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Short communication

Effects of high dietary zinc concentration and zinc sources on hematology and biochemistry of blood serum in Holstein dairy cows

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ABSTRACT

The purpose of this study was to investigate high concentration of dietary zinc (Zn) and to compare the effects of organic and inorganic zinc sources on hematological and biochemical parameters of blood serum in dairy cows. Eighteen dairy cows were randomly allocated to one of three dietary treatments in a randomized and blocked design. Animals in group 1 were treated as control (no zinc supplementation); whereas, animals in groups 2 and 3 supplemented with 500 mg of zinc/kg dry matter from either zinc sulfate monohydrate (ZnS) and zinc methionine (ZnM), respectively. The numbers of red blood cells, hemoglobin concentration, packed cell volume, and mean corpuscular hemoglobin concentration in the ZnM group was higher than the control group. Activities of lactate dehydrogenase and superoxide dismutase in ZnM and ZnS groups were higher ($P<0.05$) than the control group. There was no difference in the other metabolites between groups, but the serum zinc concentration was higher ($P<0.05$) in cows fed ZnM or ZnS. There was no difference among groups for biochemical parameters and some hormones in serum may due to the competition levels of zinc supplementation reducing availability of other minerals. Therefore, the results obtained may be beneficial in demonstrating the effects of zinc with no negative effects of high Zn concentration on hematological and biochemical parameters.

1. Introduction

Supplemental zinc (Zn) is usually added to animal diets in form of inorganic Zn, *e.g.* Zn oxide and Zn sulfate. Organically bound Zn supplements have recently been used in animal diets. Some researchers have reported Zn absorption from some organic zinc sources appears to be higher than those from inorganic zinc sources when supplemented at high concentrations (Spears, 2003).

National Research Council (2001) recommended that level of 40–60 mg of Zn/kg in the diet of lactating dairy cows is necessary. In practice, feed manufacturers supply higher levels of dietary zinc than those specified by NRC (2001) to include the effect of stress and antagonists on zinc availability. Therefore, the objectives of this experiment were to investigate the response of high concentration of organic and inorganic Zn supplements on normal hematology, biochemical parameters, hormonal concentrations, and to determine Zn concentration of blood serum in lactating dairy cows.

Abbreviations: ALB, albumin; ALP, alkaline phosphatase; BUN, blood urea nitrogen; GLU, glucose; Hb, hemoglobin concentration; HDL, high density lipoprotein; LDH, lactate dehydrogenase; LDL, low density lipoprotein; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; PCV, packed cell volume; PLT, platelet; RBC, red blood cell; SOD, super oxide dismutase; TC, total cholesterol; TG, triglyceride; TP, total protein; WBC, white blood cell; ZnM, zinc methionine; ZnS, zinc sulfate monohydrate.

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2. Materials and methods

The study was completed simultaneous with Sobhanirad et al. (2009) and so only a brief summary of the materials and methods are presented here.

2.1. Experimental animals and design

Eighteen Holstein dairy cows were randomly allocated to one of the dietary treatments (Table 1) including: (1) the basal diet without Zn supplementation; 42 mg of Zn/kg of DM, (2) the basal diet plus 500 mg Zn/kg DM as zinc sulfate monohydrate (ZnS) and (3) the basal diet plus 500 mg Zn/kg DM as zinc methionine (ZnM, Zinpro Corporation, Eden Prairie, MN, USA). Animals were housed in individual stalls and blocked by parity (2nd and 3rd). All the experimental cows were in the first phase of lactation (35 ± 3 days after parturition).

2.2. Hematological parameters

Blood samples were collected 2 h after morning feeding from coccygeal vein using vacationer tubes containing sodium heparin. The samples were placed in an ice bath to be centrifuged at $3500 \times g$ for 20 min at 5 °C. Anti-coagulated blood was analyzed after 2 h for the number of white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelet (PLT) by an automatic hematology cell counter (Celltakα, NEK6108K). Manual WBC differential counting was also performed by microscopic examination of Wright–Giemsa-stained smears (Jain, 1998).

2.3. Enzyme activity in blood serum

Blood in Zn-free no-additive tubes was allowed to clot at ambient temperature (15–21 °C), centrifuged ($3500 \times g$ for 10 min), and their serum stored at −20 °C for further analysis. Stored serum samples were analyzed for lactate dehydrogenase (LDH), alkaline phosphatase (ALP), super oxide dismutase (SOD) on a Selectra E auto analyzer (Vital Scientific NV, DIERN, Netherland).

Table 1
Dietary ingredients and chemical composition of the experimental diets.

