



Effects of source and level of zinc on haematological and biochemical parameters in Baluchi lambs

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Abstract

Thirty six growing male Baluchi lambs with an average weight 26.5 ± 0.3 kg were used to determine the level and source of zinc (Zn) supplementation in diet on performance of Baluchi lambs. Animals were divided into six groups. First group served as a control (T1) and fed the basal diet containing 33 mg/kg Zn. Second group was fed 50 mg/kg zinc propionate (T2), third group was fed 100 mg/kg zinc propionate (T3), fourth group was fed 50 mg/kg zinc sulphate (T4), fifth group was fed 100 mg/kg zinc sulphate (T5) and sixth group was fed 50 mg/kg zinc propionate and 50 mg/kg zinc sulphate. Results showed that the packed cell volume (PCV), mean corpuscular volume (MCV), and platelet distribution width (PDW) in T2 ($P < 0.05$) than other groups. Number of red blood cell (RBC), haemoglobin (Hb) concentration, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) were higher ($P < 0.05$) in T3 compared with other groups. Moreover, the number of white blood cell (WBC), platelets (PLT) and the concentration of calcium (Ca), phosphorous (P) and Zn in T6 were significantly high ($P < 0.05$) compared to the other groups. In conclusion, feeding Baluchi lambs, with organic and mixed of inorganic and organic Zn, improved haematological, biochemical parameters, and concentration of zinc in serum of lambs.

Keywords: Lambs; zinc propionate; zinc sulphate; monohydrate; growth; Hematological parameters; biochemical parameters

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Introduction

Supplemental zinc (Zn) is usually added to animal diets in the form of inorganic form. Recently, organically bound Zn supplements have gained fame in animal diet. Some researchers have reported Zn absorption from some organic Zn sources appears to be higher than from inorganic Zn sources when supplemented at high concentrations (Spear, 2003). Lambs supplemented with Zn at the level of 360 mg/kg of diet from zinc lysine had much higher Zn concentrations in kidney, liver and pancreas than lambs that received zinc sulphate, zinc oxide or zinc methionine (Roja et al., 1995). Liver and plasma zinc concentrations were higher in calves supplemented with Zn at the rate of 300 mg/kg diet from a combination of zinc lysine and zinc methionine than zinc oxide

(Kincaid et al., 1997). Higher tissue concentrations of Zn were also seen in calves (Wright et al., 2001) and lambs (Cao et al., 2000) that were fed high concentrations of zinc propionate relative to the Zn concentrations observed in animals supplemented with zinc sulphate.

According to the recommendation of NRC (2007), for optimum production, Zn requirement of sheep is 20-33 mg/kg in the diet. In practice, feed manufacturers use higher concentrations than those specified for the small ruminant (NRC, 2007) to achieve maximum production. Furthermore, little information is available in the literature regarding the toxicological effects of organic and inorganic sources of Zn. Therefore, the objectives of this experiment were to investigate the response of different concentration of organic and inorganic Zn supplements on haematology and biochemical parameters in lambs.

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Materials and Methods

Experimental animals and diets

Thirty six male Baluchi lambs with average body weight of 26.5 ± 0.3 kg were randomly allocated to six groups and each lamb was individually kept in a separate pen having concrete floor. Lambs were provided drinking water *ad libitum*. The dietary treatments were T1: the basal diet (Table 1) without supplementary Zn (BD), T2: BD+50 mg Zn/kg DM as zinc propionate (Alltech Inc., Nicholasville, KY), T3: BD+100 mg Zn/kg DM as zinc propionate) T4: BD+50 mg Zn/kg DM as zinc sulphate (Sigma-Aldrich, Seelze, Germany), T5: BD+100 mg Zn/kg DM as zinc sulphate and T6: BD+50 mg Zn/kg DM as zinc propionate+50 mg Zn/kg DM as zinc sulphate. The basal diet was provided according to NRC (2007) to meet or exceed lambs requirements except Zn (Table 1).

Haematological parameters

Blood samples were collected on day 0, 30, and 60 of the experimental period from jugular vein using vacutainer tubes containing sodium heparin. The samples were placed in an ice bath and centrifuged at $3500 \times g$ for 20 min at 5°C . Anti-coagulated blood was analyzed shortly for the number of white blood cell (WBC) count, red blood cell (RBC) count, haemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet (PLT), Platelet Distribution Width (RDW), Red Blood Cell Distribution Width (RDW), Platelet Crit (PCT), and Mean platelet volume (MPV) by an automatic haematology cell counter (Celltaka, NEK6108K).

