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Effects of Supplemental Zinc on Growth, Serum Glucose, Cholesterol, Enzymes and Minerals in Broilers

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Abstract: The effects of zinc supplementation on growth and serum parameters were investigated in Ross PM3 broiler chicks. 60 day old chicks were assigned to four treatments of 0, 20, 40, 80 ppm supplemental zinc. Blood from wing vein was analyzed for serum glucose, cholesterol, enzymes and minerals. Zinc supplementation had no significant effect on live weight and 80 ppm zinc lowered the serum glucose concentrations. Cholesterol levels slightly decreased in zinc supplemented groups. Serum alanine aminotrasferase activity remained unchanged but aspartate aminotransferase activity was increased and alkaline phosphatase activity was decreased in zinc supplemented chicks. Gamma-glutamyltranspeptidase activity was also decreased in all supplemented groups. Significant reductions occurred in serum calcium, magnesium and inorganic phosphorus levels. None of the supplemental zinc levels had significant effect on iron and copper concentrations.

Key words: Zinc, growth, serum parameters, broilers

Introduction

It is well documented that zinc (Zn) is an essential trace element for the growth and development of plants, animals and human being, needed for various physiological functions including bone formation, host defence, sexual maturity, reproduction and tissue growth (McDowell,1992).

Zinc is commonly added to all formulated animal diets. Mohanna and Nys (1999) reported that under normal commercial dietary condition, 94 % of the ingested zinc is excreted due to high amount of zinc ingested and low utilization of the element. They also indicated the risk of soil phytotoxicity, which may result from manure of poultry that contain the large amounts of zinc higher than plant requirements. Although the zinc requirement of chicks varies depending upon the nutrient contents as well as protein sources in the diet, Dewar and Downie (1984) reported that the zinc requirement of broiler for maximal live weight was 18 mg/kg diet, and 24 mg/kg diet for maximal zinc concentration in blood serum. It was reported that 40 mg Zn/kg diet is optimal for growth of chicks (McDowell,1992). Using data from a single sampling time to determine the effects of supplementation may lead to disparity. This study was designed to investigate the effects of the moderate levels of supplemental zinc on growth and serum constituents and interaction of zinc with some minerals in broilers on practical diet at repeated sampling times.

Materials and Methods

Sixty days old Ross PM3 broiler chicks were assigned to four treatments. Chicks, 15 in each group, were kept in separated pens on floor and maintained on a 24 h constant light schedule. Stainless-steel feeders and plastic waterers were used. Chicks recieved 0 (control), 20, 40 and 80 ppm supplemental zinc in reagent grade of $ZnSO_4.7H_2O$ (Berko İlaç ve Kimya San. Ltd., Şti., İstanbul, Turkey) in drinking water containing 0.048 ppm zinc and fed on commercial diet (TableI) containing 109 mgZn/kg. Body weight of chicks was measured and blood was collected from wing vein at days 20 and 35. Sera were stored at $-20°C$ until analysis, after separation by centrifugation at 3000 rpm for 10 minutes following one hour incubation at 37°C.

Serum glucose, cholesterol, calcium (Ca), inorganic phosphorus (P), magnesium (Mg) concentrations and aspartate aminotransferase (AST), alanine aminotrasferase (ALT) gamma-glutamyltranspeptidase (GGT) and alkaline phosphatase (ALP) activities were determined spectrophotometrically using commercial kits. Serum iron concentrations were measured using Buck Scientific 200A atomic absorption spectrophotometer (AAS) as described by Fairbanks and Klee (1994) and serum copper (Anonymous, 1983), water and diet zinc concentrations (AOAC, 1998) were determined by SP9 Series Pye Unicam AAS.

Statistical analyses were performed using SPSS 9.0 software (Anonymous,1998). The data were subjected to variance analysis (General Linear Model). When significant F values were obtained, comparisons were made by New Duncan's multiple range test (DMR) and all data were expressed as mean $\pm$ SEM.

Results

The results are summarised in Table 2. Zinc supplementation had no significant effect on live weight throughout the experiment, however it was slightly increased in all treatment groups on day 35. Serum glucose concentrations did not change in 20 and 40 ppm zinc supplemented groups at both sampling times, but 80 ppm zinc supplementation significantly lowered ($p<0.001$) serum glucose concentration throughout the experiment. Serum cholesterol levels slightly decreased in all treatment groups at both sampling times, but a significant decrease ($p<0.05$) was observed only in 40 ppm zinc supplemented group on day 35.

