salt	0.2
Minerals and vitamins mix [a]	0.5
Chemical composition	
Dry matter (%)	89.4
Crude Protein (%)	14.22
Ether extract (%)	2.82
Crude fiber (%)	4.79
Calcium (%)	0.59
Phosphorus (%)	0.38
Zinc (mg/kg DM)	33
Total digestible of nutrients (%)	73.5
Net energy of lactation (M cal/kg DM)	1.77

[a]Premix composition per kg: vitamin A, 500000 IU; vitamin D3, 10000 IU; vitamin E, 100mg; Ca, 190000; P, 90000; Na, 50000; Cu, 300 mg; Fe, 3000 mg; Mn, 2000 mg; I, 100 mg; Co, 100 mg; Se, 1 mg; Mg, 19000 mg; Butylated hydroxytoluene, 3000 mg.

Statistical analysis

The data was analyzed using mixed procedure of SAS (9.1) for a completely randomized block design with repeated measure records. The differences between means were investigated by PDIFF/LSMEANS statement in SAS. Differences were considered statistically significant when $P<0.05$ against control group.

Results

Zn concentration in serum and tissues are given in Table 2. Results showed that serum Zn concentration was significantly ($P<0.05$) higher in lambs fed with 100 mg/kg Zn-P and mixture of Zn-P-S at the rate of 50 mg/kg each. Zn in heart and testis was significantly ($P<0.05$) higher in lambs fed Zn-S (100 mg/kg) and Zn-P-S. In kidney, liver, muscle and spleen, Zn concentration was significantly ($P<0.05$) higher in lambs fed with Zn-P (100 mg/kg), Zn-S (100 mg/kg) and Zn-P-S at the rate of 50 mg/kg each.

Discussion

Blood plasma serves as an immediate source of stored Zn (Rojas et al., 1995). Since blood Zn

Table 2: Effect of two different zinc sources on availability of Zn in serum and different body tissues

Concentration (ppm)	Treatments						SEM
	Control	50 Zn-P	50 Zn-S	100 Zn-P	100 Zn-S	50+50 Zn-P+Zn-S	
Serum	0.96[c]	1.09[b]	1.03[c]	1.15[a]	1.09[b]	1.20[a]	0.04
Heart	44.39[b]	49.08[b]	46.13[b]	50.29[b]	53.05[a]	55.32[a]	0.05
Kidney	52.11[c]	54.4[b]	51.95[b]	56.25[a]	55.53[a]	57.78[a]	0.03
Liver	42.10[b]	43.74[ab]	50.10[ab]	54.81[a]	57.34[a]	59.22[a]	0.04
Lung	22.39[b]	26.08[b]	31.13[b]	28.29[b]	45.05[a]	55.32[a]	0.02
Muscle	62.11[c]	66.24[b]	65.95[b]	71.25[a]	73.53[a]	77.78[a]	0.03
Testis	34.39[c]	39.08[b]	38.13[b]	36.29[b]	43.05[a]	45.32[a]	0.05
Spleen	62.10[b]	65.74[ab]	66.10[ab]	74.81[a]	77.34[a]	79.22[a]	0.04

C, control, Zn-S: zinc sulfate monohydrate, Zn-P: zinc proteinate; [a,b,c]Means in the same row lacking a common superscript differ (P<0.05).

concentration is a popular Zn index in the clinical field, the increased concentration might confirm a tool to evaluate Zn status. Results of this experiment are consistent with previous reports that diets supplemented with 500 mg/kg had 1.22 µg/ml plasma Zn compared to 0.95 µg/ml in lambs kept as control (Ott et al., 1966a). Similarly, Huerta et al. (2002) reported an increase in concentration of Zn in plasma upon supplementation of 200 mg/kg of diet as Zn-methionine compared to control or $ZnSO_4$ supplemented diets in cross bred beef heifers. Other researchers observed that dietary concentration of Zn at the rate of 600 mg/kg of diet nearly doubled the concentration of Zn in plasma of calves (Stake et al., 1975; Ott et al., 1966b). Revy et al. (2002) reported the addition of 10, 20 and 30 mg/kg of Zn in comparison with no supplementation increased plasma Zn concentration by 52, 125 and 164%, respectively. Spears (1989) also found lambs fed Zn-methionine had greater serum Zn concentration than lams fed ZnO. This may imply a higher bioavailability for the organic sources of Zn. Contrary to our observations, Spears and Kegley (2002) reported no difference in serum Zn concentrations due to supplementation of 25 mg of supplemental Zn/kg diet as Zn- proteinate or ZnO in the diet of growing and finishing steers. Beeson et al. (1977) did not find any differences in the serum Zn concentration of beef cattle except when the dietary level was extremely high (300 or 620 mg/kg). The high levels of Zn supplementation used by the researchers would result in increased levels of serum Zn. Malcolm-Callis et al. (2000) also reported no differences in serum Zn concentrations among steers supplemented with 20, 100, or 200 mg/kg of dietary DM as $ZnSO_4$, Zn polysaccharide or a Zn amino acid complex. This may be due to very high levels of Zn Supplementation used by the researchers, which might have an antagonistic effect on Zn absorption.

In contrast, Spears and Kegley (2002) and Mandal et al. (2007) did not find any differences in the plasma or serum Zn concentration in steers and calves respectively in response to Zn supplementation. Therefore, since blood Zn concentration is a popular Zn index in the clinical field, the increased concentration might confirm a tool to evaluate Zn status. Limited data are available on Zn concentration in lamb tissues. The majority of bio-available Zn when supplemented in relatively high levels is stored in body organs such as liver, kidney, and pancreas, with minor storage in bone, muscle, and skin (Ott et al., 1966b).

Limited data are available on Zn concentration in lamb tissues. The results of this experiment were in agreement with previous reports that diets of rats supplemented with 1000 and 2000 mg/kg diet had higher Zn concentrations in liver, plasma, kidney and spleen (Chen et al., 1974). Unlike our results, Jeng and Sun (1981) showed little change in the tissues of spleen, kidney, skin and muscle for the fish fed group 1007 mg of dietary Zn. The results of this experiment indicated that when the dietary Zn concentration was lower than 100 mg/kg diet, the tissues of lambs will not accumulate Zn. Rojas et al. (1995) showed lambs fed organic Zn source (Zn lysine) had the highest (P<0.05) Zn accumulation (581, 389, and 340 mg/kg) in kidney, liver, and pancreas, respectively. These researchers also found Lambs fed $ZnSO_4$ and Zn methionine had higher (P<0.05) liver Zn concentrations (195 and 198 mg/kg, respectively) than the control lambs (127 mg/kg). Liver Zn concentrations in ZnO treated lambs were not different (P>0.05) from that in control, $ZnSO_4$, or Zn Methionine treated lambs. Therefore, this study found organic source of Zn have equal or greater availability than the most available inorganic source.

Conclusions

Results of this experiment showed that Zn availability in serum and tissues was higher in case of organic source when supplemented at the level of 100 mg/kg of diet. Further, the combination of organic and inorganic sources increased the bioavailability of Zn in serum and tissues.

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