Serum biochemical parameters

Blood in Zn free tubes was allowed to clot at ambient temperature (15 to 21°C), centrifuged ($3500 \times g$ for 10 min), and the serum was stored at -20°C for further analysis. Stored serum samples were analyzed for total protein (TP), glucose (GLU), blood urea nitrogen (BUN), total cholesterol (TC), triglyceride (TG), and albumin (ALB). Concentration of calcium (Ca) and phosphorus (P) were also measured. The commercial kits (Pars Azmoon kits; Pars Azmoon, Tehran, Iran) and a Selectra-E auto analyzer (Vital Scientific NV, DIERN, Netherland) were used for measuring these parameters.

Concentration of Zn in serum

Serum Zn concentration was determined by deproteinization of 0.5 ml serum samples with 1 ml of 100 ml/l trichloroacetic acid, centrifuged at $2,500 \times g$ for 10 min. The supernatant was diluted 1:5 with deionized water (Case, 2002). The concentration was

determined by atomic absorption spectrophotometer (Chemtech Analytical CTA 2000, Analytical Co., Kempston, UK).

Statistical analysis

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

Results

Haematological parameters

The results of haematological parameters are shown in Table 2. The number of WBC and MPV T6 were higher than other groups ($P < 0.05$). The number of RBC, HB concentration, MCH, and MCHC were higher ($P < 0.05$) in T3 compared with other groups. The statistical data analysis indicated that the PCV, MCV, and PDW in T4 were greater ($P < 0.05$) than other groups (Table 2). Parameters such as PLT and PCT in T2 were significantly low compared to control and other groups.

Concentration of serum biochemical parameters

In the present experiment, there was significant difference in TP, Ca, and P concentrations (Table 3). Total protein decreased significantly in T4. P and Ca concentration increased significantly in T6 compared to all the other groups.

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

^aPremix 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; BHT antioxidant, 3000 mg.

Table 2: Effects of source and level of zinc on haematological parameters in experimental lambs

Parameters	Treatments						SEM	P value
	T1	T2	T3	T4	T5	T6		
WBC ($10^3/\mu\text{l}$)	16.08 ^b	15.91 ^b	16.62 ^b	15.63 ^b	17.04 ^b	19.22 ^a	0.44	0.04
RBC ($10^6/\mu\text{l}$)	10.01 ^{abc}	10.36 ^{ab}	10.45 ^a	9.38 ^d	9.47 ^{cd}	9.77 ^{bcd}	0.16	0.05
Hb (g/dl)	9.56 ^{bc}	9.75 ^{ab}	10.12 ^a	8.89 ^d	9.09 ^{cd}	9.37 ^{bc}	0.12	0.04
PCV (%)	28.35 ^{bc}	30.00 ^a	29.37 ^{ab}	26.55 ^d	27.03 ^{cd}	27.42 ^{cd}	0.43	0.01
MCV (fl)	28.43 ^{bc}	29.12 ^a	28.18 ^{cd}	28.33 ^{bc}	28.51 ^b	28.08 ^d	0.06	0.03
MCH (pg)	9.60 ^{ab}	9.45 ^c	9.70 ^a	9.50 ^{bc}	9.60 ^{ab}	9.61 ^{ab}	0.03	0.04
MCHC (g/dl)	33.73 ^c	32.50 ^d	34.52 ^a	33.55 ^a	33.77 ^{bc}	34.27 ^{ab}	0.13	0.02
PLT ($10^3/\mu\text{l}$)	531.92 ^a	349.86 ^c	482.56 ^{ab}	432.30 ^b	482.00 ^a	467.50 ^b	14.43	0.01
RDW (%)	16.45	15.97	15.95	16.15	16.42	16.12	0.17	0.03
PCT (%)	0.17 ^a	0.12 ^c	0.14 ^{abc}	0.13 ^{bc}	0.15 ^{ab}	0.14 ^{abc}	0.007	0.05
MPV (fl)	3.23 ^{ab}	3.34 ^{ab}	3.12 ^b	3.09 ^b	3.25 ^{ab}	3.53 ^a	0.08	0.04
PDW (%)	13.20 ^c	13.57 ^a	13.16 ^c	13.25 ^{bc}	13.42 ^{ab}	13.25 ^{bc}	0.05	0.03

T1: Control, T2: 50 mg/kg Zinc propionate; T3: 100 mg/kg Zinc propionate; T4: 50 mg/kg Zinc sulphate, T5: 100 mg/kg Zinc sulphate; T6: 50 mg/kg Zinc propionate and 50 mg/kg Zinc sulphate; Hb, hemoglobin concentration; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MPV, mean platelet volume; PCT, platelet crit; PCV, packed cell volume; PDW, platelet distribution width; PLT, platelet; RBC, red blood cell; RDW, red blood cell distribution width; WBC, white blood cell; *C: control, Zn-S: zinc sulphate monohydrate, Zn-P: zinc propionate; ^{a,b,c}Means in the same row lacking a common superscript differ (P<0.05)