Zinc supplementation had no significant effect on serum ALT activity. On the other hand there was an increase in AST activity ($p<0.05$) and decrease in ALP activity ($p<0.05$ in 20 ppm zinc supplemented group at the first sampling time but no significant differences was observed thereafter. All levels of additional zinc reduced the serum GGT activity overall the experiment. The magnitude of the reduction was higher ($p<0.001$) at the first sampling time than the second one ($p<0.05$).

Significant reductions occurred in serum calcium ($p<0.01$) and magnesium ($p<0.001$) concentrations on day 20, but a fluctuation in results was observed on day 35. Zinc supplementation did not influence the inorganic phosphorus

Table1: Ingredients of diet (0, 20, 40 and 80 ppm Zinc supplemented) fed to Broilers

Ingredients	%	Calculated Nutritional Values	%
Maize	55.552	Crude protein	20.500
Full fat soya	20.000	Digestible crude protein	16.500
Soybean meal	12.718	ME(kcal/kg)	3000.018
Meat-bone mill	5.354	Crude cellulose	3.655
Cottonseed meal	3.000	Crude fat	7.813
Fish oil	1.093	Crude ash	5.910
Fish mill	1.000	Ca	1.000
Ground limestone	0.500	P	0.708
Salt	0.350	Digestible P	0.334
Vitamin-mineral mix*	0.250	Methionine	0.440
Antibacterial-anticoccidial	0.100	Sistine	0.367
Methionine	0.083	Lysine	1.305
Total	100.00	Na	0.206
Analyzed Nutritional Values			
Crude protein	21.27		
Crude cellulose	5.06		
Crude ash	6.30		
Ca	1.00		
P	1.20		
Zn (mg/kg)	109.00		

*: Vitamin-mineral premix provided per kg of the diet : vitamin A, 15 000 IU; vitamin D3, 2000 IU; vitamin E, 20 mg; vitamin K3, 5 mg; vitamin B1, 2.5 mg; vitamin B2, 7.5 mg; vitamin B6, 5 mg; vitamin B12, 0.020 mg, folic acid, 0.75 mg; calcium pantothenate, 10 mg; ascorbic acid, 50 mg; monensin sodium, 100 mg; cholin chloride, 400 mg; nicotinamide, 25 mg; D-biotin 0.05 mg; manganese, 80 mg; iron, 40 mg; zinc, 60 mg; copper, 5 mg; iodine, 0.4 mg; selenium, 0.15 mg; cobalt 0.1 mg;antioxidant,10 mg.

Table 2: Mean values of live weight and serum parameters of 0, 20, 40 and 80 ppm Zinc supplemented Broilers

Parameters and sampling days	Supplemental Zn (ppm)					
	n	0	20	40	80	p
Live Weight (g)						
20	15	486.0±7.42	480.33±4.46	472.67±11.65	472.0±11.41	
35	15	1135.33±22.40	1171.67±16.01	1188.33±30.00	1178.67±31.92	
Glucose (mg/dl)						
20	15	185.77±4.35^{a}	177.97±6.24^{a}	173.75±6.45^{a}	145.89±8.82^{b}	***
35	15	134.44±5.37^{a}	134.11±4.23^{a}	131.98±3.51^{a}	101.40±5.56^{b}	***
Cholesterol (mg/dl)						
20	15	131.60±6.33	127.70±3.93	118.91±8,97	118.81±4.23	
35	15	128.57±7.19^{a}	107.79±2.61^{ab}	92.95±4.38^{b}	111.75±6.33^{ab}	*
AST (U/L)						
20	15	220.96±21.71^{b}	278.74±14.98^{a}	217.41±8.86^{b}	232.43±18.10^{ab}	*
35	15	236.32±16.97	278.99±32.08	266.85±17.24	245.99±12.87	
ALT (U/L)						
20	15	10.81±3.08	8.87±3.08	7.42±1.80	13.74±2.51	
35	15	11.34±4.55	14.54±5.34	8.12±2.07	6.87±1.66	
GGT (U/L)						
20	15	28.47±2.73^{a}	21.92±3.12^{b}	14.99±0.99^{c}	13.67±0.98^{c}	***
35	15	34.19±5.10^{a}	19.84±2.72^{b}	21.35±3.51^{b}	21.35±1.59^{b}	*
ALP (U/L)						
20	15	4663.6±673.3	^{a}2858.03±125.34^{b}	3343.1±268.50^{ab}	3892.4±568.63^{ab}	*
35	15	2323.58±350.21	1855.05±479.32	1647.32±159.67	2133.24±218.62	
Calcium (mg/dl)						
20	15	9.23±1.57^{a}	4.68±0.29^{b}	5.34±0.66^{b}	5.94±0.52^{b}	**
35	15	6.47±0.87	6.87±0.71	8.13±0.69	5.31±0.69	
Phosphorus (mg/dl)						
20	15	11.80±0.80	10.31±1.38	10.48±0.96	12.69±0.81	
35	15	14.62±0.98^{a}	11.18±0.85^{b}	11.73±0.71^{b}	9.88±0.96^{b}	**
Magnesium (mg/dl)						
20	15	2.16±0.06^{a}	1.67±0.05^{b}	1.64±0.05^{b}	1.44±0.05^{c}	***
35	15	1.75±0.07^{b}	1.84±0.10^{ab}	1.89±0.17^{a}	1.68±0.15^{b}	*
BIron (µg/ml)						
20	10	3.26±0.32	2.59±0.19	3.08±0.55	3.30±0.33	
35	10	2.86±0.18	3.66±0.52	3.21±0.42	3.10±0.20	
Copper (µg/ml)						
20	10	0.31±0.03	0.33±0.06	0.30±0.02	0.29±0.02	
35	10	0.29±0.02	0.33±0.03	0.34±0.04	0.37±0.03	