	Diets (treatments)		
	Control	ZnS	ZnM
Ingredient (g/kg of DM)			
Alfalfa hay	15	15	15
Corn silage	23	23	23
Corn, ground	25	25	25
Soybean meal	18.50	18.50	18.50
Beet pulp	14	13.86	13.50
Protected fat	3	3	3
Urea	0.2	0.2	0.2
Dicalcium phosphate	0.3	0.3	0.3
Salt	0.3	0.3	0.3
Trace minerals and vitamins premix[a]	0.7	0.7	0.7
Zinc sulfate monohydrate[b]	–	0.14	–
Zinc methionine[b]	–	–	0.5
The calculated chemical composition (g/kg of DM)			
Dry matter	65.15	65.15	65.15
Undegradable protein of CP	36.30	36.30	36.30
Net energy of lactation (M cal/kg DM)	1.60	1.60	1.60
Non-fiber carbohydrate	43.6	43.3	43.7
Calcium	1.00	1.00	1.00
Phosphorus	0.40	0.40	0.40
Sodium	0.22	0.22	0.22
Chloride	0.40	0.40	0.40
The measured chemical composition (DM bases)			
Crude protein (g/kg)	16.80	16.80	16.90
Neutral detergent fiber (g/kg)	30.70	30.70	30.70
Acid detergent fiber (g/kg)	18.80	18.80	18.80
Zinc (mg/kg)	42	542	542

[a] Premix composition per kg: vitamin A, 500,000 IU; vitamin D3, 10,000 IU; vitamin E, 100 mg; Ca, 190,000; P, 90,000; Na, 50,000; Cu, 300 mg; Fe, 3000 mg; Mn, 2000 mg; I, 100 mg; Co, 100 mg; Se, 1 mg; Mg, 19,000 mg; BHT antioxidant, 3000 mg.
[b] Zinc sulfate monohydrate (0.14%) and zinc methionine (0.5%) were substituted for beet pulp to provide 500 mg/kg Zn.

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2.4. Some biochemical and hormonal parameters in serum

Total protein (TP), glucose (GLU), blood urea nitrogen (BUN), total cholesterol (TC), triglyceride (TG), and albumin (ALB) contents of serum samples were determined. Concentration of high density lipoprotein (HDL) and low density lipoprotein (LDL) were also measured. The commercial kits (Pars Azmoon kits; Pars Azmoon, Tehran, Iran) and Selectra E auto analyzer (Vital Scientific NV, DIERN, Netherland) were used for measuring these parameters.

Insulin, progesterone, and thyroxin concentrations were analyzed in duplicate using radio-immuno assay with the Coat-a-Count kits (Diagnostic Products Corporation, Los Angeles, CA, USA) following the manufacturer's instructions.

2.5. Concentration of zinc in blood 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 2500 × *g* for 10 min. The supernatant was diluted 1:5 with deionized water (Case and Carlson, 2002) then it was read on a flame atomic absorption spectrophotometer (Chemtech Analytical CTA 2000, Analytical Co., Kempston, UK).

2.6. Statistical analysis

The data was analyzed using the mixed procedure of SAS (9.1) for a randomized block design with repeated measures records. The differences between LS-means of treatments were investigated by PDIFF/LSMEANS statement in SAS.

3. Results and discussion

3.1. Hematological parameters and serum enzyme activity

The results of hematological parameters are shown in Table 2. The number of Hb concentration, PCV, and MCHC in the ZnM group cows were statistically higher than the control group (P<0.05). Number of RBC, Hb, and PCV were also higher (P<0.05) in the ZnM group than the ZnS group. There was no difference between treatments on WBC, MCV, MCH, PLT and leukocyte profile (P<0.05). The concentration of fibrinogen protein in cows fed ZnM was higher (P<0.05) than the allocated

Table 2
Effect of Zn sources on blood hematological parameters, serum enzyme activities, biochemical parameters and concentration of some of the typical hormones in experimental dairy cows.

Parameters	Treatments[a]			SEM	P
	C	ZnS	ZnM		
WBC (/μl)	9055.3	8467.8	9114.3	377.6	0.14
RBC (10^6/μl)	6.07ab	5.88b	6.38a	0.22	0.03
Hb (g/dl)	8.33b	8.25b	8.83a	0.21	0.04
PCV (ml/dl)	25.81b	25.50b	27.60a	0.38	0.002
MCV (fl)	42.35	44.14	43.54	0.91	0.40
MCH (pg)	13.65	14.07	13.93	0.36	0.29
MCHC (g/dl)	31.67b	32.56a	32.76a	0.30	0.05
PLT (10^3/μl)	382,233	382,762	427,946	27,561	0.60
Neutrophile (of WBC)	0.38	0.42	0.43	0.02	0.18
Lymphocyte (of WBC)	0.59	0.53	0.52	0.03	0.13
Monocyte (of WBC)	0.02	0.02	0.03	0.04	0.76
Eosinophyle (of WBC)	0.03	0.02	0.03	0.005	0.35
Fibrinogen (mg/dl)	422.2b	433.3b	627.7a	58.06	0.02
Lactate dehydrogenase (IU/l)	704.2b	733.57a	740.9a	11.57	0.05
Super oxide dismutase (IU/l)	118.31b	140.51a	144.61a	1.51	<0.0001
Cholesterol (mg/dl)	227.11	227.48	239.35	13.14	0.74
Triglyceride (mg/dl)	22.47	24.52	24.37	1.46	0.58
Blood urea nitrogen (mg/dl)	40.59	39.60	39.06	1.69	0.81
High density lipoprotein (mg/dl)	91.70	91.03	93.74	1.67	0.50
Low density lipoprotein (mg/dl)	74.86	90.19	86.60	7.49	0.35
Albumin (mg/dl)	28.00	28.10	29.33	0.46	0.12
Glucose (mg/dl)	58.19	58.60	59.74	1.35	0.70
Total protein (g/l)	66.23	66.74	67.08	2.63	0.96
Insulin (μg/dl)	0.98	1.04	1.05	0.04	0.43
Progesterone (ng/ml)	2.40	2.46	2.50	0.26	0.97
Thyroxin (ng/ml)	67.98	69.85	70.01	0.95	0.29
Cholesterol (mg/dl)	227.11	227.48	239.35	13.14	0.74