Table 3: Effects of sources and levels of zinc on biochemical parameters in experimental lambs

Parameters	Treatments						SEM	P value
	T1	T2	T3	T4	T5	T6		
Glucose (mg/dl)	71.68	77.58	72.75	75.58	73.15	72.08	2.19	0.14
Albumin (mg/dl)	3.01	3.41	3.33	3.05	3.35	3.11	0.16	3.11
Total protein (g/l)	9.06 ^a	8.55 ^{ab}	8.70 ^{ab}	7.94 ^b	8.59 ^{ab}	8.46 ^{ab}	0.22	0.04
Triglyceride (mg/dl)	17.54	18.57	18.55	13.22	14.83	17.41	2.86	0.24
Total cholesterol (mg/dl)	47.20	44.85	52.31	44.21	42.52	44.38	2.88	0.28
Phosphorus (mg/dl)	4.79 ^{bc}	4.67 ^{bc}	5.20 ^{ab}	4.50 ^c	4.85 ^{abc}	5.45 ^a	0.15	0.03
Calcium (mg/dl)	9.82 ^{ab}	9.72 ^{ab}	9.75 ^{ab}	9.44 ^b	10.20 ^a	10.27 ^a	0.15	0.03
Blood urea nitrogen (mg/dl)	52.68	53.25	50.53	51.75	50.06	50.34	2.67	0.18

T1: Control, T2: 50 mg/kg Zinc propionate; T3: 100 mg/kg Zinc propionate; T4: 50 mg/kg Zinc sulphate, T5: 100 mg/kg Zinc sulphate; T6: 50 mg/kg Zinc propionate and 50 mg/kg Zinc sulphate; ^{a,b,c}Means in the same row lacking a common superscript differ (P<0.05).

Table 4: Effect of source and levels of zinc supplementation on plasma zinc concentration in lambs

Parameter	Treatments*						SEM	P value
	T1	T2	T3	T4	T5	T6		
Zn in serum	0.96 ^c	1.09 ^b	1.15 ^a	1.03 ^c	1.09 ^b	1.20 ^a	0.04	0.04

T1: Control, T2: 50 mg/kg Zinc propionate; T3: 100 mg/kg Zinc propionate; T4: 50 mg/kg Zinc sulphate, T5: 100 mg/kg Zinc sulphate; T6: 50 mg/kg Zinc propionate and 50 mg/kg Zinc sulphate; ^{a,b,c}Means in the same row lacking a common superscript differ (P<0.05).

Concentration of Zn in serum

The relationship between Zn source and level and their concentration in serum indicated that serum Zn concentration increased significantly in T6 (Table 4).

Discussion

Since Zn supplementation has positive effects on haematological characteristics in the present study, we observed no signs and symptoms of anaemia with feeding high Zn concentration in lambs. In general, despite of some differences between the groups for haematological parameters in the present study, these parameters were in the normal range as reported for sheep (Jain, 1998).

Unlike our results, Bremner et al. (1976) reported that higher level of Zn (420 mg/kg) induced depression in haematological parameters such as blood Hb concentration and PCV when compared with diets providing 48 mg/kg Zn in sheep. Also, Southern and Baker (1983) did not find any change in blood Hb or haematocrit of the male chicks supplemented with Zn at the rate of 50 and 100 mg/kg.

Our observation for plasma Ca concentration, Khan (1978) reported an increase in the serum Ca level upon supplementation of Zn in growing calves. On the other hand, Garg and Vishal Mudgal (2008) did not find any significant difference in the serum Ca level in lambs. Therefore, besides of the existence of an antagonistic effect between Zn and some minerals (Thilising-Hansen

and Jørgensen, 2001), results of the present study suggest that supplementing organic form of Zn can decrease the antagonism and improve the retention of Ca and P in blood.

The results of this experiment were consistent with previous reports that diets supplemented with 500 mg/kg of diet had higher plasma Zn compared with control lambs (Ott et al., 1966a). Similarly, Huerta et al. (2002) reported an increase in the concentration of Zn in plasma upon supplementation of 200 mg/kg of diet Zn-methionine compared to control or zinc sulphate in crossbred beef heifers. The other researchers observed that dietary concentrations of 600 mg Zn/kg of diet nearly doubled the concentration of Zn in plasma of calves (Ott et al., 1966b; Stake et al., 1975). In contrast, Spears and Kegley (2002) and Mandal et al. (2007) did not find any difference in the plasma Zn concentration in steers and calves due to Zn supplementation, respectively. 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.

Conclusions

Results from this study suggested that supplementation of organic Zn either alone or in combination with the inorganic Zn in the diet of growing Baluchi lamb significantly improved haematological, biochemical parameters and plasma Zn.

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