^{abc}: Mean values in the same row with different superscripts differ significantly. *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$

concentrations at the first sampling time however, a decrease ($p<0.01$) was determined on day 35. None of the supplemental zinc levels had significant effect on serum iron and copper concentrations.

Discussion

Mohanna and Nys (1999) reported increased body weight gain and food intake until the total dietary zinc content raised to 45 mg/kg and they found no further responses at higher zinc concentrations. In the present study, chicks in control or treatment groups were neither depleted before the experiment nor raised on purified or semi-purified diet and zinc intake of the chicks was highly over the recommended level of 40 mg Zn/kg diet for broiler chicks even in the lowest supplemental Zinc level. It is possible that chicks, which are depleted or on purified and semi-purified diets may well respond to zinc supplementation. Cao *et al.* (2000) reported decreased feed intake and daily weight gain in chicks given 600 mg Zn/kg, while dietary zinc concentration up to 400 mg/kg had no effect on food intake and growth.

In the present experiment, lowered glucose level may either partly result from the depressed pancreatic enzyme activities by excess zinc or increased zinc uptake of pancreas with dietary zinc concentration (Lü and Combs,1988) because of the putative effect of zinc on insulin metabolism (Keen and Graham,1989) indicating the increased glucose utilization.

The relationship between dietary zinc and plasma cholesterol homeostasis is not well characterized. Zinc supplementation did not influence serum cholesterol in chicks (Lü and Combs,1988). In contrast, reduced serum total cholesterol concentrations in the present study was in agreement with the work of Boukaiba *et al.* (1993). Rupic *et al.* (1997) found a decrease in the activity of both AST and ALT in zinc depleted pigs but observed no correlation between serum zinc. In the present study, the AST activity increased significantly in only 20 ppm zinc supplemented group on day 20, while ALT activity was not elevated, as stated in the study of Lü and Combs (1988), which indicates no liver damage. In contrast to these findings, decreased GGT activity in all zinc supplemented chicks with the most pronounced decreases on the first sampling day is consistant with the findings of Rupic *et al.* (1997) who reported a negative correlation between GGT activity and serum zinc level. A study conducted in our laboratory (Uyanİk *et al.*, 2000) showed that serum zinc levels were higher in 20 and 40 ppm zinc supplemented chicks. Mohanna and Nys (1999) found no effects of 10, 25 or 40 mg/kg supplemental zinc as sulphate on ALP activity as in this study, which confirms that a severe zinc deficiency is necessary to affect the ALP activity (Mohanna and Nys, 1999).

Zinc homeostasis is regulated by a zinc binding protein, metallothionein (high zinc intake induce intestinal and liver metallothionein synthesis) (Cao *et al.*,2000) which is associated with zinc absorption. The reductions in serum calcium, inorganic phosphorus and magnesium concentrations in this experiment may be due to the diminished absorption of these elements because of the competition for similar binding sites (Prasad,1985) or incorporation of them into skeletal tissue or both.

High levels of zinc intake depresses copper absorption and iron uptake (Underwood, 1977). Tissue contents of copper and magnesium (Burch and Sullivan,1976) were affected by deficiency, tissue iron and copper (Gibson *et al.*, 1986) were affected by excess zinc in chicken. In contrast, zinc supplementation had no significant effect on serum copper and iron levels in this study. In chick studies, Stahl *et al.* (1989) showed that moderate amounts of zinc (100 mg Zn/kg) had no effect on soft tissue accumulation of copper and iron. In conclusion, since moderate levels of zinc supplementation did not improve growth but altered some serum parameters, no further zinc supplementation is of value in broilers under the practical dietary conditions.

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