Hb, hemoglobin concentration; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; PCV, packed cell volume; PLT, platelet; RBC, red blood cell; WBC, white blood cell.
(a,b) Numbers with different letters in the same row differ (P<0.05).
[a] C: control, ZnS: zinc sulfate monohydrate, and ZnM: zinc methionine.

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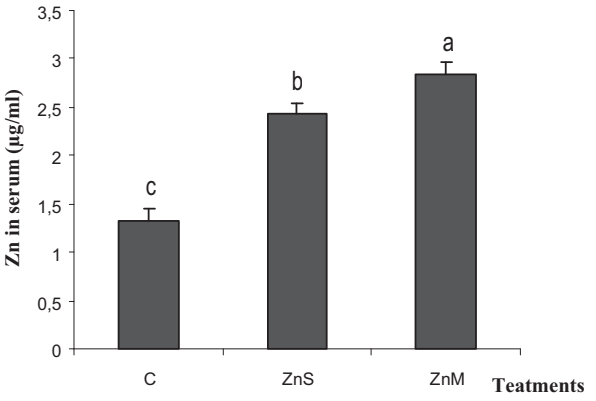

Fig. 1. Effects of Zn sources on zinc concentration of serum in dairy cows (treatments: C: control, ZnS: zinc sulfate monohydrate, ZnM: zinc methionine). [a,b,c]Numbers with different superscripts differ (P<0.05).

cows to control and ZnS groups (628 *vs.* 422 and 433 mg/dl, respectively). Similar to our results, the addition of 2000 mg Zn/kg of diet did not affect blood hemoglobin or PCV in chicks (Southern and Baker, 1983). In the present study, despite of some differences between the groups (P<0.05) for hematological parameters, these parameters were in the normal range of levels reported for cows (Jain, 1998). Difference among the groups (P<0.05) for hematological parameters increased availability of zinc from organic zinc sources.

Activities of LDH, SOD in ZnM and ZnS groups were higher (P<0.05) in comparison with the control group, but no difference was detected between ZnM and ZnS treatments for LDH and SOD activities (Table 2). Similar to our results, Xu and Wang (2001) who supplemented pigs with 3000 mg zinc/kg from zinc oxide observed that the activity of SOD enzyme is increased (P>0.01). It was known LDH and SOD have antioxidant effects; these metalloenzymes have been shown to have a protective effect, preserving cells from damage (Shaheen and El-Fattah, 1995). Therefore, in the present study, the increased activities of LDH and SOD in supplemented cows with 500 mg zinc/kg may confirm the influence of Zn supplements on the metabolic functions.

3.2. Concentration of serum biochemical parameters and hormones

Although in our study, some of the biochemical parameters were slightly (P>0.05) elevated in ZnM supplement group, there were no differences between treatments in concentrations of TP, GLU, BUN, TC, TG, ALB, HDL and LDL in blood serum (Table 2).

Liver is the main organ for the synthesis and storage of GLU, TP, ALB, TC, HDL and LDL concentrations. Therefore, no effect of treatments on serum biochemistry suggest that the Zn supplements did not effect on the enhancement or the reduction of synthesis of protein and fat metabolism in animals (Brown and Clinc, 1974).

In this experiment, no difference was detected between treatments of insulin, progesterone, and thyroxin (T4) concentrations (Table 2) although the mentioned factors were numerically (P>0.05) higher in dairy cattle received ZnM and ZnS. Therefore, in the present study, zinc supplementation had no influence on the hormonal concentrations.

3.3. Concentration of zinc in blood serum

The Zn serum concentration for ZnM (2.84 μg/ml) and ZnS (2.43 μg/ml) groups were greater than the control (1.33 μg/ml) group (P<0.05), as illustrated in Fig. 1. The concentration of Zn serum was increased when experimental diet was supplemented with ZnM compare to ZnS (P<0.05). The other researchers reported 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). Lambs fed diets with 500 mg supplemental Zn/kg of diet had 1.22 μg of Zn/ml of plasma, compared with 0.95 μg of Zn/ml of plasma in control lambs (Ott et al., 1966a). Therefore, since blood Zn concentration is a popular Zn index in the clinical field, the increased concentration might to be a tool to evaluate Zn status.

4. Conclusions

In conclusion, the data obtained may be beneficial in demonstrating the effects of Zn with no negative effects of high Zn concentration on hematological and biochemical parameters. Increased proliferation of RBC and Hb, increased concentration of fibrinogen, and increased lactate dehydrogenase and superoxide dismutase activities confirmed the effects of organic supplements on metabolic functions.

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